# Chimpanzee Self-Medication in Budongo Forest: Investigating the Use of Putative Self-Medicative Resources in the Sonso and Waibira Communities


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## GENERAL STATEMENT OF AUTHORSHIP:

By signing this statement of authorship, I certify that the candidate made substantial contributions and had either a leading or co-leading role in all co-authored publications for conceptualization, data curation, analysis, and writing, and confirm that the above information and author contributions outlined in the publications are correct.

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## ETHICAL APPROVAL AND PERMISSIONS:

Data used in this study were collected by EF with the approval by the Uganda Wildlife Authority (Uganda Wildlife Authority permit number: COD/96/05) and the Uganda National Council for Science and Technology (permit number: NS257ES). The study was purely observational and adhered to the Code of Best Practices in Field Primatology (Riley et al., 2014). All applicable international and national guidelines were followed. In accordance with the Uganda Plant Protection and Health Act (2016) and the Nagoya Protocol (2014), botanical samples were exported under permits issued by the Ministry of Agriculture (license/authority numbers: 00005033/93/PC and 00008007). A CUREC application was submitted and approved by the University of Oxford to conduct ethnobotanical interviews in the forest-edge villages of Maram and Nyabyeya (Ref No.: SAME_C1A_22_080). Consent forms were signed authorizing use of information from all interviews in future publications. The authors report no conflict of interest.

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# GLOSSARY:

| Term | Definition |
|---|---|
| **Antimicrobial Resistance (AMR)** | The ability of microorganisms, such as bacteria, viruses, parasites, and fungi, to develop resistance to the drugs (antibiotics, antivirals, antifungals, etc.) that are designed to inhibit their growth or kill them (Chapter 5). |
| **Anthelminthic** | A drug or compound with anti-parasitic properties. |
| **Antibacterial Growth-Inhibition Assays** | An assay which measures the ability of an extract to inhibit the growth of a bacterium through the production of antimicrobial compounds (Chapter 5). |
| **APRIORI Analysis** | A data mining and machine learning technique used for association rule mining in transactional databases (Chapter 4). |
| **Bark Stripping** | A behavior which describes peeling or sustainable stripping of bark to gain access to the cambium around the trunk of woody plants (Lapuente et al., 2020; Nishida, 1976). |
| **Bitter-Pith Chewing** | A self-medicative behavior in which the bitter-pith of a plant is chewed in order to access the phytochemical secondary metabolites (Huffman & Seifu, 1989). |
| **COX-2 Anti-Inflammatory Assays** | Assays which measure inhibition Cyclooxygenase-2, an enzyme which speeds up the production of prostaglandins, promoting inflammation (Chapter 5). |
| **Diameter at Breast Height (dbh)** | A standard measurement for trees referring to a tree's diameter at ~4.5 feet above ground (Chapter 6). |
| **ESKAPE Pathogens** | A highly virulent set of bacterial strains which are resistant to many available antibiotics **(**Schultz et al., 2020) (Chapter 5). |
| ***Essential Nutritional or Mineral Need Hypothesis (Related to Bark Ingestion)*** | Hypothesis which attributes bark exploitation to the targeted acquisition of specific nutrients or minerals which are otherwise environmentally scarce (Au et al., 2017; Ciani et al., 2001; Nichols et al., 2016) (Chapter 6). |
| **Fallback Food** | Resources of relatively low priority that are seasonally consumed when preferred resources are unavailable (Marshall et al., 2009). |
| ***Fallback Food Hypothesis (Related to Bark Ingestion)*** | Hypothesis which argues that bark stripping is a generalized subsistence behavior employed during periods of food shortages, classifying bark as a fallback food (e.g., Jiang et al., 2005; Nishida, 1976) (Chapter 6). |
| **Geophagy** | The intentional consumption of earth materials, including soil, earthen mounds (e.g., termite, ant cicada), nests (e.g., ovenbirds), chalk, and clay (Pebsworth et al., 2018). |
| **Huffman's Criteria for Establishing a Self-Medicative Resource** | Criteria published by Michael Huffman (1997) outlining guidelines to conclusively determine intentional self-medicative behaviors (Chapter 2). |
| **Half-maximal inhibitory concentration ($IC_{50}$ Value)** | A pharmacological term representing the concentration of a substance (such as a drug or a chemical compound) required to inhibit a specific biological or biochemical function by 50% (Chapter 5). |
| **Leaf Swallowing** | A self-medicative behavior in which a hispid leaf is folded and swallowed and is then excreted whole or undigested (Wrangham & Nishida, 1983). |
| **Medicinal Food** | Items in the diets of humans and primates, with both nutritional and medicinal properties that are ingested particularly during times of the year when the medicinal properties could be of benefit to those who ingest them (Etkin, 1996; Huffman, 1997). |
| **Minimum Inhibitory Concentration (MIC Value)** | A term used in microbiology to determine the lowest concentration of an antimicrobial agent (such as an antibiotic) that can effectively inhibit the growth of a specific microorganism, typically a bacterium or fungus. The MIC value is an important measure for assessing the susceptibility of microorganisms to antimicrobial agents and guiding antibiotic therapy (Chapter 5). |
| **Multiple Distinctive Collocation Analysis (MDCA)** | An analysis which tests the attraction between units using one-tailed exact binomial tests applied to each possible bigram combination (Gries, 2014) (Chapter 4). |
| **Non-steroidal anti-inflammatory drugs (NSAIDs)** | A class of medications commonly used to reduce inflammation, relieve pain, and lower fever. They work by blocking enzymes called cyclooxygenases (COX enzymes), which are involved in the production of substances called prostaglandins (Chapter 5). |

| | |
|---|---|
| **Plant Secondary Metabolites (PSM)** | Plant compounds that do not affect the normal growth and development of a plant but reduce the palatability of the plant tissues in which they are produced. These metabolites act as defense mechanisms, countering herbivore and insect attacks, through their toxic, repellent, and/or antinutritional effects (Krief et al., 2005). |
| **Preventive Self-Medication (Passive Prevention)** | Self-medication in which the medicating individual is not sick, but which involves the ingestion or utilization of a resource which may help prevent future illness (Huffman, 1997, 2007). |
| **Proglottid** | A segment in the strobila of a tapeworm which contains a complete sexually mature reproductive system. |
| **Putative Therapeutic Resource** | A term used in this thesis to describe unusually consumed resources that appear to have bioactive or mechanical properties and low nutritional value, but which have not yet been associated with distinctive self-medicative behaviors, or empirically proven to resolve a specific illness. |
| **Resource of Interest (ROI)** | Resource consumed rarely by chimpanzees which either possesses established medicinal properties, was consumed using uncommon food processing techniques, and/or was ingested by an individual with a high or diverse parasite load (Chapter 4). |
| **Self-Medication** | The use of natural materials or chemical substances to reduce or eliminate deleterious symptoms of parasites or pathogens (Huffman & Seifu, 1989; Huffman et al., 1993). |
| ***Self-Medication Hypothesis (Related to Bark Ingestion)*** | Hypothesis which proposes that bark may have pharmacological properties that provide consumers with therapeutic benefits (Huffman, 1997) (Chapter 6). |
| ***Self-Medicative Resource Combination Hypothesis*** | Hypothesis proposing that medicinal resource combinations could play an important role in maintaining chimpanzee well-being homeostasis (Chapter 4). |
| **Staple Foods/Preferred Foods** | Foods eaten in large amounts year-round or at specific times of year (Marshall & Wrangham, 2007). |
| ***Stressed-Tree Hypothesis (Related to Bark Ingestion)*** | Hypothesis which posits that bark of trees which have experienced ecological stress during development are selected for ingestion due to the high levels of nutrients and amino acids present in their phloem (White, 2019) (Chapter 6). |
| **Therapeutic Self-Medication** | Self-medication performed after an individual becomes infected or diseased (Huffman, 1997, 2007). |
| **Well-Being Homeostasis/ Homeostatic Perspective** | A general framework for explaining the intake of potentially harmful secondary metabolites despite high physiological costs. The model derives from the theory that animals are constantly struggling to maintain a state of equilibrium with their environment (Villalba & Provenza, 2007). |
| **Zoopharmacognosy** | The study of the use of medicinal plants by animals (Rodriguez & Wrangham, 1993). |

# Abstract

This DPhil project explores established and putative self-medicative behaviors amongst wild chimpanzees (*Pan troglodytes schweinfurthii)* in the Budongo Forest, Uganda. The study spans eight and a half months of multidisciplinary data from two wild chimpanzee communities, Sonso and Waibira, supplemented by thirty years of longitudinal data. The literature review contextualizes Zoopharmacognosy, the study of non-human self-medication, tracing its epistemic origins. Additionally, it summarizes previously established chimpanzee self-medicative behaviors and explores theoretical frameworks for understanding the evolutionary origins of non-human self-medication. Following the literature review, empirical studies are presented, reporting novel healthcare behaviors, introducing new methods for identifying putative medicinal resources, investigating pharmacological properties of suspected medicinal resources, and assessing the medicinal value of bark in chimpanzee diets.

These multidisciplinary studies not only advance our comprehension of chimpanzee self-medication but also have broader implications for paleoanthropology and conservation. Beyond enriching our general understanding of chimpanzee behavior, our findings may also lend insight into the evolutionary origins of modern human medicine. Using chimpanzees to help model the medicinal behaviors of our early hominin ancestors may provide valuable insights into our

ancestors' exploitation of natural remedies. This may also help to illuminate potential selection pressures on the development of these behaviors and more generally on modern-human healthcare systems. Finally, our research has direct implications for conservation efforts, informing the protection of medicinal plant resources in a rapidly deforested world.

# 1 Introduction

The ability to self-medicate is not unique to *Homo sapiens*. Over the last few decades, self-medication has been observed in a variety of primate and non-primate species and throughout several localities (Huffman, 2016). Non-human self-medication takes many forms and involves a variety of medicinal resources (De la Fuente et al., 2022; Huffman, 2022). In chimpanzees (*Pan troglodytes)*, there are two well-established self-medicative behaviors: *leaf swallowing* (Wrangham & Nishida, 1983), which can be performed using multiple plant species, and *bitter-pith chewing*, which specifically involves ingestion of *Vernonia amygdalina* (Huffman & Seifu, 1989). Both behaviors are considered *therapeutic* as they are performed by already infected individuals (Huffman, 2016; 2019) (see **Chapter 2: Section 2.2.1**). These behaviors meet all current criteria for intentional self-medication proposed by Huffman (1997; 2019) (see **Chapter 2: Section 2.1.2**).

Beyond the two established behaviors, many other resources have been proposed as forms of therapeutic self-medication for non-human animals. These resources are not always associated with specific processing techniques or behaviors. However, as the burden of proof for establishing novel self-medicative resources is high and these events are often difficult to directly observe in wild contexts, many proposed self-medicative resources remain putative. Throughout this project we refer to resources that are believed to ameliorate illness in infected or wounded individuals but

that do not yet meet all criteria as *putative therapeutic resources* (see **Chapter 2: Section 2.2.2**). In a meta-analysis of papers documenting cases of proposed self-medication in non-human primates, De la Fuente et al. (2022) identified 574 botanical species used by 25 primate species which classify as putative therapeutic resources.

In addition to therapeutic self-medication, Huffman (1997) proposed the term *passive prevention* to refer to prophylactic use of a resource by healthy individuals to prevent future illness. The term *medicinal food* is used to refer to resources with health-enhancing properties, usually common in the diet, that are associated with this form of self-medication (Huffman, 1997, 2019) (see **Chapter 2: Section 2.2.3**). *Passive prevention,* however, remains understudied as it is difficult to differentiate between medicinal food consumption and normal feeding behaviors. Demonstrating intentionality of passive prevention has proved particularly difficult.

There have been many methodological challenges which have delayed progress in the field of Zoopharmacognosy, a field devoted to the study of non-human self-medication. For one, as there currently are only two established self-medicative behaviors in chimpanzees, and both are relatively uncommon, gathering large enough sample sizes to run statistical analyses has been very challenging. This has prevented further investigation into important aspects of non-human self-medication. Increasing the number of established self-medicative behaviors through continued investigation into putative therapeutic resources, therefore, is crucial for the advancement of the field. However, as Huffman's (1997, 2019) criteria are inherently multidisciplinary, evaluating specific putative medicinal resources requires a holistic, collaborative, and naturalistic approach. Historically, Japanese primatology has been more open to anecdotal and qualitative studies, valuing observational data and emphasizing interconnectedness of all living things. Fundamental to this approach has been to contextualize primate behavior in the context of their ecosystems (Matsuzawa & McGrew, 2008). The epistemic culture of Western primatology has generally discouraged research that does not have a definitive, statistically significant outcome (Kutsukake, 2010).

This DPhil project, titled **Chimpanzee Self-Medication in Budongo Forest**: **Investigating the Use of Putative Self-Medicative Resources in the Sonso and Waibira Communities;** utilizes

8.5 months of multidisciplinary data collected from the Sonso and Waibira eastern chimpanzee (*Pan troglodytes schweinfurthii*) communities living in Budongo Forest, Uganda, over two field seasons as well as thirty-years of longitudinal data taken throughout the duration of the field station's operation. This thesis consists of a literature review, followed by four independent empirical studies which each seek to identify and/or evaluate site-specific putative self-medicative resources. The project takes a natural historical approach to investigating non-human self-medication, emphasizing the importance of integrating qualitative and quantitative approaches when studying behaviors with ecological components.

The ***Literature Review*** provides the reader with a general overview of the field, which includes an historical overview of zoopharmacognosy's epistemic origins and describes how criteria for establishing a non-human self-medicative resource became formalized. It then describes the two established chimpanzee self-medicative behaviors and reviews the cases for several additional putative medicinal resources proposed in the literature. To acquaint the reader with a general understanding of chimpanzee health, the chapter then provides an overview of common illnesses and sickness behaviors observed in chimpanzees across sites. It concludes with a discussion of the theoretical frameworks used to understand the evolutionary origins of these behaviors in human and non-human primates.

The first empirical chapter, titled ***Healthcare Behaviors in the Wild Chimpanzees of Budongo Forest***, reports novel findings of established and putative self-medication in Budongo, providing background and context for the following studies by establishing what is known and not known about healthcare behaviors at the site. We ask: *What is the current state of self-medication research in Budongo?* Using health data collected in the field, we provide a general overview of the current pathogenic and injury-related threats facing Budongo chimpanzees. Then, using both archival anecdotes and our own behavioral and health data, we catalog all documented cases of leaf swallowing behaviors amongst the Sonso chimpanzees and report the current absence of this behavior in Waibira. We then catalog all known cases of self-directed ingestion-based and wound care behaviors. Finally, for the first time, we establish in this chapter the presence of social-care at Budongo with observations of wound related caregiving. The chapter sets the stage for this project, emphasizing the need for further investigation into putative self-medicative resources at

Budongo. It also demonstrates the critical role of anecdotal accounts in zoopharmacognosy research, highlighting the need for more site-specific reports and cross-site collaborations.

With the historical and current self-medicative behavioral landscape established, the next empirical chapter, ***Applying Collocation and APRIORI Analyses to Chimpanzee Diets: methods for investigating non-random food combinations in primate self-medication***, interrogates how holistic and naturalistic perspectives can help advance the field of zoopharmacognosy. We address the historic limitation of small sample sizes for putative self-medicative events by proposing a paradigm-shift in how we interpret chimpanzee feeding ecology data. Instead of assessing individual feeding events in a vacuum, we argue that chimpanzees could intentionally combine resources or medicinal foods in non-random combinations to target synergistic or complementary effects. If this hypothesis holds true, then identifying dietary items which combine with established self-medicative resources could lead to the discovery of novel medicinal resources. This chapter introduces two methods, Collocation and APRIORI analyses, which can test this hypothesis, evaluating their efficacy using feeding data from Budongo as a case study. We ask: *Can non-random resource combinations be identified in the diets of Budongo Chimpanzees?* and *are Collocation and APRIORI analyses effective for testing this hypothesis*?

In the following study, titled ***Pharmacological and Behavioral Investigation of Putative Self-Medicative Plants in Budongo Chimpanzee Diets****,* we identify, collect, and test several putative therapeutic resources from Budongo to determine their pharmacological properties. This chapter provides critical evidence for assessing Huffman's sixth criteria (see **Chapter 2: Section 2.1.2**), i.e., identification of specific mechanical or chemical properties which ameliorate illness or symptoms in the medicator (Huffman, 2019). While past studies have evaluated pharmacological components of putative therapeutic resources used by chimpanzees in sites such as Kibale, Uganda (Krief et al., 2003; 2005), funding limitations and lack of interdisciplinary collaboration in Budongo has prohibited *in situ* pharmacological testing (though see Schultz, et al., 2020). We ask: *Do any of the putative therapeutic resources consumed by Budongo chimpanzees have potent bioactivity?* To test this, our assays evaluate the antibacterial and anti-inflammatory properties of these resources. While we test several plant parts from a variety of species, most tests are conducted on barks associated with the putative self-medicative behavior, bark stripping. We

interpret results from our assays based on the related behavioral observations and health data collected during the study period.

In our final study, titled ***Fallback Food Hypothesis Fails to Explain the Value of Bark in the Diet of Chimpanzees of the Budongo Forest,*** we concentrate on bark stripping behaviors at Budongo, employing both quantitative and qualitative analyses to challenge past hypotheses about its adaptive function. To make the case that ingestion of certain barks could be self-medicative, we first systematically dismantle the historically accepted hypothesis that bark is a generalized fallback food for wild chimpanzees (Nishida, 1976). This hypothesis posits that as bark is a resource of low nutritional value, this resource is only consumed during periods of relative food scarcity when preferred, highly nutritious foods are scarce. We ask: *Does the fallback food hypothesis explain bark stripping behaviors at this site?* To assess this, we provide an in-depth description of this behavior at Budongo and evaluate four predictions of the fallback food hypothesis. This chapter significantly contributes to the literature on bark ingestion in chimpanzees, as there has been a notable dearth of systematic investigation into this behavior (though see Nishida, 1976; Lapuente et al., 2020).

In sum, using multidisciplinary and holistic methods, these studies collectively aim to advance our understanding of chimpanzee self-medication with a focus on two wild chimpanzee populations in Budongo Forest. Our research has direct implications for many fields including, but not limited to, zoopharmacognosy, ethnopharmacology, paleoanthropology, and conservation. Further investigation into self-medication in our closest living relatives may help us not only model the origins of modern human medicine, but also discover and protect medicinal resources which could be mutually beneficial in a rapidly changing world.

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# 2 Literature Review

## 2.1 Zoopharmacognosy: An Historical Overview of the Field

### 2.1.1 The Scientific Beginnings of the Study of Animal Self-Medication

Humans have long looked to sympatric animals for gleaning information on medicinal uses of resources in their environment (Huffman & Seifu, 1989; Huffman, 2001). Numerous folkloric accounts and Traditional Ecological Knowledge (TEK) from around the world attribute the discovery of numerous remedies for human ailments to the observation of non-human animals' self-medicating with natural resources (Huffman, 2022). However, it was not until the late 1970s that this captured the interest of the scientific community. Richard Wrangham's (1975) PhD on chimpanzee feeding ecology provided the first scientific mention of leaf swallowing behavior, noting the presence of folded, whole leaves in the feces of chimpanzees in Gombe, Tanzania. Independently, the same behavior was observed in chimpanzees at Mahale, Tanzania by Toshisada Nishida and colleagues, 70 km further south on Lake Tanganyika (Wrangham & Nishida, 1983). A few years later, Daniel Janzen, a feeding ecologist working in Costa Rica, proposed that vertebrate herbivores could be obtaining medicinal benefits from plants (Janzen, 1978). The study of animal self-medication was later given a name, 'zoopharmacognosy' (Rodriguez & Wrangham, 1993). Since then, the field has greatly expanded. In a recent meta-analysis, Neco et al. (2019) found that self-medicative behaviors have been reported in at least seventy-one species from seven mammalian orders. Species reported to self-medicate now include, but are not limited to, elephants (Houston et al., 2001), sheep (Villalba & Provenza, 2007), European starlings (Clark & Mason, 1985), and kodiak bears (Clayton & Wolfe, 1993). Self-medication has also been identified in several insect species, including monarch butterflies (de Roode et al., 2013), a topic recently reviewed by Ashwini & Patel (2022). The presence of self-medication across different phyla in the animal kingdom suggests that the ability to self-medicate is an adaptation with strong and diverse selection pressures.

In primates, conclusive self-medication has been observed in most great ape species (Neco et al., 2019). Amongst other, non-ape primate species, ten conclusive and thirty-six inconclusive cases

have been reported. Some proposed self-medicating species include capuchin monkeys (*Cebus capucinus)* (Baker, 1996), Tibetan macaques (*Macaca thibetana)* (Huffman et al., 2020), and chacma baboons *(Papio ursinus)* (Pebsworth et al., 2012). As of 2016, established self-medicative behaviors had been observed in African and Asian great apes across sixteen field sites (Huffman, 2016). Reports of bonobo (*Pan* paniscus) self-medication have been reported in the Democratic Republic of the Congo (DRC) at LuiKotale (Fruth et al., 2014), Lamako (Dupain et al., 2002), and Wamba (Huffman, 1997). Evidence of gorilla (*Gorilla gorilla gorilla*) self-medication has predominantly come from Kahuzi-Biega (DRC) (Yamagiwa et al., 2005). White-handed gibbon (*Hylobates lar*) self-medication has been observed in Khao Yai National Park (Thailand) (Barelli & Huffman, 2017), and reports of orangutan (*Pongo pygmaeus*) self-medication (specifically fur-rubbing) have come from Central Kalimantan (Borneo) (Morrogh-Bernard, 2008; Morrogh-Bernard et al., 2017). As the list of sites with observed cases of great-ape self-medication has not been updated since Huffman's 2016 review, there are likely many unpublished and unrecognized cases.

### 2.1.2 Formalizing Self-Medication Criteria

With new evidence and increasing interest in non-human self-medication, the field of '*Zoopharmacognosy'* formalized. The term, coined by Rodriguez and Wrangham in 1992 at an AAAS symposium (Clayton & Wolfe, 1993; Rodriguez & Wrangham, 1993), refers to the study of non-human self-medication. In 1997, Michael Huffman published a review of the current evidence for botanical self-medication in non-human animals, outlining guidelines to conclusively determine intentional self-medicative behaviors (Huffman, 1997). These criteria include **1.)** infrequent intake of irregularly consumed plant species which provide no/minimal nutritional benefit **2.)** restriction of plant use to seasons associated with specific infection or ailment **3.)** presence of pathogenic or parasitic infection in the medicator at the time of self-medicative behavior **4.)** a subsequent improvement in this condition following ingestion **5.)** healthy conspecifics ignore the resource or process it in a different way. Huffman (2019) added a criteria which includes **6.)** identification of specific mechanical or chemical properties which ameliorate illness or symptoms in the medicator. Ethnomedicinal use of a putative therapeutic resources by local human communities has also been suggested as strong additional evidence (Fruth et al., 2014; Huffman, 1996, 1997, 2001; Krief et al., 2006; Lozano, 1998). Evaluating these criteria has

become standard protocol for evaluating non-human self-medicative events (e.g., De la Fuente et al., 2022; Fruth et al., 2014).

Non-human self-medication events can be categorized into four general modes: therapeutic treatment, passive prevention, behavioral avoidance, and non-ingestion based medicinal behaviors (Huffman, 2016; Huffman, 1997, 2019; Lozano, 1998). Only the first two modes, which involve ingesting organic material, will be further discussed in this review, as these are the principal modes addressed in this thesis. *Therapeutic self-medication* refers to behaviors performed by sick individuals which alleviate or ameliorate the health condition. *Passive prevention* refers to behaviors performed by healthy chimpanzees, but which may have prophylactic effects against future illness (Huffman, 1997, 2016). Resources consumed as part of passive prevention are called *medicinal foods* and may provide nutritional or energetic value to the primate diet (Etkin & Ross, 1982; Huffman, 1997).

## 2.2 Types of Self-Medicative Behaviors

### 2.2.1 Established Self-Medicative Behaviors

*Therapeutic* self-medication, as documented in the literature, has been further sub-categorized as either *mechanical* or *chemical*. Mechanical self-medication involves resources whose morphological properties induce a physiological response (e.g., reduction of gut transit time) in the consumer. For example, leaf swallowing behaviors induce the mechanical expulsion of parasites. The anthelminthic properties of leaf swallowing are a result of the *physical* properties of ingested plant material, rather than their *chemical* makeup (Huffman & Caton, 2001). Chemical self-medication involves the ingestion of resources with bioactive or pharmacological properties. The only conclusive botanical, chemical, self-medicative behavior in chimpanzees, thus far, is bitter-pith chewing of the species *Vernonia amygdalina* (Huffman & Seifu, 1989).

#### *Leaf Swallowing*

In 1983, Wrangham and Nishida described leaf swallowing behaviors at Gombe and Mahale (Tanzania), discovering feces with whole leaves in them (Wrangham & Nishida, 1983). To investigate the leaves' bioactive properties, Wrangham, Nishida, and Uehara collaborated with

Eloy Rodriguez to test for beneficial secondary compounds in *Aspilia sp.,* a plant used for leaf swallowing at these two sites. Their study reported the presence of Thiarubrine A, a secondary compound with strong anthelmintic properties (Rodriguez & Wrangham, 1993; Rodriguez et al., 1985). However, when Neil Towers and colleagues were unable to replicate this study, Rodriguez's initial findings were dismissed (Huffman et al., 1996; Page et al., 1997). Instead, Huffman and colleagues proposed that while the expulsion of parasites could not be directly attributed to chemical characteristic, the leaves' rough surfaces activate a physiological response in the host, purging parasites in approximately six hours (Huffman & Caton, 2001; Huffman et al., 1996).

The hispid leaves, swallowed whole and often in the morning (Wrangham & Goodall, 1989; Wrangham & Nishida, 1983), have a 'velcro effect,' expelling adult worms stuck to the leaves and flushing out L3 larvae before they penetrate the mucosa to develop into adults (Huffman et al., 1996) (**Figure 1**). Following expulsion, the parasites are often found in the folds of defecated leaves or attached to the hispid surfaces (Fowler et al., 2007; Huffman et al., 1996). Roughly thirty-eight plant species associated with leaf swallowing have been identified across primate field sites (De la Fuente et al., 2022). Ingestion bouts usually last 1-15 minutes and 1-100 whole leaves can be swallowed during each bout (Huffman, 2016). Using efficiency rates of this behavior on decreasing worm burden, Huffman (2001) estimated that chimpanzees need to engage in an average of ten leaf swallowing bouts during the rainy season to rid themselves of recurrent infections. Multiple bouts can take place on the same day or on consecutive days. Leaf swallowing behaviors are easily

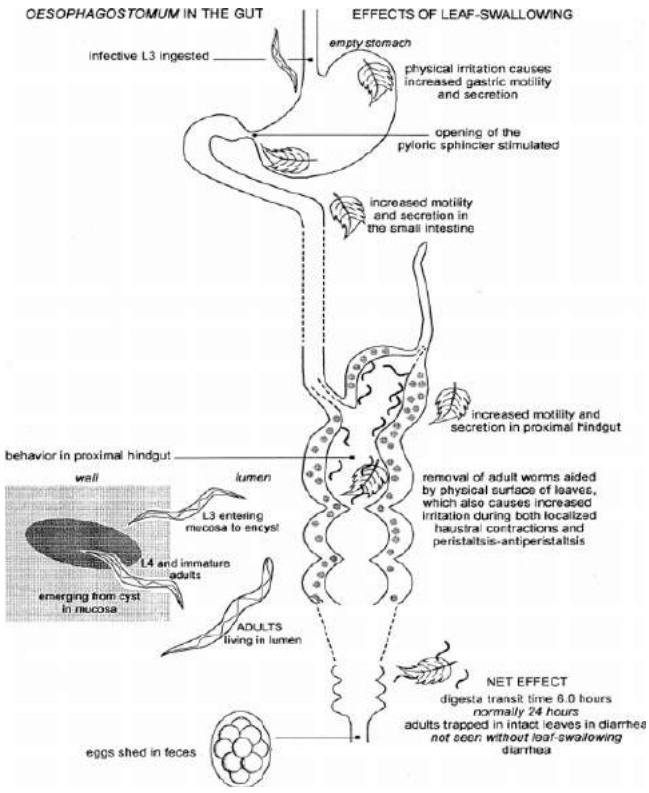


***Figure 1:*** *Diagram of leaf swallowing effects (adapted from Huffman & Caton, 2001)*

differentiated from normal feeding behaviors, as leaves processed in these contexts are individually selected, folded with the tongue and palate, pulled into the mouth, and swallowed without mastication (Huffman, 2016). **Figure 2** depicts a characteristic case of leaf swallowing from Budongo.

***Figure 2:*** *Leaf swallowing in Budongo (Image by A. Soldati)*

Amongst chimpanzees, direct observational evidence of this behavior and indirect evidence of whole leaves in fecal samples have been documented for all sub-species including *P. t. schweinfurthii* (Wrangham & Nishida, 1983; Wrangham, 1977)*, P. t. troglodytes* (Huffman, 1997)*, P. t. verus* (Boesch, 1995), and *P. t. ellioti* (Fowler et al., 2007). Leaf swallowing has also been observed in bonobos (*Pan paniscus*) (Dupain et al., 2002; Fruth et al., 2014; Huffman, 1997), eastern lowland gorillas (*Gorilla gorilla graueri*) (Yamagiwa et al., 2005), and white-handed gibbons (*Hylobates lar*) (Barelli & Huffman, 2017). Despite variation in plant species selected for leaf swallowing, all species share a characteristic hispid texture. Leaf swallowing has not yet been observed in orangutans (Menzel et al., 2013), a notable absence which may lend insight into the origin of this behavior and antiquity, if it cannot otherwise be explained by ecological variables. Based on phylogenetic analysis, leaf swallowing appears to have convergently evolved at least three different, and has been observed in several non-primate species, including brown bears (*Ursus arctos*), snow geese *(Anser caerulescens*), and Asian palm civets (*Paradoxurus hermaphroditus*) (Huffman, 2016; Huffman, 1997). The wide-spread presence of this behavior across diverse species, suggests that mechanical self-medication is wide-spread throughout the animal kingdom.

**TABLE 1:** Field sites where chimpanzee and bonobos leaf swallow

| Field Site | Country | Citation |
|---|---|---|
| **Bossou** | Guinea | Matsuzawa & Yamakoshi, 1996 |
| **Budongo** | Uganda | Observed by Chris Bakuneeta (unpublished), reported in Huffman et al., 2009 |
| **Bulindi** | Uganda | McLennan & Huffman, 2012 |
| **Fongoli** | Senegal | Pruetz, Johnson-fulton, 2003 |
| **Gombe** | Tanzania | Wrangham & Goodall, 1989; Wrangham & Nishida, 1983; Wrangham, 1977; Wrangham, 1975 |
| **Kahuzi-Biega** | DRC | Huffman, 1997 |
| **Kalinzu** | Uganda | Huffman, 2016 |
| **Kibale** | Uganda | Huffman & Wrangham, 1994; Wrangham, 1995 |
| **Kwano** | Nigeria | Fowler et al., 2007 |
| **Lamako** | DRC | Dupain et al., 2002 |
| **LuiKotale** | DRC | Fruth et al., 2014 |
| **Wamba** | DRC | Huffman, 1997 |
| **Lyema** | DRC | Huffman, 2016 |
| **Mahale** | Tanzania | Huffman & Seifu, 1989; Huffman et al., 1996; Nishida & Uehara, 1983; Wrangham & Nishida, 1983 |
| **Ndoki** | Republic of Congo | Huffman, 1997 |
| **Nimba** | Guinea | Matsuzawa & Yamakoshi, 1996 |
| **Wamba** | DRC | Dupain et al., 2002; Huffman, 1997 |
| **Taï Forest** | Ivory Coast | Boesch, 1995 |

Leaf swallowing has been empirically proven to ameliorate infections only from the nodule worm *Oesophagostomum stephanostomum* and the tapeworm *Bertiella studeri* (Huffman et al., 1996; Wrangham, 1995), both of which have distinct egestion lifecycles. When chimpanzees are infected with *O. stephanostomum,* expulsion appears to be a direct consequence of the host's response to this behavior (Huffman et al., 2009). However, when chimpanzees are infected with *B. studeri*, proglottid expulsion is a natural part of the parasite's life cycle (Huffman et al., 2009), and proglottids can be found in the feces regardless of leaf swallowing. In cases when these parasites are found together in the feces, such as in studies at Bulindi (McLennan & Huffman, 2012), the differences between parasitic egestion lifecycles are relevant for effective analysis.

### *Bitter-Pith Chewing*

In 1987, Huffman and Seifu discovered another self-medicative behavior in chimpanzees after observing a sick adult female chew on the bitter-pith of *Vernonia amygdalina* (Huffman & Seifu, 1989). This behavior, named bitter-pith chewing, involves the removal of bark and leaves from young shoots, followed by the mastication of the residual fibers and juices from the piths (5-120cm in length and 1cm in width) over the course of 1-8 minutes (Huffman, 2016). **Figure 3** shows a

Mahale chimpanzee preparing to strip the pith of *V. amygdalina.* After this initial discovery, Huffman and colleagues created CHIMPP (Huffman, 1994), the first long-term field study of non-human self-medication. Over twelve years (1985-1997), seven bouts of bitter-pith chewing were documented amongst members of the M group in Mahale (Huffman, 1997). While bitter-pith chewing events are rare, this small number of direct observations does not reflect the behavioral frequency, due to the M group's expansive home-range and the small size of the research team (M. Huffman, personal comm.). Data from this study revealed that adult chimpanzees in the vicinity of infected individuals bitter-pith chewing, rarely also ingested the pith (Huffman, 1997). Further evidence came from the discovery of bitter-pith chewing seasonality, indicating that Mahale chimpanzees engage in this behavior more frequently during the rainy season (Huffman et al., 1990; Huffman et al., 1996). The seasonality of this behavior, as well as previous findings that *Oesophagostomum* and *Strongyloides* infections and frequencies of leaf swallowing bouts all peaked in February, suggested a link between these behaviors and parasitic expulsion in Mahale (Huffman, 2015; Kawabata & Nishida, 1991). Huffman and colleagues also observed that chimpanzees took detours from normal feeding routes to access *V. amygdalina* (Huffman, 1997), suggesting procurement of this pith was intentional.

***Figure 3:*** *Bitter-pith chewing in Mahale, Tanzania (Image from* the film *Animal Doctors* by Mitsch, 2013)

To prove that bitter-pith chewing targets parasite infections and that bioactive properties in these plants reduce infections, Huffman initiated the first longitudinal chimpanzee study of intestinal parasite fauna in 1989 (Huffman, 2015; Huffman et al., 1996). First, the project analyzed fecal samples from parasitized individuals, recording both infection-type and severity. Huffman and colleagues identified three genera of nematode, one cestode, one genus of trematode, and four genera of protozoa (Huffman et al., 2009; Huffman, 1997). Second, they measured egg per gram (EPG) ratios of infectious parasites in fecal samples collected after bitter-pith chewing events to

determine whether parasite load decreased in infected individuals after bouts. Results showed that EPG levels for the parasite *O. stephanostomum* dropped dramatically for individuals who had ingested *V. amygdalina* and rose in control individuals who had not (Huffman et al., 1993). EPG did not drop in concurrent *Trichuris* infections, suggesting that bitter-pith chewing is effective against specific, rather than general parasitic infections. These studies suggested that bitter-pith chewing functions as a one-dose treatment, as multiple bouts of this behavior were not observed. These studies provided the first empirical evidence that bitter-pith chewing is a self-medicative behavior, catalyzed by specific parasitic infections (Huffman et al., 1996).

Huffman and colleagues further sought to identify the specific plant secondary metabolites in *V. amygdalina* responsible for the plant's anthelmintic properties. Plant secondary metabolites (PSMs) are organic compounds produced by plants often as a defense mechanism against predators and pathogens (Allison, 1982; Hart, 1990; Holmes & Zohar, 1990; Toft et al., 1991). PSMs have been shown to improve the health of those who ingest them (e.g., Forbey et al., 2009; Huffman, 1997; Raman & Kandula, 2008; Villalba & Provenza, 2007) and are the basis for many synthesized modern pharmaceuticals (Balandrin et al., 1985; Caldecott, 1987; McKenna, 1995; Newman & Cragg, 2020). To assess the bioactive properties of *V. amygdalina*, Ohigashi et al. (1991, 1994) conducted phytochemical analyses on samples from Mahale, finding two classes of bioactive PSMs: sesquiterpene lactones and steroid glucosides. Koshimizu et al. (1994) further found that *V. amygdalina* has anti-malarial properties. These compounds, found in other species of *Vernonia* (i.e., *Vernonia colorata*), have since been shown to be anthelmintic, anti-amoebic, anti-tumor, and antibiotic (Jisaka et al., 1992a; 1992b; Jisaka et al., 1993; Toubiana & Gaudemer, 1967). Further testing established that most active compounds are present in the plant's pith (Jisaka et al., 1992; Ohigashi et al., 1994), the part specifically targeted by infected chimpanzees, despite the demonstrated unpalatable taste of this plant part (Glander, 1982; Gustafsson et al., 2014; Vining, 1990).

Huffman and colleagues were aware that local Tongwe people, living near Mahale, used *V. amygdalina* for a variety of medicinal purposes (Huffman, 1996; Huffman & Wrangham, 1994; Huffman et al., 1993). Ethnomedicinal research revealed that doses used in these traditional human contexts were similar to those consumed by chimpanzees (Huffman, 2003). This species is also

used across other regions of Africa to treat enteritis, constipation, diarrhea, stomach upset, schistosomiasis, malaria, trematode infection, amoebic dysentery, and ringworm (Huffman, 2003). From this collective evidence, Huffman published the first scientifically tested evidence of intentional primate self-medication (Huffman, 1997), establishing both bitter-pith chewing and leaf swallowing as self-medicative behaviors.

### 2.2.2 Putative Therapeutic Resources

In addition to resources that are conclusively associated with established self-medicative behaviors, several other plant species and resource-types have been suggested as putatively self-medicative in certain chimpanzee communities. In this study I define *putative therapeutic resources* as dietary items that have been suggested but not proven to be self-medicative—in other words, those which do not yet meet all of Huffman's (1997) criteria. One commonly cited putative self-medicative resource-type is clay and/or soil, associated with a behavior called geophagy. Geophagy is defined as the intentional consumption of earth materials, including soil, nests, chalk, clay, and insect mounds (e.g., those made by termites, ants, and cicadas) (Ta et al., 2018). Various medicinal adaptive functions have been proposed for geophagy, particularly for clay eating (**Figure 4**). These include detoxification, assistance with cellulose digesting flora, cytoprotection, buffering of stomach pH, mediation of parasites, bioactivation of medicinal plants, provision of antibacterial compounds, and amelioration of diarrhea (see Krishnamani & Mahaney, 2000 and Pebsworth et al., 2019 for review of geophagy hypotheses). According to the most recent review of geophagy in non-human primates (Pebsworth et al., 2019), geophagy has been identified in at least 136 species of non-human primates and is present in nine of the seventeen primate families.

***Figure 4**: Geophagy (clay eating) in Budongo Forest, Uganda (Image by E. Freymann)*

Another putative self-medicative resource-type is tree bark, which also can include cambium and phloem. Bark ingestion often follows a behavior historically known as bark stripping which involves stripping or peeling outer bark from around the trunk of woody plants to access bark or cambium beneath (Lapuente et al., 2020; Nishida, 1976) (**Figure 5**). Huffman and Seifu first theorized that bark was a self-medicative resource in 1989 due to its low nutritional value and the common ethnomedicinal use of bark in traditional medicine. While literature on bark stripping popularly categorizes this behavior as fallback feeding strategy employed during periods of scarcity (e.g., Nishida, 1976), more recent observational and pharmacological evidence suggests that ingestion of bark may provide primates with medicinal benefits (e.g., Krief et al., 2005; Ghai et al., 2015). In Kibale, Krief and colleagues (2005) observed a highly parasitized chimpanzee ingesting *Albizia grandibracteata* bark, a resource never before observed being consumed by chimpanzees at this site. The authors also found that saponins from the bark of this species had *in vitro* anthelminthic activity and cytotoxic properties (Krief et al., 2004). Ghai et al. (2015), in a later study, found that bark ingestion of *A. grandibracteata*, as well as another species from this genus increased in red colobus monkeys (*Procolobus rufomitratus tephrosceles*) when they were infected with whipworm (*Trichuris* sp.).

***Figure 5**: Bark stripping of Syzygium guineense in Budongo, Uganda (Image by D. Sempebwa)*

Until a recent review by De la Fuente et al. (2022), there had been no cross-site collation of putative therapeutic resources, making it difficult to compare evidence of self-medication across field sites. This has likely led to many therapeutic behaviors being overlooked, mistaken for fallback foods or nutritional food items. The bioactive properties of many of these resources also remain untested. As of 2005, only 5-15 percent of the world's plant species had been tested for bioactivity (Cragg

et al., 1997; Krief et al., 2005). It is highly probable that there exist many more therapeutic resources in primate diets that remain undiscovered.

### 2.2.3 Medicinal Foods

The term 'medicinal foods' was first used by Etkin (1996) in an ethnopharmacological context, to describe food items in human diets that have general medicinal properties in addition to their nutritional value. While medicinal foods may have ethnomedicinal uses and/or known pharmacological properties, they are not consumed to ameliorate a specific pathogen or parasite infection. Huffman formally introduced this term into the field of primatology (Huffman, 1997), proposing that chimpanzee diets also contain resources with a variety of medicinal properties, which, when eaten periodically and in small amounts, could prevent against disease and illness (Huffman, 1997; Macintosh & Huffman, 2012; Petroni et al., 2017).

In fact, Huffman et al. (2020) suggest that medicinal foods are abundant in the diets of many primate species, including humans. The authors posit that 15-25% of plants in primates diets qualify as medicinal foods that have bioactive secondary compounds with pharmacological properties (Huffman, 2003; Huffman, 1997). In Mahale, 22% of food items consumed by the M-group chimpanzee community had multiple ethnomedicinal uses against parasite infections and related symptoms. Many medicinal foods identified in the diet of Tibetan macaques in China have also been suggested to improve health and reproduction, reduce stress levels, and ameliorate hormonal imbalances (Huffman et al., 2020). However, Huffman and colleagues argue that despite these medicinal properties, the M-Group chimpanzees and Tibetan macaques are likely not targeting each of these resources for their specific therapeutic benefits.

Differentiating between nutritional/medicinal foods, and therapeutic resources can be highly challenging (Kirabo et al., 2018). For example, Krief et al. (2005) observed an adult male in the Kanyawara community at Kibale with a wounded toe eating the stem of *Acanthus pubescens* (now *A. polystachyus*), a commonly consumed pith amongst members of this community (Wrangham et al., 1991). This species has many traditional uses across East Africa, best documented as a remedy for skin infections in Burindi (e.g., Krief et al. 2005). This plant has also been shown to exhibit weak antibacterial and antifungal activity (Moshi et al., 2010). While *A. polystachius* could be a

normal food, commonly consumed at Kibale for its nutritional value, it could simultaneously serve a preventative function, protecting against future infection. In either case, its ingestion by the injured individual may be purely coincidental. However, an alternative hypothesis is that this species was targeted intentionally by the individual to therapeutically ameliorate his wound. More anecdotal cases would be needed to classify this event as either a normal feeding behavior or a form of self-medication.

As demonstrated above, the line between nutritional and medicinal foods can be blurry, as can the line between staple foods and fallback foods (Marshall & Wrangham, 2007). A resource in the chimpanzee diet which exemplifies this classification challenge is figs (Krief et al., 2005). Figs, which are the fruits produced by species of the *Ficus* genus, are common feeding items across most chimpanzee communities (Harrison & Marshall, 2011). These fruits are high in sugar and fiber, and have been proposed to be both staple foods and fallback foods for wild chimpanzees (depending on site and season) (Marshall & Wrangham, 2007). Figs, however, have also been shown to contain ficin, a proteolytic and anthelminthic enzyme occurring in the latex of several species including *F. capensis* and *F. thonningii* (Etkin & Ross, 1982). Even in low concentrations of ~ 0.05%, *Ficus glabrata* latex can destroy the cuticle of certain helminths, damaging and killing harmful parasites (Huffman, 1997). While observational case studies suggest that sick chimpanzees may eat unripe *F. capensis* when visibly sick (e.g., Krief et al., 2005), until specific therapeutic medicinal uses for this species can be determined, they are better classified as medicinal foods.

## 2.3 Combining Medicinal Resources

As more is discovered about the bioactivity of resources in chimpanzee diets, it appears increasingly possible that sequential ingestion of multiple resources may enhance medicinal benefits for medicators (Krief et al., 2005). For example, Klein et al. (2008) found that certain clay types can bioactivate compounds in leaves used for drinking clay-water. These leaves, specifically of the species *Trichilia rubescens,* appear to have synergistic chemical interactions with compounds in the clay (Klein et al., 2008; Pebsworth et al., 2019). Similarly, Struhsaker et al. (1997) found that Zanzibar red colobus monkeys (*Procolobus kirkii*) consume charcoal after eating the leaves of Indian almond and mango trees, likely to adsorb the phenolics present in these

resources. Krief et al. (2005) suggest that in addition to investigating associations between bioactive plants, researchers should holistically consider the associations between plants and other resource-types like meat, honey, or soils.

## 2.4 Acquisition of Self-Medicative Behaviors

After leaf swallowing and bitter-pith chewing were established as self-medicative behaviors in chimpanzees, a wave of captive studies in the early 2000s shifted the focus of zoopharmacognosy toward establishing whether these behaviors were instinctually or socially acquired. Huffman and Hirata (2004) published the first evidence that leaf swallowing can both spontaneously emerge in naïve chimpanzees and be socially transmitted. The study exposed naïve, captive individuals to a novel local plant with hispid leaves. During the study, two individuals began spontaneously to leaf swallow, while others exhibited initial neophobia or indifference toward the plant. After a few days of observing the original models, other group members began to leaf swallow as well. The authors further noted high levels of social tolerance among the captive chimpanzees during this experiment. A follow-up study conducted by Huffman et al. (2010) used the option bias method (Kendal et al., 2009), to demonstrate that no underlying genetic code explains group-level variation in leaf swallowing behaviors amongst captive chimpanzees. In this study, two captive chimpanzee groups were once again introduced to novel plants with hispid leaves. Leaf swallowing behaviors emerged spontaneously in each group, and two group-level variants emerged. These variants (complete and partial leaf swallowing) were first "invented" by naïve innovators and consequently spread across group members with high fidelity. This study provides strong evidence that leaf swallowing variants are dispersed in communities through social transmission.

While captive studies suggest that leaf swallowing can be socially transmitted, the learning mechanisms through which chimpanzees acquire these variants remain undetermined. There are many candidate mechanisms for the social acquisition of leaf swallowing behaviors. Most recently, Huffman et al. (2010) have proposed that transmission occurs through socially mediated learning followed by associative learning. This hypothesis suggests that an observer first learns through socially observing the behavior in another, followed by selective copying (with possible modification) and subsequent acquisition of the behavior. Through associative learning, the

individual may then begin to equate use of a specific resource with alleviation of a particular symptom.

Indirect evidence suggests that bitter-pith chewing in chimpanzees is also socially transmitted. Bitter-pith chewing is rarer than leaf-swallowing in chimpanzees, and while leaf swallowing bouts can go on continuously for days (Huffman, 2016), multiple bitter-pith chewing bouts in the same individual, within the same week, have never been observed. While over-ingesting PSMs is generally deleterious to the medicator's well-being, under-ingestion appears to limit bioactive efficacy (Villalba & Provenza, 2007), as does failing to consume the bioactive resource if sick (Huffman et al., 1993). It is critical, therefore, that individuals ingest bitter-pith in the correct way at the correct time, making it highly inefficient for each individual to relearn this behavior through trial and error. As the high costs of incorrectly consuming bioactive medicinal resources seem to require high-fidelity social transmission, and medicinal behaviors can often cause no visible, immediate change in the medicator, a more directed process of social learning seems likely for chemical self-medication, one that cannot be explained by observation alone.

Although no captive studies have tested the transmission of bitter-pith chewing behaviors, anecdotal evidence suggests that young chimpanzees observe these behaviors closely in others and attempt to copy them (Huffman & Seifu, 1989; Huffman & Wrangham, 1994). However, in the two reported cases in which copying of this behavior has been reported, infants stopped after tasting *V. amygdalina's* bitter juice. One notable case, described by Huffman (1997), also suggests that models may use inhibitory teaching to transmit this behavior. During this event, a mother and infant pair co-fed near a visibly sick adult male who was bitter-pith chewing. After the male dropped the pith, the infant attempted to pick it up but was blocked by her mother. This observation suggests that mothers may be sensitive to when and how their offspring interact with bioactive resources and may use inhibitory behaviors to convey information regarding the resource's toxicity. Unfortunately, this remains an isolated observation in the zoopharmacognosy literature. Mechanisms for selective foraging and adaptive plasticity may govern the selection of beneficial plants in chimpanzees, but social transmission appears to be a prerequisite for acquiring certain types of medicinal knowledge.

## 2.5 Pathogens and Parasites

Thus far, empirical studies on self-medication have looked disproportionately at behaviors believed to mediate parasite infections. The primary reason for this has been methodological. Methods for evaluating parasite load and diversity in chimpanzees are relatively simple and non-invasive, requiring only fecal samples, a microscope, solvents, and McMaster slides. The ability to efficiently quantify individual parasite loads *in situ*, has made it possible to assess effects of specific feeding behaviors on parasite infections. However, while some endoparasites undoubtedly pose a major risk to wild chimpanzees, with some infections causing diarrhea, flatulence, anorexia, unusual sleeping hours or even death (Forbey et al., 2009), many other pathogens and ailments can also alter health states. Wild chimpanzees are highly prone to viral respiratory infections (Scully et al., 2018), bacterial infections (Hockings et al., 2020), and infection from injuries caused by other animals, inter/intra-group aggressions, and snares. Some of the common internal ailments are listed in **Table 2**, with the corresponding characteristic symptoms and methods of diagnosis.

**TABLE 2**: Examples of common sickness behaviors and corresponding methods of diagnosis

| Type of Illness | Characteristic Symptoms | Method of Diagnosis | Exemplary Studies |
|---|---|---|---|
| **Bacterial/Viral Respiratory Infection** | Cough | Observation | Krief et al., 2005(Patrono et al., 2018) |
| | Nasal Discharge | Observation | Krief et al., 2005 |
| | Sneezing | Observation | Krief et al., 2005 |
| | Lethargy | Observation | Lowenstine & Osborn, 2012 |
| | Dyspnea | Observation | Krief et al., 2005 |
| | Anorexia | Observation | Lowenstine & Osborn, 2012 |
| | Nose Blow/Pick | Observation | Kaur & Huffman, 2004 |
| | High Leukocyte, Nitrite, and pH Levels | Urinalysis | Kaur & Huffman, 2004 |
| | Presence of Bacteria/Virus in Fecal Matter | PCR Screening of Fecal Samples, Necropsy | Patrono et al., 2018 |
| **Slow-Growing Bacterial Infection (i.e., Leprosy)** | Skin Discoloration | Observation | Hockings et al., 2021 |
| | Facial Disfigurement | Observation | Hockings et al., 2021 |
| | Ulcers/Nodules | Observation | Hockings et al., 2021 |
| | Deformed Limbs | Observation | Hockings et al., 2021 |
| | Hair Loss | Observation | Hockings et al., 2021 |
| | Presence of Bacteria in Fecal Matter | PCR Screening of Fecal Samples | Hockings et al., 2021 |
| | Diarrhea | Observation, Microscopic Analysis of Fecal Samples | Ghai et al., 2015; Kaur & Huffman, 2004 |
| | Flatulence | Observation | Kaur & Huffman, 2004 |

| | | | |
|---|---|---|---|
| **Gastro-Intestinal Disorder/Parasite Infection** | Anorexia/Weight Loss | Observation | Ghai et al., 2015; Huffman & Seifu, 1989 |
| | Unusual Sleeping Hours/Day Nesting | Observation | Ghai et al., 2015; Huffman & Seifu, 1989; Kaur & Huffman, 2004 |
| | Fever | Fecal Temperature Decline | Herbert et al., 2015; Lowenstine & Osborn, 2012 |
| | Presence of Whole Leaves or Worms in Feces | Macroscopic Analysis of Fecal Samples | Kaur & Huffman, 2004 |
| | Frequent Soft Stools | Observation, Macroscopic Analysis of Fecal Sample | Kaur & Huffman, 2004 |
| | Vomiting | Observation | Kaur & Huffman, 2004 |
| | Urine Discoloration | Observation | Huffman & Seifu, 1989 |
| | Abdominal Distension | Observation | Krief et al., 2005 |
| **External Wound/Appendage Infection** | Swelling of Limb | Observation | Krief et al., 2005 |
| | Visible Laceration | Observation | Krief et al., 2005 |
| | Tooth Abscess | Observation | Krief et al., 2005 |
| | Avoidance Behavior | Observation | Lowenstine & Osborn, 2012 |
| | Fever | Fecal Temperature Decline | Lowenstine & Osborn, 2012 |
| | Change in Physical Appearance (e.g., Hair Color) | Observation | Krief et al., 2005 |
| | High Leukocyte, Nitrite, and pH Levels | Urinalysis | Kaur & Huffman, 2004 |

Methods for diagnosing non-parasite related ailments in primates, tend to be financially or logistically more challenging, involving further testing or expensive equipment. For this reason, there are no established self-medicative behaviors linked to the amelioration of viral, bacterial, or wound-related infections, despite the well-documented presence of these pathogens in primates (see **Table 2).** While this methodological limitation remains a challenge in the field, the interaction between endoparasites and chimpanzee host immune systems may have implications for host susceptibility to other pathogens. Kamal and El Sayed Khalifa (2006), demonstrate, for example, that the presence of certain helminths may impair a host's immunological response to bacterial, viral, and protozoal pathogens. Similarly, a reduced immune system and increased stress caused by co-infections could render a host more susceptible to virulent endoparasites (Walzer, & Genta, 1989; Lozano, 1998). While parasite quantification remains the primary form of health monitoring in zoopharmacognosy studies, endoparasite load and diversity can serve as a proxy for immunological health and general well-being.

## 2.6 Individual or Cultural Variation of Self-Medicative Behaviors?

Cross-site studies have suggested the presence of inter-community variation in primate diets and resource selection. Pebsworth et al. (2006) compared the diets of two chimpanzee communities in Uganda, the Kanyawara community in Kibale, and the Sonso community in Budongo. Despite an overlap of 58% of food items in both community home ranges, only 8.45% of these resources were consumed at both sites. Upon further investigation, Krief et al. (2005) found that some resources available at both sites, but consumed by only one community, included bioactive species with possible medicinal properties. *A. polystachyus* flowers, for example, have antimicrobial properties (Krief et al., 2003) and are consumed in Budongo but not Kibale, while the bioactive barks of *Albizia grandibracteata* and *Markhamia platycalyx* are consumed in Kibale but not Budongo (though see **Chapter 6**). These intergroup differences are also applicable to the selection of leaves used for leaf swallowing. While the whole leaves of *Ficus exasperata* are swallowed whole in the early morning and ingested normally in the afternoon at Mahale, at Gombe and Kibale, they appear not to be used for leaf swallowing (Newton & Nishida, 1990; Wrangham, 1975). Other examples of inter-site variation are listed in Huffman and Wrangham (1994).

Goodall (1986) first suggested that dietary differences between communities may be due to group feeding traditions and thus a product of culture. Based on differences found between sites, Pebsworth et al. (2006) also conclude that both tradition and differences in plant chemistry may play a role in medicinal resource selection, leading to unique medicinal cultures. They also add, however, that ecological variability (including resource availability and abundance) may lead to differences in parasite or pathogen patterns, and thus inter-site health differentials. Huffman and colleagues (2009) cite climatic differences like rainfall rates which affect the life cycle of *Oesophagostomum*, as factors which directly influence parasite reinfection and transmission dynamics.

## 2.7 The Evolution of Self-Medication

As ethnobotanical behaviors are often undetectable in the fossil record, reconstructing the self-medicative behaviors in our own species has been challenging (e.g., Gustafsson et al., 2016; Hardy, 2018). However, the ubiquity of self-medicative behaviors in our closest living relatives, the great apes, and across many other non-human primate species, suggests that primate modelling may be a helpful tool through which we can better understand the evolutionary origins of human medicinal

practices. The presence of these behaviors in extant *Pan* species suggests that the *Pan/Homo* last common ancestor would likely have had the technical skillset and cognitive abilities to effectively self-medicate. Evidence that *Homo neanderthalensis* used medicinal plants that are still widely used by modern humans (Hardy, 2022; Hardy et al., 2012; 2013) further supports this theory.

According to a parsimonious reconstruction of self-medication behaviors conducted by Neco et al. (2019), the ability to self-medicate has evolved at least four separate times in the mammalian lineage, with this number rising to thirty-two if inconclusive evidence is considered (Neco et al., 2019). Many species, as well as many allopatric populations of the same species in different places have developed similar self-medicative techniques and behaviors (Huffman, 1997). An evolutionary perspective offers a framework for understanding the selection-pressures and trade-offs that may have selected for a predisposition to discover or an ability to learn and socially transmit certain widely seen self-medicative behaviors. Due to scope, the following discussion of evolutionary frameworks and proximate mechanisms will focus on therapeutic self-medication, specifically related to endoparasite expulsion. This does not, however, mean that processes and mechanisms governing other kinds of self-medicative behaviors have distinctly different evolutionary origins.

It is clearly evolutionary beneficial to be able to cure oneself of sickness, especially when illness negatively impacts fitness, health, fecundity, and survival. As certain parasite infections have been shown to effect fitness and fecundity in non-human models (Nguyen et al., 2015; Reed et al., 2008; Reed et al., 2012) behaviors that mitigate these impacts would logically be selected for in any animal. While hosts may tolerate endoparasites which have no negative effects, behavioral strategies for mediating infections that decrease fitness are needed (Huffman, 2016). Having the ability to fight potentially fatal pathogens and parasites affords individuals longer lifespans and more opportunities to mate, thus increasing their chances of producing offspring. The question, therefore, is not *why* self-medication evolved, but *how* natural selection may have shaped the biological and cognitive processes and mechanisms that govern these self-medicative behaviors. Two key theories that have been used as frameworks through which to view the evolution of self-medication include: well-being homeostasis (Forbey et al., 2009; Villalba & Provenza, 2007) and

life-history theory (Neco et al., 2019; Stearns, 1992). Both perspectives offer frameworks for asking different kinds of proximate and ultimate questions.

### 2.7.1 The Homeostatic Perspective

The homeostatic perspective, also known as the well-being hypothesis, offers a general framework for explaining when/why an animal would ingest potentially harmful PSMs despite potential costs (Forbey et al., 2009). This model proposes that animals are constantly struggling to maintain a state of equilibrium with their environment, which can easily be disrupted by changes in ecological variables, illness, stress, or nutritional deficits (Forbey et al., 2009; Villalba & Provenza, 2007). It can also be disturbed by the presence of environmental toxins, like PSMs, which make certain resources deleterious to ingest (Forbey et al., 2009). When imbalances do occur, the homeostatic perspective predicts that animals will rectify these needs through selective foraging. However, for this, mechanisms are needed to recognize these needs and to know or learn how to meet them (Huffman, 2016). The presence of self-regulating mechanisms in non-human animals, were identified as early as 1936. Richter (1936), through experimental approaches using rat models, demonstrated that depletions in the availability of NaCl stimulated an increased appetite for it. The homeostatic perspective offers a general framework for evaluating the evolution of general health regulation and self-medication in both humans and animals. Specifically, this framework addresses how and why natural selection may have shaped underlying biological mechanisms governing self-medicative behaviors. It also demonstrates the extent to which our physiological needs are interwoven with our psychology (Villalba & Provenza, 2007).

*But what are the proximate stimuli which catalyze self-medication?* A possible driver for self-medication is discomfort caused by the pathogen or parasite responsible for the infection. While little is known about the internal effects on wild chimpanzees of specific pathogens, any of the common sickness behaviors (see **Table 2**) exhibited during periods of illness could also stimulate self-medicative behaviors. Since both internal parasites known to prompt leaf swallowing and bitter-pith chewing in chimpanzees (*Oesophagostomum* sp. and *Bertiella* sp.) cause abdominal and gastric discomfort in humans (Brack, 1987) this same discomfort is likely a proximate stimulus for chimpanzee leaf swallowing and bitter-pith chewing behaviors (Huffman et al., 1996; McLennan & Huffman, 2012; Wrangham, 1995). However, as leaf swallowing can take place

spontaneously in naïve individuals who are not infected with parasites, there must be additional mechanisms which activate this behavior, perhaps a mechanical response to encountering hispid leaves (Huffman & Caton, 2001; Huffman & Hirata, 2004).

Another proposed mechanism, therefore, is an evolved ability on the part of the medicator to interpret external signals (either morphological or chemical) from medicinal materials which may influence selection, frequency, or quantity (Villalba, et al., 2006). These may include flavor or visual cues in the case of chemical self-medication or physical characteristics in the case of mechanical self-medication. Visual and/or physical recognition of medicinal resources has been suggested as the primary mechanism driving specific selection of leaf species used for leaf swallowing, as all species selected for this behavior have a similar surface roughness (De la Fuente et al., 2022; Huffman, 1997).

*Which mechanisms allow for effective regulation of well-being homeostasis?* The mechanisms governing an adaptive diet that optimizes nutrition, general well-being, and sickness responses may be responsible not only for the targeted selection of medicinal PSMs when an individual is sick (Forbey et al., 2009), but also for the avoidance of toxic PSMs when an individual is healthy (Glander, 1982). One genetic mechanism which may help animals selectively target or avoid PSMs is the TAS3R8 gene, found in both human and non-human primates (Gustafsson et al., 2016; Huffman, 2016; Lalueza-Fox et al., 2009). The only known purpose of this gene is bitter taste perception linked to plant eating, and has been speculated to help warn of toxicity (Kim & Drayna, 2005). Gustafsson et al. (2016) found that this gene was present in 100% of eastern chimpanzees (*Pan troglodytes schweinfurthii),* making them sensitive to a bitter chemical called *thioureas*. One theory for the prevalence of the gene in this region, is that eastern forests contain more alkaloid-producing plant species (McKey et al., 1978). As these compounds may have medicinal properties, the ability to detect bitterness may have been fitness enhancing under more extreme selection pressures leading to the development or maintenance of this gene. It has also been suggested that taste and smell capabilities may change with animals' physiological conditions. As an individual gets sicker, its ability to perceive bitter tastes may diminish, allowing for increased ingestion of PSMs (Provenza, 1995; Provenza et al., 1996).

The homeostatic perspective also helps to explain health trade-offs which are an unavoidable consequence of certain chemical self-medicative behaviors. While PSMs can provide ample health benefits to sick individuals when consumed at correct dosages, when consumed incorrectly they can reduce fecundity, cause weight loss, lead to organ failure, alter metabolic rates, reduce digestibility of nutrients, compromise the expenditure of energy, and result in death (Forbey et al., 2009). Neco et al. (2019) speculate that these high risks and costs of self-medication may also be responsible for repeated evolutionary losses of self-medication. Sullivan et al. (2008) further argue that these costs likely deter ingestion of these compounds in healthy individuals. However, the well-being hypothesis predicts that sick individuals with advanced infections will risk these costs and ingest potentially toxic PSMs to acquire necessary dosages of pharmacologically active compounds needed for recovery.

This theoretical framework has largely been used to evaluate proximate questions about self-medicative behaviors in both human and non-human primates, specifically concerning behavioral stimuli and the adaptative mechanisms governing resource selection. It has also been used to produce new approaches for livestock management. In humans, the homeostatic perspective can help explain why we may have evolved addictions to drugs like nicotine, which have beneficial anthelmintic properties (Forbey et al., 2009; Sullivan et al., 2008). In future studies, this framework may lend insight into multi-resource self-medication through the non-random combination of dietary items which could offer synergistic or complementary advantages.

### 2.7.2. Life History Theory

The evolution of self-medication may also be theoretically evaluated through life-history theory. This theory is based on the idea that while many traits have genetically coded phenotypes, many are also affected by developmental and environmental conditions. Classical life-history theory, developed in depth by Stearns (1992), proposes that extrinsic factors such as ecological impacts on fitness and intrinsic factors such as trade-offs between life-history traits and species-specific constraints on gene expression interact to shape life-histories (Stearns, 2000). The aim of this framework is to predict which trade-offs can offer optimal outcomes and explain the variation in traits and behaviors. Identifying connections between self-medicative behaviors and life-history traits can help reveal selection-pressures acting on self-medication.

Neco et al. (2019) applied life-history theory to their phylogenetic, cross-species study of the evolution of non-human self-medication. In their meta-analysis of zoopharmacognosy literature they tested whether self-medication is a life-history adaptation associated with traits found in long-living species. The authors determined that across species, longer average lifespan predicted self-medicative abilities. They link this to the increased exposure these species have to pathogens and thus an increase in selection pressures on adaptations needed for self-medication (Neco et al., 2019). The authors also investigated correlations between self-medication and proxies for testing "life-history syndrome" (e.g., larger absolute brain size, longevity, and body mass). Results showed that, at a species-level, brain size and body size were significantly correlated with species-level self-medicative abilities, but that group sizes were not. This last find was surprising, as species which live in larger groups tend to have higher frequencies of parasite infections (Altizer et al., 2003).

Life-history theory may be an appropriate framework when investigating ultimate questions about presence or absence of self-medication across species and populations, or when seeking to determine associations and/or trade-offs between self-medication and other life-history traits. It is still unknown whether life-history can predict the presence or absence of self-medication at an individual-level. This would further help to identify specific selection-pressures or constraints acting on the natural selection of self-medicative behaviors.

### 2.7.3 Reconstructing the Evolution of Self-Medication in Humans

Our early hominin ancestors could likely not have survived without knowing or learning how to maintain basic well-being, avoid toxic vegetation, and eliminate fatal endoparasites (Hardy, 2018). While these ancestors may have had a smaller pharmacopoeia and fewer virulent diseases than we have today, they likely possessed a suite of self-medicative adaptations including taste, smell, and texture association used to identify medicinal plants (Huffman, 2016; Huffman, 2001). Some remnants of known bioactive medicinal plants have been extracted from the dental calculus of Neanderthal teeth from El Sidrón, Spain (49ka) (Hardy et al., 2012, 2013). Palaeobotanical remains from the *Boraginaceae* family, a family which includes many medicinal species, have also been found from Lower Palaeolithic Dmanisi (Gabunia et al., 2000; Messager et al., 2008),

and the Middle Palaeolithic Douara Cave (Matsutani, 1987). Palaeobotanical evidence for the presence of medicinal plants has been found at Schöningen (300 ka), the Neumark-Nord 2 site in Germany (Pop et al., 2016), and the Kebara Cave in Israel (Lev et al., 2005). Indications of increasing medicinal plant usage from the Upper Paleolithic suggest that early hominins sometimes selected plant resources for reasons other than calories and energetic efficiency (Hardy, 2018).

Huffman (2001) proposes that two major events in human evolution likely impacted the development of human-specific self-medicative behaviors, causing them to advance beyond those of non-human primates. First, the advent of language may have enabled humans to transmit detailed knowledge about plants' medicinal properties. Second, new technologies such as cooking may have allowed early hominins to detoxify plants that they previously could not use (Johns, 1990).

The ubiquity of self-medicative behaviors in our closest living relatives, and across many other non-human primates, suggests that primate modelling may be a helpful tool through which we can better understand the evolutionary origins of human medicinal practices. Specifically, which resources may have been used, what pharmacological compounds may have been targeted, and how medicinal knowledge may have been transmitted between individuals and across early hominin communities.

It is important, however, to also note the limitations of primate models. First, as great apes have undergone parallel evolution since our species split from a last common ancestor, primate models may conflate convergent and derived self-medicating behaviors. Second, since the *Pan/Homo* evolutionary split, humans and non-human primates may have influenced each other's self-medicative behaviors through observational learning in sympatric environments (Huffman, 2016). This further complicates our ability to identify convergent behaviors between species. Lastly, as self-medication could be a cultural behavior, the site where research is carried out may greatly impact the outcome of the study. These limitations must all be taken into consideration before drawing evolutionary conclusions.

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# 3 Healthcare Behaviors in the Wild Chimpanzees of Budongo Forest

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## Abstract:

Studying healthcare behaviors in non-human primates is crucial for understanding the evolution of care systems in humans. Self-care behaviors reveal how chimpanzees manage their own health, while other-directed social-care behaviors provide insight into the evolution of prosocial and empathetic behaviors in non-human primates. This paper evaluates self-directed and other-directed healthcare behaviors in the wild Sonso and Waibira chimpanzee communities of Budongo Forest using archival reports from over thirty years of observation, videos records from past researchers, and behavioral data collected over two four-month direct observational periods. We also conducted systematic health monitoring across both communities, to assess prevalence of parasites, infections, and wounds at the site. Using these combined datasets, we evaluate all known self-care behaviors at Budongo, reporting the use of two species used for leaf-swallowing by Sonso chimpanzees, and preliminarily classifying a novel technical variant observed during these events. Self-directed wound care behaviors such as wound licking, pressing fingers to wounds, dabbing wounds with leaves, and applying chewed organic material to wounds are described, as is a self-directed attempt at snare removal. We also document self-directed hygiene behaviors including post-coital genital wiping and self-cleaning after defecation using leaves. For the first time, we report the presence of social-care in Budongo, which includes physical assistance of snared individuals, other-directed wound licking, and other-directed post-coital hygiene behaviors. The presence of social-care behaviors at this site suggests that the capacity for other-directed social-care may be more widespread in chimpanzees than previously reported. Further investigation into self-care and social-care behaviors in non-human primates is needed to better understand the evolution of these behaviors in humans.

**Key Words:** *Pan troglodytes*, Prosocial, Social-Care, Animal Healthcare, Wounds, Zoopharmacognosy

## Introduction:

The ability to improve one's own health or the health of one's kin is evolutionarily advantageous, allowing individuals to live longer, reproduce more successfully, and contribute to the survival of their group (Hart, 2011; Huffman, 2001; Kessler & Aunger, 2022). In chimpanzees, it remains unknown why individuals in some communities provide social-care, while others do not. To understand the origins of our own care-behaviors, it is crucial to investigate how self-care and social-care behaviors differ between species and which factors may have influenced their evolution. This paper reports and evaluates healthcare behaviors in the Sonso and Waibira chimpanzee communities in the Budongo Forest, Uganda. Wild chimpanzees (*Pan troglodytes*), our closest living relatives, provide valuable models for studying the development of human healthcare systems; just as humans have advanced medical systems, non-human primates also exhibit complex healthcare behaviors that are essential for survival.

### A Framework for Non-Human Health Care Systems

All living beings co-evolve with pathogens in their environments (Kessler & Aunger, 2022). This evolutionary arms race has led to the evolution of complex behavioral defenses throughout the animal kingdom (Hart, 1988) which we are only beginning to understand. Kessler and Aunger (2022) refer to behavioral defenses against illness or infection as 'healthcare behaviors'. Amongst humans, healthcare behaviors are uniquely complex, with institutionalized systems like hospitals and government programs designed to elevate general health both indirectly and directly across a population. But what are the origins of these complex healthcare behaviors? In the evolutionary framework Kessler and Aunger (2022) created to better understand the origins of human and non-human healthcare systems, they subdivide these behaviors into two categories: *care behaviors* and *community-health behaviors*. Care behaviors in this context refer to actions which benefit the health of targeted sick, injured, or vulnerable individuals. Community-health behaviors refer to indirect actions which generate general benefits for the group without targeting specific individuals (e.g., latrine use to maintain a pathogen-free environment or self-isolation to reduce transmission of illness). While for scope, this paper will focus only on self and social-care behaviors amongst the Sonso and Waibira chimpanzees of the Budongo Forest, this does not preclude the possibility that community health behaviors may be present.

We elaborate on Kessler and Aunger's theoretical framework by subdividing care-behaviors into two additional categories: *self-care* and *social-care*. Self-care refers to behaviors undergone by individuals to enhance their own health states. We define social-care as behaviors which enhance the health states of others without obvious or immediate physical benefits to the caregiver. Within the category of social-care, further categories can be defined based on the relationship between the *caregiver* and *recipient* (Kessler & Aunger, 2022). Kessler and Aunger name two such categories: *kin-care* (care directed at genetically related kin) and *stranger-care* (care directed at unfamiliar individuals). We add to this framework a new category, *affiliative-care*, used to describe social-care behaviors directed toward genetically distant or unrelated, but affiliated group members. A flowchart of our theoretical framework is shown in **Figure 1.**

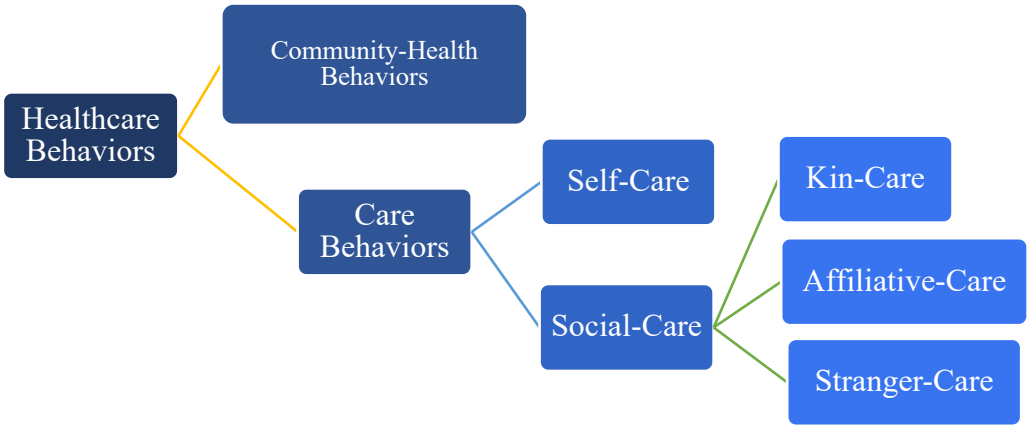


***Figure 1:*** *Adapted from Kessler & Aunger (2022), showing hierarchical relationships between terms used in this study*

## Healthcare Behaviors Across the Animal Kingdom

Non-human animals from across the animal kingdom are thought to self-medicate against several types of ailments (see Neco et al., 2019 for review). The field associated with this topic is called Zoopharmacognosy. Elephants, for example, are suggested to drink water infused with *Piliostigma thonningii* leaves to help with stomach upset (Huffman, 2022). Male great bustards have been shown to selectively forage on anthelminthic *Papaver rhoeas* and *Echium plantagineum* plants during the mating season. Recently, Indo-Pacific bottlenose dolphins have been shown to selectively rub against corals and sponges to prevent against and/or treat microbial skin infections (Morlock et al., 2022).

Social-care has also been observed in several non-human animals including elephants (*Loxodonta* sp.; Douglas-Hamilton et al., 2006), dwarf mongooses (*Helogale parvula*; Rasa, 1983), and lions (*Panthera leo*; Schaller, 2009). In primates, social wound care has been well-established in toque macaques (*Macaca sinica;* Dittus & Ratnayeke, 1989), with cases reported of healthy individuals grooming and licking the wounds of conspecifics. Tokuyama et al. (2012) report a case of social wound care in wild bonobos (*Pan paniscus*) in the form of assisted snare removal. In this case, group members helped a snared male untangle the snare wire from lianas in which it was caught, try to remove the snare from the individual's fingers, and lick his wounds. Out of all self-medicating species, however, wild chimpanzees are the most rigorously studied.

**Ingestion-Based Self-Medication in Chimpanzees**

The best established chimpanzee self-medication behaviors are leaf swallowing and bitter-pith chewing (Huffman, 2016). Leaf-swallowing, a behavior which involves the slow folding and swallowing of whole leaves, mechanically removes nodular endoparasites such as *Oesophagostomum stephanostomum* (Huffman et al., 1996; Huffman, 1997), and *Bertiella studeri* proglottids (Wrangham, 1995) as it passes through the gut. These behaviors have thus far been observed across at least sixteen field sites using over 40 different plant species (Huffman 1996). Bitter-pith chewing, which involves the stripping off the outer bark and ingestion of *Vernonia amygdalina* inner pith, provides the medicator with nematocidal plant secondary metabolites which effectively reduce *O. stephanostomum* infections (Jisaka et al., 1992; Jisaka et al., 1993; Ohigashi et al., 1991, Huffman, 1997). Since the establishment of these two self-medicative behaviors, several other behaviors, medicinal plants, and other resources have been proposed as self-medicative in non-human primates (reviewed by De la Fuente et al., 2022). These include but are not limited to geophagy (e.g., Klein et al., 2008; Krishnamani & Mahaney, 2000; Oates, 1978; Pebsworth et al., 2019; Wakibara et al., 2001), ingestion of bioactive tree barks (Huffman, 1997), and seed swallowing (Garber & Kitron, 1997).

**Self-Directed Wound Care in Chimpanzees**

In addition to ingestion-based self-medication behaviors, self-directed wound care has been reported across many chimpanzee sites (e.g., Clark et al., 2021; Watts, 2008; Goodall, 1983; Sanz

& Morgan, 2007; Mascaro et al., 2022). These behaviors can take many forms, including: 1) wound licking 2) finger licking and touching of wound 3) leaf dabbing and 4) application of chewed organic matter to wounds. **Table 1** provides operational definitions for these self-directed wound care behaviors based on past reports.

**TABLE 1:** Definitions of self-directed wound care behaviors in wild chimpanzees

| Behavior | Definition | Possible Function(s) | Reference |
|---|---|---|---|
| **Lick wound** | Licking wound directly with tongue. | Cleans or sterilizes wound by removing dirt or from antimicrobial properties of saliva | Clark et al., 2021; Hart & Powell, 1990 |
| **Lick finger and apply to wound** | Licking finger and applying or pressing finger to wound. May then lick finger and repeat. | Cleans or sterilizes wound by removing dirt or from antimicrobial properties of saliva | Clark et al., 2021 |
| **Leaf dab** | Wiping or dabbing wound with single or multiple leaves, which are then usually sniffed and dropped, but may be licked and reused. | Reduces risk of infection OR could be purely exploratory | Clark et al., 2021; Goodall, 1983 |
| **Apply chewed organic material to wound** | Masticating organic material (i.e., leaves or insects), and placing lips on wound with material still in mouth. | Promotes wound healing through possible pharmacological properties | Mascaro et al., 2022 |

The most commonly reported wound care behavior is wound licking. The function of wound licking in chimpanzees has not yet been established, however, as reported for other animals, it likely serves to remove dirt and fly eggs, as well as sterilize wounds (Hart & Powell, 1990). Antimicrobial properties have been found in the saliva of many non-human animals, likely facilitating more rapid wound-healing. Self-directed leaf dabbing has been reported in fewer sites and, when present, appears to occur infrequently (Clark et al., 2021). Whiten et al. (1999) included "leaf dab" on their list of possible cultural variants. Thus far, this behavior has been reported in Ngogo, Uganda (Clark et al., 2021; Watts, 2008), Gombe, Tanzania (Goodall, 1983), and Goualougo, DRC (Sanz & Morgan, 2007). At the time of Whiten and colleagues' (1999) study, leaf dabbing was reported as absent in Budongo.

Recently, Mascaro et al. (2022) documented a unique wound care behavior in Loango National Park in Gabon involving the application of insects to wounds. While the insect species has not yet been specified, the authors suggest that insects may have pharmacological properties which facilitate wound healing or combat infection.

## Other-Directed Wound Care Behaviors in Chimpanzees

In captive chimpanzees, social wound care has long been established. For example, captive individuals have been observed helping others remove splinters (Köhler, 1948; Yerkes, 1943). McGrew and Tutin (1972) reported chimpanzees cleaning each others' teeth. However, in the wild, reports of social wound care have been limited to a few sites, including Gombe (Goodall, 1986), Taï (Boesch, 1991), Ngogo (Clark et al., 2021), and most recently Loango (Mascaro et al., 2022). In most of these cases, reports involve healthy individuals grooming or licking the wounds of (sometimes related) group members, although other-directed leaf dabbing and the application of insects to others' wounds have also been reported (Goodall, 1986; Clark et al., 2021; Mascaro et al., 2022).

Until recently, social wound care in chimpanzees was thought to take place only between maternally-related kin (Clark et al., 2021; Goodall, 1986). Recent findings by Clark et al. (2021) and Mascaro et al. (2022), however, have established the presence of other-directed wound care (leaf dabbing and insect application) between unrelated, but affiliated individuals. While chimpanzees appear capable of at least two forms of social-care (i.e., kin-care and affiliative-care), the presence and frequency of these behaviors seem to vary between sites. Although the reason for this cross-site variation is still unknown, Boesch (1991) proposed, based on observations from Taï, that social wound care may occur more frequently amongst non-kin conspecifics in places, like West Africa, where heightened risk of predation has led to increased social cohesion.

## Health Risks Amongst Budongo Chimpanzees

Throughout the year, wild chimpanzees across field sites, harbor endoparasite infections, ranging in severity. While a baseline parasite load is expected, and may even benefit host health (Hart & Hart, 2018), when species load is too high or diverse, hosts may suffer discomfort, sickness, or reduced fitness. The presence of protozoa may also have deleterious effects on the health. Multiple studies have investigated parasite-ecology amongst the Sonso community in Budongo (e.g., Barrows, 1996; Huffman et al., 2009; Kalema, 1992, 1997; Zommers et al., 2013), the results of which are presented in **Table 2**. Waibira's parasite ecology, however, remains relatively unreported.

**TABLE 2:** Historic reports of parasite presence in the Sonso community (1992-2013)

| Scientific Name | Species | Zommers (2013) | Pebsworth (2006) | Kalema (1992;1997) | Barrows (1996) | Huffman (2009) |
|---|---|---|---|---|---|---|
| *Nematoda* | *Oesophagostomum* sp. | X | X | X | X | X |
| | *Necator* sp. | X | X | | | |
| | *Trichostrongylus* sp. | X | | | | |
| | *Probstmayria* sp. | X | | | | |
| | *Strongyloides fulleborni* | X | X | X | X | X |
| | *Trichuris* sp. | X | | | X | X |
| | *Ascaris* sp. | X | | | | |
| | *Ternidens deminutus* | | X | | X | X |
| | *Unidentified Strongyle* | X | | | X | |
| *Cestoda* | *Bertiella* sp. | X | X | | X | X |
| *Trematoda* | *Dicrocoeliidae* sp. | X | | | | |
| *Ciliates* | *Giardia lamblia* | | | | X | X |
| | *Entamoeba coli* | | | | X | X |
| | *Entamoeba histolytica* | | | | X | |
| | *Unidentified Entamoeba sp.* | | | | X | |
| | *Endolimax nana* | | | | | X |
| | *Chilomastix mesnili* | | | | X | X |
| | *Iodamoeba buetschlii* | | | | X | X |
| | *Blastocystis hominis* | | | | X | X |
| | *Troglodytella* sp. | X | X | | X | X |
| | *Balantidium coli* | | | | X | |
| | *Troglocorys cava* | X | | | | |

Another health risk at Budongo is respiratory outbreaks. While these outbreaks have not been systematically studied, Reynolds et al. (2005) reports an outbreak of Respiratory Syncytial Virus (RSV), Paramyxovirus 1, 2, or 3, or Influenza A and B which occurred in November 1999. Since this account, respiratory infections appear to be increasing, appearing seasonally in February-March in both communities (C. Hobaiter personal communication). These outbreaks could come from other animals living in the forest such as bats, but more likely spread through human-primate encounters. Similar outbreaks of fatal respiratory disease have been reported across most long-term chimpanzee field sites e.g., Mahale (Kaur et al., 2008: human-related metapneumovirus), Taï forest (Patrono et al., 2018: human coronavirus OC34), and Kibale (Scully et al., 2018: rhinovirus C).

Finally, wounds and injuries are a major health threat challenging both communities in Budongo. Severe injuries are mostly caused by intragroup aggressions, encounters with other animals (i.e., bushpigs), and—most commonly—human-laid snares (unpublished site data). Snares, which are primarily intended for duiker and bushbuck in Budongo, are mostly made from wire or nylon, though mantraps can be found in nearby forest-edge areas (Reynolds et al., 2005).

**Previously Investigated Healthcare Behaviors in Budongo**

Although chimpanzee care behaviors have been reported at various field sites, systematic, community-specific, investigations into these behaviors are still lacking. While several studies have examined specific components of chimpanzee self-medication in Sonso (e.g., Huffman et al., 2009; Pebsworth et al., 2019; Pebsworth et al., 2006), no study has directly addressed this topic in Waibira and much still remains unknown. The first documented case of chimpanzee self-medication in Budongo was observed by Chris Bakuneeta and colleagues in Sonso, who observed a chimpanzee leaf swallowing with the species *Aneilema aequinoctiale* (Commeliniaceae). Subsequent mention of *A. aequinoctiale* leaf-swallowing at Budongo, including indirect findings of whole leaves in feces, were associated with periods of increased tapeworm infection (*Bertiella sp.*) (Huffman et al., 2009; Pebsworth et al., 2006), as was also the case in Kibale (Wrangham 1995). Unlike at other sites, however, Huffman et al. (2007; 2009) found that leaf swallowing in Budongo did not appear to be associated with *Oesophagostomum* worm expulsion, and that infections involving this nematode species did not increase during wet months. The authors suggest that both findings can be attributed to the site's relatively low rainfall seasonality, which affects the reinfection biology of this parasite species. The other established self-medicative behavior, bitter-pith chewing of *V. amygdalina*, has never been reported in Budongo.

Several plant species have been suggested as putative self-medicative resources in Budongo (Pebsworth et al., 2006). Pebsworth et al. (2006), in their study on the Sonso community, report three dietary items from Budongo which they associate with unusual behaviors or altered health states in consumers. These resources include *A. boonei* bark, *F. sur* bark, and *K. anthotheca* bark and resin. Hygiene behaviors, such as self-directed and other-directed postcoital penis cleaning using leaves have been observed in the Sonso community, and were proposed to be a means to maintain cleanliness and prevent sexually transmitted diseases (O'Hara & Lee, 2006).

Geophagy as a self-medication behavior has also been investigated at Budongo, specifically involving clay (Pebsworth et al., 2019) and termite soil (Reynolds et al., 2015). In 2019**,** Pebsworth et al. found that clay consumed by Sonso chimpanzees contained elevated levels of bioavailable iron, and that it was effective at adsorbing phenolics from plant. They propose that clay could, therefore, protect against stomach upset caused by these condensed tannins. This may become increasingly important as tannin levels in chimpanzee diets are rapidly rising due to elevated $CO_2$ levels (Marsh et al., 2013). The ingestion of both active and inactive *Cubitermes speciosus* termite soil mounds amongst Sonso group members has also been proposed as a self-medicative behavior (Pebsworth et al., 2019; Reynolds et al., 2005; Tweheyo et al., 2006). Reynolds et al. (2005) found that termite soil at Budongo is rich in iron, kaolinite, halloysite and meta-halloysites. A systematic mineral assessment of termite mound soil collected *in situ,* found that soils were high in aluminum and iron but low in sodium (Reynolds et al., 2015). Tweheyo et al. (2006), in their preliminary evaluation of the health state of chimpanzees eating termite soil, found that all individuals observed consuming this resource were infected by at least two nematodes (*Oesophagostomum* sp. and *Strongyloides fullebornii)* as well as a symbiont protozoa (*Troglodytella abrassarti*). Notably, however, many chimpanzees in the community are regularly infected with these parasites. Others like Ketch et al., (2001) have suggested that termite soil could have antibacterial properties in the form of actinomycetes, a filamentous bacteria abundant in ingested termite mound clays

In this study, we aim to assess a variety of healthcare behaviors in two habituated chimpanzee communities in the Budongo Forest and discuss the evolutionary implications of these findings. By evaluating healthcare behaviors across sites, we can better determine whether specific care-behaviors are universal in this species or vary between populations.

## Methods:

### Study Site and Subjects

Established by Vernon Reynolds in 1990, the Budongo Conservation Field Station (BCFS) is located within the Budongo Forest, Uganda. Within the forest, the Sonso and Waibira communities are the only two groups habituated for research. These communities consisted of ~68 and ~105

identified individuals respectively at the onset of behavioral data collection in June 2021. Sonso's habituation began in 1990, while Waibira's began in 2011. As of 2022, Sonso's home range was ~5.33 km2 and Waibira's was ~10.28 km2 (Badihi et al., 2022). Rainfall at Budongo ranges between 1200-2200 mm and exhibits a bimodal pattern, with two historical wet seasons (March-May and September-November), and an annual dry season (December-February) (Reynolds, 2005). Temperatures remain relatively consistent throughout the year, ranging from 19°C to 32°C.

## Data Collection

### *Site Logbook*

Researchers and field staff monitor chimpanzees in Sonso daily from 07:00 to 16:30 and in Waibira from 06:30 to 17:00. In addition to party composition scans conducted every 15-minutes by field colleagues, noteworthy events are documented in community-specific logbooks. The Sonso logbook, dating back to 1993, is divided into three volumes covering September 1993-September 2021. In Waibira, daily summaries as well as noteworthy events are recorded.

### *CSMAS (Chimpanzee Self-Medicative Anecdote Survey)*

The Chimpanzee Self-Medicative Anecdote Survey (CSMAS) was activated and accepting entries from December 25$^{th}$, 2021, to September 25$^{th}$, 2022. Its purpose was to collect anecdotes from different field sites to obtain additional information on instances of primate self-medication and to promote collaboration among researchers. Any researcher who worked with wild apes could participate. Our paper includes only anecdotes submitted by researchers who have previously worked at BCFS, including reports of self-care (**Table 5**) and social-care (**Table 6**).

### *Great Ape Dictionary Video Archive*

The study also used data from the Great Ape Dictionary video database (Hobaiter et al., 2021), which contains ~35,000 catalogued video clips, updated regularly. The videos have basic meta-data, including location, date, duration, individuals present, and notable behaviors. The Budongo East African chimpanzee population contributed 13,806 videos to the current version (1.0.0) of the database. We analyzed videos from this database which were coded as including leaf swallowing and/or wound care.

***Direct Observational Period (Behavioral Data Collection and Health Monitoring***

The study employed identical methods throughout two 4-month direct observational periods, the first in Sonso (June-October 2021) and the second in Waibira (June-October 2022). We used pseudo-random focal selection, with focals selected each morning based on prioritization criteria (Hobaiter et al., 2017), including parasite load or health state, presence of sickness behavior(s), recent unusual feeding behavior(s), or inclusion in a dependent dyad (mother-infant/adopter-adoptee). If no individuals met the criteria, a focal was selected randomly. All feeding data were recorded for focal individuals, and *ad libitum* events were noted for events of interest. Individual health states were recorded opportunistically each day for all observed community members, including female estrous status, presence of sickness behaviors (including diarrhea, lethargy, day-nesting, audible respiratory symptoms, or decreased appetite), and any visible wounds. All observations of wound care behaviors were recorded and, when possible, filmed.

Age classes in this study were defined as follows: infant (0-4 years), juvenile (5-9 years), subadults (female: 10-14 years; male: 10-15 years), and adults (female: 15+; male: 16+) (Reynolds et al., 2005). Individuals were considered 'kin' if they were part of a direct maternal/paternal lineage, including maternal/paternal siblings. Individuals were considered 'affiliates' if they were group mates but not directly related, according to our operational definition. Genetic relationships at the site were determined through genetic testing, although kin relationships are better established in Sonso than in Waibira (unpublished site data).

***Fecal Collection***

In Sonso, we collected a total of 77 fecal samples from July-October 2021, from 34 individuals (female=19; m=15). Of these, only one sample was a duplicate (taken on the same day from an already sampled individual). In Waibira, a total of 168 fecal samples were collected between June-October 2022 from a total of 41 individuals (female=13; male=28), 28 of which were duplicates. To reduce bias across our community comparison, we excluded duplicate samples, using only the first sample collected per day for analyses. Remaining sample sizes numbered 76 in Sonso and 140 in Waibira.

For macroscopic fecal analyses, we visually examined all samples for the presence of adult helminths and proglottids, recording the number present. When possible, proglottids were

collected and, when possible, identified at the Natural History Museum in London (APHA authorisation no. ITIMP21.1550). We also reported stool consistency and color, as well as the presence of any identifiable plant parts (Huffman et al., 1997).

To microscopically quantify parasite load in fecal samples we used the McMaster method (Huffman et al., 1997; Huffman et al., 1993), adhering to WHO's Bench Aid Protocols (2019). Fresh samples were collected in the field with no ethanol or formalin added and refrigerated at the end of each day. Samples were tested in batches ~3 times a week. Two-chamber McMaster slides were filled with a high-density sugar and water solution and examined under a microscope at x10 magnification. The eggs per gram (EPG) of the sample was calculated by multiplying total McMaster count by 50. As eggs of *Bertiella* sp. were only identified after the Sonso data collection period, and thus not represented in our data, we excluded this species during our comparison. Micrographs of characteristic parasite eggs can be found in the **Supplementary Materials** (**SM Figures 1** and **2**). Protozoa were not systematically tested for, so were excluded from quantitative comparison.

To evaluate monthly, group-level species diversity, we averaged the number of parasite species in each fecal per month and found monthly sample means for each community. To evaluate monthly mean intensity of infection, we divided the total EPG of the species by the number of monthly samples containing that species (**SM Tables 3** and **4**).

***Urinalysis***

Urinalysis, a non-invasive diagnostic tool commonly used in veterinary health monitoring (e.g., Kaur & Huffman, 2004; Krief et al., 2005; Kurien et al., 2004; Macintosh & Huffman, 2012; Pebsworth et al., 2006), was used to opportunistically assess health and physiological status of individuals in both communities. We used multi-reagent Urine Dipstick Tests 9-RC for Urotron RL9, which can be read in 60-120 seconds, and which are easy to interpret with minimal training. Strips are used in humans to test for **leukocytes** (LEU) associated with pyuria caused by UTI, balanitis, urethritis, tuberculosis, bladder tumors, viral infections, nephrolithiasis, foreign bodies, exercise, glomerulonephritis, and corticosteroid and cyclophosphamide use; **nitrites** (NIT) associated with bacterial infection or UTI; **urobilinogen** (URO) associated with Hemolysis and hepatocellular disease; **proteins** (PRO) associated with renal disease and albuminuria;

**blood** (BLO) associated with peroxidase activity of erythrocytes; **pH** (PH) associated with UTIs and calculi; **specific gravity** (SG) associated with dehydration, glycosuria, and inappropriate antidiuretic hormone; **ketones** (KET) associated with pregnancy, carbohydrate-free diets, starvation, and diabetes; **bilirubin** (BIL) associated with liver dysfunction and biliary obstruction; and **glucose** (GLU) associated with diabetes mellitus, Cushing's syndrome, Fanconi's syndrome, and liver and pancreatic disease (Simerville et al., 2005). Urine samples were collected from wet substrates immediately after urination, including leaves, rocks, and the ground, and deposited on test strip's reagent pads. Test results were interpreted *in situ* using a colorimetric scale.

In Sonso, 52 urinalysis tests were conducted on 23 individuals (female=14; male=9); 10 were duplicate tests (from the same individual on the same day). In Waibira, 56 urinalysis tests were conducted on 24 individuals (♀=9; ♂=15); 16 were duplicate tests and two were removed due to missing measures. For our analyses, we only evaluated results from the first test conducted on each sample.

We considered a result 'abnormal' if the colorimetric scale indicated a positive result when the expected result was negative or if the result was outside the specified test parameters according to the manufacturer **(**see **Table 4)**. Equivocal or trace results of any substance were considered negative. As pH in chimpanzee urine exceeded the expected results for human urine (5-8) in all but 2 samples, we considered an abnormal pH in this study to be any result below seven, three colorimetric gradations below the sample mode of 9.

***Plant Collection and Identification***

Plants used by chimpanzees for suspected self-medication during the direct observational period were collected and, when possible, identified. After identification was confirmed by trained field staff, current scientific names of each species were confirmed on (https://powo.science.kew.org/) in October 2023. Plant family assignments were done in accordance with The Angiosperm Phylogeny Group IV guidance (2016).

### Permits and Permissions

Data recorded during the direct observational period (June-October 2021/2022) were collected under Uganda Wildlife Authority permit no. COD/96/05 and Uganda National Council for Science and Technology permit no. NS257ES. The direct observational component of this study adhered to the Code of Best Practices in Field Primatology (Riley et al., 2014). All applicable international and national guidelines were followed. The authors report no conflict of interest.

### Data Availability

Videos of several reported events are available in the supplementary materials.

## Results:

### General Health Assessment

#### *Fecal analysis*

Across all samples, including duplicates, (Sonso: n=77; Waibira: n=168), we identified 12 species of endoparasites. Taxonomic information on these species can be found in **SM Table 1**. In both communities, *Bertiella* sp. proglottids (**SM Figure 2**) were macroscopically observed in some samples. In Sonso, *Bertiella* sp. proglottids were first observed in the sample of an adult male in July 2021, and subsequently appeared in other members of the group over the following months. By October, six Sonso individuals were actively shedding proglottids (female=1; male=5). In Waibira, between June-October 2022, four Waibira individuals (♀=1; ♂=3) were observed shedding *Bertiella* sp. proglottids, however we observed no increase in frequency of occurrence throughout the study period.

When duplicate samples were eliminated (Sonso: n=76; Waibira: n=140), Sonso had a higher prevalence of *Ascaris* eggs, *Strongyloides* larvae, *Strongyloides* eggs, *Taenia* eggs, *Enterobius* eggs, and *Trichostrongyloides* eggs, while Waibira had a higher prevalence of *Ancylostoma* eggs, *Taenia* segments, and *Trichuris* eggs across samples (see **Table 3** and **SM Figure 3**). Both groups had an equal total prevalence of *Oesophagostomum* eggs across study periods (95%). In both communities, the average species richness per sample was three. We also quantified species

diversity and infection intensity across non-duplicate samples to provide a general overview of these measures in Budongo (see **SM Tables 3** and **4**).

### *Parasite Seasonality*

As fecal samples were collected for each community across the same months but during different years (Sonso: 2021; Waibira: 2022), we first evaluated seasonality across study periods to ensure comparability (**Figure 2a - 2b**). Mean monthly temperatures stayed relatively consistent from June through October across both years. In 2021 mean monthly rainfall increased in the months of September and October, while in 2022 rain increased in August and remained relatively constant throughout September and October, peaking in November.

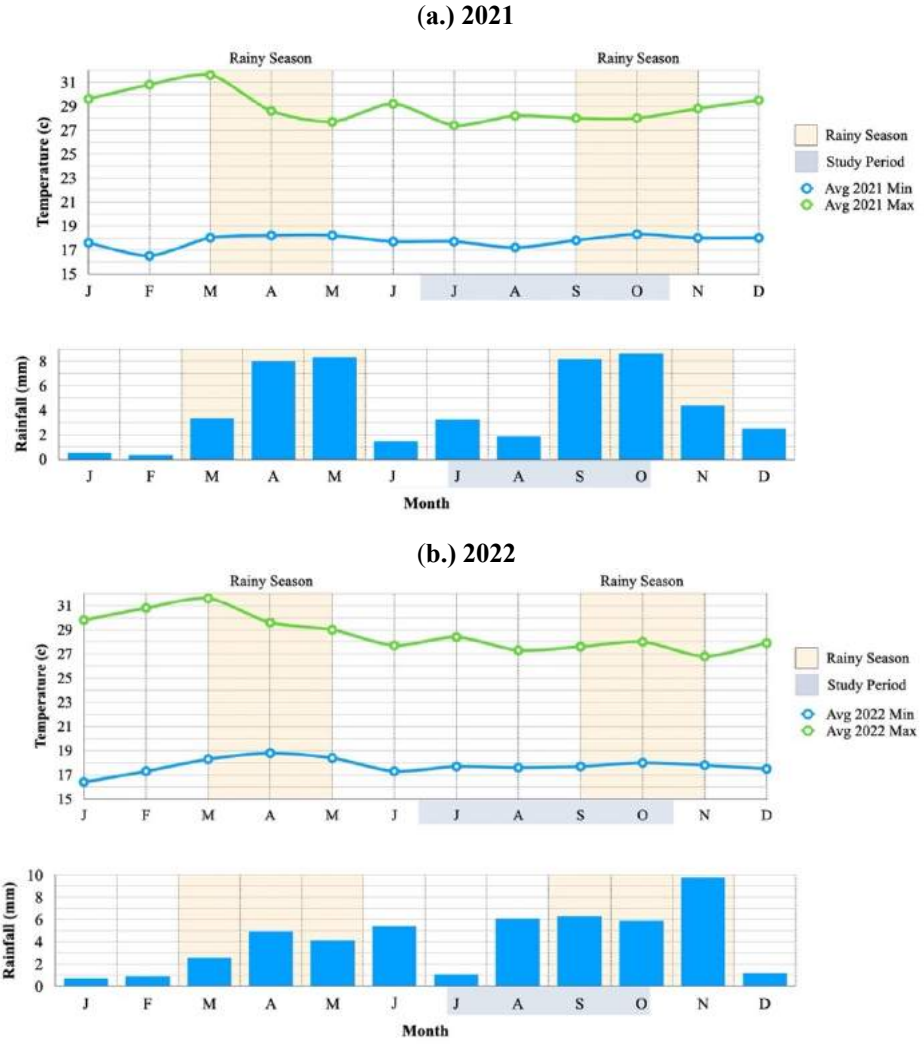


***Figure 2:*** *Monthly total rainfall and mean daily high and low temperatures*

To compare group-level sample prevalence of each parasite species, we report the percentage of fecal samples containing parasite eggs of each species per month (**Table 3)**. The number of unique individuals sampled per month is reported in **SM Table 5**.

**TABLE 3:** Monthly sample prevalence for parasites detected in Sonso (2021) and Waibira (2022)

| Month | Group | Total samples (n) | *Asc (Egg)* | *Anc (Egg)* | *Oes (Egg)* | *Stro (Larv)* | *Stro (Egg)* | *Tae (Seg)* | *Tae (Egg)* | *Ent (Egg)* | *Tricho (Egg)* | *Trichu (Egg)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***June*** | Sonso | 0 | - | - | - | - | - | - | - | - | - | - |
| | Waibira | 5 | 20% | 80% | 60% | 0% | 40% | 0% | 40% | 20% | 20% | 0% |
| ***July*** | Sonso | 18 | 78% | 50% | 100% | 28% | 28% | 16% | 0% | 0% | 28% | 6% |
| | Waibira | 52 | 21% | 71% | 94% | 0% | 21% | 0% | 0% | 0% | 29% | 8% |
| ***August*** | Sonso | 18 | 33% | 67% | 100% | 0% | 56% | 0% | 22% | 0% | 50% | 6% |
| | Waibira | 32 | 22% | 78% | 97% | 3% | 13% | 28% | 6% | 0% | 25% | 6% |
| ***September*** | Sonso | 29 | 28% | 79% | 86% | 0% | 41% | 3% | 7% | 7% | 45% | 0% |
| | Waibira | 46 | 2% | 87% | 98% | 7% | 22% | 54% | 7% | 4% | 35% | 7% |
| ***October*** | Sonso | 11 | 45% | 64% | 91% | 0% | 18% | 0% | 45% | 0% | 45% | 0% |
| | Waibira | 5 | 0% | 80% | 100% | 0% | 0% | 60% | 0% | 0% | 40% | 0% |
| ***Parasite prevalence across study period*** | Sonso | **76** | **45%** | **67%** | **95%** | **7%** | **39%** | **5%** | **14%** | **3%** | **42%** | **3%** |
| | Waibira | **140** | **14%** | **78%** | **95%** | **3%** | **19%** | **26%** | **5%** | **2%** | **30%** | **6%** |

**Asc**=*Ascaris*, **Anc**=*Ancylostoma*, **Oes**=*Oesophagostomum*, **Stro**=*Strongyloides*, **Tae**=*Taenia*, **Ent**= *Enterobius*, **Tricho**=*Trichostrongyloides*, **Trichu**=*Trichuris*, **(Larv)**=larva, **(Seg)**=segment

*Reported percentages are calculated as: $\% = \frac{\#\ of\ infected\ samples\ per\ month}{\#\ of\ collected\ samples\ per\ month\ (n)}$

To visualize variation across months and between communities, we plotted monthly sample prevalence of each parasite species (**Figure 3)**. In Sonso, the percentage of infected samples increased most notably for *Taenia* eggs toward the onset of the wet season (from September to October). During this period, the prevalence of *Ascaris* eggs and *Oesophagostomum* eggs in Sonso also marginally increased. In Waibira, the presence of *Taenia* segments increased, showing a steady incline beginning in July. *Trichostrongyloides* eggs and *Oesophagostomum* eggs also marginally increased in Waibira between September and October.

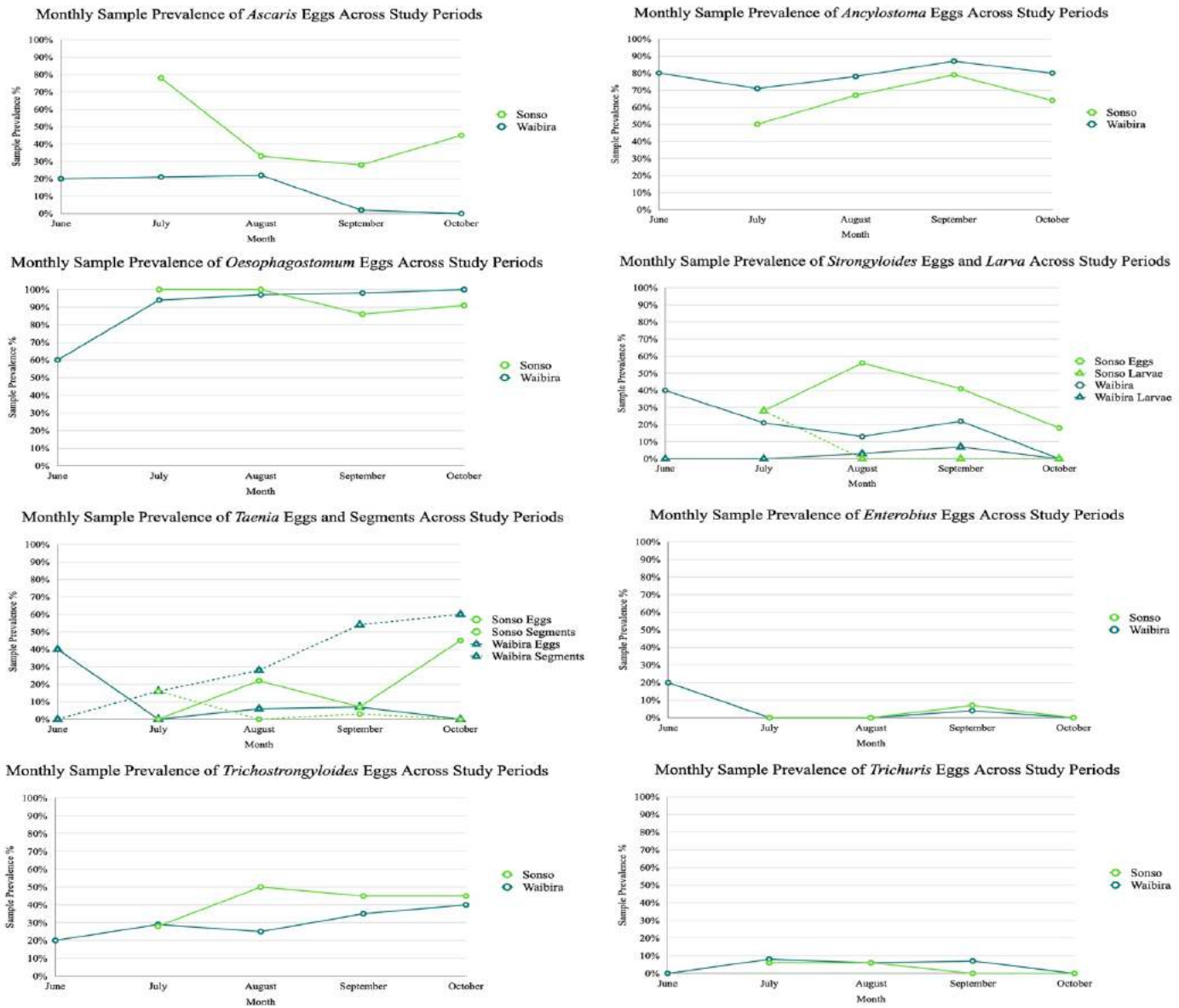


***Figure 3**: Monthly sample prevalence of each parasite species across both communities*

### *Urinalysis*

The following analysis is based on 80 samples collected from 47 total individuals (Sonso: 42 samples from 23 individuals; Waibira: 38 samples from 24 individuals). We summarize the results of these tests in **Table 4**. In Sonso, across all metrics, 28 tests (67%) had at least one abnormal value. In Waibira, 16 (42%) had at least one abnormal value. Nine tests from Sonso and four from Waibira tested positive for leukocytes. Protein and specific gravity were the other most commonly abnormal metrics. No individual from either community tested positive for bilirubin or glucose. The bilirubin pad turned orange in every test run during both study periods, which is unquantifiable on the reagent strip. Overall, Sonso had more abnormal results than Waibira across all metrics except for protein.

**TABLE 4:** Urinalysis results

| | | **# of Tests with Normal/Negative Urine Values** | | | **# of Tests with Abnormal/Positive Urine Values** | |
|---|---|---|---|---|---|---|
| **Reagent strip metric** | **Normal (Expected) Result** | **Sonso** n=14 | **Waibira** n=22 | **Abnormal Result** | **Sonso** n=28 | **Waibira** n=16 |
| LEU cacells/µl | Negative or ≤15 | 33 | 35 | ≥70+ | 9 | 3 |
| NIT | Negative | 39 | 37 | if + | 3 | 1 |
| URO µmol/l | ≤0.2(3.5) - 1(17) | 41 | 38 | ≥2(35) | 1 | 0 |
| PRO g/l | Negative or ≤15(0.15) | 34 | 30 | ≥30(0.3) | 8 | 8 |
| pH | ≥7 | 40 | 38 | ≤ than 8 | 2 | 0 |
| BLO cacells/µl | Negative or ± | 38 | 37 | if + | 4 | 1 |
| SG | ≤1.000 – 1.010 | 33 | 34 | ≥1.015 | 9 | 4 |
| KET mmol/l | Negative or ≤5(0.5) | 39 | 36 | ≥15(1.5) | 3 | 2 |
| BIL µmol/l | Negative | 42 | 38 | ≥to 1(17) | 0 | 0 |
| GLU mmol/l | Negative | 42 | 38 | if + | 0 | 0 |

**Note:** LEU = leukocytes; NIT = nitrites; URO = urobilinogen; PRO = proteins; BLO = blood; SG = specific gravity; KET= ketones; BIL= bilirubin; GLU = glucose

### *Wounds and Injuries*

In Sonso, 12 injuries (female=2; male=10) were documented over the four-month observation period, all of which were suspected to be caused by intragroup aggressions. In two cases, fresh wounds were observed in individuals the day of or after infanticides. In another case, the aftermath of an intragroup aggression event was observed. During this event, the adult female, KG, was

badly beaten by several males, resulting in severe wounds on her vulva. No new snare injuries were documented during the study period.

In Waibira we observed five injured individuals (♀=1; ♀=4) over a four-month period. A dependent juvenile female, (PAV), obtained the most severe of these injuries, caused by a wire snare. When this injury was first observed, the snare was attached to PAV's right foot, which appeared swollen and infected. After an initial sighting, PAV and her mother (PEN) were not seen again for a prolonged period (84 days). When PEN re-joined the group, she was alone, at which point PAV was assumed to have died from her wounds. The four other reported injuries in Waibira involved adult males (LKU, MAC, ALF, ILA). At least three of these were likely the result of intragroup aggressions. All wounds observed throughout both study periods are listed and characterized in **SM Table 2**.

**Healthcare Behaviors in Budongo**

Across our datasets, we report numerous cases of self-care and social-care (including both kin-care and affiliative-care) amongst the Budongo chimpanzees. Cases of self-care events include leaf swallowing (n=8) (**Table 5**), wound care (n=21), attempted snare removal (n=1), and hygiene behaviors (n=8) (**Table 6**). We also report several cases of unusual resource ingestion by unhealthy or wounded individuals observed throughout the direct observational period. Lastly, we document the presence of other-directed social-care amongst members of the Sonso community (**Table 7**), including reports of snare-related physical assistance (n=3), wound care (n=4), and postcoital hygiene (n=1).

**Self-Care**

***Leaf Swallowing***

We report eight events (involving 12 individuals) of leaf swallowing across our datasets. Individuals involved in these events include: 8 adults, 2 sub-adults, 1 juvenile, and 1 infant. As leaf swallowing is not in the site's long-term coding scheme and thus reported on an *ad libitum* basis, this is likely an underrepresentation of the frequency of this behavior. In two of these cases (**Table 5**: Observations 1 and 8) the leaves of *A. aequinoctiale* were used. In the remaining six

cases (**Table 5:** Observations 2-7), the leaves of *Broussonetia papyrifera* were selected. Throughout the direct observational period, the authors observed one case of leaf swallowing (in Sonso) (**Table 5**: Observation 8). During this event, two individuals leaf swallowed *A. aequinoctiale* (see **Supplementary Materials** for full description). On the day of this observation, one of the involved individuals, adult female (KL), had *Ascaris* (50 EPG), *Ancylostoma* (250 EPG), *Oesophagostomum* (500 EPG), and *Trichostrongyloides* (200 EPG) present in her fecal sample. No parasitology was available for other cases of leaf swallowing reported in this study. Voucher specimens of both species can be found in **SM Figure 4**.

### *Leaf Swallowing Techniques*

Of the six events involving *B. papyrifera*, we observed leaf swallowing with both unattached and attached leaves. Unattached leaf swallowing involved individuals detaching leaves from the plant before folding and swallowing. Attached leaf swallowing involved individuals folding leaves into their mouths while leaves were still attached to the rooted stem. While folding the leaves into the mouth, some individuals either elevated their hands and lowered the leaf into their mouths from above (**Figure 4** and **5**) OR held the leaf below the chin and lowered their head to fold the leaf at a downward angle (**Figure 6**). A possible technical variant was also observed across *B. papyrifera* events; in all cases, chimpanzees turned the *B. papyrifera* leaf upside down before leaf swallowing, positioning the leaf's rough upper surface downward, and the leaf's soft-haired bottom surface facing upward. However, evidence thus far for leaf swallowing of this species is based only on behavioral observations, and folded leaves have not yet been observed in fecal samples.

**TABLE 5:** Documented cases of leaf swallowing reported at Budongo

| # | Individual(s) | M/D/Y | Species | Technique | Description | Observation source |
|---|---|---|---|---|---|---|
| 1 | **Zesta** (adult, male) | 1/1998 | *A. aequinoctiale* | Unknown | Zesta pulled several plants of this species, detached either petioles or leaves from stalk, and carefully placed them in mouth, before swallowing whole. Individual chose petioles toward middle of the stalk and discarded petioles on top or bottom. | Events Book |
| 2 | **Bahati** (adult, female) | 10/1/2008 | *B. papyrifera* | **Bahati**: Attached and Unattached | Bahati leaf swallowed with unattached leaves, holding a cluster above her mouth with an elevated hand. She swallowed all leaves whole and picked another stem. When finished, she grabbed the branch and began leaf swallowing attached leaves. Alternated between attached and unattached leaf swallowing for rest of bout. | GAD **(SM Video 1-2)** |
| | **Kumi** (subadult, female) | | | **Kumi**: Unattached | Kumi leaf swallowed with unattached leaves, lowering her head to the leaf. | |
| 3 | **Karo** (juvenile, female) | 10/19/2008 | *B. papyrifera* | **Karo:** Attached and Unattached | Karo leaf swallowed from a large leaf. Instead of folding entire leaf into mouth, she held the plant in her hands and tore a piece from the leaf's center and then folded it slowly into her mouth. She then held an unattached piece of leaf in her hand, and leaf swallowed. | GAD **(SM Video 3-4)** |
| | **Karibu** (infant, female) | | | **Karibu:** Unattached | During the above event, Karo's maternal sister Karibu sat 1 m away. She attempted to leaf swallow with an unattached leaf. Efficacy of Karibu's attempt is equivocal, but her behaviors are characteristic of leaf swallowing, not normal feeding. | |
| 4 | **Hawa** (adult, male) | 6/2/2019 | *B. papyrifera* | Attached and Unattached | Hawa was within 1m of Zalu, an adult male exhibiting symptom of respiratory infection, when event occurred. Hawa repeatedly yawned throughout the bout. Hawa attempted leaf swallowing with an attached leaf, holding the branch. He then leaf swallowed with a detached leaf. | CSMAS **(SM Video 5)** |
| 5 | **Nambi** (adult, female) | 8/20/2018 | *B. papyrifera* | **Nambi:** Attached and Unattached | Nambi detached a leaf from the stem, turned leaf upside down, and began folding it into her mouth slowly. She discarded the leaf without ingestion and tried again with another smaller leaf attached to a branch. She successfully folded and swallowed whole. | CSMAS **(SM Video 6)** |
| | **Musa** (adult, male) | | | **Musa:** Attached | Musa, ~1 m, away from Nambi, slowly folded an attached leaf, turning it upside down. Other individuals waited nearby for them to finish but did not leaf swallow. | |
| 6 | **Simon** (adult, male) | 11/22/2018 | *B. papyrifera* | Attached | Simon folded leaves of an attached branch. | CSMAS **(SM Video 7)** |
| 7 | **Musa** (adult, male) | 1/10/2020 | *B. papyrifera* | Attached | Musa folded leaves of an attached branch. | CSMAS **(SM Video 8)** |
| 8 | **Kalema** (adult, female) | 8/13/2021 | *A. aequinoctiale* | Not visible | Kalema repeatedly drummed on tree buttresses prior to the event. Her son, Klauce, joined family minutes later and led the family to swampy area in the north. Kalema leaf swallowed with Kamala (infant) on her back. No technique reported due to poor visibility. | Current Study |
| | **Klauce** (subadult, male) | | | | Klauce leaf swallowed ~1 meter from Kalema. No technique reported due to poor visibility. | |

***Figure 4:*** *(**Table 5**: Observation 5)* ***(SM Video 6)*** *NB leaf swallowing sequence with attached leaf from above and unattached leaf from below (Recorded by A. Soldati)*

***Figure 5:*** *(**Table 5**: Observation 2)* ***(SM Video 1-2)*** *BH leaf swallowing sequence with unattached leaf from above (Recorded by M. Laporte)*

***Figure 6****: (**Table 5:** Observation 2)* ***(SM Video 1-2)*** *KM leaf swallowing sequence with unattached leaf from below (Recorded by M. Laporte)*

### *Bitter-pith chewing of V. amygdalina*

Bitter-pith chewing with *V. amygdalina* has not been observed in either Sonso or Waibira. While this species is available in the Sonso home range, it has only been found growing along an active road. Other, non-bitter piths (i.e., *Marantachloa leucantha, Costus* sp*., Acanthus polystachyus, Aframomum* sp.), are stripped at Budongo, but none thus far have been established as a self-medicative resource (though see **Chapter 5**).

***Resources Consumed by Unhealthy Individuals During Direct Observational Period***

Several adult females with diverse parasite loads and abnormal urological infections consumed putative self-medicative resources throughout the direct observational period. An adult, female (WM), who was experiencing moderate diarrhea, tested positive for LEU shortly before eating the bark and resin of *K. anthotheca* and the soil from an inactive termite mound. Irene (IN) tested positive for LEU, NIT, and trace levels of BLO, also while experiencing severe diarrhea. Two days later, IN again tested positive for LEU, with trace amounts of PRO and was observed consuming *K. anthotheca* bark and resin along with her infant.

Abnormal LEU levels were detected in three individuals in Waibira. A juvenile male, ROB, tested positive for both LEU and NIT. Three days later, he was the only individual in the group observed stripping and ingesting tree bark from the species *Croton sylvaticus*, while others fed on ripe fruits nearby. On another occasion, adult female BAH, tested positive for LEU while harbouring a high and diverse parasite load, including *Ancylostoma* (50 EPG), *Ascaris* (300 EPG), *Oesophagostomum* (1500 EPG), *Taenia* segments, *Bertiella* sp. (4650 EPG), and unidentified cestode eggs (200 EPG). BAH's *Oesophagostomum* load was abnormal compared to the monthly August intensity mean for Waibira (*Oesophagostomum*: 423 EPG) (**SM Table 4**). In addition, two *Bertiella* sp. proglottids were found in her sample. BAH was observed eating termite soil from an inactive mound ~1 hour after the stool sample was collected. She was also observed eating termite soil four days before in the same location, while others in the group ignored the mound. Termite soil was also consumed by an adult male in Waibira, LAF, whose urine had high levels of both KET and SG.

Adult male, ZL, exhibited abnormally low pH levels on two occasions (6 and 5 respectively). The first day this was observed, ZL actively expelled three *Bertiella* sp. proglottids in his feces and broke off from a group of males on patrol to consume *Cleistopholis patens* dead wood, followed only by an orphan male (KJ), who also briefly fed on the resource. Two weeks later, ZL was again observed with a pH of 5 and a high SG. ZL did not eat throughout the day, but again expelled a *Bertiella* sp. proglottid.

### *Self-Directed Wound Care, Hygiene Behaviors, and Snare Removal*

From historic reports and our own observations, we report 30 cases of non-ingestion-related self-care at Budongo (**Table 6)**. Of these, 28 cases of self-care took place in Sonso (20 wound care events, one attempted snare removal, six post-coital genital wiping events, and one post-defecation anal cleaning event). In Waibira we report only two cases of non-ingestion-related self-care (one wound care event and one post-coital genital wiping event). Across observations of self-directed wound care, we report individuals licking their fingers and pressing them to wounds, licking wounds directly, dabbing wounds with leaves, and applying chewed leaves to wounds. In Sonso, all above behaviors have been observed. In Waibira, the single observation of self-directed wound care involved an individual licking his fingers and pressing them to his wound as well as leaf dabbing (**Table 6: Observation 17**). Many events involved a combination of these behaviors. An observation involving both leaf dabbing and application of chewed leaves to a wound is shown in **Figure 7**. Self-directed, postcoital genital wiping and anus wiping after defecation were both observed in Sonso. In Waibira, only postcoital penis wiping has been observed.

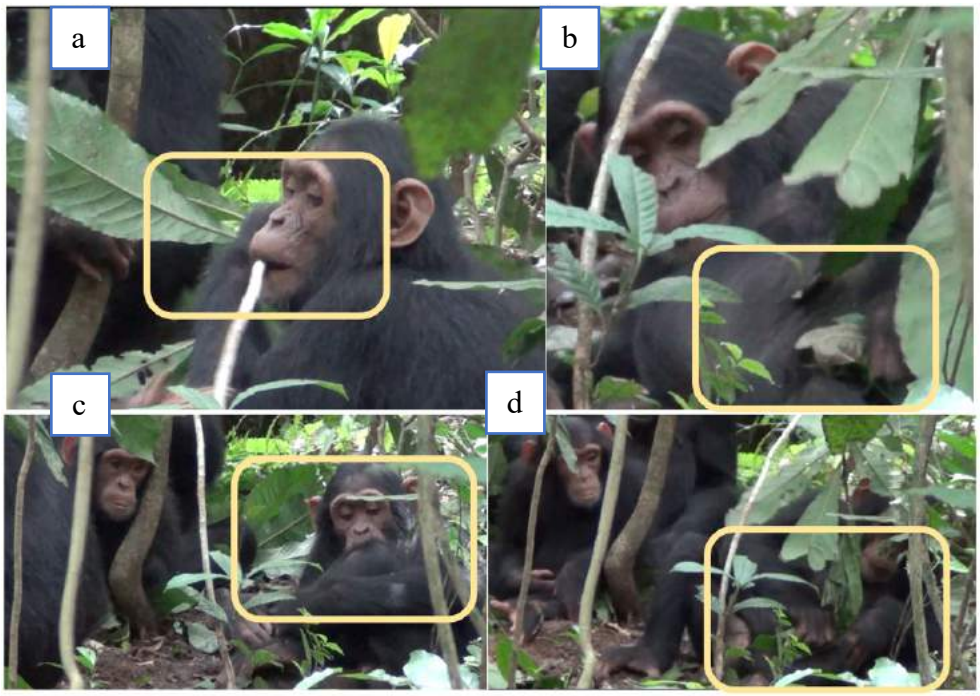


***Figure 7:*** *(**Table 6**: Observation 19)* ***(SM Video 13)*** *[**a**] KO chews stem bark of A. macrophylla [**b**] detaches leaves from stem and dabs on wounded knee [**c**] applies chewed stem bark to wound [**d**] leaf dabs with attached leaves (Images by EF)*

**TABLE 6:** Observations of self-directed wound care in Sonso

| Self-Care Type | Event # | Community S/W | Date M/DD/Y | Individual | Wound location/cause | Description | Plant species | Observer(s) |
|---|---|---|---|---|---|---|---|---|
| **Lick Fingers & Press Wound** | 1 | S | 9/10/2015 | KT (adult, male) | Unknown /suspected intragroup aggression | Licked fingers and pressed large wound on left side of back | none | Events Book |
| | 2 | S | 9/8/2021 | KC (subadult, male) | Ear/unknown | Licked finger and pressed wound | none | Current Study SM Video 9 |
| **Lick Wound** | 3 | S | 4/23/1998 | ZF (adult, male) | Puncture on left calf/unknown | Licked wound while being groomed by KW (adult, female) | none | Events Book |
| | 4 | S | 11/2002 | JM (adult, male) | Neck/unknown | Picked and licked bloody scabs from raw wound | none | Events Book |
| | 5 | S | 2/2/2003 | KN (infant, female) | Right foot/snare | Licked and touched snare wound | none | Events Book |
| | 6 | S | 12/13/2008 | JN (adult, female)<br>JT (subadult, female) | JN: Back and finger<br>JT: Right hand /unknown | Both individuals licked bleeding wounds | none | Events Book |
| | 7 | S | 2/27/2011 | ZM (adult, female) | Hand, forearm/intragroup aggression | Licked bleeding wound after being aggressed by NK (adult, male) | none | GAD |
| | 8 | S | 8/25/2011 | NT (juvenile, female) | Right hand/wire snare | Licked and touched snare wound | none | GAD |
| | 9 | S | 7/9/2012 | SQ (adult, male) | Face/unknown | Picked scab from near eye and ate it | none | GAD |
| | 10 | S | 1/23/2015 | ZG (adult, male) | Unknown/intragroup aggression | Licked wound after being injured by KT (adult, male) | none | Events Book |
| | 11 | S | 8/16/2021 | HW (adult, male) | Arm/unknown | Licked wound on lower arm | none | Current Study SM Video 10 |
| | 12 | S | 8/30/2021 | UP (adult, female) | Arm/suspected intragroup aggression | Licked wound while holding mummified carcass of dead infant. | none | Current Study SM Video 11 |
| **Lick wound & Leaf dab** | 13 | S | 2/5/2014 | MS (adult, male) | Right foot/ intragroup aggression | Licked wounds and then pressed with leaves | *Pseudopodia macrocarpa* (leaves) | Events Book |
| **Leaf dab** | 14 | S | 7/22/2008 | FK (juvenile, male) | Swollen Eye/unknown | Detached leaf and applied to eye, then discarded. Nearby juvenile (PS) picked up leaf and smelled/licked it. | Unidentified sp. | GAD |
| | 15 | S | 8/22/2011 | FK (adult, male) | Face/unknown | Detached branch of leaves, folded in mouth, pressed to forehead to stop bleeding | Unidentified sp. | GAD |
| | 16 | S | 12/19/2019 | KC (subadult, male) | Wrist/wire snare | Detached branch of leaves from tree and applied the end slowly to open wound on snared wrist | Unidentified sp. | CSMAS |
| **Leaf dab + Lick fingers & Press wound** | 17 | W | 05/27/2023 | ALF (adult, male) | Left foot | Detached leaf from shrub, licked leaf and wound dabbed with first partial, then whole leaves. Licked fresh leaves, chewed, and then finger licked and wound pressed. Repeated with old leaves from forest floor. | *Argomuellera macrophylla* (leaves) | Current Study SM Video 12a/b |
| | 18 | S | 11/20/2008 | NB (adult, female) | Vagina/intragroup aggression | Detached leaf and applied to wounded area. Folded leaf and carefully chewed it, then dabbed repeatedly on wound. Also licked fingers and applied to wound. | *Alchornia floribunda(?)* | GAD |
| **Lick wound + Leaf dab & Apply chewed material to wound** | 19 | S | 10/13/2021 | KO (juvenile, male) | Left knee/unknown | Licked wound, applied chewed stem bark to wound, and dabbed leaves of same species on wounded left knee | *Argomuellera macrophylla* (leaves) | Current Study SM Video 13 |
| | 20 | S | 2/27/2002 | MA (adult, male) | Left leg + left hand/suspected intragroup aggression | Licked wound, picked up leaves, chewed and continued to lick wound. Leaf dabbed three times, dropped leaves, and travelled | *Acalypha sp.* (leaves) | Events Book |
| **Apply chewed material to wound** | 21 | S | 10/6/2021 | PS (adult, male) | Right arm/unknown | Detached leaf from shrub, chewed leaf, and applied material to wound | Unidentified sp. | Current Study SM Video 14 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Remove snare** | 22 | S | 11/25/2015 | KT (adult, male) | Unknown/wire snare | Removed his own snare | none | Events Book |
| **Hygiene (postcoital genital wipe)** | 23 | S | 7/14/2009 | ZD (juvenile, male) | Penis/copulation | Wiped penis with leaves after copulation | Unidentified sp. | GAD |
| | 24 | S | 1/30/2011 | ZM (adult, female) | Vagina/copulation | Wiped vagina with leaves after copulation | Unidentified sp. | GAD |
| | 25 | S | 2/14/2011 | FD (adult, male) | Penis/copulation | Wiped penis with leaves in tree after copulation and then dropped them | Unidentified sp. | GAD<br>SM Video 15 |
| | 26 | S | 8/22/2011 | KM (subadult, female) | Vagina/unknown | Wiped vagina with leaves and sniffed/licked it. Unknown whether this occurred after copulation. | Unidentified sp. | GAD |
| | 27 | S | 7/14/2012 | FK (adult, male) | Penis/copulation | Wiped erect penis with leaves after copulation | Unidentified sp. | GAD |
| | 28 | S | 10/6/2021 | ZL (adult, male) | Penis/copulation | Broke branch off, wiped penis with leaves after copulation, and then licked leaves | *Lasiodiscus pervillei* | Current Study<br>SM Video 16 |
| | 29 | W | 9/30/2022 | ALF (adult, male) | Penis/copulation | Used leaves from unknown tree to wipe penis after copulation while in branches | Unknown (tree) | Current Study |
| **Hygiene (anus cleaning)** | 30 | S | 3/8/2009 | KU (adult, female) | Anus/defecation | Used leaves from unknown species to wipe anus after defecating | Unknown | Events Book |

**Social-Care**

In Sonso, social-care was not observed during the direct observation period but has previously been observed in Sonso by field staff and researchers. **Table 7** summarizes all eight recorded events of social-care in Sonso: three cases of snare-related physical assistance, four cases of social wound care, and one case of other-directed post-copulation penis wiping. The sex and age of caregivers and recipients vary across cases: three involve adult males caring for adult females, one involves a juvenile female caring for an adult female, one involves an adult female caring for a juvenile female, one involves an adult female caring for an adult male, and one involves a juvenile male caring for a juvenile male (**Figure 8**).

Of the eight total cases, three events classify as kin-care, two of which occurred between the same mother-daughter dyad (**Table 7: Observations 3** and **5**). In the first case, it is suspected that the mother (NB) provided physical assistance to her daughter (NT) who was stuck in a wire snare. In the second case, NT observed and copied her mother's self-directed wound behaviors, folding and chewing leaves and dabbing them on NB's wounds. NT also licked her fingers and applied them to NB's wound (**SM Video 18**). In the third case, an adult male (ZG), licked the wound of his maternal sibling, an adult female (KY). Of the remaining cases, four are classified as affiliative-care because they involved non-kin individuals within the same group (**Table 7: Observations 2, 6, 7, 8)**. The type of care for the remaining case is unknown as the individuals were not identified. Social-care has not yet been reported in Waibira.

***Figure 8:*** *(**Table** 7: Observation 6) Example of affiliative-care: PS licking wound of ZG (Image by L. Samuni and A. Schel)*

**TABLE 7**: Observations of social-care at Budongo

| Social-Care Type | Event # | Community (S/W) | Date M/DD/Y | Carer→Receiver | Relationship | Wound location/cause | Description | Species used | Observer |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | S | 8/15/2000 | Unknown→ Unknown | Unknown | Unknown/ mantrap | Boy in village reported seeing a chimpanzee attached to a mantrap being assisted by another chimpanzee, who was reportedly carrying the trap behind the first individual. | none | Unknown |
| **Snare-Related Physical assistance** | 2 | S | 7/18/2008 | NK (adult male)→ KW (adult, female) | Non-kin (M-F) | Unknown / nylon snare | NK helped remove a nylon snare from KW | none | Events Book Also reported by (Amati et al., 2008) |
| | 3 | S | 3/11/2009 | NB (adult, female) → NT (juvenile, female) | Kin (F-F) (Mother-Infant) | Unknown/ nylon snare | NB (mother) seen chewing nylon snare after NT (daughter) got hand stuck. Suspected that she bit nylon from NT's hand. | none | Events Book |
| **Wound care** | 4 | S | 8/13/2003 | ZG (adult, male)→ KY (adult, female) | Kin (M-F) (Maternal Siblings) | Leg/colobus attack | After attack, KY licked her wound and leaf dabbed. ZG approached and licked blood from KY's cut. | none | Events Book |
| | 5 | S | 11/20/2008 | NT (juvenile, female)→ NB (adult, female) | Kin (F-F) (Mother-Infant) | Vagina (swelling) /intragroup aggression | After attack, NB began applying a folded and chewed leaf to the wound. NT watched and then copied her mother, chewing a leaf and applying it and then licking her fingers and applying saliva. | *Alchornia floribunda(?)* | GAD SM Video 17 |
| | 6 | S | 3/8/2012 | PS (subadult, male) → ZG (subadult, male) | Non-kin (M-M) | Leg/unknown | PS groomed and sucked a deep cut on ZG's leg. | None | Events Book |
| | 7 | S | 9/5/2018 | HW (adult, male)→ RS (adult, female) | Non-kin (M-F) | Unknown/ intragroup aggression | HW licked a wound on RS after she was aggressed with her new baby. | none | Events Book |
| **Hygiene (postcoital genital wipe)** | 8 | S | 11/26/2007 | NR (adult, female)→ ZK (adult, male) | Non-kin (F-M) | Penis/copulation | NR wiped ZK's penis with leaves after copulation. | *Senna spectablilis* | Events Book |

## Discussion:

This paper provides an overview of chimpanzee healthcare behaviors at Budongo as well as established and putative self-medicative behaviors at the site. It also documents, for the first time, the presence of social-care behaviors at Budongo. Consistent with findings from Ngogo (Clark et al., 2021) and Loango (Mascaro et al., 2022), we report that Budongo chimpanzees are capable not only of recognizing and tending to their own wounds, but also to the wounds of both genetically related kin and non-related affiliates within the group.

### General Health Results

Our preliminary assessment of parasite seasonality at Budongo suggests that across both communities, *Taenia* sp. (tapeworm) infections, increased toward the onset of the wet season (September-October). In Sonso, prevalence of *Taenia* eggs increased in collected samples during this period, while in Waibira, *Taenia* segments increased in prevalence.

Our findings preliminarily suggest that diarrhea, which may be linked to geophagy (Pebsworth et al., 2019), may also be associated with high LEU levels, as several individuals who tested positive for LEU were observed eating termite soil. As LEU levels are an indicator of infection and are known to diagnose bacterial urinary tract infections in humans (Kaur & Huffman, 2004), this could offer further support for geophagy's antibiotic adaptive function (Klein et al., 2008). Additionally, our results indicate that *K. anthotheca* bark and resin ingestion could be associated with high LEU infections, as this behavior was observed in multiple infected Sonso individuals. As several individuals with positive urinalysis measures were actively shedding proglottids, we also posit that the presence of *Bertiella* sp. could impact urinalysis results. This could occur due to helminth infections affecting host immune systems, catalyzing immunomodulatory and immunoregulatory responses which compromise protection against other bacterial, viral, or protozoal co-infections (Kamal et al., 2006).

### Resources Used in Healthcare Behaviors

Sonso individuals have historically been known to use *A. aequinoctiale* leaves for leaf swallowing. We now add a second species to the community's leaf swallowing repertoire, *B. papyrifera.* From

the eight recorded videos of leaf-swallowing events, six involve *B. papyrifera*. This species, only present in the Sonso home range, is non-native to Budongo. It was likely planted in the 1950s by operators of the Budongo Sawmill, a large-scale lumbermill which operated at the site before the field station was founded (Reynolds et al., 2005). Selection of this species for leaf swallowing, therefore, must have been acquired by one or more Sonso individuals within the last two generations.

Based on our eight-months of behavioral observations and health monitoring, we also report several anecdotes involving ingestion of putative self-medicative resources by individuals in poor health. Specifically, we encourage further pharmacological testing of *Cubitermes* mound soil, *K. anthotheca* bark and resin, and *C. sylvaticus* bark.

Identified plant species used for leaf dabbing wounds include *A. macrophylla, Acalypha* sp. leaves, and *P. macrocarpa* leaves. An additional case is suspected to have included *A. floribunda* leaves. In two cases, we observed individuals chewing organic material and applying the masticated bolus to their wounds with their lips. One of the species involved in these events could not be identified, in the other, the stem bark of *A. macrophylla* was used, in combination with leaf dabbing. *A. macrophylla* leaves were also used in a self-directed wound care event in Waibira for leaf dabbing. Identified species used for postcoital penis-wiping after copulation include *S. spectablilis* and *L. pervillei* leaves.

**Intragroup and Intergroup Differences in Leaf Swallowing Behaviors**

Amongst Sonso chimpanzees, we report leaf swallowing with both "attached" and "unattached" leaves. We also report a possible leaf swallowing variant documented, in which the leaves of *B. papyrifera* are turned upside down before swallowing. Leaf swallowing has not yet been observed in Waibira. While this could be due to a true absence of the behavior, there are several variables which should be considered. First, the Sonso community has been habituated for twenty years longer than Waibira, granting far more opportunity for observation. Second, the absence of *B. papyrifera* in the majority of Waibira's home range may reduce opportunity for this behavior. The most likely possibility, however, is that because leaf swallowing is not recorded in the long-term data, the behavior may have been observed but not recognized or reported. According to our

parasitology assessment of both communities, prevalence of species targeted by leaf swallowing behaviors cannot explain the absence of this behavior in Waibira, as *Oesophagostomum* and *Bertiella* sp. infections are present and common across both communities. More parasite-ecology data is needed on chimpanzees exhibiting leaf swallowing at Budongo to better understand the site-specific catalysts for this behavior.

**Ontogeny of Self-Care Behaviors**

This study lends insight into the ontogeny of leaf swallowing, and self-care behaviors more generally. Karibu (KB), an infant female of ~22 months (in 2008) leaf swallowed *B. papyrifera* in the presence of her mother and sister (**Figure 9**) (**Table 5: Observation 3**; **SM Video 4**). While it is impossible to determine the outcome of this bout (see **Figure 9**), Karibu's leaf swallowing attempt demonstrates that this behavior can develop in infancy.

***Figure 9****: (Table 5: Observation 3) KB leaf swallows B. papyrifera (Image by M. Laporte)*

Our observations of self-directed wound care also lend insight into the development of the self-medicative skillset in chimpanzees. Our direct observation of a juvenile male, (KO), leaf dabbing and applying chewed stem bark to a wound, demonstrates that proficiency at wound related self-care is attainable in chimpanzees as early as 7yo (see **Figure 7**) (**Table 6: Observation 19**). A female, 4yo infant (IS), peered at KO throughout this bout, positioning herself nearby to watch him tend to his wound (**SM Video 14**). If peering is an index of social learning in apes, as has been suggested in orangutans (Schuppli & Van Schaik, 2019), this could suggest that horizontal

medicinal knowledge transmission or enforcement of behavioral patterns could occur between non-kin individuals from an early age.

**Socio-Ecological Variables and Healthcare Behaviors**

Chimpanzees have demonstrated a capacity for other-regarding behaviors, and recent findings show that these behaviors even appear to be expressed in group specific ways (van Leeuwen et al., 2021). *But what could cause the differences in prosocial behaviors across communities and/or sites?* While Budongo chimpanzees do not have any direct predators, they are at constant risk of human-caused injuries. In Sonso, across all known individuals to have been active members of the community, 39.2% have had confirmed snare injuries (D. Taylor personal communication). Several others have permanent disabilities which appear to have been caused by snares, but whose origins are unconfirmed. If increased predation is thought to enhance social-cohesion and cooperation (Boesch, 1991), then elevated anthropogenic risks may have a similar effect, resulting in the presence of non-kin social-care in sites with high rates of hunting. While the lack of social-care events reported in Waibira is most likely the result of observation bias, future studies should seek to systematically investigate potential correlations between social wound care and rates of snare and human-caused injuries at this site.

Instability in the social hierarchy could also impact frequency of injuries within a group, impacting opportunity for both self- or social-care, as well as for the social-transmission of these behaviors. While for scope we could not systematically investigate this in Budongo, future studies should account for social factors (e.g., rate of female migration, size of core-group, level of habituation) as well as ecological factors (e.g., availability of medicinal resources, duration of seasonal periods with increased risk) which could potentially lead to community-level differences.

**Evolutionary Implications of Social-Care**

Our observations add to the growing evidence that chimpanzees have the cognitive ability to understand both their own medical needs, and the needs of wounded or endangered group members, as evidenced by their selective aid towards them (Fábrega, 2022). The ability of chimpanzees to exhibit aid towards group members, even when they are not genetically related, strongly suggests

a capacity for empathy, a trait historically thought to be unique to humans (though see Boesch, 1991; Clark et al., 2021; Dittus & Ratnayeke, 1989).

Our observations further indicate that Sonso chimpanzees engage in social-care regardless of age, status, or relatedness, and that social-care behaviors amongst group members are highly flexible and adaptive. For example, attempts at other-directed snare removal (**Table 7: Observations 2** and **3**), suggests the presence of social-care in this community which requires innovative solutions to relatively recent environmental risks. Why social assistance with snare removal is so rare, when snare injuries remain an ongoing threat, remains unknown.

This has implications for our understanding of both non-human and human healthcare systems, as well as how these systems may have previously been impacted by social and environmental selection pressures. As chimpanzee habitats become increasingly disrupted, and primate populations inch closer to extinction, understanding the socio-ecological pressures on chimpanzee care-behaviors could play a critical role in informing conservation strategies. By identifying and protecting the resources chimpanzees need to remain healthy, and guarding against anthropogenic risks, we can help buffer our primate cousins from environmental and climatic disturbances which threaten their survival.

## Conclusion:

This paper gives an overview of self- and social-care behaviors in Budongo chimpanzees and highlights the need for further exploration into the selection pressures which drive their presence and frequency. While we currently do not know the extent to which self-medicative behaviors are socially learned or transmitted, this will be an important next step for understanding which components of non-human healthcare systems are genetically determined and which are products of culture. We strongly encourage the initiation of systematic, longitudinal studies across field sites to investigate ingestion-related and wound-related medicinal resource use by chimpanzees. Research on chimpanzee healthcare behaviors has significant evolutionary implications for our understanding of primate cognition, and highlights the need for continued research in this exciting and rapidly growing field.

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# 4 Applying Collocation and APRIORI Analyses to Chimpanzee Diets: methods for investigating non-random food combinations in primate self-medication

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## Abstract:

Identifying novel medicinal resources in chimpanzee diets has historically presented challenges, requiring extensive behavioral data collection and health monitoring, accompanied by expensive pharmacological analyses. When putative therapeutic self-medicative behaviors are observed, these events are often considered isolated occurrences, with little attention paid to other resources ingested in combination. For chimpanzees, medicinal resource combinations could play an important role in maintaining well-being by tackling different symptoms of an illness, chemically strengthening efficacy of a treatment, or providing prophylactic compounds that prevent future ailments. We call this concept the **self-medicative resource combination hypothesis**. However, a dearth of methodological approaches for holistically investigating primate feeding ecology has limited our ability to identify non-random resource combinations and explore potential synergistic relationships between medicinal resource candidates. Here we present two analytical tools that test such a hypothesis and demonstrate these approaches on feeding data from the Sonso chimpanzee community in Budongo Forest, Uganda. Using four months of data, we establish that both Collocation and APRIORI analyses are effective exploratory tools for identifying binary combinations, and that APRIORI is effective for multi-item rule associations. We then compare outputs from both methods, finding up to 60% agreement, and propose APRIORI as more effective for studies requiring control over confidence intervals and those investigating non-random associations between more than two resources. These analytical tools, which can be extrapolated across the animal kingdom, can provide a cost-effective and efficient method for targeting resources for further pharmacological investigation, potentially aiding in the discovery of novel medicines.

**Abbreviations:** Multiple Distinctive Collocation Analysis **(MDCA)**, Mutual Information Collocation Analysis **(MICA)**, Resource of Interest **(ROI),** Left-hand side of an APRIORI equation **(LHS)**, Right-hand side of an APRIORI equation **(RHS)**

**Key Words:** *Pan troglodytes,* Zoopharmacognosy*,* Food Combinations, Diet, Feeding Ecology

## Introduction:

When humans fall ill or get injured, our behaviors and diets are often impacted in a variety of ways (Prather, 2013). How and what we do to mediate illness or treat wounds largely depends on what culture we were raised in, which medical systems we participate in, availability of medicinal resources, and our own familial or cultural traditions (Kirmayer, 2004). However, irrespective of cultural or localized practice, it is unlikely that we rely on only one technique or resource to treat our symptoms (Leonti, 2013; Che et al. 2013; Ulrich-Merzenich et al. 2009; Verpoorte et al. 2009).

Across animal self-medication literature, therapeutic self-medicative anecdotes are often reported with focus placed on the ingestion of a single putative resource. Few studies investigate the broader feeding repertoires of sick individuals across the duration of an illness or interrogate whether other consumed resources may have relevant curative value (though see Krief et al. 2006; Struhsaker et al. 1997). Similarly, despite the known presence of preventative self-medication strategies across multiple primate species, involving regular ingestion of 'medicinal foods' with bioactive properties (e.g., Huffman, 1997; Huffman et al. 2020; Petroni et al. 2017), the prophylactic benefits of these resources, when combined, remain largely unknown. This is, in part, due to the high burden of proof for establishing a feeding event as therapeutically self-medicative (Huffman, 1997), or a commonly consumed resource as a 'medicinal food'.

To begin to bridge this gap in our knowledge, we propose investigation into a novel hypothesis: the **self-medicative resource combination hypothesis**, which posits that when ill, chimpanzees may therapeutically treat themselves with a combination of resources which cumulatively aid in the recovery process. This hypothesis could also apply to other self-medicative strategies, including passive prevention, through the combined ingestion of medicinal foods. However, for scope, non-therapeutic strategies are not further explored in this paper. This hypothesis is in part based on the evolutionary proximity between humans and chimpanzees, as well as the demonstrated overlap between the putative self-medicative repertoires of chimpanzees and reported ethnomedicinal practices of certain human communities (e.g., Huffman & Seifu, 1989; Petroni et al. 2017).

Medicinal resource combinations could benefit consumers in multiple ways. For example, different therapeutic resources, consumed sequentially, could mediate different parts of an illness or injury (i.e., symptom relief vs. combating the underlying infection). Consecutive medicinal resources could also have complementary operational mechanisms (i.e., mechanical vs. chemical) which attack the problem in the different ways (Huffman, 1997). Lastly, medicinal resource combinations could catalyze synergistic chemical interactions through different positive modes of combinatorial interactions. These include *Reinforcement* (when herbs have similar properties which together produce a greater effect), *Potentiation* (when herbs have different properties where one strengthens the potency of the other), and *Restraint and Detoxification* (when herbs have different properties where when one detoxifies or nullifies the negative side effects of the primary acting herb) (Che et al., 2013). If non-random medicinal resource associations are identified, and these resources are intentionally combined by wild chimpanzees, these same modes of interaction may also be present. However, to demonstrate intentionality behind these combinations, further research would need to first rule out alternative hypotheses. One such alternative hypothesis is that identified non-random food combinations are the result of an opportunistic trial-and-error self-medicative strategy (Villalba & Provenza, 2007). In this case, modes of herb-herb interactions may be present but unintentional.

We propose through the **self-medicative resource combination hypothesis** that if ill chimpanzees use a combination of resources to aid in recovery, then we may be able to identify novel self-medicative resources through identification of non-random resource combinations ingested during periods of infection. These resources may have possible mechanical, chemical, or synergistic effects against the same underlying illness, other ailments with similar symptoms, or co-infections. While proof of medicinal resource combinations will require additional behavioral data collection and targeted pharmacological analyses, a logical first step to begin exploring this hypothesis is to identify non-random resource combinations which occur above chance in existing feeding ecology datasets. These analyses can be conducted without the need for further costly or invasive data collection. However, first we need methods and analyses to quantify these associations. Despite the vast potential of this research area, to the authors' knowledge, no methods have thus far been proposed to help identify non-random food combinations in primate diets.

**Holistic Self-Medication: A New Paradigm for Studying Animal Health Maintenance Through Food Combinations**

The literature on food combinations in wild primate diets is surprisingly sparse, even in well-studied species such as chimpanzees. While wild chimpanzee diets have been evaluated at many of the long-term chimpanzee field sites (e.g., Gombe: Wrangham, 1975; Budongo: Tweheyo et al. 2004, Newton-Fisher, 1999, Villioth, 2018; Ngogo: Watts et al. 2012; Mahale: Itoh & Nakamura, 2015**;** Fongoli: Pruetz, 2006; Caiquene-Cadique: Bessa et al. 2015; Bossou: Sugiyama & Koman, 1987, Hockings et al. 2009; Goualougo: Morgan & Sanz, 2006; Taï: Goné & Wittig, 2019; Bulindi: McLennan, 2013), food combinations – for medicinal use or otherwise – have yet to be systematically studied (though see Krief et al. 2005; Klein et al. 2008; Pebsworth et al. 2019; Villalba et al. 2017). As more studies are conducted on the bioactivity of putative medicinal resources, however, it appears increasingly possible that the sequential ingestion of resources may enhance medicinal benefits for medicators through synergistic chemical interactions (Krief et al. 2005). Following observations of chimpanzees in Kibale, Uganda combining clay with *Trichilia rubescens* leaves, Klein et al. (2008) tested whether certain clay types can bioactivate botanical compounds in this species. When the interactions between these resources were modelled in gastric and intestinal compartments and assayed, the authors found that clay enhanced the antimalarial properties of *T. rubescens*. Pebsworth et al. (2019) further found that certain clays consumed by Budongo chimpanzees adsorb phenolic compounds, potentially detoxifying the consumer's diet. Ingesting clay may, therefore, allow for sequential ingestion of therapeutic plants with high concentrations of plant secondary metabolites in larger doses, without the associated high costs.

*But are chimpanzees capable of intentional sequential or combinatorial self-medication?* Janmaat et al (2014) suggested that wild chimpanzees in Taï National Park, Ivory Coast appear to plan their breakfast time, type, and location in advance, proposing that wild chimpanzees can pre-meditate future dietary decisions, and in so doing, meet their nutritional needs. Trapanese et al. (2019) suggest in their review that primates likely use mental maps to track where high-quality resources are located, and plan fitness-enhancing foraging strategies. Chimpanzees have also been found to travel far distances, taking detours from normal travel routes, to access medicinal plants in their environment (Huffman, 1997). The question, however, remains whether chimpanzees can apply dietary planning to multi-resource self-medicative practices when ill or wounded. As many

bioactive plants are costly to consume in high doses or if the consumer is healthy (due to the presence of toxic plant secondary metabolites), remedy-seeking individuals would need to develop an appropriate response to these costs and toxicity budgets (Villalba et al. 2017). If chimpanzees do employ medicinal combinations for self-medicative purposes this would open substantial new questions in the field of Zoopharmacognosy, providing evidence for the intentionality of primate self-medication, and expediting the discovery of novel self-medicative resources.

## Description:

We use two methods, never before employed in self-medicative contexts, to explore non-random food combinations in wild chimpanzee diets, as part of an early investigation into the **self-medicative resource combination hypothesis**. These methods include Collocation Analysis (Gries, 2014; Bosshard et al. 2021; Leroux et al. 2021) and APRIORI Analysis (Agrawal & Srikant, 1994). Collocation Analysis looks for binary pairs that occur above chance in a dataset. As Collocation Analysis only works with binary pairs (hereafter **bigrams**), we also employed APRIORI analysis to identify rule associations in the dataset which exceeded two food items. While these analyses are usually used with much larger datasets, in this study, we analyzed four months of feeding ecology data from the Sonso chimpanzee community living in the Budongo Forest, Uganda as a case study to identify possible resource combinations that warrant further investigation. Our example demonstrates the advantages and limitations of applying these methods to feeding ecology data. While this study aims to demonstrate the potential of these analyses in a novel context, future studies would benefit from employing larger, long-term datasets to maximize sample size, reduce seasonal or methodological biases, and increase overall accuracy.

## Methods:

### Ethical Note

Data used in this study were collected by EF with the approval by the Uganda Wildlife Authority (Uganda Wildlife Authority permit no. COD/96/05), the Uganda National Council for Science and Technology (permit no. NS257ES). The study was purely observational and adhered to the Code of Best Practices in Field Primatology (Riley et al. 2014). All applicable international and national guidelines were followed. The authors report no conflict of interest.

## Study Site and Subjects

The Budongo Conservation Field Station (BCFS), established by Vernon Reynolds in 1990, covers 793 $km^2$, 482 $km^2$ of which is populated by continuous, semi-deciduous forest cover (Eggeling, 1947). This study was conducted with the Sonso community, a community that has been studied continuously since 1990 (Reynolds, 2005), and which had ~65 individuals at the time of data collection. The small size of the Sonso home territory (5.33 $km^2$) (Badihi et al. 2022) enables efficient ecological surveys and focal follows and, as this community has been studied continuously for over thirty-years, ages, social relationships, and demographics are well documented. The diet of the Sonso community is also well established (Tweheyo et al. 2004) as is the available flora in the Reserve (Synnott, 1985).

## Data Collection

Data were collected over a four-month field season (62 field days). Behavioral data, including all feeding data, were collected between 07:00 and 16:30 using observational, day-long focal follows (*sensu* Altmann, 1974), and recorded using the program Animal Observer (AO), designed for iPad, using a custom coding scheme (see **Tables S1** and **S2**). As chimpanzee focal follows at Budongo end each day before the group nests, multiple day follows are not always possible. For this reason, we only assessed non-random food combinations for individuals which occurred in a single day. All feeding events were filmed on a Sony Handycam CX250. We also included feeding data collected from two Bushnell Trophy No-Glow camera traps during the data collection period in our analysis. These cameras were located at a clay pit site and a *Cleistopholis patens* dead wood site in the Sonso home range. To monitor health states of individuals in the community, we used internal parasite load as a metric, opportunistically collecting fecal samples and microscopically analyzing them using the McMaster Method (WHO's Bench Aid Protocols, 2019) following the methods of Huffman et al. (1993, 1997). For information on the '**Preparation of Feeding Data for Analysis**', see **Supplementary Materials**.

## Data Analysis:

### Multiple Distinctive Collocation Analysis (MDCA) of Resource Combinations

Collocation analysis was originally created by linguists to analyze lexical features and grammatical structures of natural language (e.g., Bartsch, 2004; Lehecka, 2015; Stefanowitsch & Gries, 2003; Xiao & Mcenery, 2006). More recently, this approach has been adopted by primatologists (Bosshard et al. 2021; Leroux et al. 2021) to empirically identify non-random chimpanzee call combinations. For our study, we used collocation to explore whether any resource combinations in chimpanzee diets were more common than would be expected given an assumed random baseline.

Collocation analysis is a constructional-based technique which compares the co-occurrence of specific items with one another (most frequently individual words in linguistic analyses) (Gale et al. 1991; Gries & Stefanowitsch, 2004; Kennedy, 1991; Nesselhauf, 2005). To compare co-occurrences in the dataset, collocation analysis identifies and extracts exclusive dyadic combinations, and measures the relative exclusivity of their relationship within a dataset. Put simply, the analysis quantifies each bigram's relative attraction. Leroux et al. (2021) provide a useful example for understanding this technique: for any dataset with A to E elements, if A and B are both elements that could combine into a bigram, collocation analysis compares the frequency of the A-B bigram with the frequencies of all other possible bigrams in which A and B independently appear in the dataset (i.e., A-C, A-D, A-E, B-C, B-D, B-E).

There are two types of collocation analysis, **Multiple Distinctive Collocation Analysis (MDCA)** and **Mutual Information Collocation Analysis (MICA)** (see Church & Hanks,1990; Bosshard et al. 2021; Leroux et al. 2021 for more information on MICA). MICA was not used in this study as the sample size is relatively large for this analysis (n=1409 for V1 and n=466 V2). MDCA tests the attraction between units using one-tailed exact binomial tests applied to each possible bigram combination (Gries, 2014). Results are generated as log-transformed values indicating both the **strength** and **direction** of an attraction between two units in the bigram. Positive values indicate an **attraction** (non-random co-occurrence in the corpus), while negative values indicate a **repulsion** (non-random absence of co-occurrence in the corpus). The absolute value of the pbin

values indicate the strength of these relationships. Pbin values > |**1.3**| indicate a non-random relationship: the higher the absolute value, the stronger the relationship between the units in the bigram (see Bosshard et al. 2021). More information on 'Interpreting pbin Outputs from Collocation Analysis' can be found in the Supplementary Materials.

To explore food combinations using this method, we ran two versions of MDCA (hereby referred to as V1 and V2). V1 evaluates all eligible resource combinations from feeding data across a four-month period (V1 = 1409 distinct bigrams with 98 possible food items). V2 evaluates combinations from this period that include one or more unusual/potentially self-medicative resource (V2 = 466 distinct bigrams with n = 98 possible food items). As our focus was to identify meaningful food co-occurrences, we only extracted combinations with pbins > **1.3**: $P < 0.05$ from analyses outputs.

### *MDCA Version 1 Analysis*

To create the dataset used in V1 and V2, chimpanzee feeding data from the whole group was first ordered by date. Next, within each day-cluster, we extracted available feeding data from each individual and compiled them into separate feeding lists, with consumed resources from each day ordered chronologically. Cases in which individuals consumed only one food item in a day were removed, as this excluded the possibility of food combinations. When an individual consumed the same resource in consecutive order, the second observation was removed to prevent double counting feeding events which may have been disrupted and subsequently resumed. We kept cases in which the same resource was eaten multiple times in a day, if one or more different resources were eaten between these events. In total, we excluded 512 events from the original 1324 feeding events. This left 812 usable feeding events from 52 different individuals. Next, we subdivided the complete dataset into two distinct subsets for each version of analysis (V1 and V2). For V1, we calculated every permutation of resource pairing for each individual's daily diet, preserving the order of ingestion (see **Table 1** for example).

**TABLE 1**: Example permutations from an individual's daily diet for Collocation MDCA V1

| **Example of an individual's ordered daily diet:** *Ficus exasperata* Ripe Fruit, *F. mucuso* Unripe Fruit, *Ficus variifolia* Young Fruit, and *Cubitermes* Mound Soil | |
|---|---|
| **Food 1** | **Food 2** |
| *Ficus exasperata* Ripe Fruit | *Ficus mucuso* Unripe Fruit |

| | |
|---|---|
| *Ficus exasperata* Ripe Fruit | *Ficus variifolia* Young Fruit |
| *Ficus exasperata* Ripe Fruit | *Cubitermes* Mound Soil |
| *Ficus mucuso* Unripe Fruit | *Ficus variifolia* Young Fruit |
| *Ficus mucuso* Unripe Fruit | *Cubitermes* Mound Soil |
| *Ficus variifolia* Young Fruit | *Cubitermes* Mound Soil |

Once permutations were produced, V1 consisted of 1409 resource pairs which were then run through Collocation Analysis on R (Gries, 2014). This produced a total output of 8352 distinct bigrams. Of these bigrams, 208 had non-random pbins > 1.3 ($P < 0.05$).

### *Collocation MDCA Version 2 Analysis*

While V1 included all resources in the group's diet, even those which are popularly consumed, V2 attempted to control for frequency/availability biases. The aim of V2 was to provide a more streamlined and efficient method for identifying possible synergistic resources and/or novel medicinal resources. Only combinations which included a specified, putatively self-medicative resource, hereafter referred to as a *Resource of Interest* (ROI), were included. ROI is specifically defined here as a resource consumed by Sonso chimpanzees which either possesses established medicinal properties, is consumed using uncommon food processing techniques, and/or was observed being ingested by an individual with a high or diverse parasite load. A comparison of uncommon and common processing techniques can be found in Table S3. To identify foods with known medicinal properties, we searched pre-existing ethnomedicinal and bioactivity literature prior to data collection (Kokwaro et al. 2009; PROTA4U database; Iwu, 2014). A literature review of known medicinal properties for selected ROIs can be found in the Table S4. Based on these factors, the resources in Table 2 were selected as ROIs for this study. *Water* was considered an ROI as a random factor, as it is known to combine with a "leaf sponging" behavior that was also coded as a dietary resource. While leaf sponges are not ingested, bioactive or nutritional compounds from the leaves could be ingested by chimpanzees during drinking events. Different types of *Water* (depending on the water source) were also included.

**TABLE 2**: Selected ROIs for use in Collocation MDCA Version 2

| Resource Code | Details |
|---|---|
| Cubitermes Mound Soil | *Cubitermes* spp. *termite* Soil |

| | |
|---|---|
| Afm Pith | *Aframomum* spp. Pith |
| Fe Bark | *Ficus exasperata* Bark |
| Ab Bark | *Alstonia boonei* Bark |
| Fvr Bark | *Ficus variifolia* Bark |
| Mrt Pith | *Marantachloa leucantha* Pith |
| Cos Pith | *Costus* spp. Pith |
| Cp Bark | *Cleistopholis patens* Bark |
| Acp Pith | *Acanthus polystachyus* Pith |
| Clay | Clay |
| Clay Water | Clay Water |
| Fsu Unripe Fruit | *Ficus sur* Unripe Fruit |
| Fsu Young Fruit | *Ficus sur* Young Fruit |
| Wf Young Leaf | *Whitefeldia elongata* Leaf |
| Lp Young Leaf | *Lasiodiscus pervillei* Leaf |
| Water | Drunk from Puddle or Stream |
| Water Cmi | Drunk from *Cordia mildebredii* Tree Hole |
| Water Trr | Drunk from *Trichia rubescens* Tree Hole |
| Unk Root | *Unknown* Root |
| Ka Resin | *Khaya anthotheca* Resin |
| Ptm Root | *Pterygota mildebredii* Root |
| Urc Flower | *Scepocarpus trinervis* Flower |

From our clean dataset, we only extracted feeding data on days when an individual consumed at least one ROI for our V2 subset. We then generated all resource pairs that included ROIs, taking the order of ingestion into account (see Table 3 for example).

**TABLE 3:** Example permutations with 1 ROI (***A. polystachyus* Pith**) for collocation MDCA V2

| **Example of an individual's ordered daily diet:** ***A. polystachyus* Pith**, *Ficus sur* Ripe Fruit, *Cordia millenii* Ripe Fruit, and *Saba florida* Ripe Fruit | |
|---|---|
| **Food 1** | **Food 2** |
| *Acanthus polystachyus* Pith | *Ficus sur* Ripe Fruit |
| *Acanthus polystachyus* Pith | *Cordia millenii* Ripe Fruit |
| *Acanthus polystachyus* Pith | *Saba florida* Ripe Fruit |

On days when multiple ROIs were consumed by an individual in a one-day period, all combinations which included at least one ROI were generated. Bigrams which included two ROIs were only generated once, following sequential order (see Table 4 for example).

**TABLE 4:** Example permutations with 2 ROIs (***A. polystachyus* Pith** & ***K. anthotheca* Resin**) for Collocation MDCA V2

| **Example of an individual's ordered daily diet:** *A. polystachyus* **Pith*** , *Ficus sur* Ripe Fruit, ***K. anthotheca* Resin*** and *S. florida Ripe Fruit*) | |
|---|---|
| **Resource 1** | **Resource 2** |
| *Acanthus polystachyus* Pith | *Ficus sur* Ripe Fruit |
| *Acanthus polystachyus* Pith | *Khaya anthotheca* Resin |
| *Acanthus polystachyus* Pith | *Saba florida* Ripe Fruit |
| *Ficus sur* Ripe Fruit | *Khaya anthotheca* Resin |
| *Khaya anthotheca* Resin | *Saba florida Ripe Fruit* |

## APRIORI Analysis for Resource Rules and Associations

We also used the APRIORI algorithm to evaluate chimpanzee dietary resource combinations (Agrawal & Srikant, 1994). This method reveals association rules between 'items' in a large dataset, by taking item combinations and generating all association rules that have support and confidence greater than the minimum support and minimum confidence intervals specified by the algorithm's user (Agrawal & Srikant, 1994; Al-Maolegi & Arkok, 2014). The original purpose of this algorithm was to use commercial transaction histories to improve information-driven marketing processes. Based on these transaction histories, the algorithm mines association rules and suggests additional products to customers (Hahsler, 2017; Hahsler & Karpienko, 2017). In addition to e-commerce applications, APRIORI has recently been used to unravel palaeoecological associations between extinct species in the fossil record across different geological strata (Bobe et al. 2022) and to better understand facial communication systems (Mielke et al. 2021). As far as we know, APRIORI has never been used to analyze non-human feeding behavior, offering a novel approach for testing associations between food resources.

### *APRIORI Analysis*

Feeding data from the four-month observation period were combined for all individuals and formatted identically to the Collocation Analysis V1 subset, making these two analyses efficient to run in parallel. Using the transactions() function from the arules package (Hornik et al. 2005), the long-form data set was transformed into a Binary Incidence Matrix, a format typically used for mining associations in transaction data. The dataset was then run through APRIORI on R (version

4.0.5, R Core Team, 2019), and the results outputted to our accessible interactive platform: PANacea https://osteomics.com/PANacea/. On this platform, the scientific community can interact with and interpret results of the APRIORI algorithm applied to our dataset. PANacea is an online data exploration web-app, built on top of the visualization techniques for association rules on R using the shiny (Chang et al. 2021), arules (Hornik et al. 2005; Hahsler et al. 2011), and arulesViz (Hahsler, 2017) packages. PANacea is available in the Osteomics platform.

Interpreting the results of this analysis requires an understanding of the customizable metrics: support, confidence, and lift (see Figure S1). **Support** represents the number of times the association is present in the data and serves as a popularity metric. In datasets with a high diversity of item-types such as this one (i.e., resource types), support tends to be low for most associations, as the number of times each combination occurred will likely be small. **Confidence** (scaled between 0-1) can be interpreted as percentages (0 = 0% and 1 = 100%). While confidence can give a sense of association strength, it can also be affected by dataset size. For example, if a combination between A and B is the only combination occurring for A, the confidence for that pair will be very high (1). The **Lift** metric, therefore, is crucial to consider when interpreting small datasets as it is a way of controlling for confidence. If A and B are uncommon, yet tend to be sampled in pairs, the lift will be higher. A large lift means that the confidence value is larger than the expected value and therefore this association is likely not due to chance. Lift should be >1 for confidence to be considered a usable metric. Lift can be used to indirectly control for factors like short duration of data collection. It is also useful for larger datasets that have many observations, but low frequency of occurrence for each item or combination. More detail on 'Interpreting APRIORI Data Outputs on PANacea' can be found in the Supplementary Materials.

## Results:

### MDCA Version 1 Results

Of the possible 1409 permutations of resource combinations, Collocation MDCA V1 resulted in 8352 distinct bigrams, 208 of which had pbin values > 1.3. To interpret these data, MDCA outputs were ordered by pbin value. For brevity, we present below the 25 bigrams with the highest pbin values (**Table 5**; range =3.01–17.85). The pair with the highest attraction (17.85) was *F.*

*exasperata* (Fe) Unripe Fruit & *F. mucuso* (Fm) Ripe Fruit, both popular feeding items which were fruiting simultaneously during the study period. Another result which suggests the efficacy of this analysis is the high attraction of *Leaf Sponge* and *Clay Water* (pbin=4.62), the former of which is a tool for accessing the latter.

**TABLE 5**: MDCA (V1) results: 25 bigrams with strongest attraction from complete dataset (pbins>1.3)

| Resource 1 | Resource 2 | pbin value |
|---|---|---|
| **Fe Unripe Fruit** | **Fm Ripe Fruit** | 17.85 |
| **Cgp Ripe Fruit** | **Fvr Young Fruit** | 5.53 |
| **Fsu Ripe Fruit** | **Cgp Ripe Fruit** | 5.33 |
| **Fsu Ripe Fruit** | **Fvr Young Fruit** | 4.86 |
| **Unk Leaf Sponge** | **Clay Water** | 4.62 |
| **Mie Ripe Fruit** | **Fvr Young Leaf** | 4.35 |
| **Cph Young Leaf** | **Fe Young Leaf** | 4.12 |
| **Fe Young Fruit** | **Fm Ripe Fruit** | 4.11 |
| **Cph Young Leaf** | **Avo Ripe Fruit** | 4.03 |
| **Ptm Root** | **Dd Young Leaf** | 3.97 |
| **Cph Young Leaf** | **Cgp Ripe Fruit** | 3.83 |
| **Fm Ripe Fruit** | **Gpr Ripe Fruit** | 3.73 |
| **Myh Ripe Fruit** | **Mrt Pith** | 3.62 |
| **Es Seeds** | **Dd Ripe Fruit** | 3.59 |
| **Me Ripe Fruit** | **Meat Colobus** | 3.50 |
| **Avo Ripe Fruit** | **Fe Young Leaf** | 3.32 |
| **Urc Flowers** | **Mb Mature Leaf** | 3.30 |
| **Cli Young Leaf** | **Cze Young Leaf** | 3.27 |
| **Cgp Ripe Fruit** | **Avo Ripe Fruit** | 3.15 |
| **Blu Leaf Sponge** | **Water Fe** | 3.15 |
| **Water Fe** | **Fvr Leaf Bud** | 3.15 |
| **Fvr Ripe Fruit** | **Afm Ripe Fruit** | 3.07 |
| **Cli Young Leaf** | **Cli Young Leaf** | 3.05 |
| **Fth Ripe Fruit** | **Fsu Ripe Fruit** | 3.03 |
| **Bpy Mature Leaf** | **Bpy Ripe Fruit** | 3.01 |

### MDCA Version 2 Results

In this model, created with 22 ROIs, 466 possible resource combinations were produced from 812 total eligible feeding events. Of these, Collocation Analyses resulted in 4480 bigrams, 85 of which had pbin values >1.3 (**Table 6**). The high attraction of “Leaf Sponge” and “Clay Water” (pbin =

4.13) once again indicates the test's efficacy, however, it is lower here than the pbin for this bigram in V1 (pbin = 4.62). This was expected as V2 used a smaller dataset, and thus both resources are present at higher relative rates across V2's other bigrams.

**TABLE 6**: MDCA (V2) results: 25 resource bigrams with strongest attraction which include at least 1 ROI (pbins>1.3)

| Resource 1 | Resource 2 | pbin value |
|---|---|---|
| **Myh Ripe Fruit** | **Mrt Pith** | 4.66 |
| **Cph Young Leaf** | ***Cubitermes* Mound Soil** | 4.16 |
| **Unk Leaf Sponge** | **Clay Water** | 4.13 |
| **Ptm Root** | **Dd Young Leaf** | 3.96 |
| ***Cubitermes* Mound Soil** | **Cgp Ripe Fruit** | 3.34 |
| **Water** | **Bpy Young Leaf** | 3.22 |
| **Bpy Young Leaf** | **Water Trr** | 3.18 |
| **Clay Water** | **Avo Ripe Fruit** | 3.12 |
| **Es Seeds** | **Water** | 3.12 |
| ***Cubitermes Mound Soil*** | **Fsu Ripe Fruit** | 2.99 |
| **Cli Mature Leaf** | **Cos Pith** | 2.667 |
| **Cli Young Leaf** | **Cp Bark** | 2.62 |
| **Fm Ripe Fruit** | **Ka Resin** | 2.58 |
| **Avo Ripe Fruit** | **Fsu Unripe Fruit** | 2.54 |
| **Water Trr** | **Cgp Ripe Fruit** | 2.52 |
| **Dd Ripe Fruit** | ***Cubitermes* Mound Soil** | 2.50 |
| **Sf Ripe Fruit** | **Ka Resin** | 2.45 |
| **Bpy Ripe Fruit** | **Ka Resin** | 2.41 |
| **Psg Unripe Fruit** | **Ka Resin** | 2.41 |
| **Ka Resin** | **Fvr Unripe Fruit** | 2.39 |
| **Mie Ripe Fruit** | ***Cubitermes* Mound Soil** | 2.39 |
| **Water Cmi** | **Bpy Young Leaf** | 2.37 |
| ***Cubitermes* Mound Soil** | **Cph Young Leaf** | 2.35 |
| **Es Seeds** | **Water Cmi** | 2.25 |
| **Afm Pith** | **Fm Ripe Fruit** | 2.24 |

## APRIORI Results

On PANacea, we assigned the minimum thresholds of **support** = 0.01, **confidence** = 0.6, and **lift** = 1, **rule length** = 2–5, and extracted only the top 25 rules (out of 96 total generated rules). These 25 rules are presented in Table 7 and displayed as rule networks in Figure 1, including columns for support, confidence, lift, and count (number of times this combination occurred in the dataset).

The rule with the highest lift (31.38) was Unidentified sp. (Avo) Fruit, *Celtis gomphophylla* (Cgp) Ripe Fruit, *Ficus sur* (Fsu) Ripe Fruit ⇒ *F. exasperata* (Fe) Young Leaf, which had a 75% confidence outcome. This means that throughout the study period, when Avo ripe fruit (a large fruit from an unidentified tree species resembling the genus *Gambeya*) was eaten in combination with Cgp ripe fruit and Fsu ripe fruit, it was exceedingly likely that Fe young leaves were also eaten. This is a high confidence interval for an association with the relatively long rule length of 4, as the chances of random combinations decrease as rule length increases (see section on 'Interpreting APRIORI Data Outputs on PANacea' in Supplementary Materials). Three of these species (Cgp fruits, Fsu fruits, and Fe young leaves) are commonly eaten resources amongst Sonso chimpanzees. The Unidentified spp. coded 'Avo Fruit', however, was not commonly eaten.

**TABLE 7**: Example of "Data Exploration" results, showing top 25 results with APRIORI (ordered by lift)

| rules | support | confidence | lift | count |
|---|---|---|---|---|
| {Avo Ripe Fruit, Cgp Ripe Fruit, Fsu Ripe Fruit} ⇒ {Fe Young Leaf} | 0.012 | 0.75 | 31.38 | 3 |
| {Fe Ripe Fruit, Ka Resin} ⇒ {Sf Ripe Fruit} | 0.012 | 0.75 | 26.89 | 3 |
| {Es Seeds, Fvr Ripe Fruit} ⇒ {Dd Ripe Fruit} | 0.012 | 0.75 | 20.92 | 3 |
| {Es Seeds, Fsu Ripe Fruit, Fvr Ripe Fruit} ⇒ {Dd Fruit} | 0.012 | 0.75 | 20.92 | 3 |
| {Unk Leaf Sponge} ⇒ {Clay Water} | 0.012 | 0.75 | 17.11 | 3 |
| {Avo Ripe Fruit, Cubitermes Mound Soil} ⇒ {Cph Young Leaf} | 0.012 | 1 | 16.73 | 3 |
| {Avo Ripe Fruit, Cgp Ripe Fruit, Cubitermes Mound Soil} ⇒ {Cph Young Leaf} | 0.012 | 1 | 16.73 | 3 |
| {Avo Ripe Fruit, Cubitermes Mound Soil, Fsu Ripe Fruit} ⇒ {Cph Young Leaf} | 0.012 | 1 | 16.73 | 3 |
| {Avo Ripe Fruit, Cgp Ripe Fruit, Cubitermes Mound Soil, Fsu Ripe Fruit} ⇒ {Cph Young Leaf} | 0.012 | 1 | 16.73 | 3 |
| {Acp Pith} ⇒ {Myh Ripe Fruit} | 0.012 | 1 | 14.76 | 3 |
| {Fe Ripe Fruit, Ka Resin} ⇒ {Afm Pith} | 0.012 | 0.75 | 14.48 | 3 |
| {Cgp Ripe Fruit, Fe Young Leaf} ⇒ {Avo Ripe Fruit} | 0.016 | 1 | 13.94 | 4 |
| {Cph Young Leaf, Fe Young Leaf} ⇒ {Avo Ripe Fruit} | 0.012 | 1 | 13.94 | 3 |
| {Afm Ripe Fruit, Fsu Ripe Fruit} ⇒ {Fvr Ripe Fruit} | 0.012 | 1 | 13.94 | 3 |
| {Dd Ripe Fruit, Es Seeds, Fsu Ripe Fruit} ⇒ {Fvr Ripe Fruit} | 0.012 | 1 | 13.94 | 3 |
| {Cgp Ripe Fruit, Cph Young Leaf, Fe Young Leaf} ⇒ {Avo Ripe Fruit} | 0.012 | 1 | 13.94 | 3 |
| {Cgp Ripe Fruit, Fe Young Leaf, Fsu Ripe Fruit} ⇒ {Avo Ripe Fruit} | 0.012 | 1 | 13.94 | 3 |
| {Avo Ripe Fruit, Fe Young Leaf} ⇒ {Cph Young Leaf} | 0.012 | 0.75 | 12.55 | 3 |
| {Cgp Ripe Fruit, Fe Young Leaf} ⇒ {Cph Young Leaf} | 0.012 | 0.75 | 12.55 | 3 |

| | | | | |
|---|---|---|---|---|
| {Fm Ripe Fruit, Fvr Young Leaf} ⇒ {Bpy Young Leaf} | 0.012 | 0.75 | 12.55 | 3 |
| {Avo Ripe Fruit, Cgp Ripe Fruit, Fe Young Leaf} ⇒ {Cph Young Leaf} | 0.012 | 0.75 | 12.55 | 3 |
| {Avo Ripe Fruit, Cgp Ripe Fruit, Fsu Ripe Fruit} ⇒ {Cph Young Leaf} | 0.012 | 0.75 | 12.55 | 3 |
| {Cgp Ripe Fruit, Cubitermes Mound Soil} ⇒ {Cph Young Leaf} | 0.020 | 0.625 | 10.46 | 5 |
| {Cgp Ripe Fruit, Cubitermes Mound Soil, Fsu Ripe Fruit} ⇒ {Cph Young Leaf} | 0.020 | 0.625 | 10.46 | 5 |
| {Dd Ripe Fruit, Es Seeds} ⇒ {Fvr Ripe Fruit} | 0.012 | 0.75 | 10.46 | 3 |

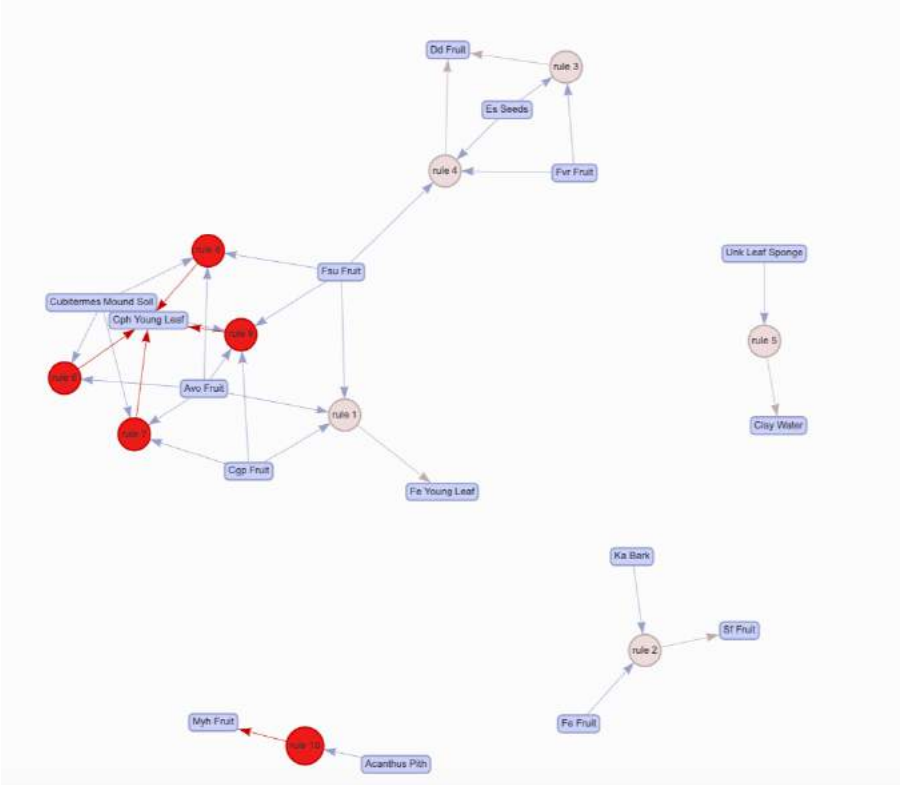


***Figure 1****: Example of a Medicinal Network result on PANacea. showing top 10 APRIORI results (ordered by lift) with* ***support*** *= 0.011,* ***confidence*** *= 0.6, and* ***lift*** *= 1,* ***rule length*** *= 2-5. NB: Rule circles gradated to red based on confidence. Blue arrows pointing toward rule circles indicate resources on left-hand side (LHS) of each equation. Red arrows pointing away from rule circles indicate resources on right-hand side (RHS). When "Fruit" is not modified, it can be assumed to be ripe.*

The APRIORI algorithm can also be tailored to generate rules which include a specified resource of interest, using the "**Find rules by food-item"** under the Data Exploration tab. To further explore this, we selected rules which included *A. polystachyus* pith with **support** =0.011, **confidence** = 0.6, **lift** = 1, and **rule length** between = 2–6. The rule with highest lift (Rule 1) for this set of criteria is displayed in Figure 2. This rule found that if *A. polystachyus* pith was consumed, then

*M. holstii* fruit was also consumed, and that this association had a 100% confidence with the notably high lift of = 14.8.

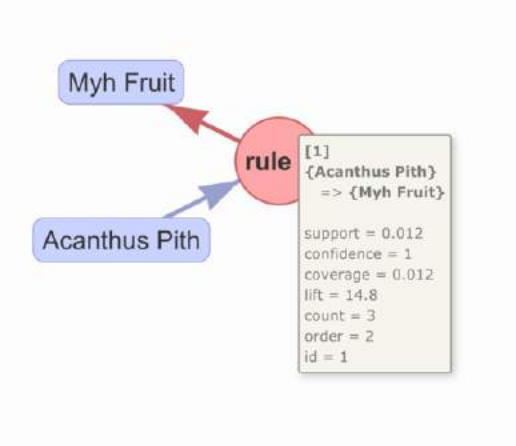


***Figure 2:*** *Top results of "Rule by food-item" search targeting A. polystachyus (ordered by lift) with* ***support*** *= 0.011,* ***confidence*** *= 0.6,* ***lift*** *=1, and* ***rules length*** *= 2-6.*

## Comparing Collocation and APRIORI Analyses

We demonstrate above how both Collocation and APRIORI can be used to analyze and evaluate food combinations in wild chimpanzee diets. To determine whether these methods produced similar outcomes, we ran the APRIORI algorithm again, this time with a rule length of 2, to produce bigrams that could be compared to bigram outputs from Collocation Analysis V1. We ran APRIORI with **support** = 0.011, **confidence** = 0.6, and **lift** = 1, **rule length** = 2, and found it produced only 17 bigrams (Table VI), although it should be noted that this minimum confidence threshold creates a bias toward rare events. Bigrams from both analyses were separately ranked and ordered and then compared to assess overall agreement between methods.

When the 17 APRIORI pairs were compared to the top 17 Collocation V1 bigrams, there were four exact matches between the two algorithms' outputs, showing only 23% agreement (Table S5). However, as these two models have different mechanisms for ranking combinations, a better assessment for agreement was how many exact matches there were between the 17 APRIORI pairs and all non-random Collocation V1 outputs with positive attraction.

$$Agreement = \frac{(total\ \#of\ exact\ matches\ across\ methods\ )}{(total\ \#\ of\ APRIORI\ pairs)}$$

When we compared all exact matches outputted by both methods, agreement was notably higher (53%) (Table S6). The clustered rule associations of these APRIORI pairs are visualized in **Figure 3**, which displays rule associations for the 17 bigram outputs which met our metric criteria.

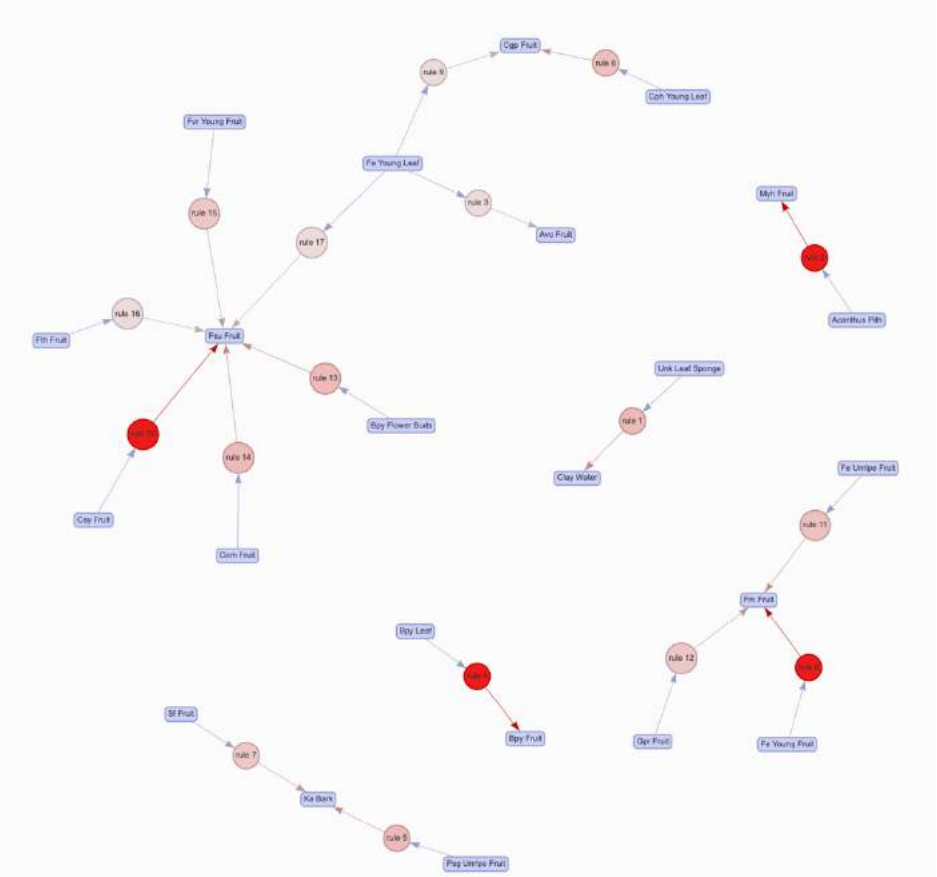


***Figure 3:*** *APRIORI Medicinal Network visualization of 17 bigrams (ordered by lift) with support = 0.011, confidence = 0.6, and lift = 1, rule length = 2.*

We also wanted to determine the role of confidence level on percentage of matched pairs between the two algorithms, as this metric is changeable in APRIORI but not in Collocation Analyses. The confidence interval was lowered from 0.6 to 0.011 on PANacea, and results were reinterpreted. Using this new minimum confidence level, PANacea produced 249 pairs, the top 25 of which were compared to the top 25 Collocation Analysis results, showing 28% agreement (**Table S7**). To better assess agreement regardless of rank, we again found the total number of exact matches between the top 25 APRIORI pairs and all non-random Collocation results with positive attraction (pbin >1.3) (**Table S8**). This yielded a 60% agreement between models, higher than agreement

found in the 0.6 confidence interval model. As the 25 top APRIORI pairs selected represent a small sample of all produced APRIORI pairs (n=249), agreement here is likely an underestimation.

## Discussion:

### MDCA Version 1

Collocation MDCA V1 explored all food combinations in the Sonso diet during a four-month observation period, resulting in non-random resource pairs which may provide relevant interpretations for future investigation. For example, V1 produced a single bigram which contained the same resource twice: Cli Young Leaf & Cli Young Leaf (pbin=3.05). As duplicate feeding events were excluded when the same resource was consumed consecutively, this means that on days when individuals ingested climber leaves, they non-randomly sought this resource out again later the same day. Results of this kind may offer valuable future insight into primate dietary decisions and spatial feeding patterns.

Another notable bigram is *U. trinervis* (Urc) Flowers & *M. bpterygocaulos* (Mb) Mature Leaf (pbin=3.30). Throughout the study period, only two chimpanzees (maternal brothers MB and MZ) were observed eating Urc Flowers, with feeding events occurring on two consecutive days. MZ was the only individual observed eating the leaves of Mb, with these events also occurring on the same two days. On the second day of ingestion, the brothers left the group to travel a far distance before consuming the resources in this bigram. MZ's fecal sample showed a high-intensity and diverse parasite load. MZ also consumed several other putative medicinal species that day. While this remains a single anecdote at present, during this event, MZ demonstrated all but one of Huffman's self-medicative criteria (Huffman, 1997).

Collocation MDCA V1 can provide a general overview of which resources are eaten in non-random combination in a given feeding dataset from a study group. When researchers have hypotheses concerning the function or benefits of a particular resource, outputted bigrams can be searched for the targeted resource. This allows for easy investigation into which other resources may be combined with the targeted resource at frequencies higher than expected by chance. For self-medication studies, this could allow for preliminary identification of putative self-medicative

resources which could then be more thoroughly evaluated. However, to produce more conclusive results about dietary choice concerning food resources in the general diet, this analysis should be re-run on long-term datasets to account for seasonality bias. Additional surveying should also be conducted to control for ecological availability and spatial distribution of resources.

**MDCA Version 2**

Collocation MDCA V2 targeted putatively self-medicative resources and aimed to identify other resources eaten in combination. Results of this analysis showed that *Cubitermes* Mound Soil was ingested in non-random combination with several other resources. Relevant bigrams include: *C. philppensis* (Cph) Young Leaf & *Cubitermes* Mound Soil (pbin=4.16); *Cubitermes* Mound Soil & *C. gomphophylla* (Cgp) Ripe Fruit (pbin=3.34); *Cubitermes* Soil & *F. sur* (Fsu) Ripe Fruit (pbin=2.99); *D. dewevrii* (Dd) Ripe Fruit & *Cubitermes* Mound Soil (pbin=2.50); *Mildbraediodendron excelsum* (Mie) Ripe Fruit & *Cubitermes* Mound Soil (pbin=2.39); *Cubitermes* Mound Soil & *C. philppensis (*Cph) Young Leaf (pbin=2.35). Food order may be relevant for *Cubitermes* Mound Soil, as the pbin of Cph Young Leaf & *Cubitermes* Mound Soil (pbin=4.16) (n=7 occurrences) differs from the pbin of *Cubitermes* Mound Soil & Cph Young Leaf (pbin=2.35) (n=5 occurrences). The number of non-random bigrams which include *Cubitermes* Mound Soil in V2, suggests that on days when individuals ate this resource, their diets were more predictable than on days when they ate other ROIs. In other words, ingestion of termite soil amongst Sonso chimpanzees is unlikely to be purely opportunistic. There are several possible interpretations of this pattern, one being that chimpanzees may target termite soil during periods of gastrointestinal distress (as was observed by Mahaney et al. 1996 in Mahale), potentially caused by ingestion of other resources in these bigrams. Future research should further explore explanations for these observed patterns.

As interpretation of collocational strength is relatively subjective in V2, it is crucial that results are contextualized with behavioral observations. For example, we observed individuals consuming *A. polystachyus* three times during the four-month study period. Each of these times, the consumer had a high parasite load. In one case, an individual consumed *A. polystachyus* pith immediately before leaf swallowing, one of the two confirmed self-medicative behaviors (Huffman et al. 1996; Huffman & Caton, 2001). In another case, an individual consuming *A. polystachyus* was observed

wadging the pith of this plant with the ROI *C. patens* (Cp) dead wood, following the ingestion of another ROI (*Cubitermes* Mound Soil). Immediately after, the individual consumed a sequence of additional ROIs (e.g., *K. anthotheca resin* and *M. bpterygocaulos leaves*) and the bark of *S. myrtina.*

*A. polystachyus* has already been suggested as a candidate self-medicative resource at Kibale (Krief et al. 2005) and is known to be used in traditional medicine to treat skin infections, dermatosis, and sterility in Burindi (Krief et al. 2005; Pebsworth et al. 2006). A leaf decoction of this species is used for treating liver and spleen problems and stems are pounded with their leaves to treat depressive psychosis (Kokwaro, 2009). Collocation MDCA V2 analysis can be employed to identify resources for further in-depth investigation of medicinal value, when some behavioral or ethnomedicinal evidence has already been attained.

**APRIORI**

APRIORI analysis offers the opportunity to explore more complex associations between more than two resources at a time, and to take data density and representativeness into account by adjusting for confidence and other factors. A result of interest was the high lift (16.73) and 100% confidence interval of the Avo Ripe Fruit, *Cubitermes* Mound Soil ⇒ Cph Young Leaf rule association. This rule maintained the same lift and confidence when two additional resources were added to the left-hand side (LHS) of this equation (i.e., Avo Ripe Fruit, **Cgp Ripe Fruit**, *Cubitermes* Mound Soil ⇒ Cph Young Leaf; Avo Ripe Fruit, *Cubitermes* Mound Soil, **Fsu Ripe Fruit** ⇒ Cph Young Leaf). This finding supports results from Collocation MDCA V2, that *Cubitermes* Mound Soil combines with several other species in a non-random way.

Of all bigrams produced by APRIORI, the pair with the highest lift (14.76) was *A. polystachyus* (Acp) ⇒ *M. holstii* (Myh) (confidence=1). This result, when interpreted in conjunction with Collocation MDCA V2's results, establishes a strong case for further investigation into a synergistic relationship between *A. polystachyus* and *M. holstii.*

## Comparison and Critique:

While our dataset was effective for a piloting these methods, Collocation and APRIORI are more typically applied to larger datasets (such as linguistic corpora or e-commerce transaction histories), and our study was short and seasonally biased. The specific examples of resource combinations provided here would now benefit from follow-up analyses using a multi-year dataset for the Sonso community. Long-term behavioral data, however, would need to be supplemented with ecological and spatial data to determine proximity of resources and fruiting synchrony. Analyses which incorporate the whole diet of each individual (e.g., Collocation MDCA V1), would benefit from additional data on feeding locations for each resource, to control for spatial and temporal factors. While Collocation MDCA V2 has some measure of control for ecological noise and popularity biases towards certain species, the species and quantity of ROIs used will substantially shape the findings and their stability. Accordingly, ROI's should be established from longer-term behavioural studies.

Collocation MDCA V1 and APRIORI also require subjective judgement when interpreting results, especially when differentiating between non-random resource associations caused by medicinal combinations and those caused by frequency/availability of the feeding items, seasonal synchronism, or geographic proximity. For example, during data collection, we observed the group feeding in a ripe *F. mucuso* (Fm) fruit tree each day for almost a full month. On most of these days, the group travelled from this tree to feed on a nearby *F. exasperata* (Fe) which was bearing unripe fruits. Likely due to the proximity and synchronism of these resources, an Fe & Fm bigram is unsurprisingly present in the top 25 Collocation results, ranking 8th. While this bigram is *not* amongst the top 25 APRIORI results when **rule length** was set between 2–5 (with **confidence** = 0.6), the combination *is* present in the top APRIORI results when **rule length** was adjusted to 2 (**confidence** = 0.6). Under these conditions, the Fe & Fm bigram was also ranked 8$^{th}$ in APRIORI (Table S6). There is, therefore, a need for ecological and spatial controls in both analyses to distinguish ecological 'noise' from potential medicinal combinations.

The similar dataset format required for running both Collocation and APRIORI makes this comparative approach easy and efficient. However, differences in result outputs must be considered. For example, we found that the level of agreement between methods increases when minimum confidence is lowered in APRIORI (thus increasing the number of generated APRIORI

outputs). When running APRIORI with rule length metrics adjusted to produce bigrams, our results differed from bigrams produced during Collocation Analysis V1. This variation is not surprising as the algorithms and analyses being run are different. Despite variation, the algorithms showed up to 60% agreement across generated bigrams, suggesting that both models can and should be considered and compared when making robust interpretations. Comparing outputs from both methods may highlight some non-random bigrams which warrant further investigation. Despite the efficacy of both approaches, APRIORI is better suited for studies which wish to customize specific metrics.

While these methods are currently insufficient for conclusively addressing the **self-medication resource combination hypothesis**, they offer an important exploratory first step which may prompt future research. Following preliminary investigation, the next stage would involve incorporation of health monitoring data into these analyses by separating 'healthy' individuals from 'unhealthy' individuals, and then statistically comparing the difference between groups. In sites where self-medicative resources have previously been established and systematically recorded, feeding data can also be extracted from days in which individuals engaged in a proven self-medicative behavior (e.g., leaf swallowing) and compared to days in which these behaviors were not observed, and individuals were healthy. Future studies should also investigate the role of food combinations in the normal diets of healthy chimpanzees, and the role bioactive medicinal food combinations may play in passive prevention strategies. While these quantitative methods may help establish meaningful medicinal resource combinations, the incorporation of behavioral, spatial, and health data will provide essential additional context before the implementation of expensive pharmacological analyses.

## Conclusion:

We argue that **Multiple Distinctive Collocation Analysis** and the **APRIORI Algorithm** can be used to effectively detect potential resource combinations and association rules in wild primate diets. These methods allow for a preliminary investigation into the **self-medicative resource combination hypothesis** and have the potential to change how we analyze and interpret long-term feeding data across field sites. If non-random medicinal combinations are identified in primate

diets, this could have important implications for the discovery of novel primate self-medicative behaviors. As a cost-effective strategy for selecting natural resources for targeted bioactivity testing, it may one day also lead to the discovery of synergistic compounds effective in treating human pathogens. Overall, employing interdisciplinary methods, such as these, to systematically study non-human feeding ecologies, seems likely to soon yield fruitful outcomes. Future studies in chimpanzee self-medication and primate feeding ecology more generally should strive to consider feeding behaviors from a more holistic perspective, remaining open to the notion that non-human medicinal diets may be more planned than previously considered.

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# 5 Pharmacological and Behavioral Investigation of Putative Self-Medicative Plants in Budongo Chimpanzee Diets

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**Conflicts of interest:** None

## Abstract:

Wild chimpanzees consume a variety of plants to meet their dietary needs and stay healthy. While some resources have obvious value, others provide minimal nutritional content and/or contain bioactive toxins which can make the plant costly to ingest. In some cases, resources with less obvious nutritional functions have been speculated to be self-medicative resources, thought to help individuals combat infections from parasites and pathogens. We present novel bioactivity results from seventeen botanical samples collected in Budongo Forest, Uganda, associated with a putative self-medicative event or behavior from wild chimpanzees in the Budongo Forest. In total, plant parts from 13 species were selected (nine trees, and four terrestrial herbaceous plants). Three extracts of different polarity were produced for each sample and introduced to antibacterial and anti-inflammatory *in vitro* models. Extracts were evaluated for growth inhibition against a panel of multidrug-resistant clinical isolates of bacterial pathogens, including ESKAPE strains, and cyclooxygenase-2 (COX-2) inhibition activity. Pharmacological results suggest that Sonso chimpanzees consume several species with potent, medicinal properties. The strongest antibacterial activity was achieved by the *n*-hexane extract of *Alstonia boonei* dead wood against *S. aureus* and *E. faecium* ($IC_{50}$: 16 μg/mL) and by the methanol-water extract of *Khaya anthotheca* stem bark with resin against *E. faecium* and pathogenic *E. coli* ($IC_{50}$: 16 μg/mL). All plant species negatively affected growth of *E. coli*. Out of 51 extracts, 17 showed promising COX-2 inhibition ($IC_{50}$ <10μg/mL). The *K. anthotheca* stem bark with resin methanol-water extract showed the most potent anti-inflammatory effects ($IC_{50}$: 0.55 μg/mL), followed by the fern *Christella parasitica* methanol-water extract ($IC_{50}$: 0.81 μg/mL). These results, integrated with associated observations from eight months of behavioral data, provide further evidence for the presence of phytochemical, self-medicative behaviors in the diet of chimpanzees.

**Keywords:** Budongo, Zoopharmacognosy, Pharmacology, Self-medication, *Pan troglodytes*

## Introduction:

'Medicinal foods' refer to resources in the diet that have potential curative value due to the presence of plant secondary metabolites (PSMs) (Etkin, 1996; Etkin & Ross, 1982). PSMs are compounds which usually occur only in special, differentiated cells (Osbourn et al., 2003) and which help plants defend against predators, pathogens, and competitors (Allison, 1982; Hart, 1990; Holmes & Zohar, 1990; Toft et al., 1991). PSMs can have a range of functions, including the inhibition of microbial, fungal, and competitor growth (Forbey et al., 2009). While some PSMs can be toxic at high doses, these compounds can also promote the health of human and non-human consumers (e.g., Forbey et al., 2009; Huffman, 1997; Raman & Kandula, 2008; Villalba & Provenza, 2007). Research suggests 15-25% of primate and other mammalian diets consist of medicinal foods (Huffman, 1997; Tasdemir et al., 2020). These seasonally consumed resources likely play a critical role in animal health-maintenance by passively preventing or reducing the impact of parasitic infections or other pathogens (Huffman, 1997; Krief et al., 2006; Huffman & MacIntosh, 2012; MacIntosh & Huffman, 2010; Petroni et al., 2017; Tasdemir et al., 2020; Villalba & Provenza, 2007).

While most animals likely consume medicinal foods as part of their normal diets, fewer species have been shown to engage in therapeutic self-medication. Huffman (2016) defines this type of self-medicative behavior as the active extraction and ingestion, by an ill individual, of medicinal resources with little nutritional value. Instead of an individual passively benefiting from a plant's medicinal properties through normal feeding, this form of self-medication requires basic awareness of the resource's healing properties. One of the best-studied animals to engage in this form of self-medication, is our closest living relative: the chimpanzee.

Wild chimpanzees (*Pan troglodytes*), across at least sixteen field sites (Huffman, 2016) have demonstrated therapeutic self-medication using two well-established self-medicative behaviors: leaf swallowing (Huffman et al., 1996; Wrangham, 1995) and bitter-pith chewing (Huffman & Seifu, 1989). Leaf swallowing, first reported by Wrangham (1977; 1975) and first described by Wrangham & Nishida (1983), involves the careful selection and ingestion of whole, hispid leaves and was later demonstrated to expel internal parasites (i.e. *Oesophagostomum* sp. and *Bertiella*

*studeri*) from the gut (Huffman & Caton, 2001; Huffman et al., 1997; Huffman et al., 1996; Wrangham, 1995). The functional mechanism responsible for this anthelminthic effect is considered primarily "mechanical" (Huffman, 1997), as the leaf's morphology, rather than a chemical compound, stimulates gut motility in the swallower (Huffman & Caton, 2001; Huffman et al., 1996; Page et al.s, 1997).

The second established behavior is bitter-pith chewing, which involves the stripping of outer bark and leaves from the soft new stem growth of the shrub, *Vernonia amygdalina,* exposing the inner pith. Individuals chew the pith and ingest only the bitter juices while spitting out the fibers (Huffman et al., 1993; Huffman & Seifu, 1989). Bitter-pith chewing is considered 'phytochemical' self-medication (Huffman, 1997), as its anthelminthic effect appears to be the result of bioactive PSMs (e.g., Jisaka et al., 1992, 1993; Kupchan et al.,1969; Ohigashi et al., 1994; Toubiana & Gaudemer, 1967). This behavior's medicinal affect was associated with a significant drop in the infection intensity of *Oesophagostomum stephanostomum* nematodes (Huffman et al., 1993), suggesting that the bitter compounds somehow directly affect the adult worms. This hypothesis was supported by *in vivo* studies conducted by Jisaka et al. (1992), demonstrating that extracts from the pith permanently paralyzed adult Schistosome parasites. The plant is also used to aid gastrointestinal discomfort and other related symptoms of parasitosis in humans and livestock, which were also displayed by sick chimpanzees observed to ingest the bitter pith (Huffman & Seifu, 1989; Huffman et al., 1993; Oyeyemi et al. 2018). The bitter piths of other species are reported to be chewed by chimpanzees at other sites but detailed studies have yet to be conducted (Huffman, 1997).

Beyond these two established behaviors, not much is known about the phytochemical self-medicative repertoires of wild chimpanzees, although some behaviors associated with the ingestion of specific plant parts or processing techniques have been recommended for further investigation (e.g., Huffman, 2016; Huffman, 1997; see De la Fuente et al., 2022 for review). One of these behaviors is bark stripping, which involves the consumption of living stem bark or cambium (Nishida, 1976), and which has been observed in at least eleven established field sites (Bessa et al., 2015; Duvall, 2008; Huffman & Wrangham, 1994; Lapuente et al., 2020; Matsuzawa et al., 2011; McGrew et al., 1988; Nishida, 1976; Pebsworth et al. 2006; Pruetz, 2006; Russak,

2013; Van Lawick-Goodall, 1968). Bark stripping has been suggested as a medicinal behavior in chimpanzees and other primates, used to aid in the chemical control of intestinal nematode infection and to relieve gastrointestinal upset (Huffman, 1997). Bark is characteristically highly fibrous, heavily lignified, sometimes toxic, relatively indigestible, and nutrient-poor (Waterman et al., 1984). However, the contribution of bark in chimpanzee diets and toward general health is still poorly understood. In this study, the bark of eight species ingested by Budongo chimpanzees (*Scutia myrtina*, *Cynometra alexandri, Alstonia boonei, Ficus exasperata, Ficus variifolia, Syzygium guineense, Desplatsia dewevrei, Khaya anthotheca)* were screened for antibiotic and anti-inflammatory properties, to better understand the function of bark stripping behaviors and the role this behavior may play in the health maintenance of chimpanzees. For the species *K. anthotheca,* we tested a mixture of bark and congealed resin, which Budongo chimpanzees were observed targeting throughout the study period.

Another putative self-medicative behavior is dead wood eating (Huffman, 1997; Huffman & Wrangham, 1994), which involves the consumption of decomposing cambium from dead trees. Most studies on this behavior in apes thus far have investigated possible mineral and nutritional uses rather than pharmacological properties (Matsubayashi et al., 2007; Reynolds et al., 2009, 2015; Rothman et al. 2006; Venable et al., 2020). Many of these studies suggest that dead wood is exploited by chimpanzees as a source of sodium in environments where this mineral is otherwise scarce (Reynolds et al., 2009; Venable et al., 2020). Our study pharmacologically evaluates two species of dead wood (*A. boonei* and *Cleistopholis patens)* consumed by the Sonso community of chimpanzees to determine whether this behavior may have multiple functions or health benefits.

The ingestion of the pith material from other species has also been suggested as putatively self-medicative (e.g., Krief et al., 2005; Krief et al., 2006; Pebsworth et al., 2006). Unlike *V. amygdalina* bitter-pith, some of these plant piths appear bland or tasteless. Several species of piths ingested by chimpanzees and proposed to have medicinal properties are reviewed by De la Fuente et al. (2022). Others, however, like Wrangham et al. (1991), have proposed that pith is likely a fallback food, providing fiber when fruit is unavailable. In this study, two species of non-bitter piths (*Marantachloa leucantha* and *Acanthus polystachyus)*, were collected for pharmacological assessment. *M. leucantha* was observed on several occasions being stripped, masticated, and spat

out after the juice was extracted from the pith, whereas *A. polystachyus* was observed being stripped, masticated, and swallowed. Both of these species are also ingested in Kibale (Wrangham et al., 1991).

The four other plant parts assayed in this study are not associated with a defined behavioral type or processing technique but were collected either to evaluate how bioactivity differs across plant parts (i.e., *F. exasperata* and *S. guineense* leaves) or based on compelling anecdotal observations during the study period (i.e., *Christella parasitica* and *Whitefeldia elongata).*

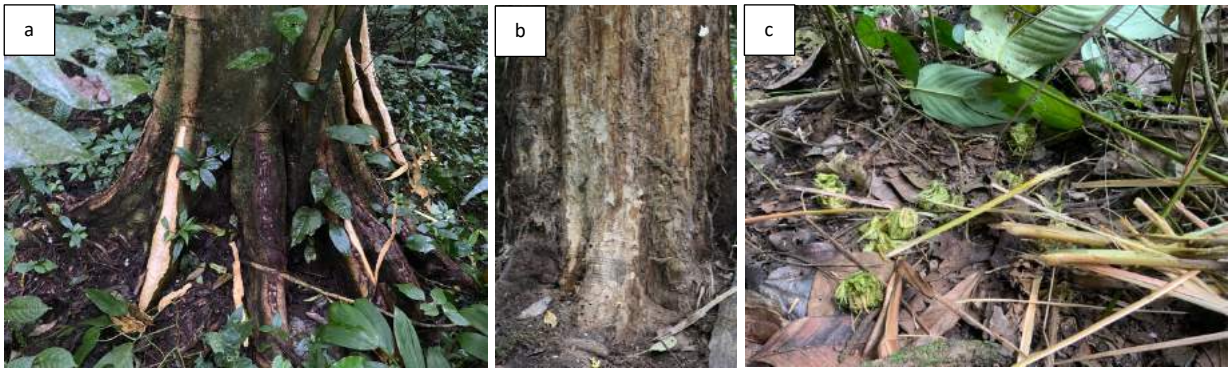


***Figure 2:*** *Evidence of [a]: F. exasperata bark stripping [b] C. patens dead wood eating [c] M. leucantha pith stripping/wadging*

Establishing phytochemical self-medicative behaviors in wild animals is difficult and time consuming, as the burden of proof is high, self-medicative events can be rare relative to other behaviors, and methods often require multidisciplinary expertise and collaboration (Huffman, 1997). Past studies have utilized ethnopharmacological methods to determine specific medicinal properties of foods consumed by primates (e.g., Tasdemir et al., 2020), greatly advancing our understanding of the relationship between primate diets and health. However, a key challenge for establishing novel self-medicative behaviors is differentiating between medicinal food consumption and therapeutic self-medication. While pharmacological data, interpreted on its own, is crucial for establishing the presence of medicinal resources in chimpanzee diets, the integration of observational and health monitoring data is needed to parse behaviors with therapeutic self-medicative functions from normal feeding with inadvertent health benefits. Furthermore, the importance of collecting *in situ* samples from the chimpanzee home ranges where putative self-medicative behaviors are observed is crucial, as ecological, climatic, and anthropogenic variables can cause variety in the bioactivity of plants across habitats (Ncube et al., 2012).

In total, we investigated the bioactivity of 51 plant extracts, produced from 17 part-specific samples (across 13 species), collected in the Budongo Forest. Each extract was tested for inhibition of bacterial growth as well as anti-inflammatory COX-2 inhibition activity. Assay results are reported and contextualized in this study, when possible, with direct behavioral evidence and health monitoring data.

## Methods:

### *Study Site and Subjects*

Behavioral data, health monitoring metrics, and botanical samples were collected from the Budongo Central Forest Reserve in Uganda (1°35′– 1°55′ N, 31°18′–31°42′ E). An overview of methodological workflow can be found in **SM Figure 1**. The Budongo Conservation Field Station (BCFS), founded by Vernon Reynolds in 1990, is composed of continuous, semi-deciduous forest and contains two, habituated Eastern chimpanzee (*Pan troglodytes schweinfurthii*) communities (Eggeling, 1947). The Sonso community has been studied continuously since 1992, and the ages, social relationships, demographics, and diet of its members are well documented (Reynolds et al., 2005; Tweheyo et al., 2004). The Sonso population was approximately 68 individuals at the time of data collection (m:23; f:45), and the home range covered an area of ~5.33 $km^2$ (Badihi et al., 2022). Waibira, a larger group of at least 105 individuals, was more recently habituated, with consistent data collection beginning in 2011. The Waibira maximum home range area was ~10.28 $km^2$ (Badihi et al., 2022).

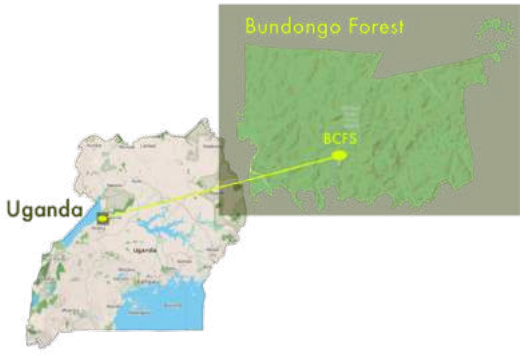


***Figure 2:*** *Map of Budongo Forest, Uganda*

### *Behavioral Data Collection*

All samples were collected in the Budongo Forest within the Sonso home range, based on behavioral observations from the study period and supporting evidence from long-term data of their use. Behavioral and health data were collected from the two neighboring chimpanzee

communities, each for one four-month field season (Sonso: June-October 2021, Waibira: June-October 2022). The data collected between June-September 2021 informed subsequent plant sample collection for pharmacological analysis in early September 2021. Behavioral data collected after sample collection provided additional behavioral context for ingestion of these species. Behavioral data were collected between 07:00 and 16:30 in Sonso and between 06:30 and 17:00 in Waibira using day-long focal animal follows (*sensu* Altmann, 1974). This data was recorded using Animal Observer (AO) on iPad Pro and *ad libitum* feeding events were recorded for any unusual feeding behaviors, including but not limited to bark stripping, dead wood eating, pith stripping, and geophagy. All feeding events were filmed on a Sony Handycam CX250. Focal follows on individuals with wounds, high or diverse parasite loads, or known ailments were prioritized. However, consecutive day follows of priority individuals were not always possible. Throughout the study, using this protocol, 27 Sonso individuals (m:11; f:16) and 24 Waibira individuals (m:14; f:10) were observed. Bushnell Trophy camera traps were also employed during the first field season at a frequently visited *Cleistopholis patens* dead wood site. Authors were blind to pharmacological results during both study periods.

***Health Monitoring***

Individual health data were recorded in both communities, including opportunistic macroscopic and microscopic fecal analysis and urinalysis testing. When helminths and/or proglottids were found in samples, they were collected and preserved in ethanol for later identification. To quantify parasite loads, fecal samples were analyzed using the McMaster Method (WHO's Bench Aid Protocols, 2019) following methods established by (Huffman, 1997; Huffman et al., 1993). Urinalysis samples were taken opportunistically using multi-reagent Urine Dipstick Test 9-RC for Urotron RL9 to assess the health and physiological status of group members following method established by Kaur & Huffman (2004).

***Plant Sample Selection for Bioactivity Testing***

Plants were selected for pharmacological testing in September 2021, after three months of data collection in the Sonso community. Based on direct observations during this period, we selected 10 samples (from nine species) for testing. These observations included individuals targeting plant parts associated with putative self-medicative behaviors (i.e., bark stripping, dead wood eating, pith stripping) or sick/wounded individuals seeking out unusually consumed resources. We then

selected an additional five species, which had not been directly observed, for testing based on their historical inclusion in Sonso chimpanzees' bark stripping repertoire. Bark stripping of these species had all been directly observed by GM, the longest serving member of the BCFS field staff who has followed chimpanzees for over thirty-years. These observations enabled collection of bark samples from trees known to have been previously stripped by the chimpanzees. In two cases, leaves were collected from tree species that were also selected for bark samples (*S. guineense* and *F. exasperata).* While neither Sonso nor Waibira chimpanzees have been observed ingesting the leaves of *S. guineense*, a sample was collected to enable comparison of bioactivity across plant parts. *F. exasperata* leaves are consumed in both communities; however, we found no behavioral evidence for use in unusual contexts. In some cases, direct observation of an event involving one of the collected species occurred after botanical collection was complete. These *post hoc* behavioral observations are reported in this paper, although they did not impact sample selection.

***Collection of Sample Material***

Plants were collected from the Sonso community home range following best practice procedures (Heinrich et al., 2018), using sustainable harvesting methods (Schultz et al., 2020). See **Supplementary Materials:** ***Methods*** for detailed information. Voucher specimens for each species were deposited at the Makerere University Herbarium in Kampala, Uganda for taxonomic identification. A duplicate set was deposited at the University of Oxford Herbarium for permanent storage. Voucher accession numbers are reported in **Table 3**. Digital images of voucher specimens can be found in **SM Figure 2**. The currently recognized scientific names of each species were confirmed on https://mpns.science.kew.org/. Plant family assignments were done in accordance with The Angiosperm Phylogeny Group IV guidance (The Angiosperm Phylogeny, 2016).

***Ethnobotanical Literature Review***

We conducted a post-hoc ethnobotanical review of all species collected for this study using Google Scholar, PROTA, and Kokwaro's (1976) ethnomedicinal pharmacopeia. To search databases, we used scientific names and synonyms for each plant as the keyword (Heinrich et al., 2018).

### *Processing of Plants and Extractions*

At Neubrandenburg University of Applied Sciences, samples were ground using a food processor. Extractions were produced using two solvents and a solvent mixture (*n*-hexane, ethyl acetate, and methanol/water (v/v 9/1)), allowing for the selective isolation of components with varying solubilities and polarities. Methanol-water, the solvent with the highest polarity in this study, generally extracts primary plant metabolites, e.g., polar compounds such as proteins, amino acids, and carbohydrates. Nonpolar solvents, like *n*-hexane, extract nonpolar compounds like lipids, making n-hexane a preferred solvent for oil or wax extraction. Extractions with each solvent were achieved through double maceration of new material (non-successively). Extraction suspensions were placed on a shaker at 80 rpm at room temperature for minimum 72h, followed by vacuum filtration. Processes were repeated with the leached material. Filtrates were then combined and dried using a vacuum evaporator, labeled, and stored at -20°C until needed for assays. More detail on sample solution preparation can be found in **Supplementary Materials:** ***Methods***. Each extract was then tested for inhibition of bacterial growth as well as anti-inflammatory COX-2 inhibition activity.

### *Antibacterial susceptibility tests*

#### Bacterial strains

For antibacterial assays, eleven multidrug-resistant clinical isolate strains from nine species were used. This process increased the studies applicability for future early-stage drug discovery, specifically pertaining to the threat of antimicrobial resistance (AMR). Seven of these strains (from six species), including *E. faecium* (DSM 13590), *S. aureus* (DSM 1104; DSM 18827), *K. pneumoniae* (DSM 16609), *A. baumannii* (DSM 102929), *P. aeruginosa* (DSM 1117), and *E. cloacae* (DSM 30054) are classified as ESKAPE pathogens, meaning they are highly virulent and resistant to antibiotics (Schultz et al., 2020). A strain of the foodborne pathogen *Escherichia coli* (DSM 498) with AMR as well as a non-resistant *Escherichia coli* strain (DSM 1576) were also included in the study. Although not an ESKAPE pathogen, *E. coli* is widely known for causing bacterial diarrhea and AMR strains are a major cause of urinary tract infections (Akhondi, 2022; Oliveira et al., 2020). Strains of *Stenotrophomonas maltophilia* (DSM 50170) and *Salmonella enterica* subsp. *enterica* (DSM 11320) were also tested. More information on specific clinical isolates/strains, their individual resistance profiles, and antibiotics used can be found in the **SM**

**Tables 1** and **2.** Clinical and Laboratory Standards Institute (CLSI) guidelines for broth microdilution testing (M100-S23) were followed (CLSI, 2020).

**Growth inhibition screening and dose-response study**

The broth dilution *in vitro* methods for bacterial susceptibility assessment have previously been described by Schultz et al. (2020). The standardized bacterial working cultures were pipetted into sterile 96-well microtiter plates (Greiner Bio-One International, CELLSTAR 655185). Extracts and antibiotic (64-1 µg/mL), vehicle and sterility controls, were then added into respective wells. Initial optical density measurement (600 nm) was performed, accounting for absorbance of extracts. Plates were incubated at 37°C for 18 h, except for *A. baumannii* which was incubated for 22h in accordance with strain characteristics (**SM Table 1)**. After incubation, a final optical density reading (600 nm) was conducted. Percent inhibition values were calculated and the $IC_{50}$ and MIC values were determined (Quave et al., 2008; Schultz et al., 2020). The $IC_{50}$ value is defined as the lowest concentration at which an extract showed ≥ 50% inhibition, and the MIC is the lowest concentration at which an extract displayed ≥ 90% inhibition. A total of 51 samples underwent single-dose pre-screening for growth inhibition (in triplicate) at the concentration of 256 µg/mL on eleven pathogens. Samples showing ≥40 % growth inhibition were further tested in a dose-response study with two-fold serial dilution at descending concentrations from 256 to 2 µg/mL. The dose-response experiments were done as biological replicates on separate days in triplicate (technical replicates) to validate reproducibility. Positive controls (antibiotics) and negative controls (vehicle control and sterile media control) were always included. Further details on bacteria standardization can be found in **Supplementary Materials:** ***Methods***. Information on plate setup for bacterial library screens and dose response assays can be found in **SM Figures 3** and **4.**

**COX-2 inhibition assay**

Anti-inflammatory assays were assessed using an *in vitro* COX inhibitor screening assay kit (Cayman Item No: 701080), with modifications previously described in Schultz et al. (2021). All extracts were first screened in duplicate for inhibition against human recombinant COX-2 at an initial concentration of 50 µg/mL. For extracts exhibiting at least 50% inhibition, the concentration was then lowered to 10 µg/mL, 5 µg/mL, and 2.5 µg/mL. The most active extracts were taken to dose-response experiments for determination of $IC_{50}$ values (**Table 5**). The assay was done in two

steps: (1) the COX reaction step in which the prostaglandin $H_2$ (PG) was produced (which was further reduced to the more stable prostaglandin $F_{2\alpha}$ by addition of stannous chloride), and (2) an acetyl choline esterase competitive ELISA step to quantify the produced prostaglandin and calculate a potential enzyme inhibition caused by the extracts. The pure compound and selective COX-2 inhibitor DuP-769 was included as a positive control. DMSO was included as the vehicle control for determining 100% enzyme activity.

# Results:

### Behavioral Observations

Several unusual feeding events and putative self-medicative behaviors were recorded over 116 total field days. **Table 1** reports all species collected for pharmacological testing and provides behavioral justifications for collection. Images of some of these ingestion events can be found in **SM Figure 5**.

Direct observations of potential injury-related ingestion included *K. anthotheca* bark and resin, *W. elongata* young leaves, *C. alexandri* bark, and *C. parasitica* ferns. Individuals exhibiting respiratory symptoms were observed ingesting *C. alexandri* bark and *K. anthotheca* bark and resin. Several resources were also consumed by individuals with positive urinalysis results including *C. patens* dead wood*, K. anthotheca* bark and resin*,* and *M. leucantha* pith. Individuals with recent cases of diarrhea were observed consuming *A. boonei* and *C. patens* dead wood, *K. anthotheca* bark and resin, and *W. elongata* leaves. Parasitological analyses further suggest individuals with varying degrees of endoparasite infections consumed *S. myrtina* and *C. alexanderi* bark, *A. boonei* and *C. patens* dead wood*, K. anthotheca* bark and resin, *W. elongata* leaves, as well as *A. polystachyus* and *M. leucantha* pith. Individuals observed leaf swallowing were also observed ingesting *K. anthotheca* bark and resin and *A. polystachyus* pith prior to this established self-medicative behavior. Ingestion of the species *F. variifolia*, *D. dewevrei*, and *S. guineense* were never directly observed during the study period.

**TABLE 1:** Relevant behavioral observations associated with plant species selected for pharmacological activity screening.

| Species | Plant part tested & associated behavior | Previously proposed as self-medicative resource[1] | Previous reports of ingestion | | Justification for collection / Notable observation(s) from study period | |
|---|---|---|---|---|---|---|
| | | | Sonso | Waibira | Sonso (Observed before sample selection) | Waibira (Observed after sample selection) |
| ***Scutia myrtina*** | **Stem Bark** (Bark Strip) | No | ✗ | ✗ | ***Species collected based on unusual feeding events.***<br><br>**Case 1:** Juvenile male (MZ) travelled a far distance from the main group with sub-adult brother (MB) on 8/26/21, and both stripped bark in periphery of home range. Both individuals also consumed several other putative medicinal resources tested in this study immediately prior (i.e., *A. polystachyus* pith, *C. patens* dead wood, *K. anthotheca* bark and resin). MZ whimpered consistently throughout the day and his fecal sample taken during the event showed six species of internal parasites including *Ascaris* (50 EPG), *Trichuris* (50 EPG), *Taenia* (50 EPG), *Strongyloides* (200 EPG), *Oesophagostomum* (250 EPG), and *Ancylostoma* (100 EPG). Plant showed evidence of previous stripping. Fresh sample was cut from plant near where bark was stripped. | ***No direct observations.*** |
| | **Stripped Stem Bark Collected from Ground** (Bark Strip) | No | ✗ | ✗ | ***Species collected based on unusual feeding events.***<br><br>**Case 1:** During event mentioned above, stripped bark was discarded by individuals during processing. A sample was collected from the ground to assess potential differences between consumed and discarded bark. | ***No direct observations.*** |
| ***Cynometra alexandri*** | **Stem Bark** (Bark Strip) | Yes | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.***<br><br>***No direct observations.*** | **Case 1:** On 9/2/22, adult male (SAM) was observed ingesting bark in a group stripping bout while coughing and sneezing. His symptoms improved in the following days. SAM's fecal analysis from this day contained *Ancylostoma* (50 EPG), *Oesophagostomum* (200 EPG), and *Trichuris* (100 EPG).<br><br>**Case 2:** On 9/2/21, during the same bark stripping event as **Case 1**, adult female (ELD) was observed bark stripping with a maximum reproductive swelling. Her fecal sample contained *Ascaris* (550 EPG), *Ancylostoma* (50 EPG), *Oesophagostomum* (250 EPG), *Strongyloides* (150 EPG), *Trichuris* (50 EPG), and Unidentified cestode eggs (500 EPG).<br><br>**Case 3:** On 9/30/22, adult male (ALF) was seen with a wounded mouth bark stripping with a group of males.<br><br>**Case 4:** On 9/24/22, sub-adult male (JNO) was observed bark stripping alone. JNO's fecal analysis from this day had *Oesophagostomum* (800 EPG). |
| ***Ficus variifolia*** | **Stem Bark** (Bark Strip) | No | ✓ | ✗ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.*** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | ***No direct observations.*** | ***No direct observations.*** |
| ***Ficus exasperata*** | **Stem Bark** (Bark Strip) | Yes | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.***<br><br>**Case 1:** On 7/13/21, while Sonso was on inter-community patrol in Waibira, two sub-adult males (ZD, MB), and one juvenile male (MZ) stripped bark for ~5 minutes. No health data is available from any of these individuals. | ***No direct observations.*** |
| | **Mature Leaves** (Ingest) | Yes | ✓ | ✓ | ***Plant part selected for cross-plant bioactivity comparison.***<br><br>***Multiple direct observations.*** | ***Multiple direct observations.*** |
| ***Desplatsia dewevrei*** | **Stem Bark** (Bark Strip) | No | ✓ | ✗ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.***<br><br>***No direct observations.*** | ***No direct observations.*** |
| ***Alstonia boonei*** | **Stem Bark** (Bark Strip) | Yes | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.***<br><br>***No direct observations.*** | ***No direct observations.*** |
| | **Dead Wood** (Dead Wood Eating) | No | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's dead wood eating repertoire.***<br><br>**Case 1:** Adult male (SM), sub-adult male (MB), and juvenile male (MZ) consumed dead wood from decaying, standing trunk on 10/6/21. Event occurred while community was outside core area. Healing bite marks indicated previous visit(s) to the tree. MB was observed with diarrhea two days before, shedding proglottids of *Bertiella* sp., and harboring unidentified protozoa. Four days after the event, SM was found to have *Ascaris* (50 EPG), *Ancylostoma* (50) *Oesophagostomum* (250 EPG), and *Trichostrongyloides* (100 EPG) eggs in his feces. | ***No direct observations.*** |
| ***Cleistopholis patens*** | **Dead Wood** (Dead Wood Eating) | No | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's dead wood eating repertoire.***<br><br>**Case 1:** On two occasions, nine days apart, adult male (ZL) consumed dead wood. On first occasion, 7/24/21, ZL travelled away from the group with a juvenile, orphaned male (OZ) to eat dead wood. A fecal analysis collected from ZL two days later showed the presence of *Ascaris* sp. (7600 EPG), *Ancylostoma* (50 EPG), *Oesophagostomum* (1050 EPG), a segment of *Taenia,* and *Strongyloide* larvae.<br><br>**Case 2:** On 8/2/21, ZL again travelled away from the group while on inter-community patrol to eat dead wood, accompanied by a different juvenile, orphan male (KJ). ZL's feces from this day had *Ascaris* (50 EPG), *Ancylostoma* (50 EPG), *Oesophagostomum* (1200 EPG), *Strongyloides* (100 EPG), | ***No direct observations.*** |

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| | | | | | *Trichostrongyloides* (50 EPG). A urine test from this day found ZL positive for leukocytes.<br><br>**Case 3:** An adult female (KL) and her two offspring broke-off from a group on 9/16/21 to eat dead wood for ~20 minutes. They were joined by adult female (DR). KL had severe diarrhea immediately following the bout and was found to have *Ancylostoma* (300 EPG) in her feces.<br><br>**Case 4:** On 8/26/21, juvenile male (MZ) was observed eating dead wood on day he consumed several other putative self-medicative resources (**see Table 1: *S. myrtina*, Case 1**). | |
| ***Syzygium guineense*** | **Stem Bark**<br>(Bark Strip) | No | ✓ | ✗ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.***<br><br>***No direct observations.*** | ***No direct observations.*** |
| | **Mature Leaves**<br>(n.a.) | Yes | ✗ | ✗ | ***Plant part selected for cross-plant bioactivity comparison.***<br><br>***No direct observations.*** | ***No direct observations.*** |
| ***Khaya anthotheca*** | **Stem Bark & Resin**<br>(Bark Strip) | Yes | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's bark stripping repertoire.***<br><br>**Case 1:** Adult male (PS) ate bark and resin on 10/6/21 while the rest of the group waited on the ground. His fecal sample had *Ascaris* (100 EPG), *Ancylostoma* (700 EPG), *Oesophagostomum* (1750 EPG), *Strongyloides* (50 EPG) and a *Bertiella* sp. proglottid. PS also had a new wound on his arm which he groomed throughout the day. Wounded individuals were observed consuming bark and resin of this species on at least two other occasions.<br><br>**Case 2:** On 7/15/21, a urinalysis test from adult female (IN) tested positive for leukocytes following ingestion of bark and resin. IN had been observed with severe diarrhea the previous day.<br><br>**Case 3:** On 7/8/21, adult female (WM) with severe diarrhea tested positive for leukocytes and trace levels of blood on urinalysis test following ingestion of bark and resin.<br><br>**Case 3:** On 8/9/32**,** a juvenile female (DB) with a persistent cough consumed bark and resin.<br><br>**Case 4:** On 8/26/21, juvenile male (MZ) ate bark and resin with several other putative self-medicative resources tested in this study (**see Table 1: *S. myrtina*, Case 1**).<br><br>**Case 5:** On 8/13/21, adult female (KL) ate bark and resin a few hours before leaf-swallowing. | ***No direct observations.*** |
| ***Whitefelida elongata*** | **Young Leaves**<br>(Ingest) | No | ✓ | ✓ | ***Species collected based on unusual feeding events.*** | **Case 1:** On 8/3/22, an adult female (NOR) consumed these leaves immediately after having severe diarrhea. She then day nested. NOR's infant did not attempt to eat leaves. |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | **Case 1:** On 8/16/21, adult male (PS) with severely injured hand ate these leaves immediately before ingesting *C. parasitica* (fern) (**See Table 1: *C. parasitica*, Case 1**).<br><br>**Case 2:** On 8/25/21, juvenile male (MZ) ate these leaves twice throughout the day. His older brother (MB), who he travelled with throughout the day, did not eat them. The following day, MZ was reported eating several putative self-medicative resources tested in this study (**See Table 1*: S. myrtina*, Case 1**). | NOR's fecal sample from this day had *Oesophagostomum* (150 EPG).<br><br>**Case 2:** On 9/26/21, an adult female (BAH) ate these leaves after day nesting. Her juvenile son, BRI, did not attempt to eat the leaves. BAH's fecal analysis contained *Oesophagostomum* (1750 EPG), *Ancylostoma (*150 EPG*), Enterobius vermicularis* (100 EPG), and *Trichostrongyloides* (100 EPG). |
| ***Acanthus polystachyus*** | **Pith**<br>(Non-Bitter Pith Strip) | Yes | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's pith-stripping repertoire.***<br><br>**Case 1:** On 8/13/21, sub-adult male, KC, observed stripping pith ~2h before leaf-swallowing.<br><br>**Case 2:** On 8/26/21, juvenile male (MZ) stripped pith on the same day he consumed several other putative self-medicative resources (**see Table 1: *S. myrtina,* Case 1**). | **Case 1:** On 9/13/22, a large group travelled into the Sonso core area to strip pith, despite proximity of vocalizing Sonso males. Waibira group travelled directly to patch of *A. polystachius*, consumed pith for ~30 minutes, and returned directly to Waibira's core area. |
| ***Marantochloa leucantha*** | **Pith**<br>(Non-Bitter Pith Strip) | No | ✓ | ✓ | ***Species collected based on known inclusion in Sonso's pith-stripping repertoire.***<br><br>**Case 1:** On 9/13/21, juvenile male (MZ) stripped pith with two unrelated adult females (NB and WM). Fecal analysis from MZ revealed presence of *Ancylostoma* (650 EPG), *Oesophagostomum* sp. (600 EPG), and *Ascaris* sp. (50 EPG). | **Case 1:** On 7/15/22, adult female (BAH) stripped and wadged pith while her juvenile son (BRI) rested. BAH's urinalysis showed high ketone levels. Her fecal sample had *Ascaris* (50 EPG), *Ancylostoma* (50 EPG), *Oesophagostomum* (650 EPG), and a *Taenia* segment.<br><br>**Case 2:** Between 7/27/22-7/31/22, adult male (FID) stripped pith three times in a four-day period. FID's fecal samples from ten and five days before the first event both had *Trichuris* sp. (whipworm), a nematode known to cause health complications. On the first day, FID was the only one to strip pith despite proximity of another adult male (MAC). The bout lasted ~20 minutes. The following day, FID stripped pith alone, and later that day stripped *Ficus saussureana* bark with two other sub-adult males, both of whom had expelled *Bertiella* sp. proglottids within four days of the event. Two days later, FID stripped *M. leucantha* again, alone. A fecal sample collected from FID the day after the final event, contained eggs of *Ancylostoma* sp. (200 EPG), *Oesophagostomum* (100 EPG), *Strongyloides* (150 EPG), and *Trichostrongyloides* (50 EPG). No *Trichuris* eggs were detected in the final sample. |
| ***Christella parasitica*** | **Fern**<br>(Ingest) | No | ✓ | ✗ | ***Species collected based on unusual feeding events.***<br><br>**Case 1:** Adult male (PS) ate leaves of *C. parasitica* on 8/16/21 with a newly injured hand while travelling outside core area. PS was the only individual in a large group to seek out and eat ferns. This was only the second report of this species being consumed in Budongo. No parasitological or urinalysis data is available for PS on this day. | ***No direct observations.*** |

[1] Species listed as a putative therapeutic resource for primates in recent review of zoopharmacognosy literature (De la Fuente et al., 2022)

## Ethnobotanical Review

According to our review of ethnomedicinal literature from various African regions between 1976-2022, 11 out of the 13 tested species also had documented medicinal uses (**Table 2**).

**TABLE 2:** Ethnobotanical literature review of selected plant species

| Species | Reported Ethnobotanical Uses | Source(s) |
|---|---|---|
| *S. myrtina* | **Roots**: Intestinal worms; Gonorrhea; Bilharzia; Fever<br>**Leaves:** Ringworm; Wounds; Parturition of placenta and childbirth | (Hedberg et al., 1983; Kokwaro, 1976, 2009) |
| *C. alexandri* | **Bark:** Wounds; Acute backache | (Howard et al., 1991; Terashima et al., 1991) |
| *F. variifolia* | None known | |
| *F. exasperata* | **General:** Hemorrhoids; Venereal disease; Arthritis; Wounds; Parasites; Diuretic for relaxing uterus; Enhancing uterine contractions<br>**Wood ash/charcoal:** Leprous ulcer; General wounds<br>**Roots:** Asthma; Dyspnea; Venereal disease<br>**Bark:** Intestinal worms; Hemorrhoids; Spleen enlargement; Heart problems; Cough; Dizziness; Facilitation of childbirth; Gonorrhea; Malaria<br>**Bark Sap:** Bleeding; Stimulant; Wounds, Sores, Abscesses; Eye ailments; Stomachache<br>**Leaves**: High blood pressure; Rheumatism; Arthritis; Intestinal pains; Epilepsy; Bleeding; Wounds; Inflammation; Bacterial infections; Fever; Edema; Leprous ulcer; Dermatosis; Abscess; Cough; Cold; Flu; Asthma; Heart disease; Thrush; Gum inflammation; Mouth/ throat ailments; Gastric ulcers; Stomachache; Poison; Kidney disease; Urinary tract infections; Headache; Tumors; Diarrhea; Intestinal parasites<br>**Leaf Shoots**: Dysentery; Jaundice (externally applied); Emetic; Diuretic.<br>**Leaf Pulp (external):** Rash; Wounds; Fungal infection; Itching; Ringworm; Rheumatism; Back pain<br>**Dried /Cooked Leaf:** Burns; Gonorrhea | (Chhabra et al., 1990; Irvine, 1961; PROTA, 2023) |
| *D. dewevrei* | **Bark:** Pain management; Nasopharyngeal infections; Febrifuges; Venereal diseases; Convulsion; Heartaches; Bodily pains | (Burkill, 1995; Ovuakporie-Uvo et al., 2019; PROTA, 2023) |
| *A. boonei* | **Bark**: Abortive; Gonorrhea; Asthma; Sores; Ulcers; Pain; Diarrhea; Dysentery; Vermifuge; Liver problems; Dropsy, Inflammation; Edema; Gout; Diabetes; Internal parasites; Dizziness; Breast infection; Nausea, Snakebites; Stomachaches; Malaria; Measles; Uterine fibroid/ovarian cysts; Gynecological lower abdominal and pelvic congestion (PID); Aches from malarial fever; Jaundice<br>**Latex**: Internal parasites; Lactation stimulant | (Adotey et al., 2012; Burkill, 1995; Kokwaro, 1976, 2009) |
| *C. patens* | **Bark/Sap:** Jaundice; Hepatitis; Stomachache; Tuberculosis; Bronchial affections; Colic; Edemas; Hunchbacks; Rickets; Headache; Pain; Pulmonary troubles; Diarrhea; Hepatitis; Malaria; Measles; Typhoid fever; Menstrual irregularities.<br>**Root:** Vermifuge | (Addo-Fordjour et al., 2008; Burkill, 1995; Irvine, 1961; PROTA, 2023) |
| *S. guineense* | **Bark**: Stomachache; Internal parasites; Purgative; Bodily weakness; Infertility; Abdominal pain; Laxative, Diarrhea; Malaria; Cough; Asthma; Throat problems; Intercoastal pain; Paralysis; Broken bones; Wounds<br>**Roots:** Internal parasites; Purgative; Stomachache; Epilepsy<br>**Leaves:** Enema; Colic; Diarrhea; Abdominal pain; Insanity; Amenorrhea; Cerebral malaria; Intestinal parasites; Stomachache; Insanity; Tonic for pregnancy; Diarrhea; Wounds; Boils; Sprains; Ophthalmia | (Amusan et al., 2002; Kokwaro, 2009; PROTA, 2023) |
| *K. anthotheca* | **Bark:** Csolds; Fevers; Pneumonia; Abdominal pain; Vomiting; Gonorrhea; Aphrodisiac; Wounds, Sores, Ulcers; Anemia; Malaria, Bilharzias<br>**Roots:** Anemia; Dysentery; Rectal prolapse | (Amri & Kisangau, 2012; Fern, 2014; PROTA, 2023) |
| *W. elongata* | **Leaves:** Bronchitis; Conception aid (women); Stomachache; Food poisoning | (Burkill, 1995; Fern, 2014) |
| *A. polystachyus* | **Leaf:** Liver/spleen problems; Scabies; Gastroenteritis; Pneumonia; Anthrax; Malaria<br>**Roots:** Gonorrhea; Syphilis; Bleeding; Stabbing pain; Pneumonia | (Boily & Van Puyvelde, 1986; Heyndrickx et al., 1992; Kokwaro, 2009; Vlietinck et al., 1995) |
| *M. leucantha* | **Roots**: Aphrodisiac<br>**Pith:** Rheumatism; Acne | (Haxaire, 1979; Hamill et al., 2003; Kokwaro, 2009) |
| *C. parasitica* | None known | |

**Production of extracts and sample information**

Taxonomic information and extraction details for the 13 plant species studied, including the plant family, local name (when available), plant part used, solvent for extraction, yield of extraction, extract identification numbers (extract IDs), herbarium accession numbers, and collection location are summarized in **Table 3**. Overall, the highest extraction yields were obtained with methanol-water (9/1) as a solvent. The yields from methanol-water extractions for *C. parasitica*, *F. exasperata* leaves, and *S. guineense* stem bark were higher than the other extractions from these samples. The plant samples which had higher yield values with *n*-hexane, such as the leaves of *W. elongata* and bark extract of *A. boonei*, likely have a higher content of lipids (i.e., fatty molecules).

**TABLE 3:** Taxonomic information and extraction details of plant samples for pharmacological assessment

| Scientific Name (Life form) | Family | Name in Runyoro & "Common Name" | Plant Part | Extraction Solvent | Extraction Yield [%] | Extract ID | Herbarium Accession Number | Collecting Location |
|---|---|---|---|---|---|---|---|---|
| ***Christella parasitica*** (L.) H.Lév. (Fern) | Thelypteridaceae | n.a. | whole plant | methanol/water (9:1, *v/v*) | 14.1 | mwE087 | Oxford: 00243122E (E001) | Budongo Forest |
| | | | | ethyl acetate | 2.7 | eE087 | | |
| | | | | n-hexane | 1.7 | hE087 | | |
| ***Khaya anthotheca*** (Welw.) C. DC. (Tree) | Meliaceae | Munyama "White Mahogany" | stem bark and resin | methanol/water (9:1, *v/v*) | 9.9 | mwE088 | Oxford: 00243123F (E002) | Budongo Forest |
| | | | | ethyl acetate | 8.5 | eE088 | | |
| | | | | n-hexane | 6.7 | hE088 | | |
| ***Scutia myrtina*** (Burm.f.) Kurz. (Scrambling Shrub/Tree) | Rhamnaceace | n.a. | stripped stem bark refuse | methanol/water (9:1, *v/v*) | 3.9 | mwE089a | Oxford: 00243128K (E007) | Budongo Forest |
| | | | | ethyl acetate | 0.6 | eE089a | | |
| | | | | n-hexane | 0.2 | hE089a | | |
| | | | stem bark | methanol/water (9:1, *v/v*) | 3.3 | mwE089b | | |
| | | | | ethyl acetate | 0.6 | eE089b | | |
| | | | | n-hexane | 0.2 | hE089b | | |
| ***Whitfieldia elongata*** (P. Beauv.) De Wild. & T. Durand (Climber) | Acanthaceae | n.a. | leaves | methanol/water (9:1, *v/v*) | 1.7 | mwE090 | Oxford: 00243129L (E009) | Budongo Forest |
| | | | | ethyl acetate | 2.8 | eE090 | | |
| | | | | n-hexane | 2.4 | hE090 | | |
| ***Cleistopholis patens*** (Benth.) Engl. & Diels. (Tree) | Annonaceae | Mubanda murogo "Salt and Oil Tree" | dead wood | methanol/water (9:1, *v/v*) | 1.3 | mwE091 | | Budongo Forest |
| | | | | ethyl acetate | 0.4 | eE091 | | |
| | | | | n-hexane | 0.1 | hE091 | | |
| ***Alstonia boonei*** De Wild. (Tree) | Apocynaceae | Mujwa/Kanji | stem bark | methanol/water (9:1, *v/v*) | 0.4 | mwE092a | Makerere: 51204 (W007) | Budongo Forest |
| | | | | ethyl acetate | 2.3 | eE092a | | |
| | | | | n-hexane | 1.6 | hE092a | | |
| | | | dead wood | methanol/water (9:1, *v/v*) | 1.2 | mwE092b | | |
| | | | | ethyl acetate | 0.4 | eE092b | | |
| | | | | n-hexane | 0.3 | hE092b | | |
| ***Ficus exasperata*** Vahl. (Tree) | Moraceae | Musomoro "Sandpaper Leaf Tree" | stem bark | methanol/water (9:1, *v/v*) | 3 | mwE093a | Oxford: 00243130D | Budongo Forest |
| | | | | ethyl acetate | 0.8 | eE093a | | |
| | | | | n-hexane | 0.4 | hE093a | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | leaves | methanol/water (9:1, *v/v*) | 11.5 | mwE093b | (E012) | |
| | | | | ethyl acetate | 3.1 | eE093b | | |
| | | | | n-hexane | 2.3 | hE093b | | |
| ***Marantochloa leucantha*** (K.Schum.) Milne-Redh. (Terrestrial Herbaceous) | Marantaceae | n.a. | pith | methanol/water (9:1, *v/v*) | 0.7 | mwE094 | Makerere: 51203 (W013) | Budongo Forest |
| | | | | ethyl acetate | 0.6 | eE094 | | |
| | | | | n-hexane | 0.2 | hE094 | | |
| ***Desplatsia dewevrei*** (De Wild. & T. Durand) Burret (Tree) | Tiliaceae | Omukoma-nyakabita | stem bark | methanol/water (9:1, *v/v*) | 3 | mwE095 | Oxford: 00243132F (E014) | Budongo Forest |
| | | | | ethyl acetate | 1 | eE095 | | |
| | | | | n-hexane | 0.6 | hE095 | | |
| ***Cynometra alexandri*** C. H. Wright (Tree) | Caesalpinioideae | Nyakaimbi "Iron Wood" | stem bark | methanol/water (9:1, *v/v*) | 14 | mwE096 | Oxford: 00243133G (E015) | Budongo Forest |
| | | | | ethyl acetate | 2.4 | eE096 | | |
| | | | | n-hexane | 1.1 | hE096 | | |
| ***Ficus variifolia*** Warb. (Tree) | Moraceae | n.a. | stem bark | methanol/water (9:1, *v/v*) | 3.1 | mwE097 | Makerere: 51195 (W005) | Budongo Forest |
| | | | | ethyl acetate | 0.5 | eE097 | | |
| | | | | n-hexane | 0.2 | hE097 | | |
| ***Syzygium guineense*** (Willd.) DC. (Tree) | Myrtaceae | n.a. | stem bark | methanol/water (9:1, *v/v*) | 9.6 | mwE098a | Oxford: 00243135I (E017) | Budongo Forest |
| | | | | ethyl acetate | 0.8 | eE098a | | |
| | | | | n-hexane | 0.3 | hE098a | | |
| | | | leaves | methanol/water (9:1, *v/v*) | 17 | mwE098b | | |
| | | | | ethyl acetate | 2.1 | eE098b | | |
| | | | | n-hexane | 0.5 | hE098b | | |
| ***Acanthus polystachyus*** Delile (Terrestrial Herbaceous) | Acanthaceae | n.a. | pith | methanol/water (9:1, *v/v*) | 8.4 | mwE099 | Oxford: 00243136J (E018) | Budongo Forest |
| | | | | ethyl acetate | 1 | eE099 | | |
| | | | | n-hexane | 0.2 | hE099 | | |

**Library screening against multidrug-resistant human and food bacterial pathogens**

Initial screening of extracts involved checking for growth inhibition against each bacterium at a concentration of 256 µg/mL. In total, 45 of the 51 plant extracts (88%) showed activity ≥40% inhibition against at least one of the 11 strains and were thus considered active and brought to dose-response experiments to determine their $IC_{50}$ value and MIC. Results from the library screening are reported in **SM Table 3**. As all tested plant species in the library screen had at least one extract that was active (*in vitro*) against at least one bacterial strain, no entire species was eliminated for further experimentation. However, as no extracts (at any potency) inhibited the growth of *K. pneumonia*, no further tests were conducted on this bacterium. The extract active against the most bacterial strains (n=11) was the methanol-water extract of *S. guineense* stem bark (mwE098a, active against eight strains), followed by the methanol-water *S. guineense* leaves (mwE098b), the ethyl acetate *P. patens* dead wood, and the *n*-hexane *A. boonei* dead wood (hE092b) extracts, which were each active against seven, seven, and six strains. The only extract that demonstrated significant inhibition against *P. aeruginosa* at the highest test concentration was the methanol-water extract from *S. guineense* bark (mwE098a). This was also the only extract to display significant inhibition at 256 µg/mL against *E. cloacae*. Of all bacteria in this study, the two strains of *E. coli* (DSM 498 and DSM 15076) were the most susceptible, with at least one extract from all plant species inhibiting their growth. The *E. coli* strain with nine known antibiotic resistances (DSM 15076) surprisingly showed growth inhibition in 80% of tested extracts.

**Dose response antibacterial experiments**

In dose response assays, 41 out of the 45 tested extracts (89%) showed activity at ≤256µg/mL, though not all extracts reached MIC values (see **Table 4**). Results are reported with standard deviations in **SM Table 4,** and a summary of how many strains each extract was active against is reported in **SM Table 6**. The strongest *in vitro* growth inhibition was reported for the methanol-water extract of *K. anthotheca* bark and resin (mwE088) against gram-positive *E. faecium* and the *n*-hexane extract of *A. boonei* dead wood (hE092b) against gram-positive *S. aureus* (DSM 1104). Both extracts had low $IC_{50}$ values of 16 µg/mL (showing strong inhibition), with MIC values of 32 µg/mL against respective strains. *E. faecium* showed the most general susceptibility to *K. anthotheca,* with all extracts of this species achieving MIC values (mwE088: 32 µg/mL, eE088:

64 µg/mL, hE088: 128 µg/mL). The ethyl acetate extract of *A. boonei* dead wood (eE092b) also strongly inhibited the growth of *E. faecium* ($IC_{50}$: 16 µg/mL; MIC: 64 µg/mL), as did the *n*-hexane extract of *A. boonei* dead wood, producing an $IC_{50}$ value of 16 µg/mL but failing to reach a MIC value. *S. aureus* (DSM 1104) was also highly susceptible to the ethyl acetate extracts of *A. boonei* dead wood ($IC_{50}$: 32 µg/mL; MIC: 128 µg/mL).

Only one extract, the methanol-water extract of *S. guineense* bark (mwE098a), was active against the gram-negative *P. aeruginosa.* This extract exhibited moderate growth inhibition ($IC_{50}$: 64 µg/mL) with no MIC value reached. Despite *E. coli* (DSM 498) being highly susceptible on the library screen, only two extracts, the methanol-water extract of *A. boonei* dead wood (mwE092b; $IC_{50}$: 256 µg/mL) and the methanol-water extract of *S. guineense* leaves (mwE098b; $IC_{50}$: 128 µg/mL), reached $IC_{50}$ values at the concentration range tested, with no MICs reached. Interestingly, the strain of *E. coli* with nine known resistances (DSM 1576) was more susceptible, with 89% of extracts achieving $IC_{50}$ values $\leq 256$. The most active extract against this strain was the methanol-water extract of *K. anthotheca* (mwE088; $IC_{50}$: 16 µg/mL; MIC: 256 µg/mL). *S. maltophilia* was most inhibited by the species *S. guineense,* with all but one extract from this species exhibiting $IC_{50}$ values of $\leq 256$ µg/mL against it. At the concentration range tested, no extracts yielded MIC values for *S. aureus* (DSM 18827), *A. baumannii*, *E. cloacae*, *P. aeruginosa* or *E. coli* (DSM 498).

**TABLE 4:** $IC_{50}$ and MIC values obtained from *in vitro* dose-response study on bacterial growth inhibition

| Scientific name | Extract ID | *S. aureus* DSM 1104 | | *S. aureus* DSM 18827 | | *A. baumannii* DSM 102929 | | *E. cloacae* DSM 30054 | | *P. aeruginosa* DSM 1117 | | *E. faecium* DSM 13590 | | *E. coli* DSM 498 | | *E. coli* DSM 1576 | | *S. maltophilia DSM 50170* | | *S. enterica subsp. enterica DSM 11320* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC |
| *C. parasitica* | eE087 | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | - | - | - | - | - | - |
| | hE087 | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **128** | **>256** | - | - | - | - |
| *K.anthotheca* | mwE088 | - | - | - | - | >256 | >256 | - | - | - | - | **16** | **32** | >256 | >256 | **16** | **256** | **64** | **>256** | - | - |
| | eE088 | - | - | - | - | - | - | - | - | - | - | **64** | **64** | >256 | >256 | **64** | **>256** | - | - | - | - |
| | hE088 | - | - | - | - | - | - | - | - | - | - | **64** | **128** | - | - | **64** | **>256** | - | - | **256** | **>256** |
| *S. myrtina* | mwE089a | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **128** | **>256** | - | - | - | - |
| | eE089a | - | - | - | - | - | - | - | - | - | - | **64** | **>256** | >256 | >256 | **256** | **>256** | - | - | - | - |
| | hE089a | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | - | - | - | - | - | - |
| | mwE089b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **256** | **>256** | - | - | - | - |
| | eE089b | - | - | - | - | >256 | >256 | - | - | - | - | **128** | **>256** | >256 | >256 | **256** | **>256** | - | - | - | - |
| | hE089b | - | - | >256 | >256 | **256** | **>256** | - | - | - | - | - | - | - | - | **256** | **>256** | - | - | - | - |
| *W. elongata* | mwE090 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **128** | **>256** | - | - | - | - |
| | eE090 | - | - | - | - | - | - | - | - | - | - | **64** | **128** | >256 | >256 | **256** | **>256** | - | - | - | - |
| *C. patens* | mwE091 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **128** | **>256** | - | - | - | - |
| | eE091 | **128** | **256** | - | - | >256 | >256 | - | - | - | - | **64** | **64** | >256 | >256 | **128** | **>256** | **256** | **>256** | >256 | >256 |
| | hE091 | - | - | - | - | | | - | - | - | - | **64** | **>256** | - | - | **128** | **>256** | - | - | - | - |
| *A. boonei* | mwE092a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **128** | **>256** | - | - | - | - |
| | hE092a | - | - | - | - | >256 | >256 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE092b | - | - | - | - | - | - | - | - | - | - | - | - | **256** | **>256** | **32** | **>256** | - | - | - | - |
| | eE092b | **32** | **128** | - | - | >256 | >256 | - | - | - | - | **16** | **64** | >256 | >256 | **128** | **>256** | - | - | - | - |
| | hE092b | **16** | **32** | **32** | **>256** | - | - | - | - | - | - | **16** | **>256** | >256 | >256 | 256 | >256 | - | - | **256** | **256** |
| *F.exasperata* | mwE093a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **128** | **>256** | - | - | - | - |
| | eE093a | - | - | - | - | - | - | - | - | - | - | **32** | **128** | >256 | >256 | **128** | **>256** | - | - | - | - |
| | hE093a | - | - | - | - | - | - | - | - | - | - | **32** | **>256** | - | - | - | - | - | - | - | - |
| | mwE093b | - | - | - | - | - | - | - | - | - | - | - | - | | | **256** | **>256** | - | - | - | - |
| | hE093b | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | - | - | - | - |
| *M. leucantha* | mwE094 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **128** | **>256** | - | - | - | - |
| | eE094 | - | - | - | - | >256 | >256 | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | - | - | - | - |
| | hE094 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **256** | **>256** | - | - | - | - |
| *D. dewevrei* | mwE095 | - | - | - | - | >256 | >256 | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | **256** | **>256** | - | - |
| | eE095 | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | - | - | - | - |
| | hE095 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | - | - | - | - |
| *C. alexandri* | mwE096 | >256 | >256 | - | - | >256 | >256 | - | - | - | - | - | - | | | **256** | **>256** | **256** | **>256** | - | - |
| | eE096 | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | - | - | - | - |
| | hE096 | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | - | - | - | - |
| *F.variifolia* | eE097 | - | - | - | - | - | - | - | - | - | - | **64** | **>256** | >256 | >256 | **256** | **>256** | - | - | - | - |
| | hE097 | - | - | - | - | - | - | - | - | - | - | - | - | >256 | >256 | **256** | **>256** | - | - | - | - |
| *S. guineense* | mwE098a | >256 | >256 | >256 | >256 | **64** | **>256** | **128** | **>256** | **64** | **>256** | - | - | >256 | >256 | **64** | **>256** | **32** | **256** | - | - |
| | eE098a | - | - | >256 | >256 | **128** | **>256** | 256 | >256 | - | - | - | - | >256 | >256 | **128** | **>256** | **64** | **256** | - | - |
| | hE098a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **128** | **>256** | - | - | - | - |
| | mwE098b | **128** | **>256** | >256 | >256 | **128** | **>256** | **128** | **>256** | - | - | - | - | **128** | **>256** | **32** | **128** | **32** | **>256** | - | - |
| | eE098b | - | - | >256 | >256 | >256 | >256 | **256** | **>256** | - | - | - | - | >256 | >256 | **128** | **256** | **128** | **>256** | - | - |
| | hE098b | - | - | >256 | >256 | >256 | >256 | >256 | >256 | - | - | - | - | | | **256** | **>256** | **128** | **>256** | - | - |
| *A. polystachius* | eE099 | - | - | - | - | - | - | - | - | - | - | **128** | **256** | >256 | >256 | **256** | **256** | - | - | - | - |
| | hE099 | **256** | **>256** | - | - | - | - | - | - | - | - | **32** | **128** | >256 | >256 | **256** | **>256** | - | - | - | - |
| **Vancomycin** | | <1 | 2 | <1 | 1 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | n.t. | | n.t. | | n.t. | | n.t | |
| **Gentamicin** | | 0.125 | 0.25 | 0.5 | 1 | >64 | >64 | n.t. | | n.t. | | 8 | >64 | <1 | 1 | n.t. | | 8 | 8 | n.t. | |
| **Ciprofloxacin** | | n.t. | | n.t. | | >64 | >64 | n.t. | | n.t. | | n.t. | | n.t. | | n.t. | | n.t. | | n.t. | |
| **Tetracyclin** | | n.t. | | n.t. | | 1 | 2 | n.t. | | n.t. | | n.t. | | <1 | 2 | n.t. | | n.t. | | 1 | 2 |
| **Chloramphenicol** | | 2 | 8 | 4 | 16 | 32 | 64 | 2 | 4 | 32 | >64 | - | 4 | n.t. | | <1 | 4 | n.t. | | 4 | 2 |

**NB:** *Only extracts showing growth inhibition ≥40% in the library screen at 256 µg/mL are listed. $IC_{50}$ and MIC values are expressed as concentration (µg/mL). The concentrations tested ranging from 256µg/mL to 4 µg/mL. (n.t: not tested).*
**NB:** $IC_{50}$Values ≤256 highighted in **bold.**

### Anti-inflammatory COX-2 inhibition library screen

Results from the *in vitro* COX-2 inhibition library screen at descending concentrations are reported in **SM Table 5**. At the initial concentration of 50 μg/mL, 43 out of 51 extracts (84%) exhibited an enzyme inhibition of at least 50%, displaying anti-inflammatory activity. This included at least one extract of every plant species. In the next stage of screening, at 10 μg/mL, 18 samples were eliminated. During the final step, at 5 μg/mL, five more were eliminated. Remaining extracts (17 extracts from 10 plant species), which displayed inhibition above 50% at 5 μg/mL, were then introduced to dose-response experiments following the library screen. The ethyl acetate extract of *S. myrtina* bark (eE089b) was taken to the COX-2 dose-response, despite not showing inhibition past 50 μg/mL, as it almost reached the selection limit during analysis and had a relatively high standard deviation. No extract from *W. elongata*, *C. patens* or *D. dewevrei* showed COX-2 inhibition at 5 μg/mL and thus these species were excluded from further testing.

### COX-2 inhibition dose-response experiments

The most active COX-2 inhibitors were extracts from *K. anthotheca* (mwE088; hE088; eE088), *C. parasitica* (mwE087; hE087), *F. exasperata* (hE093a; eE093a), *S. myrtina* (hE089a; eE089b), *F. variifolia* (eE097; hE097), *A. polystachyus* (hE099; eE099), *M. leucantha* (hE094), *S. guineense* (hE098a), *A. boonei* (hE092b), and *C. alexandri* (hE096). Results are reported in **Table 5**. The strongest COX-2 inhibitor was the *K. anthotheca* methanol-water bark and resin extract (mwE088) ($IC_{50}$ of 0.55 μg/mL), followed by the *C. parasitica* methanol-water fern extract (mwE087) ($IC_{50}$ of 0.81 μg/mL). In contrast, all extracts of the species *W. elongata*, *C. patens*, and *D. dewevrei* failed to show ≥ 50 % inhibition, mostly at the second screening concentration (10 μg/mL). *W. elongata* extracts notably showed low activity in both antibacterial and COX-2 inhibition assays.

**TABLE 5:** Results of the *in vitro* COX-2 inhibition studies, investigating plant samples from Budongo, Uganda

| | | | | IC50 ± SEM |
|---|---|---|---|---|
| **Extract ID** | **Plant Species** | **Plant Part** | **Type of Extract** | **COX-2** |
| mwE088 | *K. anthotheca* | stem bark & resin | methanol/water (9:1, *v/v*) | 0.55 ± 0.14 |
| mwE087 | *C. parasitica* | fern | methanol/water (9:1, *v/v*) | 0.81 ± 0.11 |
| hE088 | *K. anthotheca* | stem bark &resin | *n*-hexane | 1.02 ± 0.01 |
| hE093a | *F. exasperata* | stem bark | *n*-hexane | 1.06 ± 0.09 |
| hE089a | *S. myrtina* | stem bark | *n*-hexane | 1.19 ± 0.05 |
| eE097 | *F. variifolia* | stem bark | ethyl acetate | 1.20 ± 0.27 |
| eE088 | *K. anthotheca* | stem bark &resin | ethyl acetate | 1.30 ± 0.06 |
| hE099 | *A. polystachyus* | pith | *n*-hexane | 1.63 ± 0.98 |
| hE094 | *M. leucantha* | pith | *n*-hexane | 1.79 ± 0.14 |
| eE093a | *F. exasperata* | stem bark | ethyl acetate | 2.11 ± 0.08 |
| hE098a | *S. guineense* | stem bark | *n*-hexane | 2.42 ± 0.28 |
| hE092b | *A. boonei* | dead wood | *n*-hexane | 2.74 ± 0.37 |
| hE087 | *C. parasitica* | fern | *n*-hexane | 3.18 ± 0.99 |
| hE097 | *F. variifolia* | stem bark | *n*-hexane | 3.37 ± 0.59 |
| hE096 | *C. alexandri* | stem bark | *n*-hexane | 4.83 ± 0.52 |
| eE089b | *S. myrtina* | stem bark (ground) | ethyl acetate | 7.49 ± 0.52 |
| eE099 | *A. polystachyus* | pith | ethyl acetate | 7.83 ± 0.56 |
| **positive control** | *DuP-769* | | (pure compound) | 0.93 ± 0.20 |

**NB:** Extracts are sorted from highest to lowest COX-2 sensitivity; $IC_{50}$ values are provided in µg/mL (positive control: ng/mL); SEM = standard error of the mean

# Discussion:

Overall, this study provides the first pharmacological and behavioral evidence, based on *in situ* sampling, for the medicinal benefits of bark ingestion, dead wood eating, and non-bitter pith stripping behaviors in Budongo chimpanzees.

### Ingested Plant Species with High Pharmacological Activity

The following sub-sections describe and discuss the four most active plant species in further detail.

#### *Christella parasitica*

Extracts of *C. parasitica* produced notably high anti-inflammatory activity in COX-2 testing, with the methanol-water extract (mwE087) achieving an $IC_{50}$ value of 0.81 µg/mL. This same extract, however, exhibited the lowest general activity in the antibacterial library screen. The only antibacterial activity from this species was on *E. coli* (DSM 498) by the ethyl acetate and *n*-hexane extracts (eE087; hE087), and on *E. coli* (DSM 1576) by the n-hexane extract (hE087). The *n*-hexane extract reached an $IC_{50}$ of 128 µg/mL in dose-response assays with no MIC value. Prior to

this study, there had been limited pharmacological testing on *C. parasitica* (though see Paul et al., 2011), so comparison across studies is not possible.

When associated behavioral observations of *C. parasitica* ingestion are considered, we found a notable relevance to our pharmacological results (see **Table 1: *C. parasitica*, Case 1**). Ingestion of *C. parasitica* by a Sonso adult male (PS) was observed while the group was travelling outside of their core area. It was unclear if this was an inter-community patrol. PS had been observed earlier in the day with a severe hand injury which impacted his mobility, though no open wound was observed. PS separated himself from the group and moved a few meters away to a patch of ferns where he began consuming the leaflets. The bout lasted approximately ~3 m. No other group members were observed consuming this species, and this was only the second case of fern ingestion ever reported in Budongo (unpublished site data). Health states of individuals from the past event were unfortunately not recorded. Whether or not *C. parasitica's* highly anti-inflammatory properties were the principal motivator for the selection of this species remains unknown, however, regardless of intention, this plant may have benefitted PS by reducing pain and swelling in his injured hand.

***Alstonia boonei***

Numerous *in vitro* and *in vivo* studies, reviewed by Adotey (2012), have reported pharmacological activity in *A. boonei* bark. However, none of these studies investigated dead wood samples of A. boonei. Consistent with these findings, we found high levels of antibacterial and anti-inflammatory activity in the extracts of this species. Interestingly, extracts from *A. boonei* dead wood generally exhibited higher activity than living bark. This could be due either to a change in active ingredient composition, or possible fungal growth following the tree's death. While the *A. boonei* dead wood *n*-hexane extract (hE092b) exhibited strong growth inhibition against *S. aureus* (DSM 1104; DSM 18827) and *E. faecium* at low concentrations in the dose-response assays, the *n*-hexane bark extract (hE092a) showed no activity <256 µg/ml. Similarly, the ethyl acetate extract of dead wood (eE092b) also strongly inhibited *S. aureus* (DSM 1104) ($IC_{50}$: 16 µg/ml; MIC: 128 µg/ml) and *E. faecium* ($IC_{50}$: 16 µg/ml; MIC: 64 µg/ml), while the ethyl acetate bark extract of this species was not even taken to dose-response. However, the methanol-water extract of *A. boonei* bark (mwE092a) did show activity against *E. coli* (DSM 498) ($IC_{50}$: 128 µg/ml), as did the methanol-

water dead wood extract (mwE092a) ($IC_{50}$: 128 µg/ml), with no MIC values reached in either case. Overall, extracts from *A. boonei* displayed more potent activity in gram-positive bacteria, although this effect is more apparent in dead wood than stem bark. In the COX-2 inhibition assays, the *n*-hexane extract of *A. boonei* dead wood also showed strong anti-inflammatory inhibition, while the *n*-hexane extract of the bark only exhibited weak inhibition (at the highest test concentration of 50 µg/ml).

*A. boonei* is a known medicinal plant across East Africa, commonly used for a variety of reproductive, bacterial, and gastro-intestinal issues, as well as for snake bites, asthma, and dizziness (Adotey et al., 2012; Burkill, 1995; Kokwaro, 2009). The bark and latex are intensely bitter, a reliable signal of the presence of bioactive secondary compounds and toxicity (Crellin et al., 1990; Githens, 2017; Shiba, 1976). Budongo chimpanzees in both communities have been reported to consume both bark and dead wood of *A. boonei*, often travelling far distances to access these trees and only consuming small amounts of bark per bout (Freymann et al., in prep). In an observation reported in this study (see **Table 1: *A. boonei*, Case 1**), three males ingested *A. boonei* dead wood while outside the community's core area for ~1 m. Two days before the event, one of the individuals had been observed with diarrhea, while also shedding visible tapeworm proglottids (*Bertiella* sp.). This sample also contained unidentified protozoa, and *Taenia* sp. eggs. Pebsworth et al. (2006) also reported an event in which four adult males, all with diverse parasite loads, traveled to a large *A. boonei* tree and ingested bark.

In the long-term site data, *A. boonei* bark stripping was only documented 17 times between 2008-2021 (Freymann et al., in prep), although this behavior was not systematically reported. The direct observation of only one *A. boonei* dead wood eating event, and no *A. boonei* bark stripping events over the two four-month periods of observation in this study, suggest that consumption of this species is relatively rare across both communities. While specific pathogenic catalysts for selection of this species remain unknown, based on pharmacological, ethnobotanical, and behavioral data, we propose that *A. boonei* may be a therapeutic self-medicative resource for Budongo chimpanzees. The relatively strong inhibitory activity of this species against *S. aureus*, a bacteria associated with causing contamination on the skin leading to chronic wounds (Xu et al., 2021), as well as its anti-

inflammatory properties, suggests that *A. boonei* ingestion may have beneficial effects in wound care contexts.

***Khaya anthotheca***

Previous studies have demonstrated that *K. anthotheca* bark contains biologically active compounds like gedunins, mexicanolide, phragmalin, and andirobins (Olatunji et al., 2021). One limonoid identified in the species, anthothecol, has anti-cancer properties (Verma et al., 2015). *K. anthotheca* has also been shown to cause cell lysis in some gram-negative bacteria by targeting cytoplasmic membranes (Dougnon et al., 2021). A study by Obbo et al. (2013) on *K. anthotheca* bark collected in Budongo Forest, found strong antiprotozoal activity against *Plasmodium falciparum* ($IC_{50}$ 0.96 µg/ml) and *Trypanosoma brucei rhodesiense* ($IC_{50}$ 5.72 µg/ml).

In our antibacterial library screen, of all extracts tested, only the methanol-water extract inhibited growth of *A. baumannii* (although no $IC_{50}$ values were reached in dose-response). The methanol-water extract also inhibited the growth of *E. coli* (DSM 498) in the library screen, as did the ethyl acetate (eE088) extract, though again no $IC_{50}$ values were reached. In our antibacterial dose-response assays, all extracts of *K. anthotheca* stem bark and resin exhibited strong inhibition against the gram-positive *E. faecium.* The most active extract against this strain, which was also the strongest antibacterial result reported in this study, was the methanol-water (mwE088) ($IC_{50}$: 16 µg/mL; MIC: 32 µg/mL). All extracts of this species were also found to inhibit *E. coli* (DSM 1576) in the dose-response experiments, with the methanol-water extract once again also showing the strongest inhibition ($IC_{50}$: 16 µg/mL; MIC: 256 µg/mL). This extract also inhibited the growth of *S. maltophilia* ($IC_{50}$: 64 µg/mL) in the library screen. Only weak inhibition was found against the food pathogen *S. enterica* (*n*-hexane extract, $IC_{50}$: 256 µg/mL).

*K. anthotheca* exhibited potent anti-inflammatory activity. Of all extracts tested, the methanol-water *K. anthotheca* extract (mwE088) displayed the strongest COX-2 inhibition activity ($IC_{50}$: 0.55 µg/mL). Past phytochemical studies on methanol and ethanol-water stem bark extracts from the related species, *K. senegalensis*, revealed many phenolic compounds, including flavonoids and tannins (Dougnon et al., 2021; Salih et al., 2014; Ugoh, et al., 2014). Flavonoids act on the inflammatory response, and may block molecules like COXs, cytokines, nuclear factor-κB and

matrix metalloproteinases (Fawole et al., 2009). Some tannins have also been proven to have strong free radical-scavenging and antioxidant activities (Diouf et al., 2009). These compounds are antagonists of particular hormone receptors or inhibitors of particular enzymes such as COX enzymes (Fawole et al., 2009). If *Khaya* species are phytochemically similar, this could help explain *K. anthotheca*'s strong COX-2 inhibitory activity.

Across Africa, *K. anthotheca* is traditionally used for ailments including allergies, fever, headaches, jaundice, bacterial infections, and as a disinfectant for bleeding wounds (Akoègninou et al., 2006; Issifou et al., 2018; Orwa et al., 2009). Our behavioral observations suggest this species is also a common resource for Sonso chimpanzees, with a total of 65 feeding events recorded throughout the first field season. Of these events, several involved individuals with altered health states (see **Table 1: *K. anthotheca*)**. On at least three independent occasions, *K. anthotheca* bark and resin was consumed by wounded individuals. Two adult females, on different days, tested positive on urinalysis tests for leukocytes within hours of ingesting *K. anthotheca*, suggesting the presence of infection. One of these individuals was also experiencing severe diarrhea the day prior. A juvenile female with a persistent cough was also observed consuming *K. anthotheca* bark. On several occasions individuals with high or diverse parasite loads were observed targeting this resource while shedding tapeworm proglottids (*Bertiella* sp.). An elderly female was also observed eating bark and resin a few hours prior to leaf-swallowing, a well-established self-medicative behavior known to rid the gut of endoparasites (Huffman & Caton, 2001; Huffman, 1997). The frequency of *K. anthotheca* ingestion in the Sonso diet during this period, suggests that individuals have consistent exposure to the antibacterial and anti-inflammatory compounds present in this species. Whether this is a case of passive prevention through intake of a medicinal food, or therapeutic self-medication for a common and wide-spread condition will need further investigation. If used therapeutically, our results suggest this species could be used for treating wounds, treating bacterial infections, and/or reducing internal parasite loads.

***Syzygium guineense***

*S. guineense* bark and leaves have both previously been found to exhibit a range of pharmacological activity, reviewed by Uddin et al. (2022). The antioxidant, analgesic, and anti-inflammatory activities of this plant have been attributed to flavonoids, tannins, saponins,

carbohydrates, alkaloids, and cardiac glycosides in the extracts (Dharani, 2016; Ior, 2012; Oladosu et al., 2017; Ssegawa & Kasenene, 2007; Uddin et al., 2022). In our assays, *S. guineense* bark exhibited high antibacterial growth inhibition effects *in vitro*. The methanol-water bark extract (mwE098a) showed some level of inhibition against all bacteria tested in the dose-response assays, except for *E. faecium* and *S. enterica*. This was also the only extract, out of all tested, to inhibit growth of *P. aeruginosa* ($IC_{50}$: 64 µg/mL; MIC: >256 µg/mL) a pathogen known to cause infections in the blood, lungs, and other body parts after surgeries (CDC report, 2019), and was one of two extracts to reach a MIC value against *S. maltophilia* ($IC_{50}$: 32µg/mL; MIC: 256 µg/mL). The other extract to reach a MIC value was the ethyl acetate *S. guineense* bark extract (eE098a; $IC_{50}$: 64 µg/mL; MIC: 256 µg/mL). All bark and leaf extracts showed strong inhibition against *E. coli* (DSM 1576) in the dose-response assays, with the strongest results coming from the methanol-water extracts (mwE098a and mwE098b). All bark and leaf extracts of this species, except for the *n*-hexane bark extract (hE098a), inhibited *E. cloacae,* and were the only extracts in the study to do so. *E. cloacae,* while part of normal intestinal flora, can cause UTI's and respiratory infections in humans (CDC report, 2019). *S. guineense* extracts were also the only extracts to inhibit *A. baumannii* at a concentration <256 µg/mL, with the methanol-water bark extract showing the strongest inhibition. *A. baumannii* can cause infections in wounds, blood, urinary tracts, and lungs (CDC report, 2019). The efficacy of methanolic extracts from this species suggests that the active compounds are polar molecules. In the anti-inflammatory COX-2 inhibition dose-response assays, only the *n*-hexane bark extract displayed strong inhibitory effects ($IC_{50}$: 2.42 µg/mL), while the other extracts failed to exhibit significant activity during the pre-screening or ≥ 50 % inhibition at 10 µg/mL. The COX-2 inhibition assays showed no inflammatory inhibition amongst leaf extracts at tested concentrations.

*S. guineense* can be found throughout Sub-Saharan Africa and is a common traditional medicine, for malaria (Oladosu et al., 2017). The bark is also used for stomachaches, diarrhea, internal parasites, and infertility (Kokwaro, 2009; Uddin et al., 2022). Bark stripping of *S. guineense* is rare in Budongo, with no direct observations in either community throughout the study period, and only six total cases between 2008-2021 documented in the site's long-term data. No observations of leaf ingestion of this species have ever been reported. The infrequent ingestion of *S. guineense* bark implies a more targeted use, making it unlikely to be a medicinal food. Instead, our

pharmacological findings make this resource a strong candidate as a putative, therapeutic self-medicative resource. Unfortunately, as there is currently no health data associated with individuals who have recently consumed *S. guineense* bark, we do not yet know which properties chimpanzees may be targeting. However, based on pharmacological results, we recommend further investigation into this species as a curative agent for respiratory-related infections.

**Assessment of Putative Self-Medicative Behaviors**

We synthesized pharmacological and behavioral evidence to specifically assess therapeutic use of species associated with bark stripping, dead wood eating, and pith stripping behaviors. A simplified summary of the antibacterial and anti-inflammatory results for each species is reported in **SM Table 6**. Overall, stem bark and dead wood samples were notable for their activity. Bark samples from every species showed >40% antibacterial inhibition against at least one bacterial strain. This activity was also true of the dead wood samples. When plant parts of the same species were tested (*S. guineense* and *F. exasperata*), barks generally exhibited more potent antibacterial and COX-2 inhibition activity than the leaves, likely to do with the higher concentration of plant secondary metabolites in bark. Our findings offer strong support that bark stripping and dead wood eating of *certain* species could constitute novel self-medicative behaviors in wild chimpanzees. We also encourage more investigation into the bioactivity of non-bitter pith stripping, as the pith of *A. polystachius* showed strong antibacterial activity against *E. faecium* (hE099; $IC_{50}$: 32 µg/mL; MIC: 128 µg/mL), and the piths of both *A. polystachius* and *M. leucantha* demonstrated significant anti-inflammatory properties at low concentrations. Future primatological research should prioritize the establishment of multi-disciplinary long-term projects that look systematically at health states of individuals who engage in bark stripping, dead wood eating, and pith-stripping behaviors. We also encourage further pharmacological testing on other species used for these behaviors in Budongo and across primate field sites.

**Drug Discovery**

Multidisciplinary studies on this topic have potential to lead to the discovery of new medicines which may benefit our own species (Clayton & Wolfe, 1993; Cowen, 1990; Huffman et al., 1998; Rodriguez & Wrangham, 1993). Historically, PSMs have played a major role in the development

of modern human medicine, and even today, a large portion of medicines are derived either directly or indirectly from plants and other natural materials (Balandrin et al., 1985; Caldecott, 1987; McKenna, 1995; Newman & Cragg, 2020; Schultz & Garbe, 2023). Antimicrobial resistance is rising to dangerously high levels according to the World Health Organization (2021) requiring the rapid creation of new antibacterial treatments. Infections caused by multi-drug resistant bacteria kill hundreds of thousands of people annually. Our findings of strong antibacterial growth inhibition across numerous species growing in Budongo have promising implications for our ability to discover novel compounds in existing forest habitats. Further laboratory work is now needed to isolate and elucidate the plant's specific bioactive compounds and determine pharmacological selectivity and toxicity, while also taking potential synergistic effects into account. Extracts should also be tested against additional bacteria and for anti-virulence effects, e.g., inhibition and disruption of biofilm formation, quorum sensing and toxin production, pursuing development of new therapeutic strategies that apply less evolutionary pressure, likely resulting in emergence of less antibiotic resistances in the future.

Simultaneously, we are currently faced with a pressing need for more effective treatments to combat symptoms of acute inflammation and mediate long-term consequences of chronic inflammatory diseases (Dinarello, 2010). The prostaglandin-producing cyclooxygenase-2 (COX-2) mediates and regulates pain, fever, wound inflammation, and many other medical disorders, as it plays a crucial role in the host organism's defense against pathogens and injury. COX-2 inhibition has the same mechanism of action as non-steroidal anti-inflammatory drugs (NSAIDs). While inflammation is a normal part of the body's defense against injury or infection, it can be damaging when occurring in healthy tissues or over a protracted period. Chronic inflammation can lead to cardiovascular diseases (CVD) and cancer, the two leading global causes of death (Roser, 2018). Past studies have shown that the $IC_{50}$ values of Aspirin and ibuprofen (pure compounds and common NSAIDs) are 210 µg/mL and 46 µg/mL respectively for COX-2, and 5 µg/mL and 1 µg/mL respectively for COX-1 (Mitchell et al., 1993; Mitchell & Warner, 1999). The *in vitro* COX-2/COX-1 selectivity ratio for Aspirin and ibuprofen is 42 and 46 respectively. Surprisingly, the 17 most active extracts in our COX-2 assays display lower $IC_{50}$ values than these popular NSAIDs, meaning our extracts have more potent inhibitory effects on the inhibition of COX-2 than the most common anti-fever and anti-pain drugs on the market. In future studies, COX-1

inhibition activity of these 17 extracts should be determined to calculate COX-2/COX-1 selectivity ratios. Doing so will allow for preliminary assessment of potential side effects, selectivity, and efficacy before future *in vivo* experiments can commence.

### Future Directions

Future research on this topic would benefit from the inclusion of control samples (plants or plant parts not consumed by chimpanzees); however, in this study, assay costs were a prohibiting factor. Additional nutritional and mineral content testing are also needed from species tested in this study to better understand incentives for ingestion. However, bioactivity and nutritional/mineral content are by no means mutually exclusive. It is, therefore, highly likely that these resources provide multiple benefits to consumers.

Future studies should also consider ecological variables. For example, different individual plants of the same species should be tested across habitat types to determine whether bioactivity varies based on location, age, life history, or time of harvest. Situating samples in their ecological context will provide a better understanding of whether chimpanzees select resources based on species alone, or other more nuanced criteria. Lastly, climatic studies in combination with pharmacological testing should examine how climate change may impact bioactivity of these plants, as shifting weather patterns have already been shown to alter nutritional content (Rothman et al., 2015). This information will be critical for the establishment of protected habitats that can sustain healthy, wild, primate populations.

## Conclusion:

As we learn more about the pharmacological properties of plants ingested by wild chimpanzees, we can expand our understanding of their health maintenance strategies. Our results provide pharmacological evidence, from *in vitro* assays of plant parts consumed by wild chimpanzees collected *in situ*, for the presence of potent bioactive secondary plant metabolites in Budongo chimpanzee diets. Whether these resources are consumed intentionally as a form of therapeutic self-medication or passively as medicinal foods, must be assessed on a case-by-case basis, taking behavioral observations into account.

For zoopharmacognosy to progress, we encourage continued multidisciplinary collaboration between primatologists, ethnopharmacologists, parasitologists, ecologists, and botanists (Huffman, 1997). Beyond generally improving our understanding of chimpanzee health maintenance, multidisciplinary studies will benefit our own species, potentially leading to the discovery of novel human medicines to combat the looming problem of growing drug-resistance. For this to happen it is imperative that we urgently prioritize the preservation of our wild forest pharmacies as part of the world's natural and cultural heritage, as well as our primate cousins who inhabit them.

# Declarations:


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**Conflict of interest**

The authors declare that they have no conflicts of interest.

**Ethical Note**

Behavioral data used in this study was collected by EF with the approval by the Uganda Wildlife Authority (Uganda Wildlife Authority permit number: COD/96/05), the Uganda National Council for Science and Technology (permit number: NS257ES). Export of samples for pharmacological testing were conducted under UNCST permit number: NS104ES. Behavioral data collection adhered to International Primatological Society's Code of Best Practice for Field Primatology (Riley et al., 2014). No exported samples were listed under CITES. Plant samples were exported in collaboration with Makerere University (permit number: UQIS00005033/93/PC, issued by the Ugandan government) and transported to Neubrandenburg University of Applied Sciences in accordance with the Nagoya Protocol. The authors report no conflict of interest.

**Data Accessibility Statement**

Data can be accessed in the **Supplementary Materials.** Further information is available upon reasonable request from the corresponding author.

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# 6 Fallback Food Hypothesis Fails to Explain the Value of Bark in the Diet of Chimpanzees of the Budongo Forest

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## Abstract:

The ingestion of bark has been observed across the animal kingdom and is well documented in wild chimpanzees. The most popular hypothesized adaptive function for this behavior is the fallback food hypothesis which asserts that chimpanzees consume cambium during periods of food scarcity when preferred foods are unavailable. However, alternative explanations exist, notably the stressed-tree hypothesis, the essential nutrient and mineral hypothesis, and the self-medication hypotheses. Here, we tested whether the fallback food hypothesis can explain bark ingestion at Budongo by assessing four predictions, using long-term data from two chimpanzee communities in Uganda, combined with 8-months of direct observations. Contradicting our predictions, we found that bark-ingestion efforts varied seasonally between tree species, with some species stripped most frequently when staple food sources were abundant. We also demonstrate the presence of species-specific quantities of bark targeted per ingestion event and report anecdotal evidence of chimpanzees prioritizing bark over high-value foods in certain contexts. Lastly, we found that chimpanzees often ingest the bark of certain species outside their core area, expending energy and risking dangerous encounters with neighboring groups to obtain this resource. Overall, we argue that the fallback food hypothesis cannot explain bark ingestion across all tree species and instead present evidence which supports several of the alternative hypotheses, challenging the widely accepted function of this dietary item. We conclude that, moving forward, the adaptive function of bark ingestion should be assessed on a tree species level and discuss the relevance of these findings for evolutionary theories related to this behavior, including its origins in our own species.

**Key Words:** *Pan troglodytes*, Diet, Bark Ingestion, Cambium, Self-Medication, Fallback Food

## Abstract:

The ingestion of bark has been observed across the animal kingdom and is well documented in wild chimpanzees. The most popular hypothesized adaptive function for this behavior is the fallback food hypothesis which asserts that chimpanzees consume cambium during periods of food scarcity when preferred foods are unavailable. However, alternative explanations exist, notably the stressed-tree hypothesis, the essential nutrient and mineral hypothesis, and the self-medication hypotheses. Here, we tested whether the fallback food hypothesis can explain bark ingestion at Budongo by assessing four predictions, using long-term data from two chimpanzee communities in Uganda, combined with 8-months of direct observations. Contradicting our predictions, we found that bark-ingestion efforts varied seasonally between tree species, with some species stripped most frequently when staple food sources were abundant. We also demonstrate the presence of species-specific quantities of bark targeted per ingestion event and report anecdotal evidence of chimpanzees prioritizing bark over high-value foods in certain contexts. Lastly, we found that chimpanzees often ingest the bark of certain species outside their core area, expending energy and risking dangerous encounters with neighboring groups to obtain this resource. Overall, we argue that the fallback food hypothesis cannot explain bark ingestion across all tree species and instead present evidence which supports several of the alternative hypotheses, challenging the widely accepted function of this dietary item. We conclude that, moving forward, the adaptive function of bark ingestion should be assessed on a tree species level and discuss the relevance of these findings for evolutionary theories related to this behavior, including its origins in our own species.

**Key Words:** *Pan troglodytes*, Diet, Bark Ingestion, Cambium, Self-Medication, Fallback Food

## Introduction:

Bark stripping, the primate feeding behavior associated with the exploitation of bark for subsequent ingestion or mastication, is characterized by the removal of bark, cambium, and phloem through stripping or sustainable peeling practices (Lapuente et al., 2020). In this study, we operationally add to this definition, including cases of primates removing bark by use of any technique, including biting, which has yet to be formally described in chimpanzees. Tree bark and cambium, mostly composed of cellulose, lignin and other compounds that are indigestible by humans, are considered to have low nutritional value (Huffman, 1997; Krief et al., 2006) and in some cases, deleterious costs. Due to high levels of plant secondary metabolites (PSMs) concentrated in the bark, some species are highly toxic and can be fatal if over-consumed (Forbey et al., 2009).

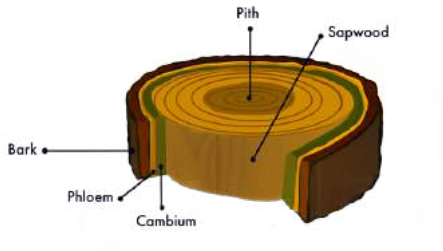


***Figure 1:*** *Cross section of a tree trunk (Image by E. Freymann)*

### Adaptive Function Hypotheses

Several hypotheses for the adaptive function of ingesting bark have been proposed across a variety of non-human species. For primates, the most popularly accepted of these hypotheses is the ***Fallback Food Hypothesis***, which argues that bark is a generalized subsistence food item sought after during periods of food shortage. This hypothesis classifies bark as a fallback food (e.g., Jiang et al., 2005; Nishida, 1976). According to Marshall et al. (2009), fallback foods are resources of relatively low priority that are seasonally consumed when preferred resources are unavailable. This hypothesis is largely supported by primate studies which show a seasonal correspondence between periods of increased bark exploitation and periods of food scarcity. Bark ingestion has been proposed as a fallback food behavior in several ape species including chimpanzees (*Pan troglodytes*) (Nishida, 1976), Gorillas (*Gorilla* spp.), (Rogers et al., 1994) and (Campbell-Smith et al., 2011), as well as non-ape primates including Japanese macaques (*Macaca fuscata*) (Hanya

et al., 2006). It has also been proposed in numerous non-primates including, but limited to, Sika deer (*Cervus nippon*) (Jiang et al., 2005).

A less popularly accepted, alternative hypothesis is the ***Stressed-Tree Hypothesis***, reviewed by White (2019), which posits that the bark of trees which undergo ecological stress during development caused by poor soil quality, exposure to excess sun, fire, drought, or nitrogen fertilizers are selected due to the high levels of nutrients and amino acids present in their phloem (Brockley & Elmes, 1987; Ciani et al., 2001; Mori et al., 2016; Nichols et al., 2016; Pulliainen & Tunkkari, 1987; Rousi et al., 1987). This hypothesis is based on non-primate studies showing that stressed trees have higher levels of stress-associated proteins (Eberhardt, 2000; Faber, 1996; Pakkala et al., 2017; Rousi et al., 1987; White, 1984). Evidence includes reports of Eurasian three-toed woodpeckers (*Picoides tridactylus*) and bark beetles feeding on stressed trees while avoiding non-stressed neighbors (Månsson & Jarnemo, 2013; Pakkala et al., 2017; White, 2015, 2019) and of bark exploitation by animals during protein-scarce periods (Andreev, 1988; Beeson, 1987; White, 2019).

Another alternative hypothesis is the ***Essential Nutritional or Mineral Need Hypothesis****,* which attributes bark ingestion to the targeted acquisition of specific nutrients or minerals which are otherwise environmentally scarce (Au et al., 2017; Ciani et al., 2001; Nichols et al., 2016). This hypothesis, which is non-mutually exclusive with the *Stressed-Tree Hypothesis,* has been proposed for numerous primate species including barbary macaques (*Macaca sylvanus*) (Ciani et al., 2001), and chacma baboons (*Papio ursinus)* (Erasmus, 1993) as well as koalas (*Phascolarctos cinereus*) (Au et al., 2017) and grey squirrels (*Sciurus carolinensis*) (Nichols et al., 2016). This hypothesis has also been proposed as the adaptive function of several related feeding behaviors like dead wood eating (Reynolds et al., 2009) and pith chewing (Krief et al., 2006). Evidence comes primarily from studies which identified high levels of minerals and nutrients such as calcium, sodium, zinc, and water in tree species whose bark is ingested (Au et al., 2017; Ciani et al., 2001; Erasmus, 1993; Levia & Herwitz, 2005; Nichols et al., 2016).

Shortly after the discovery of chimpanzee self-medication, another alternative hypothesis for the function of bark ingestion was proposed: bark ingestion as a self-medicative behavior (Huffman,

1997). The **Self-Medication Hypothesis** proposes that barks of certain species may have pharmacological properties that provide consumers with therapeutic benefits. This hypothesis is primarily based on the low nutritional value of bark relative to other feeding items (Huffman 1997; Krief et al., 2006), the antibacterial and anthelminthic properties found in many barks ingested by chimpanzees (e.g. Krief et al., 2003), and behavioral observations of sick primates stripping bark (Ghai et al., 2015; Krief et al., 2006; Ndagurwa, 2013; Pebsworth et al., 2006). Ndagurwa (2013) provided evidence for this hypothesis by linking terpene rich pine bark consumption with seasonal episodes of coughing and other symptoms of throat infection in chacma baboons (*Papio hamadryas ursinus*). Ghai et al. (2015) further found that wild red colobus monkeys (*Procolobus rufomitratus tephrosceles*) in Kibale consumed significantly more bark (from the genus *Albizia)*, when shedding whipworm eggs (*Trichuris* sp.).

**The Adaptive Function of Bark Ingestion Amongst Wild Chimpanzees**

While chimpanzee feeding behaviors are known to be complex and flexible, few studies have investigated the role of specific food types in the overall diet. While some common food types have obvious caloric and nutritional value, bark has less evident dietary benefits. Nishida (1976), published a report on bark ingestion at Mahale, which remains one of the few empirical assessments of this behavior's adaptive function in chimpanzees. In this report, Nishida argues that bark is a fallback food, citing evidence that seasonal exploitation corresponded with periods of food scarcity at this site.

In Budongo, chimpanzees have long been known to remove bark and ingest it (Reynolds et al., 2005; 2009). Reynolds et al. (2009), in their study exploring the increasing frequency of *Raphia farinifera* dead wood ingestion in the Sonso community, assessed the mineral composition of barks from several species collected at the site. They conclude that bark and dead wood of *C. patens* may be targeted for sodium, however, they do not address possible reasons for exploitation of any other species. Since these reports, few studies have challenged Nishida's (1976) claim that bark ingestion is a fallback food behavior, and the dietary function of bark remains relatively unexplored in site-specific contexts (though see Pebsworth et al., 2006; Reynolds et al., 2009; Lapuente et al., 2020).

### Bark Ingestion Seasonality

In chimpanzees, seasonality in bark ingestion has been reported across field sites (Nishida, 1976; Nishida & Uehara, 1983; Pruetz, 2006; Van Lawick-Goodall, 1968). In Mahale, bark ingestion events increased from January to mid-March (mid-rainy season) and in mid-May to August (start of dry season), both periods in which staple foods were less abundant (Nishida, 1976). In Comoé National Park, Lapuente et al. (2020) found that bark ingestion of *Ceiba pentandra* occurred only during the rainy season (with highest rates in October-December) when fruits were scarce.

To establish the adaptive function of bark ingestion, we first need to determine whether ecological variables drive seasonality of the behavior and consider potential variation across chimpanzee communities. Budongo presents an appropriate case study as, unlike other sites, these chimpanzees appear less affected by periods of food shortage (Newton-Fisher, 1999; Plumptre & Reynolds, 1994; Reynolds, 1992). If bark ingestion across species is a general fallback food strategy, we would expect bark feeding to increase when preferred foods are scarce and decrease when preferred foods are abundant.

### Technical Variation in Bark Extraction/Removal

Lapuente et al. (2020) recorded bark removal behaviors in Comoé National Park, comparing chimpanzee techniques to those of sympatric baboons. The authors found that bark removal techniques varied between primate species; chimpanzees used their fingernails or front teeth to scrape cambium of *C. pentandra* longitudinally, while baboons either bit (rather than stripped) the bark of this species or scraped the cambium transversely with their teeth. Technical variation between these primate species not only looks different in practice, but also leaves behind distinctive tree damage. Lapuente et al. (2020), however, only address bark removal of a single tree species, and did not report any technical variation between tree species in either primate species.

While never systematically evaluated, intra-species bark removal technique variation has been documented across wild chimpanzee field sites. While Gombe chimpanzees have been observed cutting bark with their teeth, pulling large strips of bark using their hands, and scraping the bark with their teeth horizontally (Goodall 1968), this description does not match the techniques

employed by Comoé chimpanzees (Lapuente et al., 2020). It remains unclear whether variation in bark removal technique is related to morphological tree characteristics, cultural variation, individual preferences, dosage, amount required to reach satiety, or other properties in the bark that this behavior targets.

As bark ingestion provides low nutritional value (e.g., Krief et al., 2006) and is both energetically costly and time consuming compared to other feeding behaviors (e.g., eating figs), for the benefits to outweigh the costs, we expect that relatively large amounts of cambium/bark should be consumed. If chimpanzees indiscriminately ingest the bark of all species to supplement nutrition or energy budgets in times of food scarcity, there should be limited variation in cambium quantities targeted across tree species, unless nutritional value greatly varies between species. While nutritional value for most species whose bark is ingested in Budongo remains unreported (though see Reynolds et al., 2009), we would expect that if great nutritional variation existed, low value barks would be eschewed in favor of high value barks, if species availability was equivalent.

**Bark Ingestion Site Spatial Distribution**

Understanding the distribution of bark ingestion sites across a chimpanzee community's home range is critical for evaluating this behavior's adaptative function. Lapuente et al. (2020) assessed the spatial distribution of bark ingesting events at Comoé National Park, and identified hotspots. Trees were revisited yearly by members of the same community and exploited 'sustainably,' which allowed for bark regeneration. However, spatial analyses in this study measured the distribution of a single species (*C. pentandra*) and were not interpreted in relation to the broader ecological context. If bark is a fallback food and trees targeted for use are available in the core area of a community's home range, individuals should not have to travel far distances or into areas of high-risk to exploit bark, as this resource offers minimal nutritional returns.

**Evolutionary Implications of Bark Ingestion's Adaptive Function**

Many modern human communities around the world also consume stem bark and cambium for both subsistence (e.g., Niklasson et al., 1994; Östlund et al., 2009) and medicine (e.g., Gottesfeld, 1992; Kokwaro, 1976). This reliance on bark is not new, as evidence in the fossil record suggests

that various bark removal behaviors may have been present in our early hominin ancestors, including *Australopithecus sediba* (Henry et al., 2012) and *Homo neanderthalensis* (Sandgathe & Hayden, 2003). *But why did our early ancestors eat bark?*

If bark was used as a fallback food, this may have implications for our understanding of our early ancestors' subsistence strategies and ability to adapt to changing environments. Laden and Wrangham (2005) argue that the divergence in morphological characteristics between our species and African great apes may even be attributed to changes in dietary reliance on fallback foods. They contend that the transition from a chimp-like diet of herbaceous leaves and piths to a reliance on fallback underground storage units led to the emergence of large jaw muscles and robust dental adaptations in *Australopithecus* and *Paranthropus* (Wood & Strait, 2004). These theories highlight the potential impact of resource availability on hominin evolution.

If, instead, our ancestors used bark as a curative agent or as a source of scarce minerals, as it is commonly used today in traditional human healing practices, this would have substantial implications for the evolution of modern human medicine. Investigating the adaptive function of bark ingestion in our own species as well as in our primate relatives, therefore, may shed further light on bark's broader influence on human evolution.

**Assessing Predictions of the Fallback Food Hypothesis in Wild Chimpanzees**

As consumption of bark is relatively rare compared to other food items, we employed 14 years of long-term site data from two neighboring chimpanzee communities in Budongo (Sonso and Waibira), as well as behavioral and spatial data from two four-month field seasons, one in each community. We also conducted forest transects in both community core areas to investigate availability of both targeted and non-targeted tree species. Below, we assess whether bark ingestion meets the following predictions of the fallback food hypothesis:

***Prediction 1****: Bark ingestion increases during periods of food scarcity and decreases during periods of ripe fruit availability.*
***Prediction 2:*** *Quantity of bark ingested is relatively consistent across tree species.*
***Prediction 3****: Staple fruits are prioritized over bark when both are available.*

***Prediction 4:*** *Individuals conserve energy and minimize risk by exploiting trees for bark within or near their core areas, if trees are distributed throughout the home range.*

## Methods:

### Study Site and Subjects

The Budongo Central Forest Reserve (CFR), located in the Masindi District of Western Uganda, covers a 793 $km^2$ area, 482 $km^2$ of which is made up of semi-deciduous forest (Eggeling, 1947). Historically Budongo CFR has two wet seasons, usually occurring between March-May and September-November (Reynolds et al., 2005); however, over the past several years rainfall patterns have become more variable. Rainfall at Budongo ranges between 1200 to 2200 mm (average 1600 mm), and temperatures generally remain consistent throughout the year, with daytime highs ranging from 19°C to 32°C.

This study was conducted with the neighboring Sonso and Waibira wild Eastern chimpanzee (*Pan troglodytes schweinfurthii*) communities, habituated for research at the Budongo Conservation Field Station (BCFS). Sonso, which included ~68 individuals at the time of the current study, has been continuously studied since 1990 (Reynolds et al., 2005). Waibira, a larger group of at least 105 current individuals, was more recently habituated, with consistent data collection beginning in 2011. As of 2022, the Sonso maximum home range was 5.33 $km^2$, and the Waibira maximum home range was 10.28 $km^2$ (Badihi et al., 2022). In this paper we define 'home range' as the specific geographic region that a chimpanzee group uses to meet its collective needs over a defined time span (Martínez-Íñigo et al., 2021). This definition encompasses peripheral regions the group may visit less frequently. We use 'core area' to mean areas within the home range that are heavily used or visited by the community, and excludes areas that are rarely visited or which may overlap with neighboring community home ranges (Martínez-Íñigo et al., 2021).

A trail system is cut across the main study area forming a labelled grid for each community which covers the majority of both home ranges (**SM Figure 1**). While parts of the home range may, in some cases, extend outside of the grid-system, all core areas for both communities are located within respective grid-systems (**Figures 10** and **12**). Travel outside of the grid is relatively

infrequent. Sonso trails are cut 100m apart forming 100m x 100m blocks. In the southern and eastern regions of the grid, several blocks are >100x100m for geographic or historic reasons (see **SM Figure 3**). The Waibira grid system is less standardized, with some 200 x 500m, 200 x 200m, and 100 x 200m blocks, as trails were added over time. For this study, we standardized Waibira block sizes for easier comparison (see **Supplemental Materials**). Trails in both communities are up to 0.5m wide. The Sonso diet (Tweheyo et al., 2004) and flora (Synnott, 1985) have been intensively studied, whereas systematic investigation into the Waibira diet and surrounding flora is still ongoing.

## Data Collection

### *Long-Term Site Data*

Long-term site data were used in this study, including **a**) behavioral focal follow data including all bark ingestion events recorded in Sonso between 2008-2021 and in Waibira between 2015-2021 **b**) party composition data from these same periods **c**) site-specific annual rainfall and temperature data and **d**) six years of fruit availability index (FAI) data from fruiting trees known to be of importance to Budongo chimpanzees (2013-2016). For information on how FAI was calculated, see **Supplemental Materials**.

### *Behavioral Data Collection During the Study Period*

For this study, we conducted two four-month field seasons between mid-June and mid-October (2021 and 2022). The first of these collection periods was with the Sonso community, the second with the Waibira community. Identical methods were used throughout the study period to ensure a comparative dataset. During both field seasons, pseudo-random focal animal selection was used (Hobaiter et al., 2017), where parties were initially opportunistically searched for in the morning according to a random schedule, and focal animals were then selected according to prioritization criteria (listed in the **Supplementary Materials**). Behavioural and video data were collected on focal individuals. Direct observations of bark ingestion recorded by other researchers at the field station during the field study were also included in this study.

### *Indirect Evidence*

To investigate bark ingestion techniques, we took *ad libitum* photographs and tree scans of trees with characteristic traces of the behavior, encountered during focal follows. Scans were taken on an iPad Pro using a 3D Scanner (Laan Labs). Only bark removal sites that could be accessed from the ground were measured and considered for this analysis.

### *Forest Transects*

To assess tree species availability in each community home range, we conducted eight line-transects were walked between September 1st and October 10th, 2022, four in each of the two habituated communities (**SM Figure 2**). Each line-transect was 400m long and was located within the pre-cut trail system. Four transects ran north-south and four ran east-west (following, Hedges & McGrew, 2012). Transects were selected at random from samples stratified by cardinal direction in each home range. Trees were measured on both sides of the trail within 5m of the trail center. See **Supplemental Materials** for details.

## Botanical Information

All tree species mentioned in this study were identified by trained BCFS field staff. The most current scientific names for each species were confirmed for this paper as of May 2023, according to Kew's Plants of the World Online (https://powo.science.kew.org/). For more details on botanical data collection see **Supplementary Materials**.

## Ethical Statement

Data used in this study was collected with approval from the Uganda Wildlife Authority (permit no: COD/96/05), the Uganda National Council for Science and Technology (permit no: NS257ES). The study was observational only and adhered to the guidelines for best practice in field primatology (Riley et al., 2014). All applicable international and national guidelines were followed. The authors report no conflict of interest.

## Analysis:

### Seasonality

FAI was used as a proxy for general food abundance at the site, as ripe fruit is the most relevant high value food in Budongo chimpanzee diets. While other dietary items are consumed throughout the year (e.g., pith, meat, agricultural field cultivars) not captured by this index, ripe fruit abundance in Budongo provides the best approximation of general food abundance and scarcity. We plotted both FAI and direct observations of bark ingestion events in the long-term data by species using R (version 4.0.5, R Core Team, 2019) to compare seasonality of this data. For this comparison, we excluded species with fewer than six associated events, as small sample sizes limited ability to detect seasonal patterns.

### Technical Variation

Technical variation of bark removal techniques was described from *in situ* photographs and 3D scans of stripped trees. When possible, measurements of the area of bark removed were taken in the field or else measured *post hoc* on 3D Scanner. Measurements were taken from the longest strip on each tree and averaged across species (**SM Table 1**). These measurements were used as a metric to compare the amount of bark or cambium consumed across species. We calculated mean diameter at breast height (dbh) in the long-term data across all trees used for bark feeding, when data was available, as well as mean dbh for all stripped trees reported along transects (**SM Table 1**). The healing stage of feeding scars were assessed and quantified using the 1-4 scale established by Lapuente et al. (2020).

### Forest Transects

To calculate relative tree species abundance within each community's home rnage, we divided the number of reported trees of each species by the total number of trees reported within each community's home range.

### Heat Maps

Following Badihi et al. (2022), we used the 'adehabitatHR' package (Calenge, 2020) in R to create heat maps for bark ingestion events reported in the long-term data. Heat maps were made using the 'heatmap.2' function to map events across each community's home range using the site's current grid system. In Sonso, the grid was considered to extend between lines J to 12 (which run north/south) and H to 20 (which run east/west). In Waibira the grid was considered to extend between lines 10 to 40 (which run north/south) and X to 6 (which run east/west). Each block was color-coded by number of events. Separate heat maps were produced for each community, and for individual tree species. When events took place outside of the grid-system (e.g., 'off-grid South') it was not possible to assign them to a specific block. We therefore grouped all off-grid events based on the direction relative to the grid in which they were observed and assigned separate labelled blocks along the map's outer edge for each of these groups. Species-level heat maps were interpreted and compared, taking habitat-type and context into consideration.

To determine core areas, we also created community activity heat maps representing the proportion of scans where chimpanzees were observed. In Sonso we used data between 2008-2021 and in Waibira between 2015-2021. Grids including scan numbers per block can be found in **SM Figures 3** and **4**. For this analysis, we quantitatively define core area blocks as those with scan numbers ≥ (1/3)*Highest Value Block for Each Community, and define "near" a core area as ≤500m from a core area block. Data came from party composition scans taken every 15-minutes by trained field staff as part of the site's long-term data. In total, we analyzed N=113,851 Sonso scans and N=23,237 Waibira scans. Grid parameters were the same as those used for bark ingestion event heat maps. To calculate proportions for larger Sonso blocks, we divided the number of observations per block by the number of 100x100m blocks they encompassed. We excluded scans recorded as 'off-grid', which eliminated n=18,464 Sonso scans and n=588 Waibira scans from analysis. Grids are positioned in a north-up orientation.

## Results:

### Species whose Bark was Ingested in Budongo

In total, bark ingestion from 27 tree species was reported in the long-term data, between 2008-2021 in Sonso (n=24) and 2015-2021 in Waibira (n=18) (**Table 1**). The barks of two additional

species (*Lannea welwitschia* and *Celtis gomphophylla*) were reported as a dietary item in Sonso by Newton-Fisher (1999), but remain unreported in the long-term data and were thus omitted from analysis. Of these species, four are solely targeted for bark in Budongo (*Eucalyptus* sp., *Trichilia* sp., *Albizia glaberrima*, *Dombeya kirkii).* Throughout our eight-month study period, bark ingestion of 16 identified species were directly observed from across N=39 events, either by the authors or other researchers at the site. Four of these species had been reported as used for bark feeding. In total, indirect evidence of bark ingestion was collected from 18 species, three of which were the first records of this behavior in either group. We collected indirect evidence *ad libitum* from N=65 trees during routine behavioral data collection, and from N=27 trees while conducting transects.

**TABLE 1**: All bark ingestion events in long-term data (2008-2021)

| Species | Site Code | Family | Life Form | Sonso Event Total | Waibira Event Total | Present in Home Range | | Other Parts Eaten (Sonso) | Months of bark stripping events |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | S | W | | |
| *Broussonetia papyrifera* | BPY | Moraceae | Tree | *29* | *0* | ✓ | X | L, Fl, F | 9,10 |
| *Cleistopholis patens* | CP | Annonaceae | Tree | *34* | *9* | ✓ | ✓ | Dw, F | 1,2,3,4,5,6,7,8,9,10,11,12 |
| *Eucalyptus* sp. | EUC | Myrtaceae | Tree | *5* | *0* | ✓ | X | | 3,7,10,11 |
| *Cynometra alexandri* | CYA | Fabaceae | Tree | *12* | *72* | ✓ | ✓ | L, Fl, S | 1,4,5,6,7,8,9,10,11,12 |
| *Alstonia boonei* | AB | Apocynaceae | Tree | *17* | *2* | ✓ | ✓ | L | 1,2,3,4,5,6,7,8,10, 11 |
| Various Unidentified Trees | UNK | | Tree | *11* | *41* | | | | 1,2,3,4,5,6,7,8,9,10,11,12 |
| *Ficus variifolia* | FVR | Moraceae | Tree | *9* | *0* | ✓ | ✓ | L, F | 3,5,6,7,8 |
| *Ficus sur* | FSU | Moraceae | Tree | *6* | *0* | ✓ | ✓ | F, L | 4,5,11 |
| *Syzygium guineense* | SZG | Myrtaceae | Tree | *6* | *0* | ✓ | X | F | 4,6,9,10,11 |
| Various Unidentified Climbers | CLI | | Climber | *7* | *13* | | | | 1,2,3,5,7,8,9,10,11,12 |
| *Khaya anthotheca* | KA | Meliaceae | Tree | *5* | *0* | ✓ | ✓ | R | 3,4,9 |
| *Cordia millenii* | COM | Boraginaceae | Tree | *4* | *4* | ✓ | ✓ | F, Fl | 6,9,10,11,12 |
| *Raphia farinifera* | RF | Arecaceae | Tree | *3* | *0* | ✓ | ✓ | Dw | 2,4,12 |
| *Antiaris toxicaria* | ANT | Moraceae | Tree | *3* | *1* | ✓ | ✓ | F, L | 2,7 |
| *Desplatsia dewevrei* | DD | Malvaceae | Tree | *3* | *1* | ✓ | ✓ | F, L | 5,7,11 |
| *Ficus exasperata* | FE | Moraceae | Tree | *4* | *3* | ✓ | ✓ | F, L, R | 7,8 |
| *Lasiodiscus pervillei* | LP | Rhamnaceae | Tree | *2* | *0* | ✓ | ✓ | L, Fl | 3,7 |
| *Ficus saussureana* | FSS | Moraceae | Tree | *1* | *3* | ✓ | ✓ | F | 5,6,10,12 |
| *Trichilia* sp. | TRI | Meliaceae | Tree | *1* | *0* | ✓ | ✓ | | 2 |
| Various Unidentified Terrestrial Herbaceous Plant | THV | | Herb | *1* | *0* | | | | 3 |
| *Celtis zenkeri* | CZE | Cannabaceae | Tree | *1* | *0* | ✓ | ✓ | F, L | 11 |
| *Celtis mildbraedii* | CMI | Cannabaceae | Tree | *1* | *1* | ✓ | ✓ | F, L | 1,8 |
| *Psidium guajava* | PSG | Myrtaceae | Tree | *1* | *0* | ✓ | ✓ | F | 10 |
| *Celtis gomphophylla* | CGP | Cannabaceae | Tree | *0* | *2* | ✓ | ✓ | F, L | 9 |
| *Cola gigantea* | COG | Malvaceae | Tree | *0* | *1* | ✓ | ✓ | F, S, W | 7 |
| *Albizia glaberrima* | AGL | Fabaceae | Tree | *0* | *1* | ✓ | ✓ | | 8 |
| *Ficus natalensis* | FN | Moraceae | Tree | *0* | *5* | ✓ | ✓ | F | 7,8,9,10 |
| *Gambeya albida* | GAL | Sapotaceae | Tree | *0* | *1* | ✓ | ✓ | F | 8 |
| *Dombeya kirkii* | DOK | Malvaceae | Tree | *0* | *44* | X | ✓ | | 1,4,5,6,9,10,11,12 |
| *Ficus mucuso* | FM | Moraceae | Tree | *0* | *1* | ✓ | ✓ | F, L, Fl | 8 |
| **Community Totals** | | | | **168** | **205** | | | | |

✓ = Present in the core area (reported along forest transects or established as present from *ad libitum* observations)
X = Absent or rare in the core area (unreported along forest transects and no *ad libitum* observations of presence)
**F**=Fruits, **L**=Leaves, **Fl**=Flowers, **Dw**=Dead wood, **S**=Seed

**Prediction 1:** *Bark ingestion increases during periods of food scarcity and decreases during periods of ripe fruit availability.*

***Seasonality of Bark Ingestion and Rainfall***

When all bark eating events in the site's long-term data were combined and seasonally evaluated, we identified multiple seasonal peaks (**Figure 2**). Across both communities, bark ingestion peaked in October. Sonso also had an additional peak in February and smaller peaks in May and July. Waibira data indicate a second bark stripping peak in July. When bark stripping seasonality is compared to rainfall seasonality (**Figure 3**), there appears to be some relationship, with the most bark stripping events and greatest average rainfall (mm) also occurring in October. This relationship, however, does not apply as obviously to the other months in which bark ingestion frequencies increase, which have historically been considered drier periods or months of seasonal transition. Across years, we also note an increase in maximum temperature and decrease in temperature minimum in both February and December **(Figure 3)**, whereas the rest of the year stays relatively consistent.

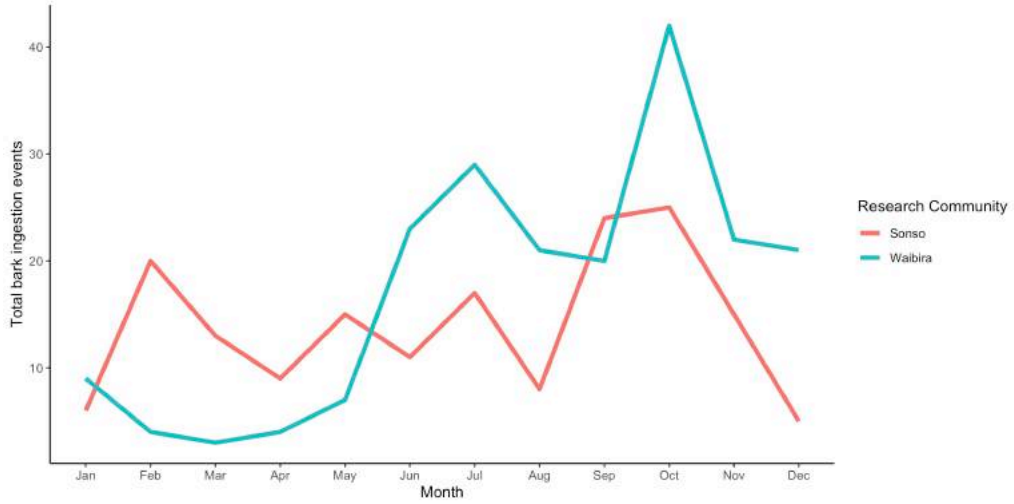


***Figure 2:*** *Seasonality of bark events events across both communities*

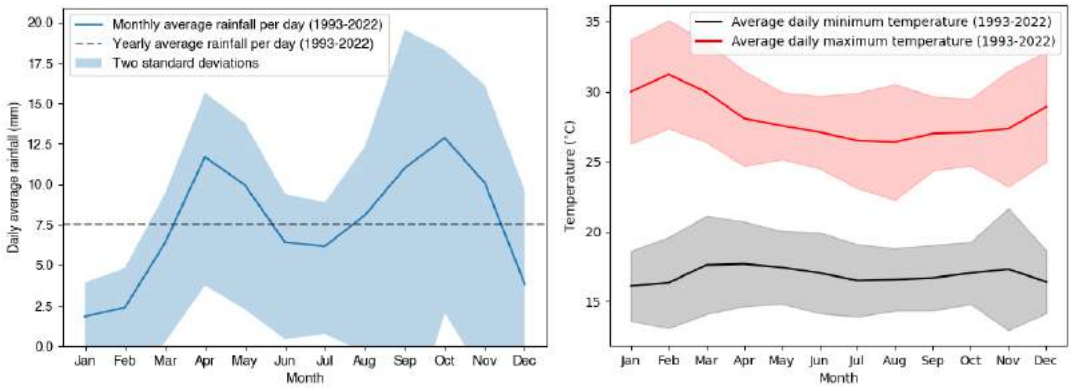


***Figure 3:*** *Average rainfall (mm) and average daily temperature (c) in Budongo between 1993-2022*

We plotted all bark ingestion events from the long-term data across years to see whether bark ingestion seasonality is inter-annually consistent (**Figure 4**). Sonso events are reported between 2008-2021, while Waibira events are only reported between 2015-2021. Our plot showed relative consistency across years with notable event increases in October 2012 and 2018.

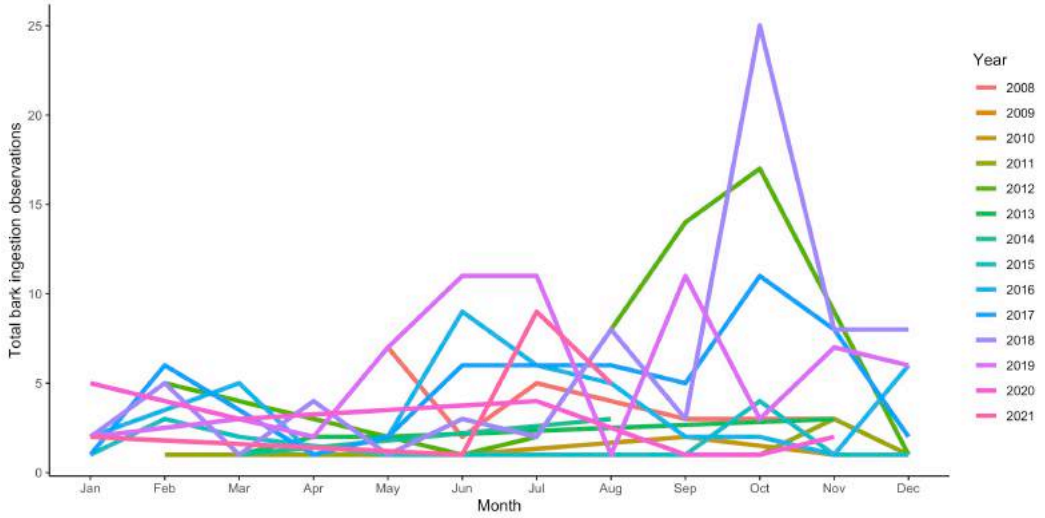


***Figure 4:*** *Seasonality of bark ingestion between 2008-2021*

### *Species Specific Seasonality and Ripe Fruit Abundance*

To determine seasonality of ripe fruit availability, we plotted FAI (see **Figure 5**) at the site from 2013-2019 and found that ripe fruit peaks in February and December, with periods of relative scarcity in July and October. When we compared this to the seasonality of all bark ingestion events, without taking tree species variation into account (**Figure 2**), the increases in October and July (across both communities) correspond closely to periods of relative food scarcity. To determine

whether this pattern extended across all tree species, we assessed bark ingestion seasonality on a tree-species-level. To do this, we created individual plots for each species with ≥6 associated events across both communities (**Figure 5)** and compared species-specific bark ingestion seasonality with FAI seasonality.

Counter to the fallback food hypothesis prediction, only certain species in the repertoire fit this pattern. *C. alexandri,* the species with the most reported observations (n=84) and the most notable seasonality, peaked in October and July, the months when ripe fruit availability is lowest. *C. alexandri* has never been reported to be ingested in February (in either community) when ripe fruits are maximally abundant. *D. kirkii* (n=44) bark ingestion also peaked in October and June when fruits are scarce, and also had no cases reported in February. *B. papyrifera* bark*,* only known to be available in Sonso, has also only been observed being fed on in September and October.

However, other species did not fit the predicted pattern. The barks of *C. patens* and *A. boonei* are both most frequently ingested in February, when fruits are relatively abundant, with 43 and 19 observed events in this month, respectively. Unlike the patterns of bark ingestion for *C. alexandrii* and *D. kirkii*, the frequency of *C. patens* bark ingestion fluctuates throughout the year, with smaller peaks in June, July, September, and November. *A. boonei* is more consistently targeted throughout the year with an additional, smaller peak in October.

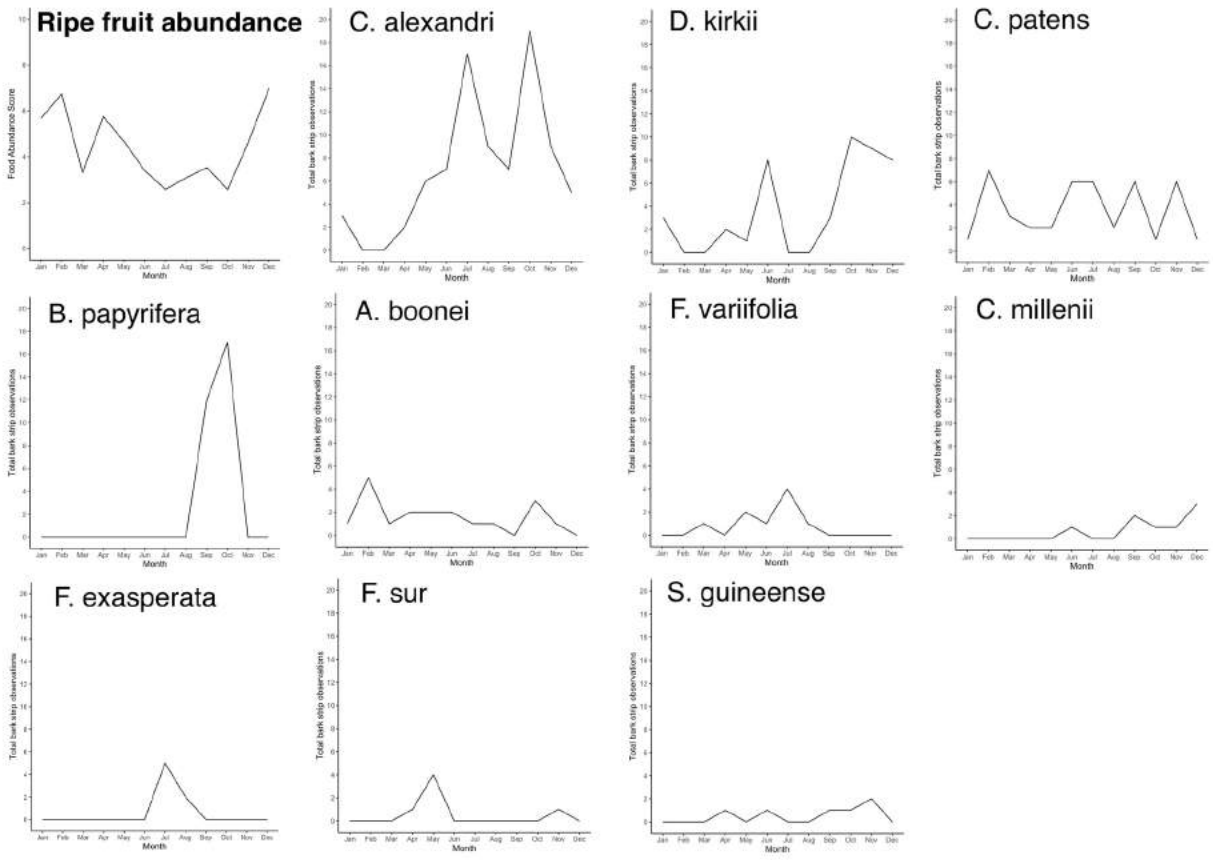


***Figure 5:*** *Comparing ripe fruit availability (2013-2019) to bark ingestion seasonality across tree species (2008-2021)*

**Prediction 2:** *Quantity of bark targeted is relatively consistent across tree species.*

***Bark Removal Techniques***

We recorded multiple bark removal techniques throughout the study period. Based on these observations, we classified bark removal into two broad categories: *terrestrial* and *arboreal*. Chimpanzees removed and ingested bark while on the ground, on a buttress, or within one meter off the forest floor. Removal techniques used during such cases in both communities commonly involved **a.)** peeling bark from the trunk or buttresses in long strips and **b.)** trunk/buttress biting. In most such cases, bark and cambium were then masticated and swallowed. On several occasions, however, we also observed individuals licking the juice from exposed cambium on the trunk after the bark was removed. We never observed bark wadging during terrestrial bark stripping events.

Arboreal bark ingestion occurred off the ground while individuals sat on branches or in the canopy and was associated with **a.)** branch stripping **b.)** trunk stripping or **c.)** bark/resin scraping. Throughout the study period we observed *C. alexandri*, *F. sur, K. anthotheca*, *D. kirkii,* and *S. dawei* associated with arboreal bark exploitation. Arboreal bark processing techniques, following its removal, often included wadging or scraping strips with teeth to remove cambium. As indirect evidence of arboreal bark ingestion is often hard to identify and measure due to poor visibility, we excluded it from the following analyses.

***Variation in Ingested Quantities of Bark***

Though sample sizes were limited (n=46), we found evidence for species-specific variation between quantities consumed during bark ingestion events. We plotted mean length of bark strips or bark bites for each species, with variance shown for one standard deviation in both directions (**Figure 6**). If only one measurement was obtained for a species, it appears as a single point with no variance. Of the 14 species measured, *Ficus mucuso* (μ=146.3cm), *F. exasperata* (μ=79.6cm), and *F. sur* (μ=68.8cm), had the longest average strip lengths. The species with the smallest mean (with calculable variance) was *A. boonei* (μ=15.9cm). Two species from the same genus, *Albizia glaberrima* and *A. zygia,* also had single measurement lengths well under the sample mean (63.2cm). Mean strip/bite lengths of all measured species can be found in **SM Table 2**.

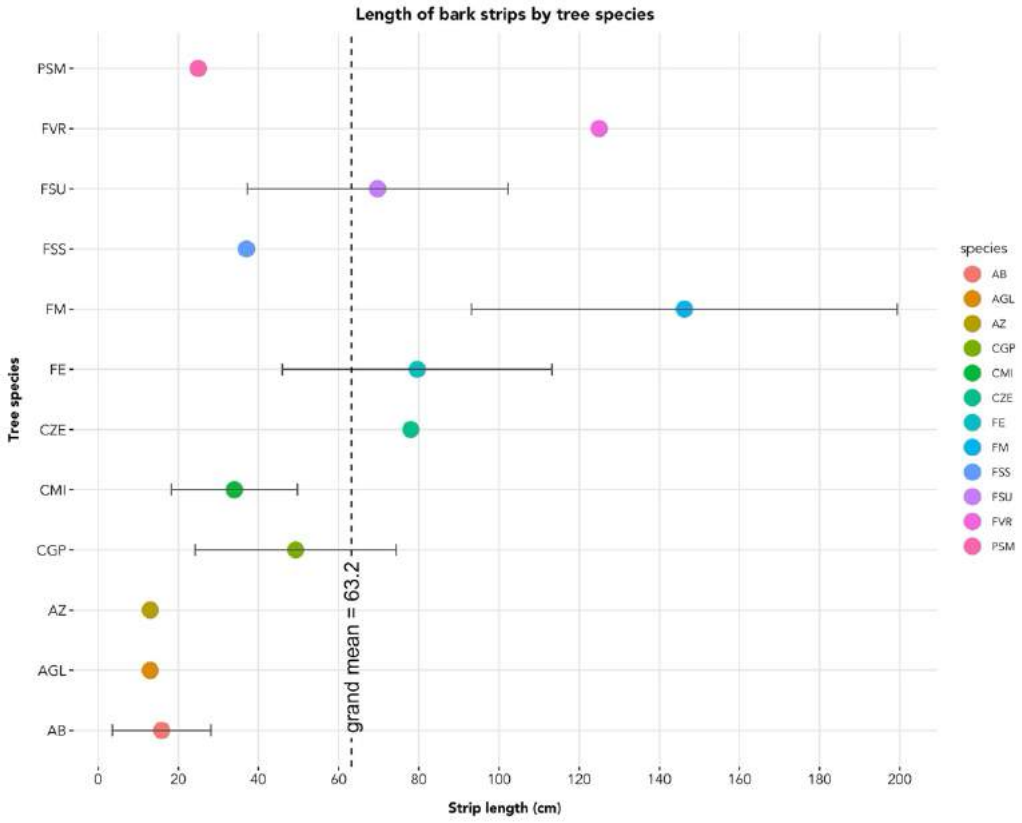


***Figure 6:*** *Mean length of areas stripped of bark across species*

Variation in bark removal techniques across tree species must also be considered. The *Ficus* species with the longest mean strip lengths, were all vertically stripped along their buttresses, while the *A. boonei, A. zygia,* and *A. glaberrima* trees were instead all bitten on either their buttresses or trunks. A comparison between *A. boonei* and *F. mucuso* bark removal wounds are shown in **Figure 7**. To determine whether low targeted bark yields from certain species could be associated with relative tree size, we calculated the mean dbh of all bark stripped *A. boonei*, *A. glaberrima*, and *A. zygia* trees reported throughout the study period (μ=89.2cm, μ=95.5cm, and μ=46.2cm respectively). In all cases, the mean dbh of these species exceeded the mean dbh of all trees targeted for bark ingestion in the long-term data (μ dbh=48.3). In other words, chimpanzees select relatively large trees to strip in small amounts, suggesting that low targeted yields from these species cannot be explained by lack of available bark.


***Figure 7:*** *[Left] 3D scan of A. boonei bark bite (8cm) [Right] 3D scan of F. mucuso bark strip (110cm)*

Using strip length as a metric for quantity of targeted cambium in this analysis may underestimate the total quantity of bark/cambium ingested during *Ficus* bark feeding bouts, as *Ficus* trees usually had multiple bark removal wounds in the same stages of healing. This could imply either multiple strips per event by the same individual or multiple individuals visiting at the same time. *A. boonei*, *A. glaberrima*, and *A. zygia* trees had, on average, fewer removed patches per tree of the same healing stage, although samples sizes are smaller. Variation in amount of bark/cambium consumed per event across species could, therefore, be even greater than reported here.

**Prediction 3:** *Staple fruits are prioritized over bark when both are available.*

***Bark vs. Staple Food Prioritization***

On multiple occasions we anecdotally observed chimpanzees selecting bark over staple fruits, despite availability and accessibility of both. A notable example was recorded between field seasons by DS in March 2022 (**Figure 8** and **SM Video 1**). A Sonso sub-adult male (MB) was observed feeding on *A. boonei* cambium, following a successful blue duiker (*Philantomba monticola*) hunt. MB's juvenile brother (MZ) sat 1m away holding the carcass, eating the meat (Figure 8a). After a short time, ingesting bark, MB travelled out of sight. MZ dropped the carcass and checked his surroundings (Figure 8b), then moved toward the *A. boonei* tree (Figure 8c). After feeding on the cambium for approximately 4 seconds (Figure 8d), MZ descended the tree, retrieved the carcass (Figure 8e), and travelled off in the direction of MB (Figure 8f).

***Figure 8:*** *MZ drops a blue duiker carcass to ingest A. boonei cambium*

In a series of group feeding events during the study period, we also observed groups of Waibira individuals in the canopy of an *F. sur* tree, arboreally stripping and ingesting bark from both the branches and the trunk while others ate ripe fruits in the same tree. Over the course of multiple days individuals returned to this tree and alternated between eating bark and eating ripe fruit. Both high and low-ranking individuals were observed removing and ingesting bark during these events, as well as individuals of both sexes, and all age classes. Several other staple fruit trees were bearing ripe fruit during this period.

On several other occasions, we observed single individuals ingesting bark, despite members of the group simultaneously feeding on staple foods nearby. On one occasion, a Waibira juvenile male (ROB) ingested the bark of *C. sylvaticus* while other group members ate *C. millenii* fruit in a large tree nearby. On another occasion, a juvenile female (ASM) ingested *F. elastica* bark while others in the group, including her mother, ate ripening *C. millenii* fruits in a nearby tree. Subsequently, ASM joined the group and began feeding on *C. millenii.* In Waibira, we observed a group feeding event in a large *S. dawei* tree, in which an unidentified individual arboreally removed and wadged bark while all others ate young leaves. In Sonso, a juvenile and sub-adult male separated from a group after feeding on two popular food items (*F. mucuso* ripe fruits and *F. exasperata* unripe fruits), and travelled together to a *Scutia myrtina* scandent vine, from

which they then both removed and ingested bark. Direct observations from both communities suggest that bark is exploited when staple foods are available, and in certain contexts, may even be prioritized over highly nutritious, nearby, staple foods.

**Prediction 4:** *Individuals conserve energy and minimize risk by exploiting trees for bark within or near their core areas, if trees are distributed throughout the home range.*

***Spatial Distribution of Bark Feeding Events***

Contrary to **Prediction 4**, Sonso's bark ingestion event heat map shows a strong pattern of events taking place outside the Sonso community grid-system (**Figure 9**), and far from core areas (**Figure 10**). To quantify this, we mapped core areas for each community according to our operational definition, including blocks with ≥1379 scans in Sonso and ≥451 scans in Waibira. Core areas are delineated in blue in **SM Figures 3** and **4.** In both communities, all core area blocks were ≥500m from the edge of the grid, except for one block in Waibira which bordered the community's western edge.

The number of bark ingestion events which took place in each block is coded by color gradient, with exact values specified in the color key. Of all Sonso bark ingestion events (n=168), 36 (21%) took place outside Sonso's grid system, far from core areas. Core area blocks in Sonso (see **SM Figure 3**) were a minimum distance of 900m from the eastern edge of the grid (line 12), 800m from the western edge (line J), 700m from the northern edge (line H), and 1700m from the southern edge (line 20). Species targeted by Sonso for such events include *A. boonei, C. mildbraedii, C. millenii, C. patens, C. alexandri, D. dewevreii, F. exasperata, F. sur, K. anthotheca, L. pervillei, R. farinifera,* as well as unidentified climber and unidentified tree species. Each of these species except for *C. patens* were recorded along Sonso transects, within the grid-system, though frequencies varied. In Sonso, five out of the 11 species ingested off-grid, were within the top 24 most abundant species represented throughout the Sonso grid-system, suggesting a wide distribution of these species within the home range (**SM Figure 5**). It is therefore unlikely that far and off-grid travel was necessary for access to selected species.

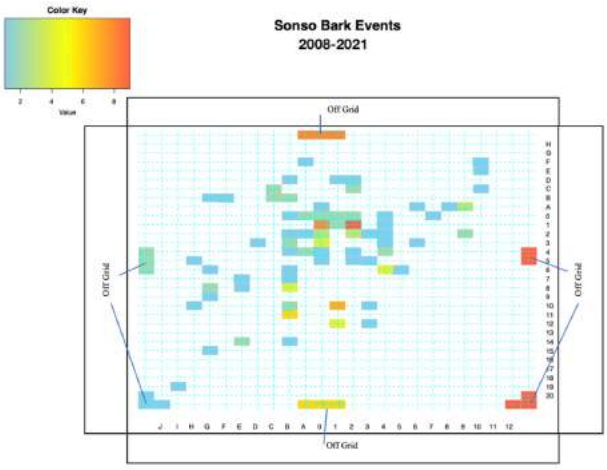


***Figure 9:*** *Heat map of Sonso bark ingestion events (2008-2021)*
**NB:** White blocks are regions with no bark ingestion event

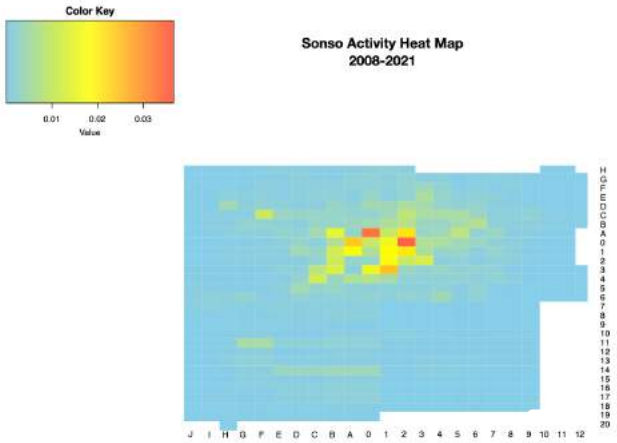


***Figure 10:*** *Heat map of Sonso focal activity scans (2008-2021)*
**NB:** White blocks are regions where group was never reported on scans

In Sonso, bark ingestion events within the community's core areas occurred most frequently (see orange-red coloration) in block 2-1 (**Figure 9**). This block is nested in a cluster of frequently visited, core area blocks toward the center of the home range and only 100m south of block 2-0, the block with the highest number of recorded scans (n=4137). Trees stripped in this block include *B. papyrifera* (n=7), *P. guajava* (n=1), and *S. guineense* (n=1). The second most common (on-grid) block where bark ingestion occurred was in 0-1, not technically within a core area (n=749 scans), but 100m from core area blocks on both the east and west and only 200m south of the popularly frequented block 0-A (n=3871 scans). Within the home range, but south of the central core area, bark ingestion also appears to have occurred at relatively high frequencies in two blocks (1-10 and B-11), located in a swampy forest region. None of these blocks are in a core area, with only n=102 and n=192 scans per block, respectively. Both blocks are at least 600m south of any core area block, and no other block to the south, east, or west classify as core area. Bark was ingested in 1-10 and B-11 from *A. boonei* (n=4), unidentified climber species (n=2), *C. patens* (n=2), *F. exasperata* (n=1), *R. farinifera* (n=1), unidentified terrestrial herbaceous species (n=1), and unidentified tree species (n=3).

In Waibira, the community's core area was larger and more contiguous. All core area blocks were a minimum distance of 500m from the eastern edge of the grid-system (line 40) and except for the core area block 10-N, were also ≥500m from the western edge (line 10). The closest core area blocks to both the north and south of the grid were a minimum distance of ≥600m. Only two Waibira bark ingestion events were reported off-grid in the long-term data, one in the north targeting *C. millenii,* and one in the west targeting *F. saussureana*. The latter event was recorded in the neighboring Sonso home range (block 5-4), 1000m south and 200m west of the

closest Waibira core area block (20-0). While *C. millenii* was not recorded along transects in this community, suggesting relative scarcity, this fruit is considered a staple food in both communities and was observed being fed on by chimpanzees regularly throughout the study period. It can be assumed, therefore, that Waibira chimpanzees know the location of *C. millenii* trees within their core areas. *F. saussureana* was recorded along a singular transect in Waibira and is also considered a staple fruit for this community.

We found strong overlap between Waibira's bark ingestion heat map (**Figure 11**) and our heat map of the group's core areas based on proportion of time spent in each block (**Figure 12** and **SM Figure 4**). Waibira's bark ingestion heat map shows the most activity in block 30-R, where the core group spends the highest proportion of their time (n=1353 scans). Bark ingestion events, reported in this block, involved *C. alexandri* (n=12), *D. kirkii* (n=3), *C. millenii* (n=1), unidentified tree species (n=4), and *G. albida* (n=1).

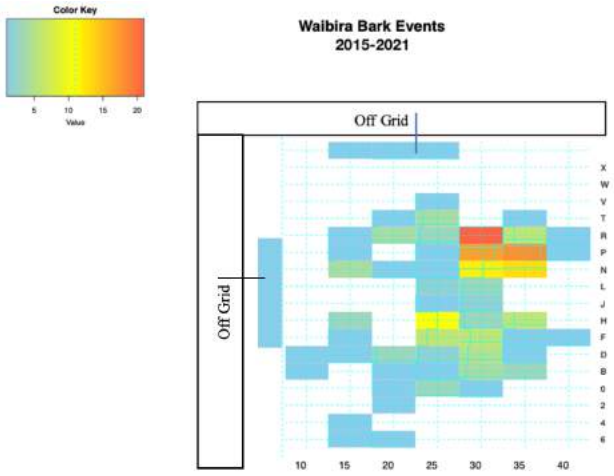


***Figure 11:*** *Heat map of Waibira bark ingestion events (2015-2021)*
**NB:** *White blocks are regions with no bark ingestion events*

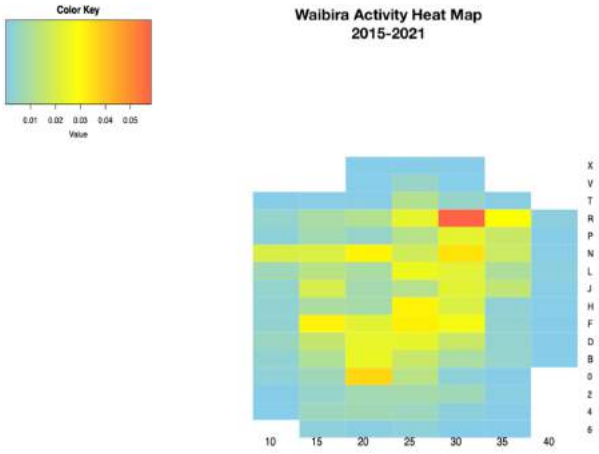


***Figure 12:*** *Heat map of Waibira focal activity scans (2015-2021)*
**NB:** *White blocks are regions where group was never reported on scans*

### *Species-Level Heat Maps*

In Waibira, the species with the most bark ingestion events was *C. alexandri* (n=72), with the majority of events concentrated in the most frequented block of the core area (block 30-R) as well as some surrounding blocks (**SM Figure 6**). This suggests that Waibira chimpanzees travel minimal distances to access this species. *C. alexandri* is also the fourth most abundant species in Waibira's grid-system, comprising 6% of the community's forest composition, according to transects.

Again, this is not the case for all species. In Sonso, where off-grid bark ingestion events were more common, the two species with the highest number of off-grid events were *C. patens* (n=9) and *A. boonei* (n=8*)*. Of all Sonso *C. patens* (CP) bark ingestion events (n=34), 9 (26%) took place outside of the grid-system **(Figure 13)**. Of all *A. boonei* (AB) bark ingestion events (n=17), 8 (47%) took place off-grid (**Figure 14).** For both species, few bark ingestion events were observed near the group's central core area, except for several *C. patens* bark ingestion events which took place in block 4-6, 400m from the closest core area block. A relatively high concentration of events involving both tree species occurred in block B,11, located in the south of the grid in a less frequented region, far from the central core area. *A. boonei* was reported along all transects in the Sonso and Waibira home ranges, comprising 1% of overall forest composition in both communities (**SM Figure 7**). *C. patens* was not observed along any Sonso transects, suggesting its relative scarcity in the Sonso home range. All off-grid bark ingestion events in the long-term site data are reported by species in **SM Table 3**.

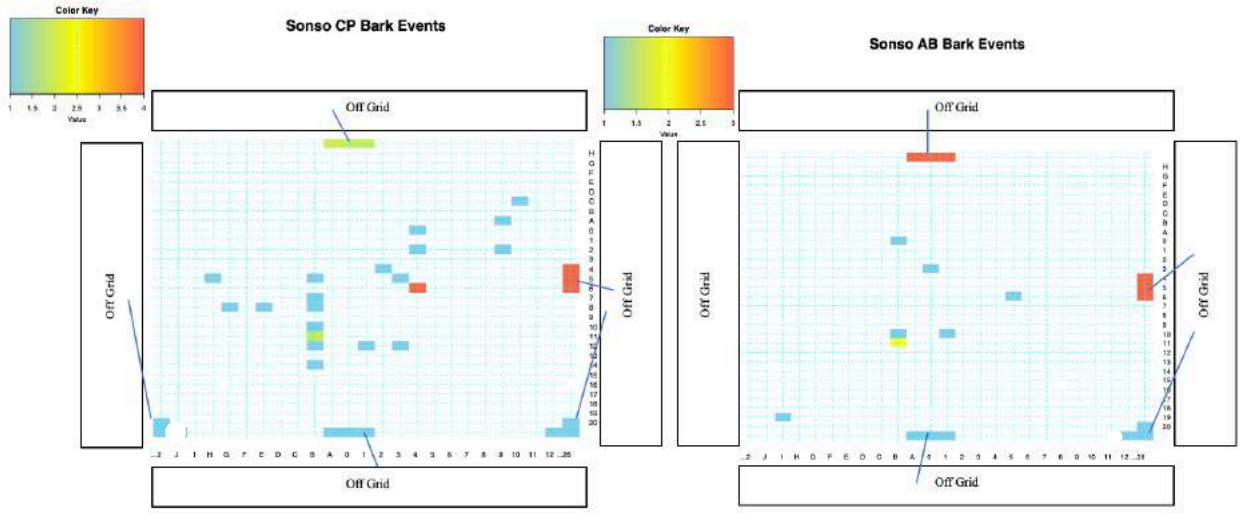


***Figure 13:*** *C. patens bark ingestion events in Sonso*




***Figure 14:*** *A. boonei bark ingestion events in Sonso*

### *Direct Observations of Bark Ingestion Events Outside the Core Area*

Throughout the study period, we directly observed individuals ingesting bark far from core area blocks, both at the edge of the grid-system, and in other community home ranges. In Sonso, we observed three individuals ingesting bark of a *F. exasperata* tree at the eastern Sonso-Waibira border (block 9-4) while on a group-wide patrol, before immediately travelling into Waibira with the rest of the group. In the previously described *S. myrtina* bark ingestion event, two Sonso males travelled to the periphery of Sonso's grid (block 9-A), to exploit a previously stripped scandent trunk, despite nearby calls from the Waibira community. We also observed a

sub-adult male in Waibira ingest bark from a buttress of *C. millenii* (in block 30-F) immediately before travelling off-grid (east) with the rest of the group.

## Discussion:

This paper challenges the historically accepted fallback food hypothesis as the singular adaptive function of bark ingestion. We systematically assessed and challenged four predictions of this hypothesis using long-term data and behavioral observations from an 8-month study period. We found that bark ingestion behaviors appear to differ not only in form, but also in function across tree species. Overall, our results suggest the presence of species-specific variability in bark ingestion behaviors regarding seasonality, quantity of material targeted, degree of preferential selection, and location of individual trees selected for use. Given this variability, we suggest the existence of multiple, non-mutually exclusive, tree-species-level adaptive functions for bark ingestion in chimpanzees.

**Prediction 1:** *Bark ingestion increases during periods of food scarcity and decreases during periods of ripe fruit availability.*

While the frequency of bark ingestion for some species (*C. alexandri, D. kirkki,* and *B. papyrifera)* peaked during periods in which food was at its scarcest, bark feeding on other species (*C. patens* and *A. boonei*) increased during periods of relative fruit abundance. Unlike Nishida's (1976) findings from Mahale, bark feeding seasonality of certain tree species failed to satisfy **Prediction 1**.

***What could drive bark ingestion during periods of ripe fruit food abundance*?** If the seasonality prediction for bark as a fallback food cannot be met across all tree species, alternative hypotheses should be explored. Variability in seasonality of the behavior across both wet and dry seasons suggests that rainfall and/or temperature is likely not uniformly driving this behavior. If ecological stress is enhancing protein or chemical production in the bark of trees, as predicted by the stressed-tree hypothesis, the source of this stress is also likely not seasonally driven, unless trees are experiencing species-specific seasonal stress based on differing ecological factors.

Based on bark ingestion seasonality, the essential nutrient and mineral hypothesis cannot be ruled out, as different species could provide critical dietary components that become scarce in the diet at different periods throughout the year. To further test this, all species targeted for bark

ingestion should be tested for nutritional and mineral composition, including those which have already been assessed (e.g., Reynolds et al., 2009). While we were unable to assess the water content of selected barks (Klich, 2017), it is unlikely that water is the target of this behavior as ingestion most commonly occurred in October, the month with the highest average rainfall (**Figure 3**).

The self-medication hypothesis can also not be ruled out based on bark ingestion seasonality. Since bark feeding generally appears to peak during the wet season, or periods of seasonal transition, this could correspond with an increase in internal parasite load or outbreaks of respiratory diseases associated with rainy seasons in sites like Mahale (Huffman, 1997; Kaur et al., 2008). However, seasonal variation in endoparasites is more nuanced in Budongo (Huffman et al., 2009), and further long-term investigation into host parasite-ecology is needed. While several species failed to satisfy **Prediction 1**, *C. patens* and *A. boonei* warrant particular attention, as bark ingestion from these species notably increased in February when fruit was most abundant. In the last few years, severe respiratory infections at Budongo have repeatedly occurred in February-March (C. Hobaiter, personal comm.), suggesting a possible relationship between bark ingestion and the presence of seasonal viral infections. The ingestion of these barks could, therefore, be an adaptive self-medicative behavior for combatting illness during periods of increased susceptibility to infections of various kinds.

**Prediction 2:** *Quantity of bark ingested is relatively consistent across tree species.*

Chimpanzees ingest different quantities of bark and cambium depending on the tree species, using honed, species-specific techniques. While Budongo chimpanzees strip and ingest large quantities of species like *F. exasperata*, other species like *A. boonei* are consumed in much smaller quantities. These latter species are likely not fallback foods, as bite-sized quantities of nutrient-poor bark would likely not justify the costs of exploitation. While morphological or nutritional differentials in bark structure could explain technical variation for bark removal across species, if this is the case, then hard-to-strip or less nutritional bark types would likely be abjured in favor of more efficient and beneficial species. Rather, another possibility is that bark quantities targeted may be related to the presence and density of plant secondary metabolites (PSMs) in some species. While PSMs can negatively impact the wellbeing of healthy individuals if ingested in large amounts (Forbey et al., 2009), in small doses, some species may provide medicinal benefits to consumers. For example, the bark of several species ingested by chimpanzees (e.g., *A. boonei* and *A. toxicaria*) are traditionally considered toxic by

people living in villages surrounding Budongo, but are commonly used in small doses as medicine (E. Freymann, unpublished data).

While we use the length of bark strips and bites as a proxy for quantity of ingested bark, this measure has limitations. As previously mentioned, measurable strip length may be influenced by morphological characteristics such as easiness to peel, or differences in cambium thickness which could mean similar rates of return between small, but deep incisions and long, shallow strips. Additional work to establish the nutritional, mineral, and medicinal content of each species' bark, and to what extent exposed area (length) accurately predicts quantity of constituent compounds consumed, will be helpful for further refining our assessment of this hypothesis.

***Why expend energy ingesting bark of certain species if not for nutritional gain?*** Chimpanzees may target certain tree barks, especially those consumed in small quantities, for their pharmacological properties. If so, targeting bioactive barks in small doses may allow an individual to attain pharmacological benefits while avoiding toxic side-effects. Further support for the self-medicative hypothesis comes from taste tests conducted on barks mentioned in this study. Species with the largest quantities targeted (*F. mucuso, F. exasperata, F. variifolia*) were sweet or neutral in flavor, while the species with the lowest quantities targeted (*A. boonei, A. zygia, A. glaberrima)* were highly bitter. Bitterness is a commonly cited proxy for PSM density and pharmacological activity (e.g., Behrens et al., 2018; Dragoș et al., 2022; Ntie-Kang, 2019). To further investigate this, systematic bioactivity testing should be done on targeted species to determine specific pharmacological properties and toxicity levels (e.g., Freymann et al., in prep.).

**Prediction 3:** *Staple fruits are prioritized over bark when both are available.*

Counter to **Prediction 3**, direct observations of bark ingestion indicate that regardless of staple food availability, certain bark species are sometimes prioritized over nutritious resources. This finding is surprising given the comparatively complex processing techniques needed to access bark versus those needed to access fruit. The observation of a juvenile Sonso male prioritizing *A. boonei* bark over protein-rich meat simultaneously undermines assumptions of the fallback food hypothesis *and* the stressed-tree hypothesis, as it suggests that bark is not targeted for protein. To further assess this prediction, the seasonality of hunting and acquisition of other

protein-rich foods should be systematically compared to seasonality of bark ingestion across species.

***Why is bark of certain species prioritized over staple foods?*** The occasional prioritization of bark over staple foods suggests that the adaptive function of ingesting bark is not always related to nutrition. Rather, in these cases, selected barks may provide chimpanzees with other mineralogical or pharmacological benefits that cannot be found in staple fruits or meat.

**Prediction 4:** *Individuals conserve energy and minimize risk by exploiting trees for bark within or near their core areas, if trees are distributed throughout the home range.*

Counter to **Prediction 4**, Sonso individuals often leave the safety of their core areas, travelling far distances to the periphery of, or outside, their known home range to ingest bark. This expends energy and elevates risk of intergroup encounters. In Sonso, 21% of all bark ingestion events took place off-grid, despite the availability of targeted species within the community's home range. This disproportionately occurred for certain species, including *C. patens* and *A. boonei.* This result is even more notable when the low targeted quantity of *A. boonei* bark biting (μ15.8 cm) is considered. It is highly unlikely that nutritional benefits of ingesting this small quantity of *A. boonei* bark could outweigh the high costs of travel or justify the risk of intergroup encounters. We once again reject the generalized application of the fallback food hypothesis and encourage species-level exploration of alternative hypotheses.

Unlike in Sonso, Waibira bark ingestion events more commonly occurred in frequently visited blocks, with only two off-grid events reported in the long-term data. We, therefore, cannot yet reject **Prediction 4** in Waibira. While our initial results suggested that Waibira group members may opportunistically be selecting bark to ingest based on abundance of preferred trees (e.g., *C. alexandri)* in frequented blocks, we cannot rule out the possibility that the group commonly visits these blocks *because* of the abundance of preferred species in these areas. Another possibility is that bark feeding events outside of the grid-system may be underreported for this community as dense thickets and valleys along boundary areas make focal follows more difficult in these areas.

***Why do Sonso chimpanzees disproportionately strip bark outside of their core areas?*** Preferred habitat type for each species should be considered when interpreting heat map results. For example, both *A. boonei* and *C. patens* prefer wet tropical biomes, and are often found along rivers or in swamps (POWO, 2023). While this could suggest that Sonso individuals travel to

specific habitat-types in search of preferred species only found in these regions, transect data reveal that *A. boonei* is consistently distributed throughout Sonso's core area (**SM Figure 7)** which spans multiple habitat types and elevations. While *A. boonei* may grow at higher densities in wet forest regions, this species is also accessible in dry and central regions of the community's core area. The distribution of *C. patens* remains less known as it was unreported along Sonso transects.

An alternative hypothesis is that Sonso chimpanzees are preferentially selecting specific individual trees (rather than targeting trees solely based on species) which have been subjected to certain environmental conditions that are rare or absent in their core area. These conditions could uniquely influence the chemical composition of individual tree barks, making them more desirable than others of the same species. In Sonso, *A. boonei* and *C. patens* bark ingestion events within the community's grid, clustered in block B-11, a block made up of primarily swamp with a river running through it. While the stressed-tree hypothesis contends that a stressful life-history causes trees to increase production of certain proteins (White, 2019), stressful or harsh habitats, like swamps, may also promote production of bioactive PSMs (e.g., Ramakrishna & Ravishankar, 2011; Selmar, 2008; Selmar & Kleinwächter, 2013) enhancing the bark's medicinal value. Chimpanzees may, therefore, be targeting specific habitats when selecting trees for bark ingestion, to exploit elevated levels of medicinal compounds.

A non-mutually exclusive hypothesis is that chimpanzees may target trees which grow in certain soils (e.g., clay), as this may alter mineral composition of the bark (Reynolds et al., 2009). To investigate this further, habitats where bark ingestion is common should be systematically classified and recorded. Comparative bark samples should also be taken across habitat-types to test for compositional differences. Behaviors that co-occur with travel to blocks far from core areas or outside of the home range should also be further studied to assess whether these parties travelled to ingest bark, or whether ingestion of bark opportunistically co-occurred with other activities due to increased accessibility (e.g., boundary patrols or exploiting other spatially restricted resources).

**General Discussion**

We identify several tree species, used for bark ingestion, which fail to meet one or more of the above predictions, including *A. glaberrima*, *A. zygia*, *C. millenni*, *F. saussureana, F. sur*, *S. myrtina*, *C. sylvaticus*, *C. patens*, and *A. boonei*. We recommend targeted investigation into

these species and suggest that *A. boonei* bark ingestion, which fails to meet all predictions of the fallback food hypothesis, can be best explained by the self-medicative hypothesis. However, identifying the specific pathogens or ailments affecting chimpanzees which may predict and/or explain this behavior will require further study.

Our findings have several evolutionary implications. As the adaptive function of bark ingestion in chimpanzees cannot be generalized across tree species, we must exercise caution when interpreting evidence of this behavior in the fossil record. If we find that chimpanzees are ingesting bark of certain species at increased frequencies in certain habitat-types, this may also inform future paleoenvironmental and palaeobotanical studies. If future studies determine that the bark of trees growing in certain habitats have enhanced pharmacological properties, we may also gain a better sense of the role specific habitats played in the survival of our hominin ancestors. With rapid advancements in zooarchaeological, paleoanthropological, and palaeobotanical methods, we can now identify bark removal traces as far back as the early Holocene (Edvardsson et al., 2021). Using recent morphometric and machine learning methods, new techniques have enabled identification of percussive signatures on wooden tools used by chimpanzees (Luncz et al., 2022), suggesting that ancient bark removal techniques may also have left distinctive signatures. Using chimpanzees as phylogenetic models provides a valuable opportunity to better understand the role bark and cambium may have played in the diets of early hominins and how they may have adapted to dynamic environments.

## Conclusion:

We provide an in-depth, site-specific evaluation of bark's role in the diets and well-being of Budongo chimpanzees, challenging the long-held assumption that bark is primarily ingested as a fallback food during periods of food scarcity. Instead, we argue for a species-specific approach when assessing the adaptive function of bark feeding. This requires an overarching paradigm shift when assessing the adaptive functions of dietary behaviors involving multiple plant species, parts, or techniques. Instead of generalizing food items, we must investigate dietary behaviors at a species level, taking taxonomic, morphologic, pharmacologic, and life-history characteristics into account. Cross-site collaborations will be crucial for progress on this topic, as will sharing of forest composition and chimpanzee feeding data. We encourage future field studies on technical characteristics of bark removal, as well as systematic documentation of indirect evidence. These data will be valuable for establishing standard metrics for future studies and for ensuring cross-site replicability.

Investigation into the adaptive function of bark ingestion may also be critical for the survival of our primate cousins. As the climate warms and once-common trees grow scarce or are put under ecological stress, a better understanding of how and why trees are exploited by chimpanzees could inform conservation strategies. As more is discovered about the role of certain species in primate diets, we encourage collaborations with conservation and government agencies to ensure these species are kept protected and available in chimpanzee home ranges.

### Data Availability Statement

Data from this study is available from the corresponding author upon reasonable request. Images of terrestrial and arboreal bark exploitation can be found in **Appendix G**.

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# 7 Conclusion

In this thesis, I use several approaches, both quantitative and qualitative, to investigate putative self-medicative resources amongst the wild chimpanzees of Budongo Forest. As no in-depth studies on primate self-medication have been conducted at this site in the last two decades, this project provides critical methodological, behavioral, pharmacological, and theoretical information which can enable the implementation of future zoopharmacognosy research at this site. This chapter presents an overview of the key findings, including several putative self-medicative resources identified across the various studies. From these resources I highlight *Alstonia boonei,* which stands out as a species of particular interest. Drawing on our findings, I make a holistic case for *A. boonei* as a therapeutic medicinal resource amongst Budongo chimpanzees. The chapter then addresses several ethical concerns and limitations which arose throughout this research and discusses the implications of the findings on primate conservation and chimpanzee habitat management. The thesis concludes with future directions and suggests ideas for further research on this topic.

## Key Findings and Associated Putative Self-Medicative Resources

In **Chapter 3** we collated historical anecdotes with our own behavioral observations and health monitoring data to assess what is currently known about healthcare behaviors in Budongo. Before this study, limited systematic research on this topic had been conducted at the site, and putative self-medicative anecdotes had never been compiled or shared. This prevented researchers interested in conducting zoopharmacognosy projects from asking complex or comparative questions about self-medication, and generally impeded research on this topic at this site. This chapter, therefore, provides the first overview of self-medication amongst Sonso and Waibira chimpanzees, identifying plant species used for leaf swallowing, wound care, and putative ingestion-based self-care. Based on reports and observations from this study, we propose *Cubitermes* termite soil, *K. anthotheca* bark and resin, *C. sylvaticus* bark, and *C. patens* dead wood as putative self-medicative resources in Budongo. A key finding from this study is that Sonso chimpanzees engage in social care through snare-related physical assistance, wound care, and hygienic behaviors. Furthermore, we report social care between non-kin individuals, a discovery which has only been reported at a few other primate sites and thus an important contribution to the field. Methodologically, this study emphasizes the importance of data sharing between researchers when studying rare behaviors such as self-medication and

highlights the significance of recording anecdotal observations which may one day lead to unforeseen discoveries.

In **Chapter 4** we piloted two quantitative methods, Collocation Analysis and APRIORI, never before used on primate feeding ecology data, to explore non-random food combinations in the diets of Budongo chimpanzees. We demonstrated that these methods can effectively identify resource combinations which occur in individual diets above or below chance. Our methodological findings contribute to the field of zoopharmacognosy two-fold. First, they offer new tools to future researchers which can assess a novel hypothesis we introduce in the chapter: the **self-medicative resource combination hypothesis**. This hypothesis holds that when ill or wounded, chimpanzees may consecutively ingest more than one medicinal resource throughout the course of the day, challenging the unarticulated assumption that self-medication events take place in a vacuum. The formulation of this hypothesis was based on behavioral observations made throughout our first field season. Second, these methods seek to quantitatively tackle an historic challenge in zoopharmacognosy: identifying novel self-medicative resources in primate diets given the relative rarity of self-medication behaviors. Based on our preliminary investigations, we propose *A. polystachyus* pith, *M. holstii* fruit, *Cubitermes* termite soil, *M. bpterygocaulos* young leaves, *U. trinervis* flowers, *K. anthotheca* bark and resin, and *Aframomum* sp. pith as putative self-medicative resources. With future refinement of these methods, we believe they will be effective for use on long-term datasets to identify novel medicinal resources, or medicinal resource combinations. Our exploratory investigation contributes new quantitative tools to the field of zoopharmacognosy.

In **Chapter 5,** we collaborated with researchers at Neubrandenburg University of Applied Sciences, to pharmacologically evaluate putative self-medicative resources, collected *in situ,* based on behavioral observations made throughout the study period. Using anti-bacterial growth inhibition and COX-2 assays on plant extracts from Sonso's home range, we report both strong antibacterial and anti-inflammatory properties amongst several putative self-medicative resources. Species which showed particularly strong bioactivity in this study include *S. guineense* bark, *A. boonei* bark, *C. parasitica* fern leaves, *K. anthotheca* bark and resin, and *A. polystachius* pith. For several of these species we also report behavioral evidence of unusual ingestion events associated with these resources. This study, for the first time in Budongo, combines pharmacological results with primatological observations to establish the presence of medicinal plants within the Budongo chimpanzee diet. Our results identify highly bioactive

medicinal species which could lead to the discovery of novel human medicines, as well as incentivize more drastic conservation measures in chimpanzee inhabited areas.

In **Chapter 6** we further explored bark ingestion as a putative self-medicative behavior, evaluating and challenging the popularly accepted adaptive function of this behavior. While bark has historically been considered a fallback food, by examining bark consumption at the tree species level, this chapter revealed that the fallback food hypothesis does not account for all instances of bark ingestion. This challenges a traditional view in chimpanzee feeding ecology and underscores the importance of evaluating botanically based behaviors at a species level. Furthermore, the research demonstrates the presence of bark ingestion techniques which differ by tree species, and which leave behind classifiable traces. These findings may have implications for primate archaeology, helping to shed light on past primate behaviors. In **Chapter 6** we identify *A. boonei*, *C. patens*, and *S. guineense* as species which fail to meet most predictions of the fallback food hypothesis. We argue instead that bark feeding on these species may be better explained by the self-medication hypothesis. This is strongly supported by our pharmacological results reported in **Chapter 5**.

## Interdisciplinarity in Zoopharmacognosy

Across all studies, a panoply of methods from several disciplines, including primatology, anthropology, pharmacology, parasitology, and botany, were employed—all of which were necessary for answering the research questions posed in this thesis. To do this, I forged multidisciplinary collaborations which were critical for gathering expertise and diversifying the scope of this project. As therapeutic self-medication is a relatively rare behavior, much of this project relied on observational anecdotes and historical reports, none of which (at this point) can be statistically tested—but nonetheless offer invaluable information which move the field forward. More broadly, this research required adopting a natural historical perspective and resisting specialization in one field, an approach which we argue is necessary for the study of zoopharmacognosy.

## *Alstonia boonei:* Evidence for a Novel Therapeutic Self-Medicative Behavior

Across the chapters of this thesis, I repeatedly encountered evidence suggesting that *A. boonei* bark is not consumed as a "normal food" by Budongo chimpanzees and instead may be consumed as a therapeutic self-medicative resource. *A. boonei*, a tree species native to West and Central Africa, has a long history of traditional use in African folk medicine for the

treatment of a variety of health conditions (Adotey et al., 2012; Burkill, 1995; Kokwaro, 1976, 2009). This species has also been previously shown to possess several pharmacological properties (reviewed in Adotey et al., 2012). Pebsworth et al. (2006) also mentioned this species as a putative therapeutic resource in Budongo based on their behavioral observations. Below, all relevant evidence produced in this thesis related to *A. boonei* is reviewed and evaluated in regards to of Huffman's (1997) criteria for establishing novel self-medicative behaviors (see **Chapter 2: Section 2.2.1**).

**Criterion 1:** ***Infrequent intake of irregularly consumed plant species which provide no/minimal nutritional benefit.***

In **Chapter 6** we report only 17 cases of *A. boonei* bark stripping in Sonso's long-term data (from 2008-2021) and two cases in Waibira's (from 2015-2021). Relative to other food sources, such as ripe fruits, young leaves, or even other bark stripped species such as *C. alexandri* (n=84 observations), *A. boonei* appears to be infrequently consumed. Historically, tree bark is considered a poor-quality resource which provides little nutritional value (see **Chapter 6**), though this may vary by species. While we did not test the nutritional yield of *A. boonei* bark, we did report a species-level pattern which suggests that the bark of this species is unlikely to provide much nutritional value. Unlike the bark of other species like *F. exasperata* which is exploited in long strips, *A. boonei* is consumed in small bites (**Figure 1**). Unless *A. boonei's* bark is nutritionally rich, it is unlikely that these targeted quantities can provide individuals with enough energy to justify the energetic and temporal costs of accessing the cambium beneath the hard bark.

***Figure 1****: Examples of A. boonei bark bites in budongo*

**Criterion 2:** ***Restriction of plant use to seasons associated with high rates of infection.***

In **Chapter 6** we assessed the general seasonality of bark stripping behaviors in the long-term site data and show that bark ingestion of *A. boonei* peaks in February and October (**Figure 2**). If bark stripping were a fallback food, it should decrease, not increase, in February when food is relatively abundant. However, as reported in **Chapter 3**, February is also the month when serious, and often fatal respiratory outbreaks have been increasing across both communities (C. Hobaiter personal communication). If *A. boonei* is a therapeutic medicine for Budongo chimpanzees, it may help them combat these outbreaks.

In **Chapter 3** we also reported the annual rainfall and temperatures for 2021 and 2022. Relative to other months, February had low rainfall (≤1mm), and high maximum temperatures (31˚C across both years). Conversely, October had relatively high rainfall (between 6mm-8mm) and low maximum temperatures (28˚C across both years). While we do not have year-long parasite ecology data to assess parasite seasonality at Budongo, we do report an increase of *Taenia* segment prevalence in October (relative to prevalence in June-September) from health monitoring data reported in **Chapter 3.** Chimpanzees may, therefore, increase *A. boonei* bark stripping in October to combat specific pathogens or parasites which could increase at the onset of the colder rainy season. The relationship between rainfall and parasite load has been demonstrated in chimpanzees at several sites (Huffman et al., 2009). This relationship, however, has yet to be proven at Budongo (Huffman et al., 2007; 2009).

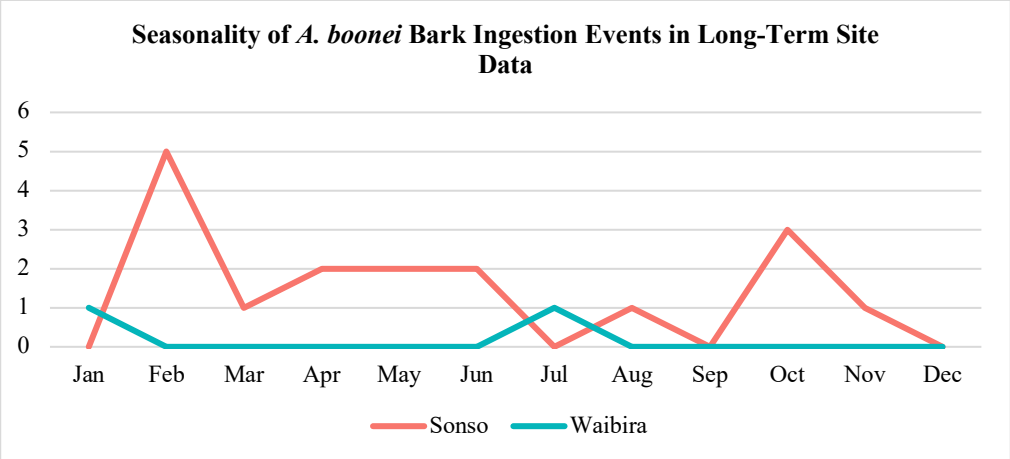


***Figure 2:*** *Seasonality of all A. boonei bark stripping events reported in long-term data across months in both communities*

**Criterion 3-4:** ***Presence of pathogenic or parasitic infection in the medicator at the time of self-medicative behavior AND a subsequent improvement in this condition following ingestion.***

The third and fourth criteria are combined here, as they both involve health state of the consumer. Unfortunately, in this thesis we did not have the scope to fully address these criteria as we only directly observed a singular *A. boonei* dead wood eating event during our eight months of behavioral data collection. During this event (described in **Chapter 5**) three Sonso individuals ate *A. boonei* dead wood, one of had diarrhea two days before as well as unidentified protozoa and *Bertiella* sp. proglottids in his stool. As we do not have any health information on individuals who consumed living bark of *A. boonei,* we are currently unable to fully address these criteria.

**Criterion 5:** ***Healthy conspecifics ignore the resource or process it in a different way.***

As observations were limited, more data is needed to assess this criterion.

**Criterion 6:** ***The identification of specific mechanical or chemical properties which ameliorate illness or symptoms in the medicator.***

In **Chapter 5** we assessed the antibacterial and anti-inflammatory properties of *A. boonei* from samples collected within the Sonso home range and found that bark extracts inhibited growth in numerous bacteria including *A. baumannii* and two strains of E. coli (DSM 15076, DSM 498). In anti-inflammatory COX-2 assays, *A. boonei* bark showed inhibition ≥50% at 50 µg/ml in dose-response. While we have not yet identified a specific illness associated with *A. boonei* bark ingestion, our results suggest that this species possesses numerous chemical properties which could likely ameliorate certain illnesses in an infected chimpanzee. This is also true of *A. boonei* dead wood, which showed even stronger pharmacological properties on our assays.

In **Chapter 6**, we further propose that the substrate in which *A. boonei* trees grow (e.g., clay), could alter the pharmacological properties of the bark. We made this hypothesis based on our findings that Sonso chimpanzees travel far distances into other community home ranges to access *A. boonei* bark. Further pharmacological assays and ecological mapping will need to be done, however, before this hypothesis can be tested.

Although *A. boonei* does not currently meet all of Huffman's criteria, our amalgamated evidence provides compelling support for *A. boonei* as a therapeutic medicine for Budongo chimpanzees.

Disturbingly, however, the future of *A. boonei* is uncertain in Budongo. According to our informal discussion with field staff and management, *A. boonei* trees are some of the most frequently targeted by illegal loggers in the area, harvested to make coffins and rain gutters. The primary attraction for logging this species is its lightweight wood, facilitating illicit transport out of the forest. Throughout both field seasons, we encountered multiple logging sites containing felled *A. boonei* trees. The continued exploitation of this medicinal tree poses a significant risk, and without intervention, *A. boonei* may soon vanish from Budongo. Urgent conservation measures are warranted to safeguard this species and its potential therapeutic benefits for both chimpanzees and local people.

***Figure 3:*** *[Left] Illegal A. boonei logging camp [Right] felled A. boonei tree in Waibira home range (October 2022) (Images by E. Freymann)*

## Limitations

This thesis, while comprehensive in its approach, is not without its limitations, primarily stemming from temporal and financial constraints. A notable limitation throughout our studies was our reliance on parasitology as the predominant health measurement. Parasitology is popularly favored as a health metric in zoopharmacognosy studies due to its non-invasive nature and efficiency in the field. However, this reliance introduces a potential bias. When a chimpanzee appears to have a low parasite load, researchers may incorrectly interpret this as a sign of overall health, potentially overlooking unusual feeding behaviors that could be indicative of medicinal plant use. The absence of easily accessible, inexpensive, non-invasive, and efficient tests for other pathogens further exacerbates this limitation. While we attempted to address this issue through urinalysis testing, collection of urine samples was not always

feasible. While conducting microbiome studies on fecal samples would have provided valuable insights into individual gut health and potential bacterial infections, the establishment of the necessary collaborations and permits for this undertaking was logistically challenging within the time frame. Furthermore, as the initial field season coincided with the Covid-19 pandemic, we were restricted in our ability to export fecal samples. Consequently, we were reliant on *in situ* microscopic identification methods, rather than more precise methods (e.g., DNA metabarcoding). Overall, our health monitoring methods, while informative, were not as robust as envisioned.

Additionally, this thesis could have benefited from a more extensive collection of ethnobotanical interviews to enrich analysis. However, due to a delay in permit amendments, we were restricted to only two interviews, limiting the depth of our ethnobotanical insights. We thus chose not to report our results. Lastly, as nest-to-nest focal follows are not carried out at BCFS, it was often challenging to locate priority focal individuals on consecutive days. This limitation hindered our ability to obtain consecutive health monitoring measurements from individuals involved in ingestion events of interest. Several other minor methodological limitations are reported in **Appendix B: General Materials and Methods**. Despite these constraints, we believe this thesis advances the field of zoopharmacognosy, and offers a foundation upon which future research can build.

## Evolutionary Implications

The study of self-medication in chimpanzees provides a unique insight into the origins of modern human medicine and healthcare systems. By investigating behavioral adaptations linked to health and healing in our closest living relatives, we may better understand the medicinal practices and related cognitive mechanisms which allowed our ancestors to survive.

***Medicinal Species:***

The identification of specific plant resources with antibacterial and anti-inflammatory properties in chimpanzee diets has implications for our understanding of medicinal plant knowledge in primates. These findings suggest that chimpanzees could possess a level of pharmacological knowledge which subsequently dictates resource selection. This knowledge may have played a role in the health and survival of ancestral primates, offering insight into the origins of medicinal plant-use in modern humans (Huffman, 2001). Though still unknown,

determining whether non-human self-medicative knowledge is intergenerationally transmitted through social learning or repeatedly innovated through individual learning strategies, will also inform our understanding of the spread and transmission of medicinal information in our own species (e.g., Salali et al., 2016). Further investigation into the ecological and pathogenic factors that drive chimpanzee self-medication will also provide insights into the selective pressures that shaped our own medicinal plant use, and help us reconstruct the pathogen-host co-evolutionary history of our own lineage (Huffman, 2001).

***Social Wound Care in Primates:***

The discovery of social wound care in Sonso chimpanzees, even between unrelated individuals, contributes to our understanding of social cooperation and altruism in primates (Mascaro et al., 2022). Specifically, it further demonstrates that this behavior is widely prevalent in our closest primate relatives, suggesting its presence in our common ancestor. This behavior may have evolved as a means of enhancing group cohesion, highlighting the importance of social relationships in the evolutionary history of non-human primates, as well as in our own species. From a paleoanthropological perspective, this finding may inform future interpretations of early hominin fossils found with indications of treated injuries or signs of wound care.

***Bark-Stripping Behaviors and Primate Archaeology:***

The study of bark-stripping behaviors in chimpanzees, including the discovery of variation in stripping techniques across tree species which leave signature traces, has direct implications for primate archaeology. The ability to classify bark-stripping traces across primate species and field sites could aid in the interpretation of any similar traces found in the fossil record (e.g., Edvardsson et al., 2021). This may provide future insight into the emergence and evolution of this behavior in the primate lineage. As technologies and palaeobotanical methods develop which allow us to uncover percussive signatures on organic materials (Luncz et al., 2022), we may soon need primate modelling to help us reconstruct and interpret the behaviors which left these traces behind.

These findings collectively contribute to our understanding of our own evolutionary history, by shedding light on our closest living relatives' health-related adaptations to ecological challenges. These include medicinal resource selection strategies, self-medicative behaviors, and care-giving. More broadly, this thesis illustrates the complex interplay between ecology, behavior, and health in the evolutionary trajectory of primates.

## Ethical Considerations

The study of chimpanzee self-medication raises numerous complex ethical dilemmas that demand thoughtful consideration. One such dilemma revolves around the disclosure through publication of self-medicative resources used by chimpanzees. The question arises: *Is it ethical to publish the scientific names of these resources*? While revealing such information may enhance scientific understanding, it also poses risks. It could potentially make these trees targets for pharmaceutical companies or loggers seeking to exploit them commercially on an unsustainable scale, as was the case with the medicinal tree *Prunus africana* in Uganda (see Bodeker et al., 2014). This could not only clear the forest of trees that are necessary for chimpanzee health-maintenance, but also eradicate species used for medicine by local people. However, there is a counterargument that publicizing these medicinal resources might increase their living value, potentially deterring destructive logging practices in favor of more sustainable harvesting techniques. Sharing findings on this topic could also lead to beneficial revision of land use policy and influence rewilding programs.

In this study, we chose to publish scientific names of identified species because our unpublished ethnobotanical interviews indicated that all trees mentioned were already locally thought to have medicinal properties. All but two resources, pharmacologically tested in **Chapter 5**, already had widely reported ethnomedicinal uses and/or had already undergone some form of prior pharmacological testing. Furthermore, several species, such as *A. boonei, K. anthotheca,* and *C. millenii* are already heavily logged in the forest for non-medicinal, commercial reasons. We believe that in this case, publicizing the potential medicinal importance of these trees for the chimpanzees offers a pathway for a novel conservation strategy. However, we emphasize the importance of carefully considering the implications of publication for resources which are less well-known.

Another crucial ethical concern relates to how pharmacological results are reported back to local healers without inadvertently altering traditional medicinal practices. This point is also addressed by Schultz et al. (2021). Even in cases when our pharmacological assays did not identify bioactivity in plant samples, this does not rule out the presence of bioactive properties in the extracts. However, the misinterpretation of technical findings could inadvertently affect the way medicinal healers prescribe plants to patients, potentially eroding traditional knowledge

systems. Another risk is that as many healers rely on traditional ethnobotanical knowledge as their primary source of income, distributing results of these studies irresponsibly may compromise the traditional healers' practices and economic well-being. We will, therefore, follow the protocols established by Schultz et al. (2021) for dissemination of results, ensuring it is done in a way that respects and supports these healers and their practices.

However, despite the plethora of potential ethical dilemmas arising from zoopharmacognosy studies, there remains a clear lack of ethical oversight in the field. Given its inherently multidisciplinary nature, ethics boards responsible for approving research projects should also be interdisciplinary. Furthermore, students engaging in such research should receive appropriate and comprehensive ethical training. To structure this training, we stress the need for an articulated ethical protocol in this field to help those of us navigating these issues.

## Implications for Conservation

As climate change and habitat destruction continue to disrupt natural processes, humans and non-human primates are being displaced and losing access to familiar local flora (Hockings et al., 2015). To preserve human and non-human primate access to medicinal resources and create better management programs for protecting these resources, we must start by prioritizing research into the distribution of self-medicative resources across chimpanzee field sites.

The identification, in this thesis, of several established and putative self-medication behaviors amongst Budongo chimpanzees, including the ingestion of medicinal tree barks, has significant implications for primate conservation in the Masindi region of Uganda and across chimpanzee habitats more generally. Despite the apparent importance of certain trees for the health maintenance of our primate cousins, rapidly increasing agricultural and commercial activities threaten many of the species mentioned here. While forest habitats most obviously provide chimpanzees with shelter, food, and a buffer from anthropogenic encroachment—these forest environments may also serve as remedy repositories—vital to the well-being of wild apes. With the increasing incidence of virulent anthropogenic pathogens spreading from humans to non-human primates (e.g., Devaux et al., 2019; Scully et al., 2018), there is new urgency for protecting these resources, as the loss of these natural remedies may further imperil already vulnerable chimpanzee populations.

Conserving medicinal trees may not only benefit our primate cousins, it may also benefit us. The recent global pandemic served as a stark reminder of our urgent need for new medicines to tackle the rapidly growing problem of novel pathogens and drug-resistance. By preserving chimpanzee habitats and studying their self-medication practices, we may discover invaluable reservoirs of knowledge that could help solve future global health crises. From a psychological perspective, continuing to identify and conserve medicinal resources shared between species may also create a global incentive to help preserve forested habitats in these regions. Optimistically, this alignment of interests could foster collaboration between conservation groups, local stakeholders, and global industry, with the shared aim of safeguarding vital ecosystems.

## Future Directions

The findings presented in this study open numerous promising avenues for future research, underscoring the dynamic and multifaceted nature of chimpanzee self-medication. Several ongoing and planned research endeavors will expand upon the insights gained in this thesis. Currently, I am developing several additional manuscripts that incorporate data extending beyond the scope of this thesis. One such manuscript seeks to assess the validity of *A. boonei* as a novel therapeutic self-medicative resource more thoroughly. This work incorporates additional behavioral evidence observed after the study period and integrates ethnomedicinal information gathered during interviews with local healers. I am also working on a comprehensive report that describes and compares bark stripping repertoires in both Sonso and Waibira. Leveraging long-term data alongside our own direct and indirect observations, this paper will offer an overview of tree species targeted for bark stripping, the techniques employed, and the indirect evidence left behind. In addition, I have collected and exported another15 bark samples from Budongo. These samples are earmarked for further pharmacological testing at Neubrandenburg University of Applied Sciences. This initiative will enable us to ascertain the bioactivity of nearly all trees in the Sonso and Waibira bark stripping repertoires.

Ethical considerations remain paramount, and I am actively crafting a paper dedicated to addressing the specific ethical dilemmas that have surfaced during my research. Moreover, my commitment to ethical rigor in zoopharmacognosy has led to ongoing work focused on developing the field's inaugural ethical protocol.

More generally, there is great scope in this field to empirically investigate potential community-level differences in bark stripping behaviors, including selection preferences and techniques, both within neighboring communities and across field sites. Such research should involve comparison of bark stripping frequencies and species selection, while considering ecological availability within community home ranges. Building upon our argument for the self-medicative function of bark stripping established in preceding chapters, a vital next step is to explore whether wild chimpanzees may possess unique medicinal cultures. Future efforts should also address the nutritional aspects of bark stripped trees, to further understand differences between bark stripping techniques and quantities targeted. Lastly, the documentation of wounds left behind on bark stripped trees should be expanded to advance the field of primate archaeology.

Overall, I hope this research collectively serves as a catalyst for a diverse range of future investigations which further contribute to our understanding of chimpanzee self-medication, its ecological implications, and its ethical dimensions.

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# APPENDICES

# APPENDIX A:
# Study Site and Subjects

## Budongo Conservation Field Station:

The Budongo Conservation Field Station (BCFS), established by Vernon Reynolds in 1990, covers 793-km2, 482 km2 of which is populated by continuous, semi-deciduous forest cover (Hobaiter et al., 2017). There are three habituated communities in the Budongo Forest, Sonso, Waibira and Kaniyo-Pabidi and three known non-habituated communities, Busingiro, Waisoke, and The Nature Reserve community.

## Demographic Information:

**TABLE 1:** Sonso individuals (2021)

| Name | Site Code | Community | Sex | Age Class |
|---|---|---|---|---|
| **Anna** | AN | Sonso | Female | Adult |
| **Alice** | AC | Sonso | Female | Infant |
| **Arcus** | AR | Sonso | Female | Adult |
| **Cindy** | CD | Sonso | Female | Adult |
| **Deli** | DL | Sonso | Female | Adult |
| **Dembe** | DB | Sonso | Female | Infant |
| **Dora** | DR | Sonso | Female | Adult |
| **Edinburgh** | ED | Sonso | Female | Adult |
| **Eve** | EV | Sonso | Female | Adult |
| **Eris** | ER | Sonso | Female | Infant |
| **Frank** | FK | Sonso | Male | Adult |
| **Faith** | FH | Sonso | Female | Juvenile |
| **Gladys** | GL | Sonso | Female | Adult |
| **Geoffre** | GF | Sonso | Male | Juvenile |
| **Ghana** | GH | Sonso | Female | Infant |
| **Heri** | HR | Sonso | Female | Sub-Adult |
| **Harriet** | HT | Sonso | Female | Adult |
| **Hawa** | HW | Sonso | Male | Adult |
| **Harmoni** | HM | Sonso | Female | Juvenile |
| **Hadue** | HD | Sonso | Male | Infant |
| **Irene** | IN | Sonso | Female | Adult |
| **Ishe** | IS | Sonso | Female | Infant |
| **Juliet** | JL | Sonso | Female | Adult |
| **Jacob** | JB | Sonso | Male | Juvenile |
| **Jacintha** | JA | Sonso | Female | Infant |
| **Janie** | JN | Sonso | Female | Adult |
| **James** | JS | Sonso | Male | Sub-Adult |
| **Katia** | KA | Sonso | Female | Adult |
| **Kaqwa** | KQ | Sonso | Male | Infant |
| **Karibu** | KB | Sonso | Female | Sub-Adult |
| **Kigere** | KG | Sonso | Female | Adult |
| **Kathy** | KH | Sonso | Female | Sub-Adult |
| **Kalema** | KL | Sonso | Female | Adult |
| **Klauce** | KC | Sonso | Male | Sub-Adult |
| **Kirabo** | KO | Sonso | Male | Juvenile |
| **Kamala** | KM | Sonso | Female | Infant |
| **Kutu** | KU | Sonso | Female | Adult |

| | | | | |
|---|---|---|---|---|
| **Kato** | KT | Sonso | Male | Adult |
| **Kefa** | KF | Sonso | Male | Juvenile |
| **King** | KI | Sonso | Male | Infant |
| **Kwera** | KW | Sonso | Female | Adult |
| **Kaija** | KJ | Sonso | Male | Juvenile |
| **Kewaya** | KY | Sonso | Female | Adult |
| **Kox** | KX | Sonso | Female | Sub-Adult |
| KX1 | KX1 | Sonso | Female | Infant |
| **Kavera** | KV | Sonso | Male | Juvenile |
| **Linda** | LD | Sonso | Female | Adult |
| **Mukwano** | MK | Sonso | Female | Adult |
| **Marion** | MI | Sonso | Female | Sub-Adult |
| **Mbotella** | MB | Sonso | Male | Sub-Adult |
| **Muhumuza** | MZ | Sonso | Male | Infant |
| **Nambi** | NB | Sonso | Female | Adult |
| **Musa** | MS | Sonso | Male | Adult |
| **Ozzie** | OZ | Sonso | Male | Juvenile |
| **Pamoja** | PJ | Sonso | Female | Adult |
| **Pascal** | PS | Sonso | Male | Adult |
| **Rafia** | RF | Sonso | Female | Sub-Adult |
| **Ruhara** | RH | Sonso | Female | Adult |
| **Rose** | RS | Sonso | Female | Adult |
| **RS4** | RS4 | Sonso | Male | Infant |
| **Ramula** | RM | Sonso | Female | Adult |
| **R**udisha | RA | Sonso | Unsexed | Infant |
| **Simon** | SM | Sonso | Male | Adult |
| **Tanja** | TJ | Sonso | Female | Adult |
| **Upesi** | UP | Sonso | Female | Adult |
| **Waseme** | WM | Sonso | Female | Adult |
| **Yuliyo** | YL | Sonso | Female | Adult |
| **YL1** | YL1 | Sonso | Unsexed | Infant |
| **Zambe** | ZB | Sonso | Female | Adult |
| **Zalu** | ZL | Sonso | Male | Adult |
| **Zed** | ZD | Sonso | Male | Adult |

**TABLE 2:** Waibira individuals (2022)

| Name | Side Code | Community | Sex | Age Class |
|---|---|---|---|---|
| **Akiki** | AKK | Waibira | Female | Adult |
| **Ardbeg** | ARD | Waibira | Male | Adult |
| **Aimee** | AIM | Waibira | Female | Juvenile |
| **Arua** | ARU | Waibira | Female | Adult |
| **Andrua** | AND | Waibira | Male | Sub-Adult |
| **Asimwe** | ASM | Waibira | Female | Juvenile |
| **Bahati** | BAH | Waibira | Female | Adult |
| **Brian** | BRI | Waibira | Male | Juvenile |
| **Byazi** | BYZ | Waibira | Female | Adult |
| **Baraka** | BAR | Waibira | Male | Juvenile |
| **Chapati** | CHP | Waibira | Female | Adult |
| **Caelidh** | CAE | Waibira | Female | Sub-Adult |
| **Ciska** | CIS | Waibira | Female | Juvenile |
| **CHP3** | CHP3 | Waibira | Unsexed | Infant |
| **Dada** | DAD | Waibira | Female | Adult |
| **Dun-Edin** | DUN | Waibira | Male | Adult |
| **DAD2** | DAD2 | Waibira | Female | Adult |
| **David** | DAV | Waibira | Male | Juvenile |
| **Daima** | DAI | Waibira | Female | Adult |
| **Daudi** | DAU | Waibira | Male | Adult |
| **Darwin** | DAR | Waibira | Male | Sub-Adult |

| | | | | |
|---|---|---|---|---|
| **Dawa** | DAW | Waibira | Female | Infant |
| **Eili** | EIL | Waibira | Female | Sub-Adult |
| **Hobaiter** | HOB | Waibira | Female | Adult |
| **Harris** | HAR | Waibira | Male | Sub-Adult |
| **HOB2** | HOB2 | Waibira | Male | Infant |
| **Jinja** | JIN | Waibira | Female | Adult |
| **Jino** | JNO | Waibira | Male | Sub-Adult |
| **Jake** | JAK | Waibira | Male | Infant |
| **Ketie** | KET | Waibira | Female | Adult |
| **Kalinzu** | KLZ | Waibira | Female | Infant |
| **Kidepo** | KID | Waibira | Female | Adult |
| **Ila** | ILA | Waibira | Female | Adult |
| **Karamoja** | KAR | Waibira | Female | Sub-Adult |
| **Kibale** | KIB | Waibira | Female | Infant |
| **Kipepeo** | KIP | Waibira | Female | Adult |
| **Kilimanjaro** | KIL | Waibira | Male | Juvenile |
| **Liran** | LIR | Waibira | Female | Adult |
| **Lahni** | LAN | Waibira | Male | Adult |
| **Lokuyu** | LKU | Waibira | Male | Sub-Adult |
| **Lamon** | LAM | Waibira | Male | Juvenile |
| **Lotty** | LOT | Waibira | Female | Adult |
| **Liz** | LIZ | Waibira | Female | Juvenile |
| **Lotus** | LTS | Waibira | Female | Infant |
| **Masindi** | MSD | Waibira | Female | Adult |
| **Merkley** | MER | Waibira | Male | Sub-Adult |
| **Micky** | MIK | Waibira | Male | Juvenile |
| **MSD4** | MSD4 | Waibira | Female | Infant |
| **Mathaai** | MAT | Waibira | Female | Adult |
| **Monika** | MON | Waibira | Female | Adult |
| **Muko** | MUK | Waibira | Female | Infant |
| **Ndito-Eve** | NEV | Waibira | Female | Adult |
| **Natan** | NAT | Waibira | Male | Sub-Adult |
| **Noah** | NOA | Waibira | Male | Sub-Adult |
| **Nimba** | NIM | Waibira | Female | Juvenile |
| **Ngogo** | NGO | Waibira | Male | Infant |
| **Nora** | NOR | Waibira | Female | Adult |
| **Nalala** | NAL | Waibira | Male | Juvenile |
| **NOR4** | NOR4 | Waibira | Female | Infant |
| **Onyofi** | ONY | Waibira | Female | Adult |
| **Penelope** | PEN | Waibira | Female | Adult |
| **Philipo** | PHI | Waibira | Male | Sub-Adult |
| **Pavela** | PAV | Waibira | Female | Juvenile |
| **Pili pili** | PLI | Waibira | Female | Infant |
| **Queen** | QEN | Waibira | Female | Adult |
| **Rachna** | RAC | Waibira | Female | Adult |
| **Rita** | RIT | Waibira | Female | Adult |
| **Tibu** | TIB | Waibira | Male | Adult |
| **Robert** | ROB | Waibira | Male | Sub-Adult |
| **Rwenzori** | RWZ | Waibira | Male | Infant |
| **Santa** | SAN | Waibira | Female | Adult |
| **Scovia** | SVA | Waibira | Female | Infant |
| **Shay** | SHY | Waibira | Female | Adult |
| **Sula** | SUL | Waibira | Female | Sub-Adult |
| **Shon** | SHO | Waibira | Male | Juvenile |
| **Lafroig** | LAF | Waibira | Male | Adult |
| **Spini** | SPN | Waibira | Female | Sub-Adult |
| **Soldati** | SDT | Waibira | Male | Juvenile |
| **Tatu** | TAT | Waibira | Female | Adult |
| **TAT1** | TAT1 | Waibira | Female | Infant |
| **Tibu** | TIB | Waibira | Female | Adult |

| | | | | |
|---|---|---|---|---|
| **Tembaya** | TBY | Waibira | Male | Infant |
| **Vesta** | VET | Waibira | Female | Adult |
| **Victor** | VIC | Waibira | Male | Juvenile |
| **Virunga** | VIR | Waibira | Female | Adult |
| **Vincent** | VIN | Waibira | Male | Juvenile |
| **Velu** | VEL | Waibira | NA | Infant |
| **Abooki** | ABO | Waibira | Male | Adult |
| **Alf** | ALF | Waibira | Male | Adult |
| **Atayo** | ATA | Waibira | Male | Adult |
| **Ben** | BEN | Waibira | Male | Adult |
| **Bright** | BRT | Waibira | Male | Sub-Adult |
| **Chandia** | CHN | Waibira | Male | Adult |
| **Douglas** | DOU | Waibira | Male | Adult |
| **Fiddich** | FID | Waibira | Male | Adult |
| **Gerald** | GER | Waibira | Male | Adult |
| **Kasongoire** | KAS | Waibira | Male | Adult |
| **Kevilee** | KEV | Waibira | Male | Adult |
| **Lillo** | LIL | Waibira | Male | Sub-Adult |
| **Macallan** | MAC | Waibira | Male | Adult |
| **Masariki** | MAS | Waibira | Male | Adult |
| **Morangie** | MOR | Waibira | Male | Adult |
| **Mugisha** | MUG | Waibira | Male | Adult |
| **Sam** | SAM | Waibira | Male | Adult |
| **Talisker** | TAL | Waibira | Male | Adult |
| **Tristan** | TRS | Waibira | Male | Adult |
| **Ursus** | URS | Waibira | Male | Adult |

## Ecological Information:

**TABLE 3:** Sonso and Waibira feeding list

| Scientific Name | Site Code | Community |
|---|---|---|
| *Albizia ferruginea* | Af | Sonso |
| *Albizia glaberrima* | Agl | Waibira |
| *Alstonia boonei* | AB | Sonso, Waibira |
| *Antiaris toxicaria* | Ant | Sonso, Waibira |
| *Antrocaryon micraster* | Anc | Sonso, Waibira |
| *Balsamocitrus dawei* | Bd | Sonso, Waibira |
| *Bombax buonopozense* | Bob | Sonso |
| *Broussonetia papyrifera* | Bpy | Sonso |
| *Caloncoba crepiniana* | Clc | Sonso, Waibira |
| *Carica papaya* | paw | Sonso |
| *Celtis gomphophylla* | Cgp | Sonso, Waibira |
| *Celtis mildbraedii* | Cmi | Sonso, Waibira |
| *Celtis philppensis* | Cph | Sonso, Waibira |
| *Celtis zenkeri* | Cze | Sonso, Waibira |
| *Cleistopholis patens* | Cp | Sonso, Waibira |
| *Climbers general* | Cli | Sonso, Waibira |
| *Cola gigantea* | Cog | Sonso |
| *Cordia africana* | Coa | Sonso, Waibira |
| *Cordia millenii* | Com | Sonso, Waibira |
| *Croton megalocarpus* | Cmg | Sonso |
| *Croton macrostachyus* | Cmc | Sonso |
| *Croton sylvaticus* | Csy | Sonso, Waibira |
| *Cynometra alexandri* | Cya | Sonso, Waibira |
| *Desplatsia dewevrei* | Dd | Sonso, Waibira |
| *Dombeya kirkii* | Dok | Waibira |
| *Dovyalis macrocalyx* | Dvm | Sonso |

| | | |
|---|---|---|
| *Drypetes gerrardi* | Dgr | Waibira |
| *Engleropytum oblanceolatum* | Eno | Sonso |
| *Entandrophragama angolense* | Ena | Sonso, Waibira |
| *Entandrophragama cylindricum* | Enc | Sonso |
| *Entandrophragama utile* | Enu | Sonso |
| *Erythropyleum suaveolens* | Es | Sonso, Waibira |
| *Ficus asperifolia* | Fa | Sonso |
| *Ficus ottoniifolia* | Fo | Sonso, Waibira |
| *Ficus barteri* | Fb | Sonso, Waibira |
| *Ficus saussureana* | Fss | Sonso, Waibira |
| *Ficus spp.* | Fic | Sonso, Waibira |
| *Ficus exasperata* | Fe | Sonso, Waibira |
| *Ficus mucuso* | Fm | Sonso, Waibira |
| *Ficus natalensis* | Fn | Sonso, Waibira |
| *Ficus polita* | Fpo | Sonso, Waibira |
| *Ficus sur* | Fsu | Sonso, Waibira |
| *Ficus thonningii* | Fth | Sonso, Waibira |
| *Ficus variifolia* | Fvr | Sonso, Waibira |
| *Ficus vallis-choudae* | Fvl | Sonso |
| *Ficus lingua* | Fl | Sonso, Waibira |
| *Ficus sansibarica* | Fsa | Sonso, Waibira |
| *Gambeya albida* | Gal | Sonso, Waibira |
| *Gambeya gorungosana* | Ggo | Sonso, Waibira |
| *Gambeya perpulchra* | Gpr | Sonso, Waibira |
| *Gambeya muerensis* | Gmu | Sonso, Waibira |
| *Irvingia gabonensis* | Ig | Sonso, Waibira |
| *Khaya anthotheca* | Ka | Sonso, Waibira |
| *Klainedoxa gabonensis* | Klg | Sonso, Waibira |
| *Lannea welwitschia* | Lw | Sonso, Waibira |
| *Lasiodiscus pervillei* | Lp | Sonso, Waibira |
| *Lovoa trichilioides* | Lt | Sonso |
| *Macaranga schweinfurthii* | Ms | Sonso |
| *Maesopsis eminii* | Me | Sonso, Waibira |
| *Mammea africana* | Ma | Sonso, Waibira |
| *Mangifera indica* | Min | Sonso |
| *Mildbraediodendron excelsum* | Mie | Sonso, Waibira |
| *Milicia excelsa* | Mex | Sonso, Waibira |
| *Monodora myristica* | Mom | Sonso |
| *Morus mesozygia* | Mrm | Sonso, Waibira |
| *Myrianthus holstii* | Myh | Sonso, Waibira |
| *Parkia filicoidea* | Pf | Sonso |
| *Picralima nitida* | Pn | Sonso |
| *Pseudospondias microcarpa* | Psm | Sonso, Waibira |
| *Psidium guajava* | Psg | Sonso |
| *Psydrax parviflora* | Psp | Sonso |
| *Raphia farinifera* | Rf | Sonso |
| *Ricinodendron heudelotii* | Rh | Sonso |
| *Sterculia dawei* | Std | Sonso, Waibira |
| *Strychnos mitis* | Sm | Sonso, Waibira |
| *Syzygium guineense* | Szg | Sonso |
| *Trichilia spp.* | Tri | Sonso, Waibira |
| *Trichilia rubescens* | Trr | Sonso, Waibira |
| *Trichilia dregeana* | Trd | Sonso |
| *Uvariopsis congensis* | Uc | Sonso, Waibira |
| *Vepris nobilis* | Vn | Sonso |
| *Alaphia spp.* | Alp | Sonso, Waibira |
| *Basella alba* | Ba | Sonso |
| *Piper guineense* | Ppg | Sonso |
| *Urera camaroonensis* | Urc | Sonso, Waibira |
| *Aframomum spp.* | Afm | Sonso |

| | | |
|---|---|---|
| *Epiphyte* | Epi | Sonso, Waibira |
| *Leptosporangiate spp.* | Fer | Sonso |
| *Marantochloa* | Mrt | Sonso |
| *Megafaunium* | Mgf | Sonso, Waibira |

**TABLE 4:** Species totals and relative abundance from transects conducted in both chimpanzee core areas

| Species | Sonso Species Total | Relative species abundance in Sonso (n=962) | Species | Waibira Species Total | Relative species abundance in Waibira (n=818) |
|---|---|---|---|---|---|
| *Funtimia elastica* | 211 | 22% | *Celtis mildbraedii* | 100 | 12% |
| *Celtis mildbraedii* | 123 | 13% | *Lasiodiscus pervillei* | 98 | 12% |
| *Celtis zenkeri* | 48 | 5% | *Funtimia elastica* | 85 | 10% |
| *Cynometra alexandri* | 47 | 5% | *Cynometra alexandri* | 53 | 6% |
| *Khaya anthotheca* | 42 | 4% | *Celtis zenkeri* | 38 | 5% |
| *Lasiodiscus pervillei* | 42 | 4% | *Rinorea beniensis* | 35 | 4% |
| *Trichilia prieuriana* | 39 | 4% | *Croton sylvaticus* | 33 | 4% |
| *Croton sylvaticus* | 28 | 3% | *Celtis gomphophylla* | 31 | 4% |
| *Celtis gomphophylla* | 21 | 2% | *Pouteria altissima* | 20 | 2% |
| *Trilepisium madagascariense* | 20 | 2% | *Trichilia prieuriana* | 20 | 2% |
| *Margaritaria discoidea* | 19 | 2% | *Khaya anthotheca* | 18 | 2% |
| *Tapura fischeri* | 19 | 2% | *Alchornea laxifora* | 16 | 2% |
| *Antiaris toxicaria* | 17 | 2% | *Margaritaria discoidea* | 15 | 2% |
| *Trichilia rubescens* | 16 | 2% | *Trichilia rubescens* | 15 | 2% |
| *Lychnodiscus cerospermus* | 15 | 2% | *Alstonia boonei* | 12 | 1% |
| *Caloncoba crepiniana* | 14 | 1% | *Celtis philppensis* | 11 | 1% |
| *Ficus exasperata* | 13 | 1% | *Tapura fischeri* | 10 | 1% |
| *Gambeya albida* | 13 | 1% | *Ehretia cymosa* | 9 | 1% |
| *Rinorea beniensis* | 12 | 1% | *Myrianthus holstii* | 9 | 1% |
| *Alstonia boonei* | 10 | 1% | *Psidium guajava* | 9 | 1% |
| *Tabernaemontana pachysiphon* | 10 | 1% | *Lychnodiscus cerospermus* | 8 | 1% |
| *Albizia glaberrima* | 9 | 1% | *Caloncoba crepiniana* | 7 | 1% |
| *Myrianthus holstii* | 9 | 1% | *Ficus sur* | 7 | 1% |
| *Entandrophragama angolense* | 8 | 1% | *Gambeya albida* | 7 | 1% |
| *Maesopsis eminii* | 8 | 1% | *Gambeya muerensis* | 7 | 1% |
| *Ricinodendron heudelotii* | 8 | 1% | *Maesopsis eminii* | 7 | 1% |
| *Alchornea laxifora* | 7 | 1% | *Trilepisium madagascariense* | 7 | 1% |
| *Ehretia cymosa* | 7 | 1% | *Albizia zygia* | 6 | 1% |
| *Ficus sur* | 7 | 1% | *Antiaris toxicaria* | 6 | 1% |
| *Tetrapleura tetraptera* | 7 | 1% | *Unknown* | 6 | 0% |
| *Vepris nobilis* | 7 | 1% | *Vepris nobilis* | 6 | 1% |
| *Alangium chinense* | 6 | 1% | *Belonophora coffeoides* | 5 | 1% |
| *Baphia wollastonii* | 5 | 1% | *Desplatsia dewevrei* | 5 | 1% |
| *Cordia millenii* | 5 | 1% | *Entandrophragama angolense* | 5 | 1% |
| *Holoptelea grandis* | 5 | 1% | *Holoptelea grandis* | 5 | 1% |
| *Milicia excelsa* | 5 | 1% | *Maerua duchesnei* | 5 | 1% |
| *Pouteria altissima* | 5 | 0% | *Albizia glaberrima* | 4 | 0% |
| *Ficus variifolia* | 4 | 0% | *Bombax buonopozense* | 4 | 0% |
| *Gambeya perpulchra* | 4 | 0% | *Lannea welwitschii* | 4 | 0% |
| *Glenniea africana* | 4 | 0% | *Leplaea cedrata* | 4 | 0% |
| *Unknown* | 4 | 0% | *Tetrapleura tetraptera* | 4 | 0% |
| *Entandrophragama cylindricum* | 3 | 0% | *Alangium chinense* | 3 | 0% |
| *Leplaea cedrata* | 3 | 0% | *Gambeya perpulchra* | 3 | 0% |

| | | | | | |
|---|---|---|---|---|---|
| *Morus mesozygia* | 3 | 0% | *Greenwayodendron suaveolens* | 3 | 0% |
| *Strombosia scheffleri* | 3 | 0% | *Klainedoxa gabonensis* | 3 | 0% |
| *Albizia zygia* | 2 | 0% | *Macaranga barteri* | 3 | 0% |
| *Antidesma laciniatum* | 2 | 0% | *Macaranga monandra* | 3 | 0% |
| *Blighia unijugata* | 2 | 0% | *Pseudospondias microcarpa* | 3 | 0% |
| *Desplatsia dewevrei* | 2 | 0% | *Cleistopholis patens* | 2 | 0% |
| *Erythrina excelsa* | 2 | 0% | *Cordia millenii* | 2 | 0% |
| *Greenwayodendron suaveolens* | 2 | 0% | *Ficus mucuso* | 2 | 0% |
| *Macaranga barteri* | 2 | 0% | *Ficus variifolia* | 2 | 0% |
| *Trichilia dregeana* | 2 | 0% | *Glenniea africana* | 2 | 0% |
| *Turraea vogelioidestrobusta* | 2 | 0% | *Manilkara dawei* | 2 | 0% |
| *Vitex ferruginea* | 2 | 0% | *Paropsia guineensis* | 2 | 0% |
| *Albizia ferruginea* | 1 | 0% | *Tetrorchidium didymostemon* | 2 | 0% |
| *Antidesma membranaceum* | 1 | 0% | *Thecacoris lucida* | 2 | 0% |
| *Antrocaryon micraster* | 1 | 0% | *Vitex ferruginea* | 2 | 0% |
| *Belonophora coffeoides* | 1 | 0% | *Albizia ferruginea* | 1 | 0% |
| *Bridelia micrantha* | 1 | 0% | *Balanites aegyptiaca* | 1 | 0% |
| *Canarium schweinfurthii* | 1 | 0% | *Balsamocitrus dawei* | 1 | 0% |
| *Celtis philppensis* | 1 | 0% | *Blighia unijugata* | 1 | 0% |
| *Coffea spp.* | 1 | 0% | *Bridelia brideliifolia* | 1 | 0% |
| *Erythropyleum suaveolens* | 1 | 0% | *Engleropytum oblanceolatum* | 1 | 0% |
| *Fagaropsis angolensis* | 1 | 0% | *Fagaropsis angolensis* | 1 | 0% |
| *Ficus mucuso* | 1 | 0% | *Ficus lingua* | 1 | 0% |
| *Ficus saussureana* | 1 | 0% | *Ficus saussureana* | 1 | 0% |
| *Gambeya muerensis* | 1 | 0% | *Glyphaea brevis* | 1 | 0% |
| *Lepisanthes senegalensis* | 1 | 0% | *Kigelia africana* | 1 | 0% |
| *Leptactina arborescens* | 1 | 0% | *Leptonychia mildbraedii* | 1 | 0% |
| *Leptonychia mildbraedii* | 1 | 0% | *Majidea fosteri* | 1 | 0% |
| *Lovoa trichilioides* | 1 | 0% | *Monodora angolensis* | 1 | 0% |
| *Mallotus oppositifolius* | 1 | 0% | *Oxyanthus speciosus* | 1 | 0% |
| *Mammea africana* | 1 | 0% | *Premna angolensis* | 1 | 0% |
| *Mildbraediodendron excelsum* | 1 | 0% | *Ritchiea albersii* | 1 | 0% |
| *Monodora angolensis* | 1 | 0% | *Rothmannia urcelliformis* | 1 | 0% |
| *Oxyanthus speciosus* | 1 | 0% | *Tabernaemontana pachysiphon* | 1 | 0% |
| *Picralima nitida* | 1 | 0% | *Treculia africana* | 1 | 0% |
| *Pseudospondias microcarpa* | 1 | 0% | *Uvariopsis congensis* | 1 | 0% |
| *Raphia farinifera* | 1 | 0% | | | |
| *Sterculia dawei* | 1 | 0% | | | |
| *Strychnos mitis* | 1 | 0% | | | |

**TABLE 5**: General tree abundance across forest transects (September 2022)

| Transect # | Community (Location in Home Range) | Lines | Total Trees in Transect |
|---|---|---|---|
| **T1** | Sonso (East) | 4,1 to 8,1 | 173 |
| **T2** | Sonso (West) | F,B to F,2 | 266 |
| **T3** | Sonso (North) | B,F to 2,F | 333 |
| **T4** | Sonso (South) | B,8 to B,12 | 190 |
| **T5** | Waibira (North) | 27,N to 27, J | 216 |
| **T6** | Waibira (East) | 37,H to 37,D | 206 |
| **T7** | Waibira (West) | 16,F to 20,F | 237 |
| **T8** | Waibira (South) | 25,2 to 29,2 | 159 |

## Maps and Figures:

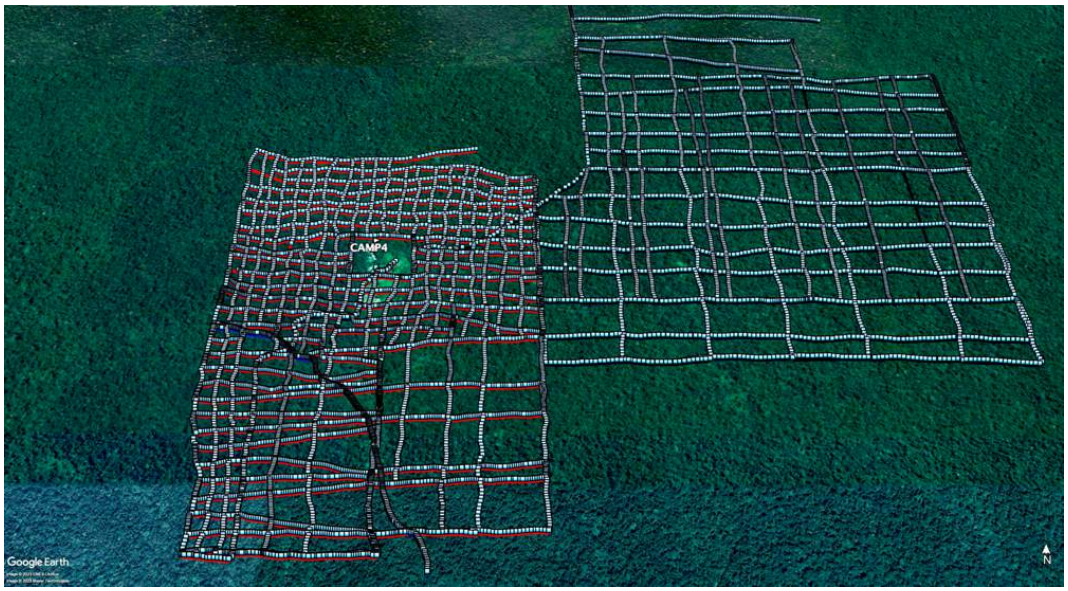


***Figure 1**: Map of Sonso (red) and Waibira (blue) home ranges*

***Figure 2:** Illustrations of the BCFS basecamp, July 2021*

# APPENDIX B:
# General Materials and Methods

## 1. Introduction

To better understand self-medication amongst wild chimpanzees in Budongo Forest I conducted a comparative behavioral study, working with two wild communities of chimpanzees at the Budongo Conservation Field Station in Uganda, Sonso and Waibira. To identify putative self-medicative feeding events I employed a three-prong approach, using behavioral data, health monitoring, and pharmacological testing. To collect behavioral data, I filmed all feeding bouts and unusual events and live coded data on the Animal Observer application for iPad. I also recorded GPS coordinates of every observed feeding event to track spatial distribution of these resources. To monitor general health in the community as well as the health state of individuals exhibiting unusual behaviors, I collected fecal samples and used microscopic and macroscopic parasitology methods to quantify parasite loads. I also conducted opportunistic urinalysis testing. To better understand the pharmacological properties of these resources, I collected, identified, and tested suspect plants for their bioactive properties. A high-level and comprehensive workflow of my general methods can be found in **Figures 1** and **2**.

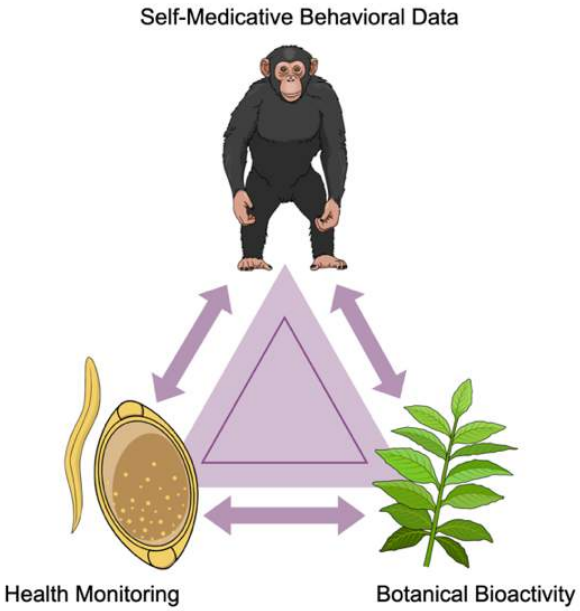


***Figure 1:*** *High-level methodological workflow*

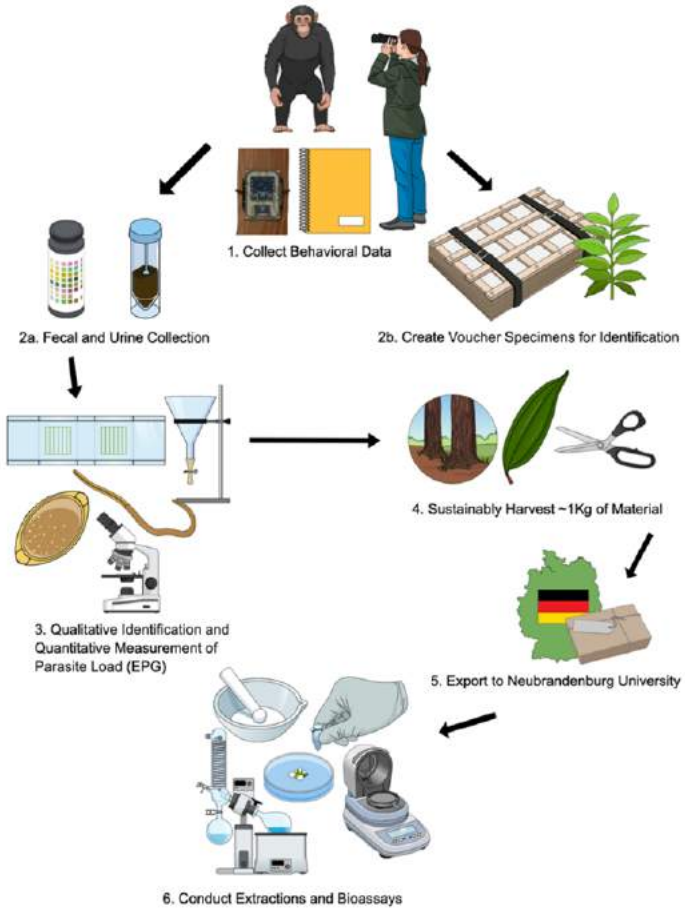


***Figure 2:*** *Comprehensive methodological workflow*

My first four-month field season was completed with the Sonso chimpanzee community (June-October 2021). My second four-month field season was completed with the neighboring Waibira chimpanzee community (June-October 2022). Field seasons were scheduled for the same months across both years to control for temperature and rainfall, food availability, and parasite infection rates. The decision to run this study in neighboring communities was made to enable comparison between dietary repertoires of communities which share an ecological niche. Budongo was an ideal field site to conduct this research as the Sonso and Waibira communities have overlapping home ranges, and both are well habituated to human researchers. Below is a detailed explanation of the methods I employed during this project.

## 2. Behavioral Data Collection

Focal sampling (sensu Altmann, 1974) was used to collect behavioral data. Data was recorded using three techniques: video recording, live coding, and ethnographic notetaking for contextual information. Throughout Field Season 1, I worked with staff primatologist, Geresomu Muhumuza (GM) who has worked in Budongo since 1991 and lived at the research station since

1993. GM instructed me on the identification and behaviors of the Sonso chimpanzees and shared with me his encyclopedic botanical knowledge. Throughout Field Season 2 I worked with Eguma Robert Yikii. ERY's background in forestry was critical for my investigation into chimpanzee bark stripping behaviors, as well as his insight into motivations behind the illegal logging operations which are pervasive in the forest. Below are descriptions of methods used for selecting focal individuals, what information was recorded, and how data was taken.

**2.1 Focal Follows**

To collect behavioral data, I employed observational, pseudo-random, day-long focal follows (sensu Altmann, 1974). Nest-to-nest focalling is not practiced at Budongo, so focals are left each evening and new focals are selected the next morning. When an individual exhibited sickness behaviors or ingested a putative, unusual, and/or established medicinal resource, consecutive follows could not be guaranteed. To select a focal, I compiled a sample group of the dependent adult-infant/adult-juvenile dyads in each community. My methodology originally called for the prioritization of mother-infant pairs, but as the Sonso community in Budongo had three dependent male orphans who travel with adult males, I decided upon arrival to include orphan-adult pairs in my prioritized focal group. From these target pairs, I opportunistically focalled the first pair I found each morning, avoiding consecutive-day follows unless at least one member of the pair was considered sick, wounded, or had recently exhibited unusual feeding behaviors. When adults had more than one dependent offspring, I selected one principal dyad to focal but also took ad libitum data on the third individual. Of my original Sonso target individuals, I collected behavioral data from 13 out of 14 priority dyads.

In Waibira, due to under-habituation of females and shyness from mothers with dependent offspring, consistently focalling mother-infant pairs proved impossible. When present, mother-infant pairs were prioritized, however, data is dramatically skewed toward males and Sonso-migrant females who were highly habituated. This bias is also present in the Waibira parasite data as fecal samples were difficult to attain from females. Priority focals in Waibira, therefore, tended to be more often chosen based on observation of unusual feeding behaviors and health assessments.

When a sick individual was identified in the group, and all members of the adult-offspring sample group appeared healthy, I followed that individual until the focal period ended or the individual was lost. Sick individuals were defined in this project as individuals exhibiting any of the sickness behaviors classified in **Chapter 1: Table 2**, those who were actively shedding

cestode proglottids, or those who had a diverse species richness (>3 parasite species). If the individual continued to exhibit sickness behaviors over multiple days or showed a sustained diverse or intense infection, the individual was considered a '**priority focal**.' I continued to prioritize these focals until symptoms subsided, I was unable to find them, or I obtained three consecutive focal follows. If I could not find a priority individual on a consecutive day by 11:00am, I selected the first mother-infant, mother-juvenile, male-adopted-orphan pair I encountered. If I still could not find a pair in the focal sample group, I selected a random individual based on the first opportunistic encounter. When I had multiple priority focals, I randomly selected one to follow.

When I made *ad libitum* observations of a non-focal individual ingesting a self-medicative or unusual resource and/or demonstrating a self-medicative or unusual behavior, I recorded the event but stayed with my focal individual, as focal switching is not permitted at BCFS. If multiple non-focal individuals simultaneously began self-medicating or feeding on unusual resources independently, I recorded behaviors for the closest individual and attempted to record as many of the other events as possible.

### 2.2 Live Coding on Animal Observer (AO)

During focal follows, directional behaviors, and self-directed behaviors were live coded on Animal Observer (AO), a behavioral software designed for iPad. I chose AO for its efficiency, user interface, and ability to code social interactions as directional behaviors. Directional behaviors were defined as social behaviors that involved inherent interaction between multiple individuals (i.e., grooming, begging, feeding, copying, nursing etc.). Self-directed behaviors included non-interactive behaviors (i.e., travel, feeding, day nesting, etc.). If observer(s) were present and made any directional behaviors (ie. begging, peering, inspecting, retrieving a dropped resource etc.) toward the individual engaged in the feedings event, these behaviors were recorded on AO. Throughout the day, I noted relevant independent variables for the focal under 'Global Settings' including whether a fecal sample had been collected, presence/absence of visible worms, presence of sickness and/or self-medicative behaviors in the past seven days, current presence/absence of sickness behaviors, presence of day nest, reproductive status on a scale from 1-4 (when applicable). The coding scheme on AO was programmed prior to the arrival in the field in June 2021 and amended throughout the first month of data collection. Data used in this thesis was recorded using the V.6 version of my AO coding scheme.

### 2.3 Camcorder Footage

All feeding events which fulfilled one or more of following conditions was filmed using a Sony HandyCam CX250 with 50x zoom:

1. An adult was feeding within 3m of an infant or juvenile
2. An individual was feeding on an unusual food (i.e., bark, dead wood, animal, invertebrate product, plant pith, whole leaf, termite soil, clay, water)
3. An individual was feeding on a food when others around him/her were not
4. An individual was feeding alone
5. An individual or multiple individuals travelling to an unusual location to feed
6. An individual consuming food was exhibiting sickness behaviors or was known to have an altered health state

When filming an event, I narrated as much information as possible into the camera, including which individuals were present, to ensure more accurate *post hoc* analysis. If I was unable to film an event of interest, I recorded the resource after it had been dropped and dictated relevant details.

### 2.4 Focal Follow Index and Census Data

Each day I completed a **Focal Follow Index** spreadsheet with information on which individual was focalled. This data also included whether the focal exhibited sickness behaviors, their reproductive state (when applicable), any unusual behaviors, and a complete daily feeding list for the individual, which also included plant part and processing technique. Any relevant *ad libitum* information from non-focal individuals was also recorded, including unusual resources they ate or any unusual behaviors. A **Census Data Sheet** was also completed each day which included information on which individuals were seen, reproductive status of any observed females, cases of diarrhea, sickness behaviors, or wounds.

### 2.5 Master Event List and Unusual Feeding List

Each day I wrote an ethnographic report of everything I observed in the forest, including in-depth descriptions and context of interesting events observed throughout the day. In these descriptions I tried to record as many qualitative variables as possible, including but limited to: weather changes, anthropogenic sounds coming from the villages, and seasonal information on fruiting trees. I recorded notes in a notebook which I digitally transcribed at the end of each day. All unusual feeding behaviors were also summarized each day in a separate **Unusual Feeding List** which including the date, the consumer ID, the resource, the processing type, and whether

the event was recorded on video. This list included all events, both focal and *ad libitum*. Events in which peering, watching, or begging were also noted in this list.

### 2.6 Camera Traps

To increase sample size, motion and infrared triggered Bushnell Trophy camera traps were installed at two respective sites during Field Season 1. Following (Pebsworth et al., 2019), these camera traps were placed ~20 cm above the ground near fixed locations and operated continuously for 2.5 months (July-October 2021), recording 59s videos with 1s intervals. Once retrieved, these videos allowed for assessment of which faunal species utilize these sites and whether sites are ever occupied by multiple species simultaneously in addition to supplemental self-medicative behavioral and demographic data. Weekly checks were scheduled to change batteries, memory cards, and download data.

The first camera trap was installed at a *Cleistopholis patens* dead tree site after two individuals were observed stripping and eating the dead wood. The second camera was installed at one of the four clay sites in Sonso where individuals were repeatedly observed consuming clay-water during early focal follows in June 2021. Over the course of a three-month period, over 190 videos were captured, 83 of which included chimpanzees.

## 3. Spatial Data

I collected GPS waypoints for all sites in which normal feeding and medicinal events took place allowing for *post hoc* spatial analysis (**Chapter 6**). To do this, I collected GPS waypoints on a Garmin Rhino 650 for all locations in which a focal individual was observed ingesting, drinking, or processing resources. The location of all normal feeding events, putative therapeutic events, and established self-medicative events were saved in UTM format. All rare or unusual events (ie. interspecies interactions, major aggression events, leaf sponging) were also saved. When it was impossible to reach the exact point of the resource, I got as close as I could before marking the waypoint. If I missed a GPS point, I marked the time of the event and added the waypoint during data backup, by locating the location from the corresponding time stamp on the GPS track for the day. Tracks were also recorded every day to trace routes taken to each resource.

## 4. Health Monitoring

According to Huffman's (1997) criteria, to establish a novel self-medicative resource, health data should be available from before and after a putative medicinal event, to demonstrate an

improvement in the individual's health following ingestion. Therefore, throughout the study, we conducted opportunistic health monitoring, which included macroscopic and microscopic fecal analysis, as well as urinalysis testing.

### 4.1 Macroscopic Analysis

To conduct macroscopic monitoring on fecal samples, all chimpanzee fecal samples observed in the field were visually examined for presence of visible helminths and proglottids. If present, the number of visible organisms were counted and recorded, and the sample was photographed. For all samples, stool consistency, color, and the presence of any whole leaves or identifiable plant parts was recorded (McLennan & Huffman, 2012)

### 4.2 Microanalysis and Fecal Samples Collection

Conducting microanalysis required fecal sample collection. My methodology called for opportunistic collection of samples from all group members, provided I had a positive ID on the individual. Collected samples were taken back to camp at the end of each field day and placed in the freezer. Identification of parasite eggs and quantification of parasite load were carried out in the veterinary lab at BCFS by D. Sempebwa. Two techniques were used in microanalysis: qualitative identification of parasite eggs, and quantitative measurement to assess EPG.

***Figure 3**: Preparation of McMaster slide*

#### 4.2.1 McMaster Method for Parasite Quantification

Parasite loads were assessed using the McMaster flotation technique, a method commonly used in zoopharmacognosy studies (e.g., Huffman et al., 1997; 1993). To do this, we followed the BCFS standard protocols created by D. Sempebwa in 2020. This technique is used to identify and quantify the number of parasitic eggs per gram (EPG) in each fecal sample. The specimens we tested were fresh with no ethanol or formalin added. Methods were followed from WHO's Bench Aid Protocols (2019). McMaster slides were prepared by creating a high-density solution of sugar and water (128 g sugar to 1 L of water) (**Figure 3**). Next, 3 grams of fecal sample were mixed into the solution and the new solution was filtered through two layers of gauze. The high density of the solution caused the eggs in the fecal sample to rise. A pipette was then used to fill the McMaster slide with solution from the top of the sample, making sure that there were

***Figure 4**: D. Sempebwa using the McMaster Method at BCFS*

no bubbles. The slide was left for 5 minutes to allow for the eggs to rise to the surface. When the slide was prepared, it was placed under the microscope at x10 magnification and examined, the highest magnification possible (**Figure 4**). This magnification was x40 less than the magnification used by Gillespie et al., (2010) who called for a compound microscope at 400x to the nearest 0.1 µm with an ocular micrometer. For each parasite egg seen, one count was marked. Once both chambers were assessed, the total counts were multiplied by 50 to calculate the EPG of the sample. When only one chamber was counted, EPG was determined by multiplying the number of parasitic elements by 100.

### 4.2.2 Quantifying Protozoa

The McMaster method was also used to identify and quantify certain species of protozoa, but as they are small and not colorful protozoa can be easily missed during microanalysis. Therefore, the number reported for protozoa in these results likely underrepresent the actual frequencies. As our lab lacked the solution required for identifying specific protozoa, we only conduct only high-level qualitative analysis.

## 4.3 Procedure for Collection of Visible Helminths or Proglottids

When worms or proglottids were found in samples during macroanalysis, they were placed in prepared tubes containing 60% ethanol solution, and the fecal sample was collected. When possible, photographs were taken of the sample *in situ.* In the lab, the worm or proglottid was kept in ethanol for two weeks, then dried in a sealed container with silica beads. After another two weeks these samples were placed in the refrigerator. For identification, proglottids were sent to the Natural History Museum in London (Authorization number: ITIMP21.1550).

## 4.4 Urinalysis Testing

Urinalysis is commonly used in veterinary health monitoring practices as a non-invasive diagnostic tool (Kurien et al., 2004; Macintosh & Huffman, 2012). Following the methodology of Kaur and Huffman (2004), I opportunistically used rapid urine screening tests (multi-reagent Urine Dipstick Test 9-RC for Urotron RL9) to assess the health and physiological status of chimpanzees. These tests measure pH levels, leukocytes, urobilinogen, bilirubin, nitrites, specific gravity, blood, protein, glucose, and ketones. In addition to being cheap, portable, and rapid (60-120 seconds), results are easy to interpret using a provided colorimetric scale. For specific implications of each parameter, I referred to Simerville et al. (2005).

To conduct urinalysis, I swabbed tests on wet substrates, immediately after micturition by an identified individual. As substrate did not appear to affect samples (Kaur & Huffman, 2004; Krief et al., 2005), urine samples were taken from leaves, rocks, and the ground, but not from feces (Macintosh & Huffman, 2012) or samples which had changed in color post-micturition. After swabbing, the color of each pad was compared with the colorimetric chart calibrated to various levels of the chemical parameters and interpreted *in situ*. If the test could not be taken immediately, urine was collected using a sterilized pipette and transferred to a sterile specimen container where it was tested within a 5-hour window (Kaur & Huffman, 2004).

## 5. Botanical Collection Methods

Throughout both field seasons I collected botanical specimens for identification and pharmacological analyses. Samples collected for bioactivity testing (see **Chapter 6**) were exported to Neubrandenburg University of Applied Sciences in Germany. For every batch of exported samples, I also produced a set of voucher sample (in duplicate) for identification. Protocols for botanical collection are outlined below.

### 5.1 Creating Voucher Specimens

***Figure 5:*** *Voucher specimen of Scepocarpus trinervis*

To create voucher specimens in the field, I collected two representative samples and stored them in a plastic bag. When possible, samples included intact material of leaves, reproductive organs, and/or seed pods which could aid in taxonomic identification. Bags were labelled with the time, date, and GPS coordinates (UTM).

At the field station, I photographed the samples and pressed them in the station's herbarium. When the sample was sufficiently dry (~4 days), plants were removed from dryer sheets and adhered to thick paper with a corresponding label (**Figure 5**). Specimens were then stored in a cool, dark place.

### 5.2 Collecting Plants for Pharmacological Testing

During the second week of September 2021 and 2022, I conducted targeted botanical collecting for bioactivity testing. For each sample, we aimed to collect ~1kg of material, however, in many

cases we were not able to collect this much due to the size of the sample. To select which resources became candidates for bioactivity testing, I used anecdotal data from putative therapeutic events I had observed (see **Chapter 5**). I also systematically collected all barks historically observed in bark stripping events in the Sonso and Waibira communities. This list was compiled using the long-term site data.

All material for each sample was taken from the same tree or plant and placed in the same bag for storage. Bags were labelled with the date of collection, species, GPS coordinate, and collection ID. If different plant parts from the same individual plant were collected, each part was given a unique collection ID. I photographed all trees from which we harvested plant material. To reduce contamination, we removed moss and lichen from bark before harvesting. Each plant was considered potentially toxic and thus all collection was done wearing protective gloves using a machete.

### 5.2.1 Sustainable harvesting

Sustainable harvesting was practiced when collecting plant material. When we need to collect a whole plant, I only collected from areas in which there were numerous plants of that species remaining. When collecting bark, I only collected from trees old enough to survive debarking.

***Figure 6:*** *EF collecting botanical specimens in Budongo Forest*

### 5.2.2 Drying and Processing Plants in the BCFS Herbarium

After collection, plant material was dried in the field station's herbarium under a shaded roof, with open-air, screened-in, windows. To prevent samples from becoming moldy or desiccated before export, plants had to be completely dry. To avoid contamination, each type of plant part was prepared separately. To do this, I cut material into small pieces with gardening clippers. As I processed samples, I removed remaining debris or insects that were accidently collected with the material. Plant material was then laid out to dry on newspapers in the shade as bioactive

substances could be lost to evaporation, and as exposure to UV easily damaged bioactive compounds.

***Figure 7:*** *Barks dry in the station's herbarium, September 2022*

Throughout drying period, plants were turned several times a day to ensure that the drying was uniform, and that mold could not begin to grow. When material was fully dried, samples were stored in individual paper bags and sealed with tape. Bags were labelled with the collection ID, the scientific name and/or the local name. Sealed bags were kept in a sealed plastic box until export.

### 5.2.3 Exporting Plant Samples

Botanical samples from Field Season 1 were exported under the Authority License to Export Plant/Soil Materials of Uncertain Health Status permit (UQIS 00005033/93/PC) issued on 24/9/2021 and followed all stipulations of the Nagoya Protocol (2014). Botanical samples from Field Season 2 were exported under the Authority License to Export Plant/Soil Materials of Uncertain Health Status permit (UQIS 00008007/93/PC) issued on 20/10/2022.

## 5.3 LIDAR Tree Scanning

During the second field season (June-October 2022), when indirect evidence of bark stripping was opportunistically observed or found on transects, LiDAR scans were taken of the tree using 3D Scanning App on iPad. Measurements made from these scans were reliability tested in the field.

***Figure 8****: [Left] LiDAR scan of a stripped F. mucuso tree, indirectly observed in Waibira [Right] EF scanning stripped tree*

## 6. Forest Transects

With Moses Businge (MB), I walked eight line-transects (four in each of the two habituated community home ranges) between September 1st and October 10th, 2022. Each line-transect was 400m long and was located within the pre-cut trail system, four ran north-south and four ran east-west (Hedges & McGrew, 2012). Transects were selected at random from samples stratified by cardinal direction in each home range. Trees were measured on both sides of the trail within 5m of the trail center. All trees >12cm dbh (diameter at breast height) were measured, as no evidence of bark stripping had been observed on trees smaller than this. All living trees that met criteria were measured for dbh and identified by MB, director of the phenology team at the station. When trees showed evidence of bark stripping (fresh wounds, teeth marks, wadges, or characteristic signs of regrowth) we took GPS points, counted the number of strips, measured the longest strip, and quantified stages of bark healing over past strips on a 1-4 scaling, using the photographic reference metric established by (Lapuente et al., 2020). Trees were then scanned using 3D Scanner on iPad for *post hoc* measurements. If tree was not known or visibility too poor for ID it was marked as 'UNK' and dbh was taken when possible. For interobserver reliability, a random sample of photographs from each transect, including both stripped and non-stripped trees were taken to an independent member of field staff and scored for stripping status. If original status and reliability status matched, the tree remained marked as *stripped*, if there was disagreement, the tree was marked *equivocal*.


***Figure 9:*** *Map of Sonso (white) and Waibira (orange) core areas where transects were conducted;* ***NB****:* Yellow rectangles indicate the locations of 400m transects

***Figure 10:*** *B. MB measuring an MDI tree during transects*

## 7. Pharmacological Assays

In January 2022 I travelled to Neubrandenburg University of Applied Sciences to conduct pharmacological testing on exported plant samples from Field Season 1. Further details on these tests, results, and discussion can be found in **Chapter 5**. All protocols for the follow bioactivity

assays were created and formalized by Dr. Fabien Schultz in 2016 and updated in 2021. These methods were sourced from a protocol originally produced by Quave et al. (2008).

### 7.1 Extractions

Extractions were performed to separate potentially bioactive compounds from the rest of the botanical material. Extractions were achieved by means of maceration in three different solvents: n-hexane (h), methanol-water (mw), and ethyl acetate (e). In total we created 51 crude extracts from a total of 17 plant samples (13 total species). Extractions took ~1 month to complete. See **Figure 11** for diagram of extraction workflow.


***Figure 11:*** *Workflow for sample extraction conducted at the Neubrandenburg University of Applied Sciences*

#### 7.1.1 Preparation of retention voucher

Before beginning extractions, retention vouchers were prepared. To do this we defrosted the sample bag which had been stored in the freezer (-20°C) since arrival at the University (~5 months before). A small sample of the dried material was placed in a bag (1-50 g) and sealed, ensuring that the voucher was a homogenic representation of the sample. The retention voucher was then placed in another zip bag and vacuum sealed, for storage at -20 °C in the retention voucher library.

### 7.1.2 Processing Raw Samples

Remaining plant material was ground up using a food processor. Processing was done in short pulses to ensure the processor did not get too hot. Processing continued until the whole sample was chopped into fine pieces or a powder.

### 7.1.3 Weighing the Processed Material

The processed material was then weighed, after the scale was tared with an empty Erlenmeyer flask. Three glass bottles were prepared, and an appropriate amount of sample material was transferred into each container using a powder funnel and spoon (lab scale, non-analytical). Extraction flasks/bottles were labelled with collection ID.

### 7.1.4 First Maceration

Extractions were done with three organic solvents, each with a different polarity, resulting in three crude extracts per sample: *n*-hexane, ethyl acetate and methanol-water (9:1, v/v). Once each flask was weighed, ten to thirty times the amount of the respective solvent was added to the corresponding flask. Each flask was then covered with aluminum foil, labeled, and placed in a shaker at 60rpm for at least 72 hours under a fume hood.

### 7.1.5 First Filtration

Next the material underwent a first filtration in vacuo, using filter paper and Buchner funnel. If any solids remained in the solution, we filtrated a second time using a filter with a finer pore size. Remaining sample material was then transferred back into the flask containing the plant material for later use. The filtrate was transferred into a round bottom flask and labeled. Collection ID was written on both sides of the flask and sealed with a lid. When the filtrate was not immediately vacuum evaporated, the lid was sealed with parafilm, and the filtrate was stored at 4° C.

***Figure 12**: An ethyl acetate extract after first filtration step*

### 7.1.6 First Vacuum Evaporation

To complete the first vacuum evaporation, machine temperature must have reached 5°C. The water bath was then turned on and set to 40°C. When methanol-water or *n*-hexane extracts were being evaporated, the evaporator had to be fully under the fume hood. Through rotation, the filtrate was concentrated in the evaporator until the sample was almost dry. The solvent, which had evaporated into a flask attached to the evaporator was recovered and transferred back into flasks with original plant material to begin the second maceration. The lid was put on the round bottom flask containing the sample concentrate (formerly the filtrate) and sealed with parafilm. Sample concentrates were then stored in the fridge at 4°C.

### 7.1.7 Second Maceration

After the solvent was recovered from the evaporator, it was added back into the flask containing the original plant material (section **7.1.6**). We then measured the amount of the recovered solvent and added fresh solvent if needed. The flasks were then relabeled and covered with aluminum foil. These flasks were placed on the shaker at 60 rpm for an additional 72 hours under the fume hood.

***Figure 13:*** *Plant material in solvents on the shaker, awaiting 2nd Maceration stage*

***Figure 14:*** *Extract being finalized in evaporator after transfer from round bottom flask*

***Figure 15:*** *Pipetting the extract from round bottom flask into extract container*

### 7.1.8 Second Filtration

During the second filtration step, the sample was fine filtered in vacuo using filter paper and Buchner funnel. If any solids remained in the solution, a second filtration was conducted, this time using a filter with a smaller pore size. The plant material was then transferred into the Buchner funnel to ensure complete solvent collection, and it was left under the fume hood for drying, making disposal easier. The second filtrate was then combined with the first concentrate. If there was no immediate plan for evaporating the combined filtrate, it was sealed with parafilm and stored in the refrigerator at 4°C. Lastly, to maintain clarity in labeling, the round-bottom flask was marked with "2nd Maceration," with the instruction to remove the "1st Maceration label" only.

### 7.1.9 Second Vacuum Evaporation

Steps from First Vacuum Evaporation were repeated.

### 7.1.10 Drying and Extract Finalization

An empty 50ml screw-lid extract container was first weighed and then labelled. The concentrated sample was then transferred into a container using a Pasteur pipette (for rinsing flask with acetone) and spatula (for scraping residual off flask walls). Slightly different procedures were followed for oily and waxy concentrates. Samples were then put back in the vacuum evaporator until fully dry. Extracts were then weighed in the container and the final extract mass and yield was calculated by subtracting the weight of the empty container. Extract was then once again transferred from 50ml containers to smaller tubes for placement in the extract library Labels were applied to tubes, lids were sealed, and extracts were placed in a freezer at -20 °C for permanent storage at the University of Neubrandenburg.

***Figure 16:*** *Finalized extraction tubes being prepared for the Library Screen*

### 7.2 Antibacterial and Anti-inflammatory Assays

After extractions, antibacterial and anti-inflammatory assays were carried out between March-September 2022. Methods used for these extracts are detailed in **Chapter 5**.

## 8. Data Management

To protect democratic access and avoid reproducibility issues, I ensured that all methods employed in this project were transparent and publicly accessible, both in this thesis and in all ongoing publication manuscripts. Raw datasets have been uploaded to Google Drive and are stored on external hard drives. Feeding ecology, behavioral, transect, and health data collected during this project will be shared upon appropriate request.

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# APPENDIX C:
# Supplementary Material Chapter 3:
## Healthcare Behaviors in the Wild Chimpanzees of Budongo Forest

### Supplementary Information:

**List of Contributing Observers:**

***[Table 5: Leaf Swallowing Observations]***

Observation 1. Events Book: Zephyr and Clea
Observation 2. GAD: M. Laporte
Observation 3. GAD: M. Laporte
Observation 4. CSMAS: A. Soldati
Observation 5: CSMAS: A. Soldati
Observation 6. CSMAS: A. Soldati
Observation 7. CSMAS: A. Soldati
Observation 8. Current Study: E. Freymann

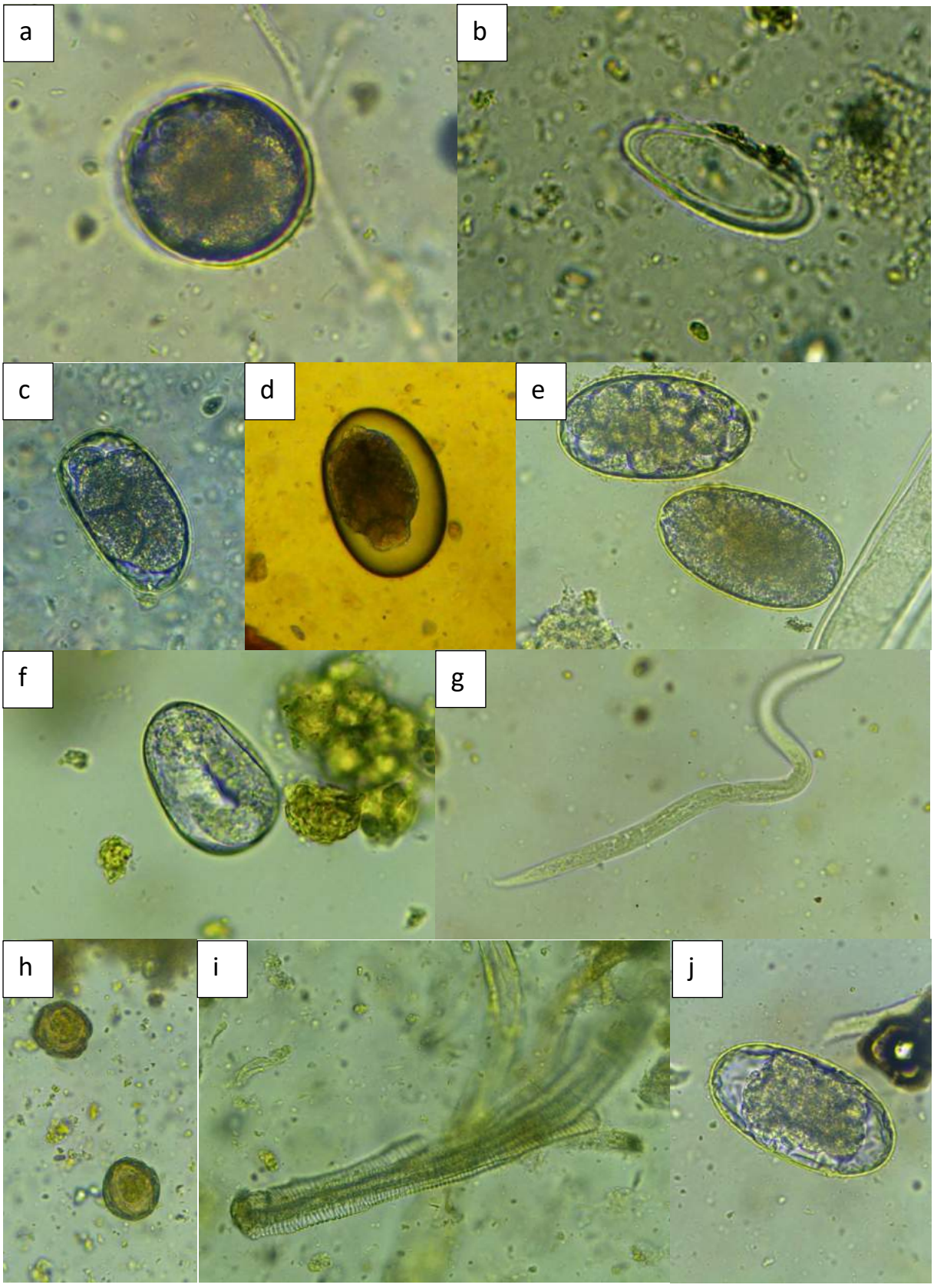
***[Table 6: Self-Care Observations]***

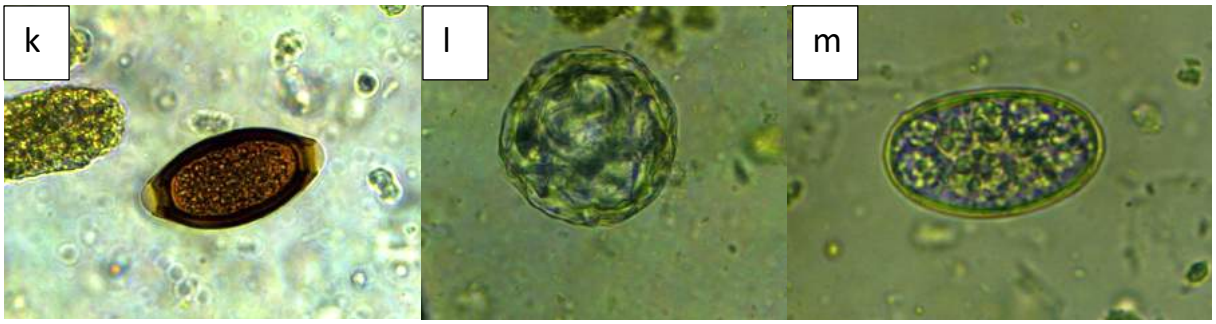
Observation 1: Events Book: G. Muhumuza, C. Siever
Observation 2: Current Study: E. Freymann
Observation 3: Events Book: K. Fawcett. G. Muhumuza
Observation 4: Events Book: S. O'Hara
Observation 5: Events Book: L. Bates
Observation 6: Events Book G. Muhumuza, A. Stephen
Observation 7: GAD: C. Hobaiter
Observation 8: Events Book: Jackson Okuti, P. Fedurek, Mayanja, G.
Observation 9: GAD: C. Hobaiter
Observation 10: Current Study: E. Freymann G. Muhumuza
Observation 11: GAD: C. Hobaiter
Observation 12: Current Study: E. Freymann G. Muhumuza
Observation 13: Events Book: G. Muhumuza, Gerald, C. Bosco
Observation 14: GAD: C. Hobaiter
Observation 15: GAD: C. Hobaiter
Observation 16: CSMAS: A. Soldati, G. Muhumuza
Observation 17: CSMAS: H. Klein, K. Vincent
Observation 17: GAD: C. Hobaiter
Observation 18: Current Study: E. Freymann, G. Muhumuza
Observation 19: Events Book: Zephyr
Observation 20: Current Study: E. Freymann, G. Muhumuza
Observation 21: Events Book: Unknown
Observation 22: GAD: C. Hobaiter
Observation 23: GAD: C. Hobaiter
Observation 24: GAD: C. Hobaiter
Observation 25: GAD: C. Hobaiter
Observation 26: GAD: C. Hobaiter
Observation 27: E. Freymann, G. Muhumuza
Observation 28: E.Freymann, R.E. Yikii
Observation 29. Steven, T. Gruber

***[Table 7: Social-Care Observations]***
Observation 1. Events Book: Unknown
Observation 2. Events Book: A. Stephen
Observation 3. Events Book: M. M. Gideon
Observation 4. Events Book: Joseph K., Ofen, R.
Observation 5: GAD: C. Hobaiter
Observation 6. Events Book: L. Samuni, Anne
Observation 7. Events Book. G. Muhumuza, A. Soldati
Observation 8. Events Book: G. Muhumuza, M. Laporte

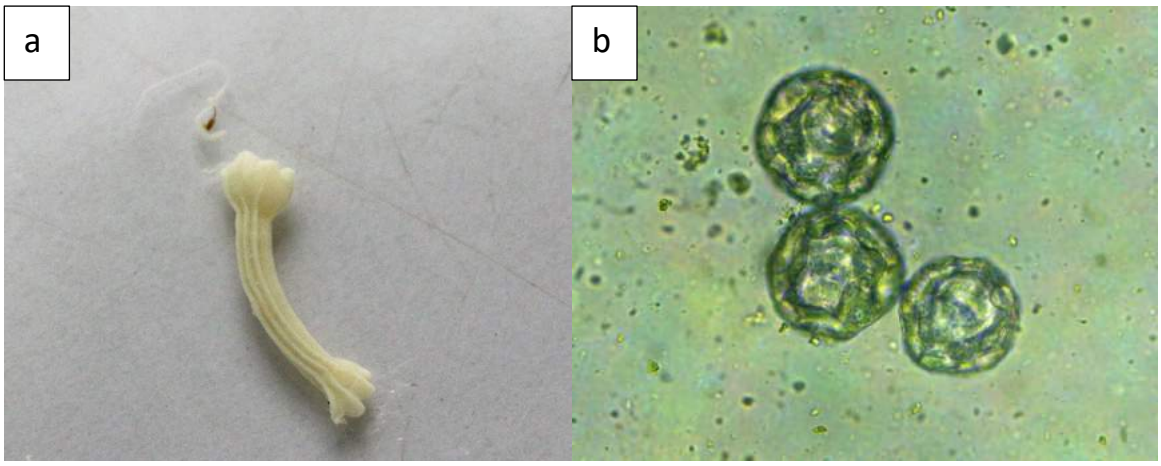
**Leaf Swallowing Event During Study Period [Full Description]**
**August 16th, 2021:** Adult female, KL, and two of her offspring, KO (juvenile, male) and KA (infant, female), had been traveling in the west. Many of the adult males had left on patrol earlier in the day, including KL's sub-adult son KC. At around 12:45 KL, KO, and KM hear vocalizations and started moving, we could hear individuals on both sides of us. KL drummed on a tree (*C. zenkeri)* twice. This was the first time we had seen a female drum. Kirabo (KO), her juvenile offspring drummed with her the first time, but not the second. They both climbed separate trees—KL in a *Cynometra alexandrii* tree, and KO in a *Funtimia elastica* tree. At 12:53 KO moved into a *Khaya anthotheca* tree and at 13:01 I noticed him eating the resin of this species. Meanwhile, across the trail, KL groomed KM in the *C. alexandrii.* At 13:06 after a vocalization from close by, KO stopped, descended his tree and crossed toward his mother. KL and KM descended, and the family travelled, stopping and starting until they encountered KC on line 5F. They moved into the block and all groomed. At 13:36 KC began travelling, and the others followed, heading north. At around 13:31 they passed a vine with *Saba florida* fruits, and KC and KL ate a few bites from fallen fruit before continuing to travel. KO did not eat the fruit. They kept moving and entered some vines around 13:36. When they came back into view it was 13:45, KL was sitting with KM on her back leaf swallowing *A. aequinoctiale*. KC was sitting across from her, also with his back to us leaf swallowing the same species. At this point we were north of line I (off the grid) in a swampy area. KC moved out of sight into some vines. KL transitioned to eating the leaves of an unidentified climber. KO was seated 1m away from KC stripping and eating the pith of *Costus sp.,* but then switched to eating the leaves of the same climber as KL. KL switched back to leaf swallowing *A. aequinoctiale* at 13:51. KM could be seen nibbling on a climber vine, she did not appear to express interest in her mother's leaf swallowing behavior. KL heard something and shifted into the vines, grabbing *Aframomum* sp. fruit from the ground and travelling with it. The family was out of sight for about 10 minutes, before coming back into view on the trail and rejoining the group.

## Supplementary Figures:

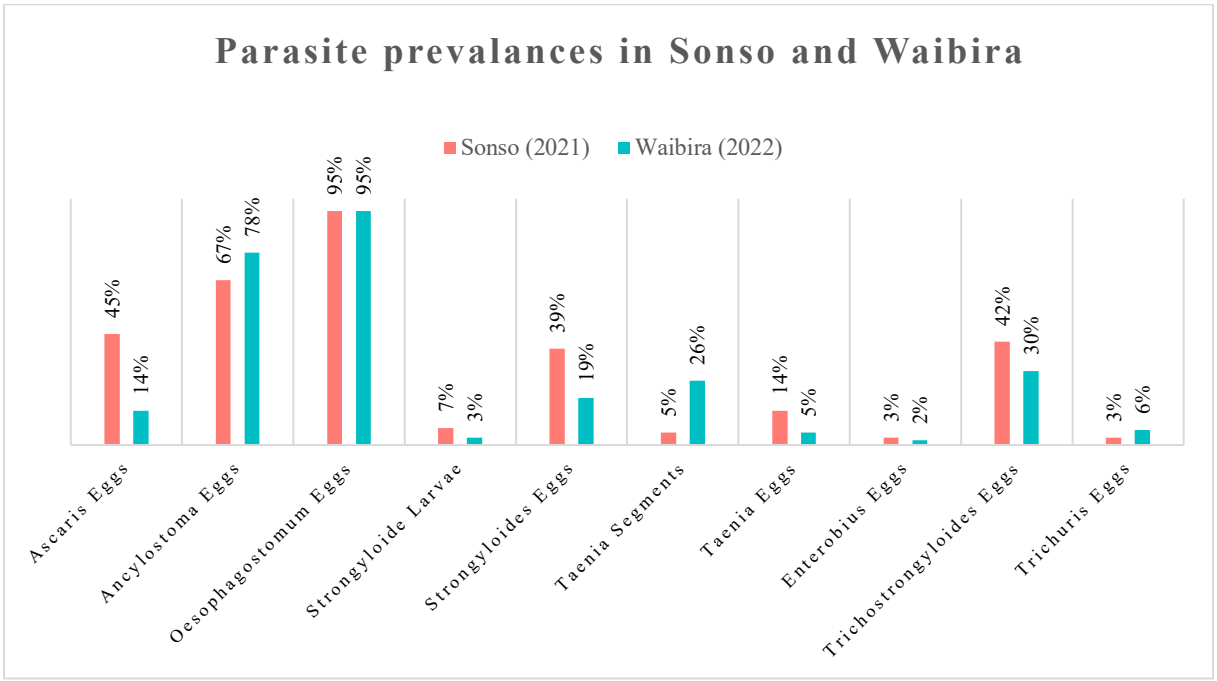





***SM Figure 1**: Parasites from left to right: (a.) Ascaris (b.) Enterobius (c.) Ancylostoma (d.) Mite Eggs (e.) Oesophagostomum (f.) Strongyloides (g.) Strongyloides Larvae (h.) Taenia (i.) Taenia Segment (j.) Trichostrongylus (k.) Trichuris (l.) Bertiella sp., (m.) Unidentified Cestode*

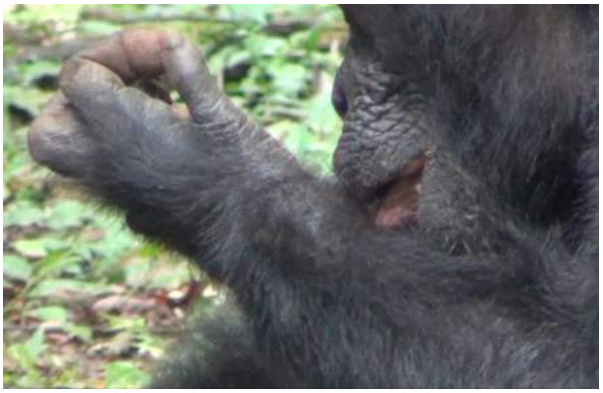



***SM Figure 2:** (a.) Bertiella sp. proglottid (b.) Bertiella sp. eggs*



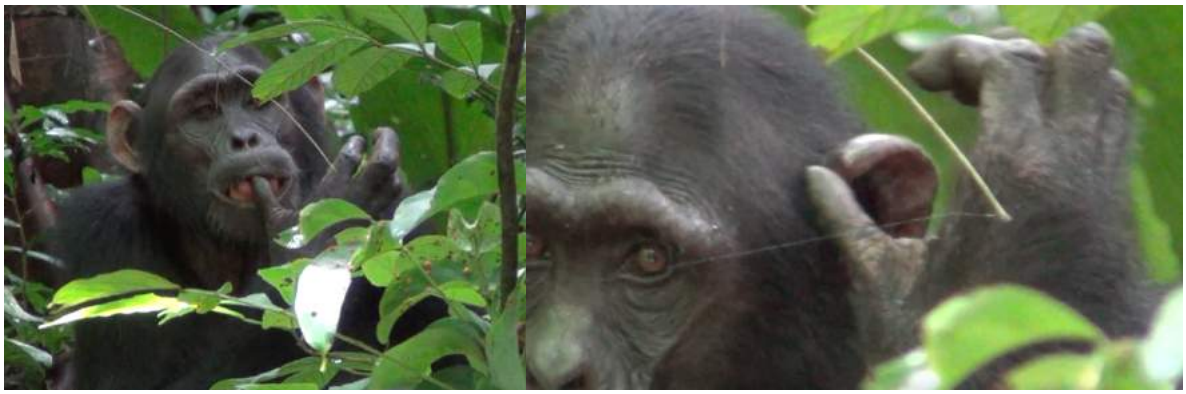

***SM Figure 3**: Percentage of infected individuals by parasite species across all samples in each community*


***SM Figure 4:*** *(a.) Voucher specimen* of A. aequinoctiale (EFZO.01) (b.) Voucher specimen of B. papyrifera (EFZO.02)



***SM Figure 5:*** *(Table 6: Observation 11) Hawa licking wound on lower arm*

***SM Figure 6:*** *(Table 6: Observation 12) UP licking wound while carrying corpse of dead-infant*



***SM Figure 7:*** *(Table 6: Observation 2) [Left] KC licks finger [Right] KC presses finger to wounded ear*

***SM Figure 8:*** *(Table 6: Observation 20) [Left] PS chews leaves of unknown species in the front of his mouth [Right] PS applies chewed leaves to wound with mouth*

## Supplementary Tables:

**SM TABLE 1:** Parasite species identified in Sonso and Waibira chimpanzees over the study period

| Phylum | Class | Family | Common Name | Genus | Sonso (n=77) | Waibira (n=168) |
|---|---|---|---|---|---|---|
| Roundworms **Nematoda** | Roundworms Chromadorea | *Strongylidae* | Hookworm | *Ancylostoma* sp. | **X** | **X** |
| | | | Nodule-worm | *Oesophagostomum* sp. | **X** | **X** |
| | | | Thread-worm | *Strongyloides* sp. | **X** | **X** |
| | | *Trichostrongylidae* | | *Trichostrongylus* sp. | **X** | **X** |
| | | *Ascarididae* | Giant Intestinal Roundworm | *Ascaris* sp. | **X** | **X** |
| | | *Oxyuridae* | Pinworm | *Enterobius* sp. | **X** | **X** |
| | | *Trichuridae* | Whip-worm | *Trichuris* sp. | **X** | **X** |
| Flatworms **Platyhelminthes** | Tapeworms Cestoda | *Taeniidae* | Tapeworm | *Taenia* sp. | **X** | **X** |
| | | *Diphyllobothriidae* | Fish Tapeworm | *Diphillobothrium* sp. | | **X** |
| | | *Hymenolepididae* | Dwarf Tapeworm | *Hymenolepsis* sp. | | **X** |
| | | *Anoplocephalidae* | Tapeworm | *Bertiella* sp. | **X** | **X** |
| | Trematoda | *Paragonimidae* | Lung fluke | *Paragonimus* sp. | **X** | |

**SM TABLE 2:** Observed wounds throughout study periods

| Community | Individual | Demographic | Wound Placement | Cause |
|---|---|---|---|---|
| **Sonso** | RH | Adult, female | Arm | Suspected intragroup aggression |
| | MB | Sub-adult, male | Leg | Suspected intragroup aggression |
| | KC | Sub-adult, male | Ear | Suspected intragroup aggression |
| | ZL | Adult, male | Hand | Suspected intragroup aggression |
| | HW | Adult, male (alpha) | Arm | Suspected intragroup aggression |
| | PS | Adult, male | Arm | Suspected intragroup aggression following infanticide |
| | KC | Sub-adult, male | Hand | Suspected intragroup aggression |
| | MB | Sub-adult, male | Hand | Suspected intragroup aggression |
| | KG | Adult, female | Vulva | Observed intragroup aggression |
| | PS | Adult, male | Arm | Suspected intragroup aggression |
| | UP | Adult, female | Head, Arm | Suspected intragroup aggression following infanticide |
| | KO | Juvenile, male | Knee | Unknown |
| **Waibira** | LKU | Adult, male | Foot (Bottom) | Unknown |
| | MAC | Adult, male | Ankle | Suspected intragroup aggression |
| | ALF | Adult, male | Mouth | Suspected intragroup aggression |
| | ILA | Adult, male | Leg | Suspected intragroup aggression |
| | PAV | Juvenile, female | Hand | Snare (wire) |

## **SM TABLE 3:** Parasite richness monthly mean

| Month | Parasite species per sample | |
|---|---|---|
| | S | W |
| ***June*** | - | 3 |
| ***July*** | 3 | 3 |
| ***August*** | 3 | 3 |
| ***September*** | 3 | 3 |
| ***October*** | 4 | 3 |

**NB:** To evaluate monthly mean species diversity, we averaged the number of parasite species in each fecal per month and found monthly sample means for each community.

## **SM TABLE 4:** Monthly mean for parasite intensity (EPG) across species

| Parasite species | June | | July | | August | | September | | October | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S | W | S | W | S | W | S | W | S | W |
| *Ascaris* eggs | - | 100 | 1854 | 50 | 150 | 57 | 119 | 550 | 160 | 0 |
| *Ancylostoma* eggs | - | 275 | 75 | 204 | 117 | 242 | 237 | 160 | 279 | 513 |
| *Oesophagostomum* eggs | - | 283 | 535 | 680 | 531 | 423 | 571 | 564 | 445 | 520 |
| *Strongyloides* eggs | - | 50 | 75 | 77 | 130 | 75 | 88 | 110 | 75 | 0 |
| *Taenia* eggs | - | 150 | 0 | 0 | 63 | 100 | 50 | 117 | 50 | 0 |
| *Enterobius vermicularis* eggs | - | 50 | 0 | 0 | 0 | 0 | 50 | 75 | 0 | 175 |
| *Trichostrongyloides* eggs | - | 250 | 120 | 127 | 128 | 188 | 131 | 94 | 370 | 0 |
| *Trichuris* eggs | - | 0 | 50 | 175 | 50 | 75 | 0 | 117 | 0 | 0 |
| *Bertiella* sp. eggs | - | - | - | 4300 | - | 13350 | - | 100 | - | 0 |

**NB:** To evaluate monthly mean intensity of infection, we divided the total EPG of the species by the number of monthly samples which contained that species.

## **SM TABLE 5:** Number of unique individuals sampled per month

| Month | S | W |
|---|---|---|
| ***June*** | - | 5 |
| ***July*** | 13 | 21 |
| ***August*** | 14 | 20 |
| ***September*** | 20 | 24 |
| ***October*** | 10 | 5 |

## Supplementary Videos:

All videos are accessible on Google Drive by following the below link:
https://drive.google.com/drive/folders/1MTSv6zQp7g9_m4QJpESPTLVMdMMg7Aot?usp=sharing

## Video Index:

**SM Video 1:** [Table 5 Observation 2] BH Leaf Swallowing
**SM Video 2:** [Table 5 Observation 2] KM Leaf Swallowing
**SM Video 3:** [Table 5 Observation 3] KR Leaf Swallowing
**SM Video 4:** [Table 5 Observation 3] KB Leaf Swallowing
**SM Video 5:** [Table 5 Observation 4] HW Leaf Swallowing
**SM Video 6:** [Table 5 Observation 5] NB/MS Leaf Swallowing
**SM Video 7:** [Table 5 Observation 6] SM Leaf Swallowing
**SM Video 8:** [Table 5 Observation 7] MS Leaf Swallowing
**SM Video 9:** [Table 6: Observation 2] KC Self-Care
**SM Video 10:** [Table 6: Observation 11] HW Self-Care
**SM Video 11:** [Table 6: Observation 12] UP Self-Care
**SM Video 12a/b:** [Table 6: Observation 17] ALF Self-Care
**SM Video 13:** [Table 6: Observation 19] KO Self-Care
**SM Video 14:** [Table 6: Observation 21] PS Self-Care
**SM Video 15:** [Table 6: Observation 25] FD Self-Care
**SM Video 16:** [Table 6: Observation 28] ZL Self-Care
**SM Video 17:** [Table 7: Observation 5] NT-NB Social-Care

# APPENDIX D:
# Supplementary Materials Chapter 4:
## Applying Collocation and APRIORI Analyses to Chimpanzee Diets: methods for investigating non-random food combinations in primate self-medication

## Supplementary Information:

### Methods:

***Preparation of Feeding Data for Analysis***

To prepare data for analysis, all feeding data were pooled and, when applicable, food type was combined with part eaten and ripeness/age (i.e., *Ficus exasperata* Unripe Fruit or *Broussonetia papyrifera* Young Leaves). The dataset included feeding data from both focal individuals and *ad libitum* feeding observations from non-focal group members recorded during daily follows. *Ad libitum* data were included to increase sample size but were only considered if the *ad libitum* individual was observed consuming >1 food item within a single day. In this dataset, each species-plant-part was considered a unique food (Hiraiwa-Hasegawa, 1990). If the food item had an age/ripeness qualifier, these were also considered a unique food (e.g., *Ficus mucuso* Ripe Fruit is *different* from *Ficus mucuso* Unripe Fruit). We made this distinction between maturity stages as phytochemical, nutritional, and mineral properties have been shown to vary noticeably based on fruit and leaf maturity in many species (e.g., Oszmiański et al. 2018; Adegbaju et al. 2020; Flyman & Afolayan, 2008). As Budongo chimpanzees appear to factor maturity stage into their feeding choices, this a relevant factor to consider. For example, Sonso chimpanzees have clear preferences for the young leaves of *Ficus variifolia* species and the young fruits of *Ficus exasperata* while noticeably not favoring these items in their mature or ripe stages respectively. This distinction, however, reduced the sample size of each resource. Additionally, assuming that resources of the same species but of differing maturity stages are independent from one another might not optimize the signal-to-noise ratio (the measure of the strength of the desired signal relative to background noise) in all cases.

***Interpreting pbin Outputs from Collocation Analysis***

To arrive at these pbin values, the MDCA analysis statistically contrasts all possible bigram combinations to determine whether each bigram occurs at frequencies higher or lower than expected by chance (Gries & Stefanowitsch, 2004; Hilpert, 2006). The MDCA analysis provides a superficial estimate of bigram ordering that helps to determine whether each combination is sensitive to the position of the individual element (e.g., is A-B as frequent as B-

A) (Bosshard et al. 2021). As pbins correspond to p estimation significance levels, then if the absolute value of pbin was > 3: P < 0.001; > 2: P < 0.01; > 1.3: P < 0.05; < 1.3: NA (Bosshard et al. 2021). The assumptions needed for running MDCA are not constrained by usual sampling assumptions, and thus the analysis can be run with skewed, non-random, and small datasets (Gries, 2014; Gries & Stefanowitsch, 2004; Hilpert, 2006).

***Interpreting APRIORI Outputs on PANacea***

The interactive interface of **PANacea** allows users to customize metrics using an interactive side panel and a main panel with three menu tabs: "**Data Exploration**," "**Medicinal Network**," and "**Clustered Rules**." On the "Data Exploration" tab users can control, using the side panel, Minimum Support, Minimum Confidence, Minimum Lift, and Rule Length (1–10) (Figure S1). The first three of these criteria, allow the user to filter the feeding data in different ways and define the strength of the analysis, while the rule length allows the user to define the number of foods in the left-hand side (LHS) of the if—then rule. The right-hand side (RHS) of the equation will always only generate a singular food item. For example, if the rule length is 3, the output may look like: **IF:** A, B, C food items were eaten → **THEN:** D was likely also eaten. In this example, if both confidence and lift are high for this association rule, it would suggest that it is extremely likely that if an individual consumes A, B, and C then they would also consume D. The number of items (rule length) in the LHS increases the strength of the combination as it decreases the likelihood that the individual would have opportunistically encountered and ingested each of the resources.

The landing pageof PANacea is the "Data Exploration" tab. Rules and outputs are summarized here as they are generated by the APRIORI algorithm running on an online RStudio Server hosted by Osteomics, according to the parameters selected on the side panel. At any point, results dependent on the adjustable metrics can be downloaded as a .csv table allowing for easy comparison between different settings.

The next tab, titled "Medicinal Network," can be used for visualizing rule networks and associations in an interactive format. In the default display settings, foods are connected to "rule" nodes, which are depicted as circles. These circles are numbered according to their order in the data output according to their "lift". The lower the rule number, the higher the lift value (i.e., Rule 1 = the association with the highest lift in the dataset). To facilitate easier visualization of these associations, circle sizes increase with "support" and circle shading saturates (to red) as "confidence" increases. While lift is likely the most reasonable metric due to the dataset's high

diversity of items relative to its size, settings can be changed in R to have rules numbered according to confidence or support. The tab titled “Clustered Rules” is helpful if there are too many rules for efficient visualization. Users can use this tab to see a summarized version of the network rule visualization.

## Supplementary Figures:

$$Support = \frac{\#\ of\ Associations\ between\ A\ and\ B}{Total\ \#\ of\ Associations} = P(A \cap B) \quad \text{(eq. 1)}$$

$$Confidence = \frac{\#\ of\ Associations\ between\ A\ and\ B}{Total\ \#\ of\ Associations\ with\ A} = \frac{P(A \cap B)}{P(A)} \quad \text{(eq. 2)}$$

$$Lift = \frac{Confidence}{Expected\ Confidence} = \frac{P(A \cap B)}{P(A) \times P(B)} \quad \text{(eq. 3)}$$

***SM Figure 1****: Equations for Support, Confidence, and Lift customizable metrics in PANacea web-app*

## Supplementary Tables:

**TABLE SI:** Budongo resource list (with site codes) used in AO coding scheme

| Scientific Name | Site Code |
|---|---|
| *Acanthus pubescens* | Acp |
| *Alstonia boonei* | Ab |
| *Broussonetia papyrifera* | Bpy |
| *Celtis gomphophylla* | Cgp |
| *Celtis mildbraedii* | Cmi |
| *Celtis philppensis* | Cph |
| *Celtis zenkeri* | Cze |
| *Cleistopholis patens* | Cp |
| *Climbers general* | Cli |
| *Cordia millenii* | Com |
| *Costus* spp. | Cos |
| *Croton sylvaticus* | Csy |
| *Crepiniana coloncoba* | Cc |
| *Cynometra alexandri* | Cya |
| *Desplatsia dewevrei* | Dd |
| *Erythropyleum suaveolens* | Es |
| *Ficus exasperata* | Fe |
| *Ficus mucuso* | Fm |
| *Ficus sur* | Fsu |
| *Ficus thonningii* | Fth |
| *Ficus variifolia* | Fvr |
| *Gambeya perpulchra* | Gpr |
| *Khaya anthotheca* | Ka |
| *Klainedoxa gabonensis* | Klg |
| *Lasiodiscus pervillei* | Lp |
| *Maesopsis eminii* | Me |
| *Merremia bpterygocaulos* | Mb |
| *Mildbraediodendron excelsum* | Mie |
| *Myrianthus holstii* | Myh |
| *Psidium guajava* | Psg |
| *Trichilia rubescens* | Trr |
| *Scepocarpus trinervis* | Urc |
| *Aframomum spp.* | Afm |
| *Marantochloa* | Mrt |
| *Unidentified* spp. | Avo |

| | |
|---|---|
| *Cubitermes* spp. Termite Soil | Termite Soil |
| Clay | Clay |
| Clay Water | Clay Water |
| Soil | Ground Soil |
| Water | Drink from Puddle or Stream |
| Water (Sp.) | Drink from Tee Hole |
| Leaf Sponge | Leaf Sponge |
| Honey | Honey |

**TABLE S2:** Plant part and part maturity used in coding scheme

| Part | Maturity |
|---|---|
| Fruit | Ripe Fruit |
| | Young Fruit |
| | Unripe Fruit |
| Pith | |
| Leaf | Young Leaf |
| | Mature Leaf |
| Seed | |
| Flower | |
| Flower Bud | |
| Bark | |
| Dead Wood | |
| Other | |

**TABLE S3:** Common vs. Uncommon Processing Techniques

| Status | Name | Definition |
|---|---|---|
| **Common** | Eat Fruit | Select fruit, masticate, swallow |
| | Wadge Fruit | Insert fruit into mouth and pack in lip to extract juice from resource |
| | Eat Leaves | Select leaf, masticate, swallow |
| | Eat Meat | Hunt and kill prey species and subsequently consume meat |
| **Uncommon** | Strip Pith | Remove fibrous outer layer of plant stem with teeth and consume pith |
| | Strip Bark | Remove bark from tree exposing cambium and consume either inner or outer bark |
| | Eat Dead Wood | Remove wood from dead tree (either standing or fallen) and consume cambium |
| | Geophagy | Use mouth or organic tool to consume clay, clay-water or termite soil |
| | Eat Resin | Use mouth or hands to peel resin from bark of tree, and consume in combination with tree bark (only known to be done in Sonso for *K. anthotheca* tree) |
| | Root eating | Dig for roots below base of tree and subsequently bite and ingest subterranean plant material |

**TABLE S4**: Some known medicinal properties for ROIs used in this study

| Resource | Ethnomedicinal Uses | Sources |
|---|---|---|
| *Aframomum* sp. Pith | **Fruit:** Aphrodisiac; Sexual stimulation<br>Leaf: Measles; Leprosy<br>**Root:** Excessive lactation in nursing mothers, Post-partum hemorrhage; Postpartum hemorrhage<br>**Rhizome:** Infertility<br>**General:** Purgative; Galactogogue; anthelminthic; Hoemostatic agent<br>**Seeds:** External tumors; Sleeping sickness | Iwu, 2014 |
| *Ficus exasperata* Bark | **General:** Hemorrhoids; Venereal disease; Arthritis; Wounds; Parasites; Diuretic for relaxing uterus; Enhancing uterine contractions<br>**Wood ash/charcoal:**<br>Leprous ulcer; General wounds<br>**Roots:** Asthma; Dyspnoea; Venereal disease<br>**Bark:** Intestinal worms; Hemorrhoids; Spleen enlargement; Heart problems; Cough; Dizziness; Facilitation of childbirth; Gonorrhea; Malaria (locally applied)<br>**Bark Sap:** Bleeding; Stimulant; Wounds, sores, abscesses; Eye ailments; Stomachache<br>**Leaves**: High blood pressure; Rheumatism; Arthritis; Intestinal pains; Epilepsy; Bleeding; Wounds; Inflammation; Bacterial infections; Fever; Edema; Leprous ulcer; Dermatosis; Abscess; Cough; Cold; Flu' Asthma; Heart disease; Thrush; Gum inflammation; Mouth and throat ailments; Gastric ulcers; Stomachaches; Poison; Kidney disease; Urinary tract infections; Headache; Tumors; Diarrhea; Parasites (Mechanical)<br>**Leaf Shoots**: Dysentery; Jaundice (externally applied); Emetic; Diuretic.<br>**Leaf Pulp (externally applied):** Rash; Wounds; Fungal infection; Itching; Ringworm; Rheumatism; Back pain<br>**Dried /Cooked Leaf:** Burns; Gonorrhea | PROTA4U |
| *Alstonia boonei* Bark | **Bark**: Abortive; Gonorrhea; Asthma; Sores; Ulcers; Painkiller; Diarrhea; Dysentery; Vermifuges; Liver problems; Dropsy, Inflammation; Edema; Gout; Hypoglycemic effect on diabetics; Internal parasites; Dizziness; Breast infection; Nausea, Snakebites; Stomachaches; Malaria; Measles; Uterine fibroid or ovarian cysts; Gynecological lower abdominal and pelvic congestion (e.g., PID); Aches from malarial fever; Jaundice<br>**Latex**: Internal parasites; Lactation stimulant | Kokwaro, 2009; PROTA4U |
| *Marantachloa leucantha* Pith | **Roots**: Aphrodisiac<br>**Pith:** Rheumatism; Acne | Kokwaro, 2009; PROTA4U |
| *Cleistopholis patens* Bark | **Bark/Sap:** Jaundice; Hepatitis; Stomachache; Tuberculosis; Bronchial affections; Colic; Edemas; Hunchbacks; Rickets; Headaches; Pain; Pulmonary troubles; Diarrhea; Hepatitis; Malaria; Measles; Typhoid fever; Menstrual irregularities.<br>**Root:** Vermifuge | PROTA4U |
| *Acanthus pubescens* Pith | **Leaves and root:** Pneumonia; Bleeding; Scorpion sting; Stabbing pain; Anthrax<br>**Leaves**: Malaria | Kokwaro et al. 2009 |
| *Ficus sur* Fruit | **Latex**: Wounds; Toothache; Eye problems, General body pain, Lung problems; Throat problems, Gonorrhea; Anti-emetic; Burns<br>**Root:** Cough; Sore throat; Diarrhea, Stomach pain in babies, Chest pain, Infertility, Uterine pain, Gonorrhoea, Oedema, Emmenagogue; Emetic<br>**Bark:** Pain; Rheumatism; Diarrhea; Stomach problems; Oedema in children, Infertility; Galactagogue; Fever; Cough; Skin rashes; Mouth sores<br>**Leaves:** Peptic ulcers; Chest problems; Tonsilitis; Stomach pain; Disinfectant body wash for ophthalmia<br>**Sap: W**ounds; Gonorrhea<br>**Seed:** Lactogenic<br>**Fruit:** Infertility, Tuberculosis, Abscesses and Sores; Lactogenic, Purgative; Aphrodisiac | PROTA4U |
| *Khaya anthotheca* Resin | **Bark:** Colds; Fevers; Pneumonia; Abdominal pain; Vomiting; Gonorrhea; Aphrodisiac; Wounds, Sores, Ulcers; Anemia; Malaria, Bilharzias<br>**Roots:** Anemia; Dysentery; Rectal prolapse | PROTA4U |

**TABLE S5**: Comparing Collocation (V1) and APRIORI results (confidence=0.6)

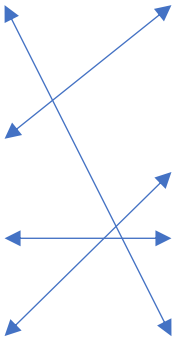
| # | Collocation Pairs | | APRIORI Pairs (**support** = 0.011, **confidence** = 0.6, and **lift** = 1, **rule length** = 2) |
|---|---|---|---|
| 1 | Fe Unripe Fruit ⇒ Fm Ripe Fruit |  | Unk Leaf Sponge ⇒ Clay Water |
| 2 | Cgp Fruit ⇒ Fvr Young Fruit | | Acp Pith ⇒ Myh Ripe Fruit |
| 3 | Fsu Ripe Fruit ⇒ Cgp Ripe Fruit | | Fe Young Leaf ⇒ Avo Ripe Fruit |
| 4 | Fsu Ripe Fruit ⇒ Fvr Young Fruit | | Bpy Mature Leaf ⇒ Bpy Ripe Fruit |
| 5 | Unk Leaf Sponge ⇒ Clay Water | | Psg Unripe Fruit ⇒ Ka Resin |
| 6 | Mie Ripe Fruit ⇒ Fvr Young Leaf | | Cph Young Leaf ⇒ Cgp Rip eFruit |
| 7 | Cph Young Leaf ⇒ Fe Young Leaf | | Sf Ripe Fruit ⇒ Ka Resin |
| 8 | Fe Young Fruit ⇒ Fm Ripe Fruit | | Fe Young Fruit ⇒ Fm Ripe Fruit |
| 9 | Cph Young Leaf ⇒ Avo Ripe Fruit | | Fe Young Leaf ⇒ Cgp Ripe Fruit |
| 10 | Ptm Root ⇒ Dd Young Leaf | | Csy Ripe Fruit ⇒ Fsu Ripe Fruit |
| 11 | Cph Young Leaf ⇒ Cgp Ripe Fruit | | Fe Unripe Fruit ⇒ Fm Ripe Fruit |
| 12 | Fm Ripe Fruit ⇒ Gpr Ripe Fruit | | Gpr Ripe Fruit ⇒ Fm Ripe Fruit |
| 13 | Myh Ripe Fruit ⇒ Mrt Pith | | Bpy Flower Buds ⇒ Fsu Ripe Fruit |
| 14 | Es Seeds ⇒ Dd Ripe Fruit | | Com Ripe Fruit ⇒ Fsu Ripe Fruit |
| 15 | Me Ripe Fruit ⇒ Meat Colobus | | Fvr Young Fruit ⇒ Fsu Ripe Fruit |
| 16 | Avo Ripe Fruit ⇒ Fe Young Leaf | | Fth Ripe Fruit ⇒ Fsu Ripe Fruit |
| 17 | Urc Flowers ⇒ Mb Mature Leaf | | Fe Young Leaf ⇒ Fsu Ripe Fruit |

**TABLE S6:** Comparing exact matches between APRIORI pairs (confidence = 0.6) and all non-random Collocation (V1) bigrams

| **APRIORI Pairs (support** = 0.011, **confidence** = 0.6, and **lift** = 1, **rule length** = 2) | **APRIORI Rank** | **Collocation Bigram Rank** |
|---|---|---|
| Unk Leaf Sponge ⇒ Clay Water | #1 | #5 |
| Acp Pith ⇒ Myh Ripe Fruit | #2 | #183 |
| Fe Young Leaf ⇒ Avo Ripe Fruit | #3 | Bigram not above chance |
| Bpy Mature Leaf ⇒ Bpy Ripe Fruit | #4 | #25 |
| Psg Unripe Fruit ⇒ Ka Resin | #5 | #28 |
| Cph Young Leaf ⇒ Cgp Ripe Fruit | #6 | #11 |
| Sf Ripe Fruit ⇒ Ka Resin | #7 | #88 |
| Fe Young Fruit ⇒ Fm Ripe Fruit | #8 | #8 |
| Fe Young Leaf ⇒ Cgp Ripe Fruit | #9 | Bigram not above chance |
| Csy Ripe Fruit ⇒ Fsu Ripe Fruit | #10 | Bigram not above chance |
| Fe Unripe Fruit ⇒ Fm Ripe Fruit | #11 | #1 |
| Gpr Ripe Fruit ⇒ Fm Ripe Fruit | #12 | Bigram not above chance |
| Bpy Flower Buds ⇒ Fsu Ripe Fruit | #13 | Bigram not above chance |
| Com Ripe Fruit ⇒ Fsu Ripe Fruit | #14 | Bigram not above chance |
| Fvr Young Fruit ⇒ Fsu Ripe Fruit | #15 | Bigram not above chance |
| Fth Ripe Fruit ⇒ Fsu Ripe Fruit | #16 | #24 |
| Fe Young Leaf ⇒ Fsu Ripe Fruit | #17 | Bigram not above chance |

**TABLE S7:** Comparing Collocation (V1) and APRIORI results (confidence = 0.011)

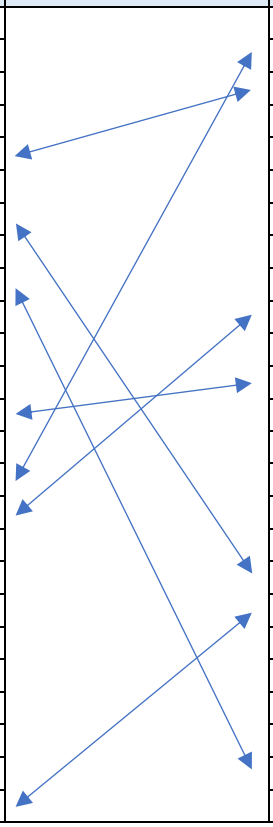
| # | **Collocation Pairs** | | **APRIORI Pairs (support** = 0.011, **confidence** = 0.011, and **lift** = 1, **rule length** = 2) |
|---|---|---|---|
| 1 | Fe Unripe Fruit ⇒Fm Ripe Fruit |  | Meat Colobus ⇒ Me Ripe Fruit |
| 2 | Cgp Ripe Fruit ⇒Fvr Young Fruit | | Me Fruit ⇒ Meat Colobus |
| 3 | Fsu Ripe Fruit ⇒Cgp Ripe Fruit | | Unk Leaf Sponge ⇒ Clay Water |
| 4 | Fsu Ripe Fruit ⇒Fvr Young Fruit | | Clay Water ⇒ Unk Leaf Sponge |
| 5 | Unk Leaf Sponge ⇒ Clay Water | | Acp Pith ⇒ Myh Ripe Fruit |
| 6 | Mie Ripe Fruit ⇒Fvr Young Leaf | | Myh Ripe Fruit ⇒ Acp Pith |
| 7 | Cph Young Leaf ⇒Fe Young Leaf | | Sf Ripe Fruit ⇒ Fe Ripe Fruit |
| 8 | Fe Young Fruit ⇒Fm Ripe Fruit | | Fe Ripe Fruit ⇒ Sf Ripe Fruit |
| 9 | Cph Young Leaf ⇒Avo Ripe Fruit | | Fe Young Leaf ⇒ Avo Ripe Fruit |
| 10 | Ptm Root ⇒Dd Young Leaf | | Avo Ripe Fruit ⇒ Fe Young Leaf |
| 11 | Cph Young Leaf ⇒Cgp Ripe Fruit | | Mrt Pith ⇒ Myh Ripe Fruit |
| 12 | Fm Ripe Fruit ⇒Gpr Ripe Fruit | | Myh Ripe Fruit ⇒ Mrt Pith |
| 13 | Myh Ripe Fruit ⇒Mrt Pith | | Cmi Young Leaf ⇒ Me Ripe Fruit |
| 14 | Es Seeds ⇒Dd Ripe Fruit | | Me Ripe Fruit ⇒ Cmi Young Leaf |
| 15 | Me Ripe Fruit ⇒Meat Colobus | | Fth Ripe Fruit ⇒ Cph Young Leaf |
| 16 | Avo Ripe Fruit ⇒Fe Young Leaf | | Cph Young Leaf ⇒ Fth Ripe Fruit |
| 17 | Urc Flowers ⇒Mb Leaf | | Fe Young Leaf ⇒ Cph Young Leaf |
| 18 | Cli Young Leaf ⇒ Cze Young Leaf | | Cph Young Leaf ⇒ Fe Young Leaf |
| 19 | Cgp Ripe Fruit ⇒ Avo Ripe Fruit | | Bpy Mature Leaf ⇒ Bpy Ripe Fruit |
| 20 | Blu Leaf Sponge ⇒ Drink Fe | | Bpy Ripe Fruit ⇒ Bpy Mature Leaf |
| 21 | Drink Fe ⇒ Fvr Leaf Bud | | Psg Ripe Fruit ⇒ Fvr Young Fruit |
| 22 | Fvr Ripe Fruit ⇒ Afm Ripe Fruit | | Fvr Young Fruit ⇒ Psg Ripe Fruit |
| 23 | Cli Young Leaf ⇒ Cli Young Leaf | | Avo Ripe Fruit ⇒ Cph Young Leaf |
| 24 | Fth Ripe Fruit ⇒ Fsu Ripe Fruit | | Cph Young Leaf ⇒ Avo Ripe Fruit |
| 25 | Bpy Leaf ⇒ Bpy Ripe Fruit | | Dd Ripe Fruit ⇒ Fvr Ripe Fruit |

**TABLE S8:** Comparing Exact Matches Between APRIORI Pairs (confidence = 0.011) and All Non-Random Collocation (V1) Bigrams

| **APRIORI Pairs (support** = 0.011, **confidence** = 0.011, and **lift** = 1, **rule length** = 2) | **APRIORI Rank** | **Collocation Bigram Rank** |
|---|---|---|
| Meat Colobus ⇒ Me Ripe Fruit | #1 | Bigram not above chance |
| Me Fruit ⇒ Meat Colobus | #2 | #15 |
| Unk Leaf Sponge ⇒ Clay Water | #3 | #5 |
| Clay Water ⇒ Unk Leaf Sponge | #4 | #87 |
| Acp Pith ⇒ Myh Ripe Fruit | #5 | #182 |
| Myh Ripe Fruit ⇒ Acp Pith | #6 | Bigram not above chance |
| Sf Ripe Fruit ⇒ Fe Ripe Fruit | #7 | #34 |
| Fe Ripe Fruit ⇒ Sf Ripe Fruit | #8 | Bigram not above chance |
| Fe Young Leaf ⇒ Avo Ripe Fruit | #9 | Bigram not above chance |
| Avo Ripe Fruit ⇒ Fe Young Leaf | #10 | #16 |
| Mrt Pith ⇒ Myh Ripe Fruit | #11 | Bigram not above chance |
| Myh Ripe Fruit ⇒ Mrt Pith | #12 | #13 |
| Cmi Young Leaf ⇒ Me Ripe Fruit | #13 | Bigram not above chance |
| Me Ripe Fruit ⇒ Cmi Young Leaf | #14 | #43 |
| Fth Ripe Fruit ⇒ Cph Young Leaf | #15 | Bigram not above chance |
| Cph Young Leaf ⇒ Fth Ripe Fruit | #16 | #84 |
| Fe Young Leaf ⇒ Cph Young Leaf | #17 | Bigram not above chance |
| Cph Young Leaf ⇒ Fe Young Leaf | #18 | #7 |
| Bpy Mature Leaf ⇒ Bpy Ripe Fruit | #19 | #25 |

| | | |
|---|---|---|
| Bpy Ripe Fruit ⇒ Bpy Mature Leaf | #20 | Bigram not above chance |
| Psg Ripe Fruit ⇒ Fvr Young Fruit | #21 | #204 |
| Fvr Young Fruit ⇒ Psg Ripe Fruit | #22 | Bigram not above chance |
| Avo Ripe Fruit ⇒ Cph Young Leaf | #23 | #119 |
| Cph Young Leaf ⇒ Avo Ripe Fruit | #24 | 9 |
| Dd Ripe Fruit ⇒ Fvr Ripe Fruit | #25 | 31 |

# Appendix D References: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# APPENDIX E:
# Supplementary Materials Chapter 5:
## Pharmacological and Behavioral Investigation of Putative Self-Medicative Plants in Budongo Chimpanzee Diets

### Supplementary Information:

### Methods:

#### Collection of Sample Material (Additional Information)

When possible, bark samples were collected from trees with traces of previous bark stripping. In all but one case (*S. myrtina*), stem bark samples were cut directly from the trunk of the tree. For this sample, stripped bark refuse was taken from the base of the tree. After harvesting, plants were cut into small pieces and dried in the shade for two weeks. Samples were turned twice a day to prevent mold. Once dry, samples were transferred to paper bags and stored in a dark, dry room until export.

#### Sample Solution Preparation

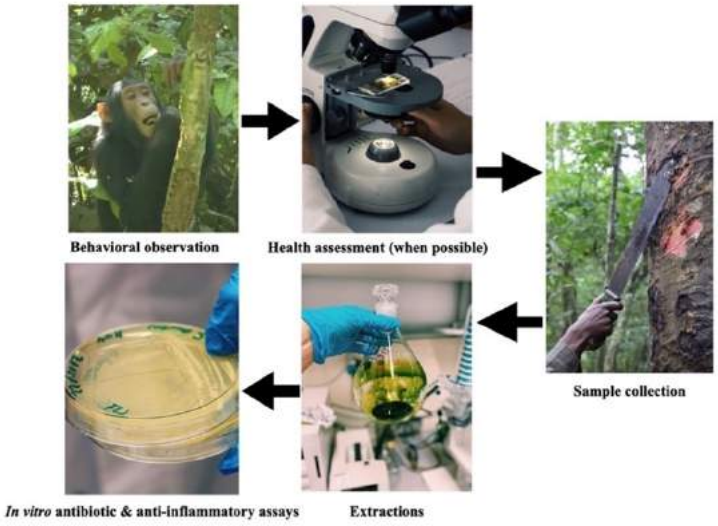
Each crude extract was dissolved in DMSO (Carl Roth) at a concentration of 10 mg/mL. To ensure a homogenous solution, samples were mixed with a vortex mixer and, if necessary, treated with sonication at room temperature or up to 55°C for samples with low solubility. The solutions were stored at -20°C until needed for further testing.

## Antibacterial susceptibility tests:

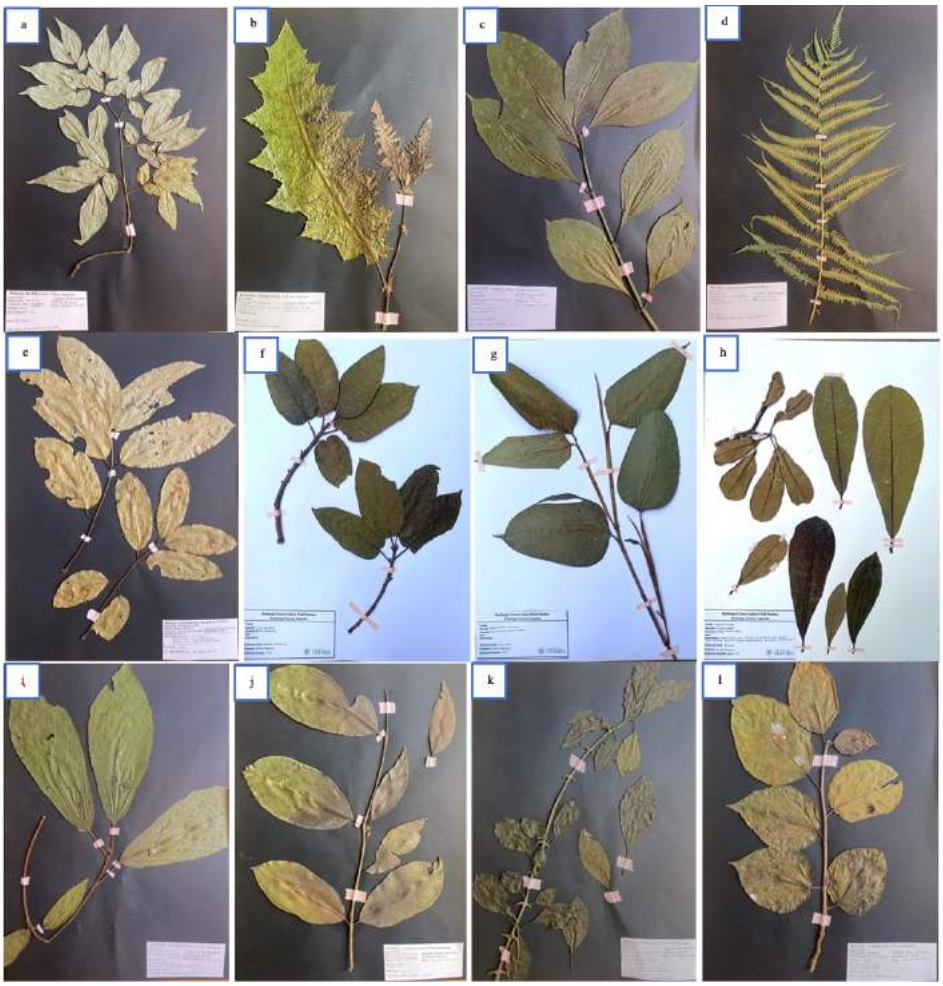
#### *Bacterial culture preparation and bacteria standardization*

Methods for antibacterial assays used in this study have previously been described in Schultz et al. (2020). Strains were streaked from freezer stock, maintained on tryptic soy agar (TSA) and incubated overnight at 37°C. To prepare the working suspensions for the bioassays, 14 mL tryptic soy broth (TSB) were inoculated with a bacterial colony and incubated overnight at 37°C. Working suspensions were placed at an angle and were constantly shaking at 200 rpm. The bacterial suspension was standardized via determination of the overnight culture's optical density at 590 nm using a UV-vis spectrophotometer and to a confluence of $5x10^5$ CFU/mL, ensuring individual experiments were carried out using the same bacterial count. To create the standardized working culture, calculated quantities of assay growth media (cation-adjusted Müller-Hinton broth (CAMHB)) and overnight culture were combined.

## Supplementary Figures:




***SM Figure 1:*** *Generalized multi-method workflow used in this study*



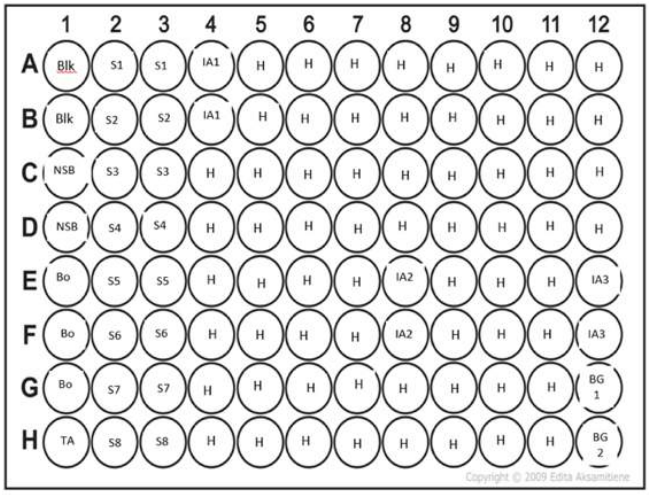

***SM Figure 2****: Voucher samples collected in duplicate:* ***a.)*** *Cynometra alexandri* ***(00243133G) b.)*** *A. polystachius* ***(00243136J) c.)*** *W. elongata* ***(00243129L) d.)*** *C. parasitica* ***(00243122E) e.)*** *K. anthotheca* ***(00243123F) f.)*** *F. variifolia* ***(51195) g.)*** *M. leucantha* ***(51203) h.)*** *A. boonei* ***(51204) i.)*** *D. dewevrei* ***(00243132F) j.)*** *S. guineense* ***(00243135I) k.)*** *S. myrtina* ***(00243128K) l.)*** *F. exasperata* ***(00243130D)***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | DMSO | eE087 | hE089a | mwE091 | eE092b | mwE094 | hE096 | mwE098b | | | | |
| B | mwE085 | AB 1 | mwE089b | eE091 | hE092b | eE094 | mwE097 | eE098b | | | | |
| C | eE085 | hE087 | AB 2 | hE091 | mwE093a | hE094 | DMSO | hE098b | | | | |
| D | hE085 | mwE088 | eE089b | AB3 | eE093a | mwE095 | eE097 | DSMO | | | | GC1 |
| E | mwE085 | eE088 | hE089b | mwE092a | hE093a | eE095 | hE097 | mwE099 | | | | GC1 |
| F | eE085 | hE088 | mwE090 | eE092a | mwE093b | hE095 | mwE098a | eE099 | | | | |
| G | hE085 | mwE089a | eE090 | hE092a | eE093b | mwE096 | eE098a | hE099 | | | | |
| H | mwE087 | eE089a | hE090 | mwE092b | hE093b | eE096 | hE098a | | | | | |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Conc. 256µg/ml | Sample 1 | | Conc. 256µg/ml | Sample 2 | | Conc. 256µg/ml | DMSO | | Conc. 64µg/ml | Antibiotic | |
| B | 128 | | | 128 | | | 128 | | | 32 | | |
| C | 64 | | | 64 | | | 64 | | | 16 | | |
| D | 32 | | | 32 | | | 32 | | | 8 | | |
| E | 16 | | | 16 | | | 16 | | | 4 | | |
| F | 8 | | | 8 | | | 8 | | | 2 | | |
| G | 4 | | | 4 | | | 4 | | | 1 | | |
| H | GC | | | MB | | | GC | | | GC | DMSO | Blk |

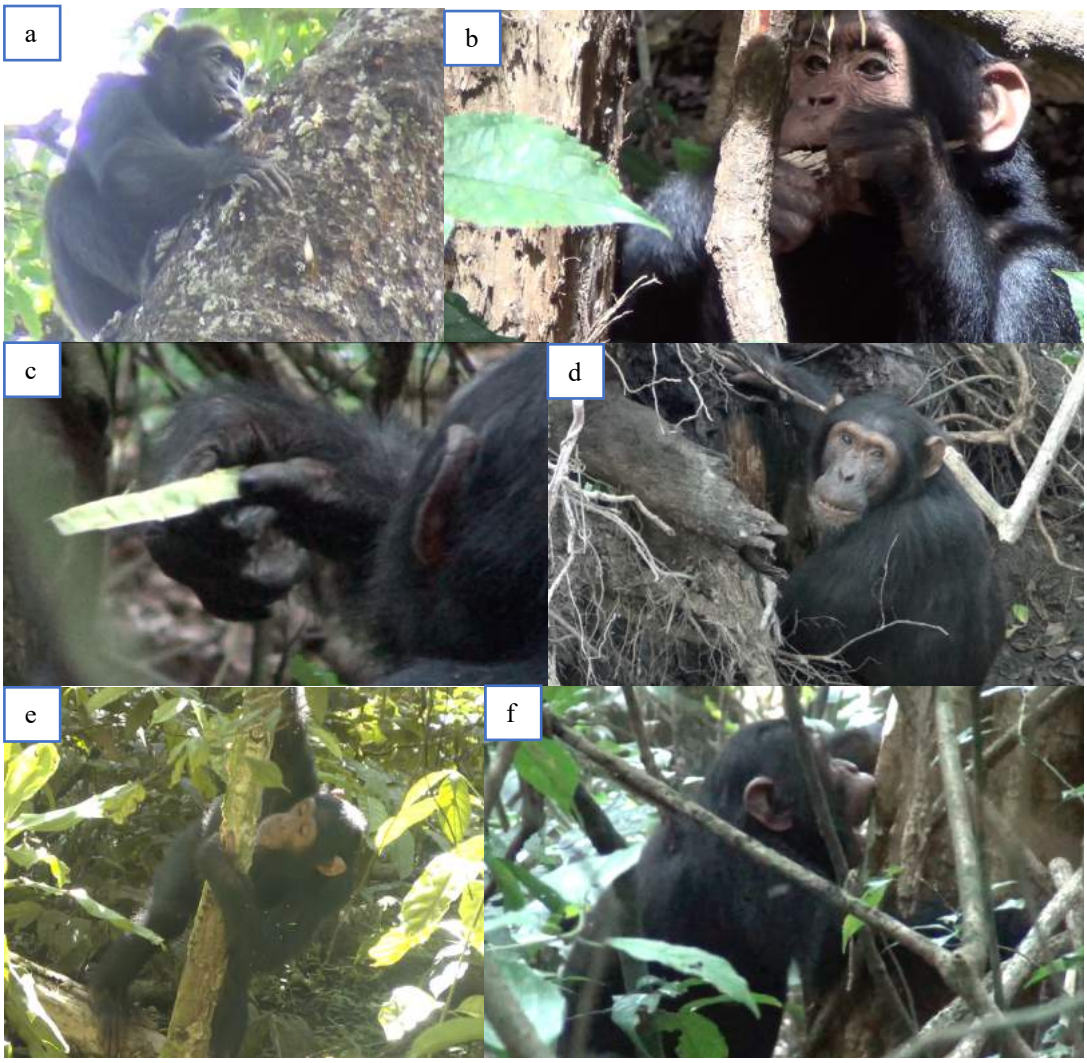
***SM Figure 3**: Plate layout growth inhibition assay; above: Library Screen, below: Dose Response*

**Note: Library Screen:** screening against human and food pathogens, done in 96-wells-mikrotiterplate; AB: Antibiotic as positive control; DMSO: vehicle control / negative control; GC: growth control: containing working culture, to check whether the bacterium grew/active
**Dose response:** done in descending concentration of samples, DMSO and antibiotic. MB: Media blank, consisted of CAMHB as negative/ sterile media control; DMSO as negative/ vehicle control; GC: growth control, consisted of working culture.




**Legend:**
Blk– Blank
TA– Total Activity
NSB– Non-Specific Binding
Bo– Maximum Binding
S1-S8– Prostaglandin Standards 1-8
BG 1-2– Background COX-2
IA 1-3– 100% Initial Activity Samples
H– COX Inhibitor Samples in duplicate (C2)

***SM Figure 4**: ELISA assay setup for anti-inflammatory assay*




***SM Figure 5: a.)*** *IN stripping K. anthotheca bark and resin* ***b.)*** *MZ stripping S. myrtina bark* ***c.)*** *KC stripping A. polystachyus pith* ***d.)*** *MB eating C. patens dead wood* ***e.)*** *OZ stripping S. guineense bark (outside study-period)* ***g.)*** *MZ stripping F. exasperata bark*

## Supplementary Tables:

**SM TABLE 1:** Information on strain ID for each bacteria

| Scientific Name | Strain ID | Resistances | Incubation length (h) | Type of isolate |
|---|---|---|---|---|
| *Acinetobacter baumannii* | DSM 102929 | Penicillin G, Oxacillin, Ampicillin, Ticarcillin, Mezlocillin, Cefazolin, Cefotaxime, Vancomycin, Gentamicin, Ciprofloxacin | 22 | clinical isolate: human, skin, thigh |
| *Enterobacter cloacae* | DSM 30054 | Penicillin G, Oxacillin, Ampicillin, Cefazolin | 18 | clinical isolate: spinal fluid |
| *Enterococcus faecium* | DSM 13590 | Penicillin G, Oxacillin, Ticarcillin, Cefazolin, Cefotaxime, Aztreonam | 18 | clinical isolate: human faeces, rectal swab |
| *Escherichia coli* K12 | DSM 498 | Penicillin G, Oxacillin | 18 | unknown |
| *Escherichia coli* | DSM 1576 | Oxacillin, Vancomycin, Lincomycin, Bacitracin, Clindamycin, Linezolid, Nystatin, Quinupristin/Dalfopristin, Teicoplanin | 18 | human faeces |
| *Klebsiella pneumoniae* | DSM 16609 | Penicillin G, Oxacillin, Ampicillin, Ticarcillin, Mezlocillin | 18 | blood |
| *Pseudomonas aeruginosa* | DSM 1117 | Chloramphenicol, Vancomycin, Penicillin G, Oxacillin, Ampicillin, Cefazolin | 18 | clinical isolate: blood culture; Human blood |
| *Staphylococcus aureus* | DSM 1104 | Aztreonam | 18 | clinical isolate: Human |
| *Staphylococcus aureus* | DSM 18827 | Penicillin G, Oxacillin, Cefazolin, Cefotaxime, Aztreonam, Imipenem | 18 | clinical isolate: tracheal secret; 65-year-old male |
| *Stenotrophomonas maltophilia* | DSM 50170 [ATTC: 13637] | Penicillin G, Oxacillin, Ampicillin, Ticarcillin, Cefalotin, Cefazolin, Aztreonam | 18 | clinical isolate: Oropharyngeal region of patient with mouth cancer |
| *Salmonella enterica* subsp. *enterica* | DSM 11320 | Wildtype | 18 | unknown |

**NB:** Permanent culture created in 2020; Source: Bacdive.dsmz.de and lab data

**SM TABLE 2:** List of antibiotics used

| Abbreviations | Antibacterial agents |
|---|---|
| CIP | ciprofloxacin |
| TET | tetracycline |
| GEN | gentamicin |
| VAN | vancomycin |
| CHL | chloramphenicol |

**SM TABLE 3:** Pre-screening results of the *in vitro* antibacterial trials at 256 µg/mL (library screen)

| | | Inhibition [%] ≥ 40 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant species** | **Extract ID** | ***S. aureus* DSM 1104** | ***S. aureus* DSM 18827** | ***A. baumannii* DSM 102929** | ***E. cloacae* DSM 30054** | ***K. pneumoniae* DSM 16609** | ***P. aeruginosa* DSM 1117** | ***E. faecium* DSM 13590** | ***E. coli* DSM 498** | ***E. coli* DSM 15076** | ***S. maltophilia* DSM 50170** | ***S. enterica* subsp. *enterica* DSM 11320** |
| *C. parasitica* | mwE087 | - | - | - | - | - | - | - | - | - | - | - |
| | eE087 | - | - | - | - | - | - | - | + | - | - | - |
| | hE087 | - | - | - | - | - | - | - | + | + | - | - |
| *K. anthotheca* | mwE088 | - | - | + | - | - | - | + | + | + | + | - |
| | eE088 | - | - | - | - | - | - | + | + | + | - | - |
| | hE088 | - | - | - | - | - | - | + | - | + | - | + |
| *S. myrtina* | mwE089a | - | - | - | - | - | - | - | + | + | - | - |
| | eE089a | - | - | - | - | - | - | + | + | + | - | - |
| | hE089a | - | - | - | - | - | - | - | + | - | - | - |
| | mwE089b | - | - | - | - | - | - | - | - | + | - | - |
| | eE089b | - | - | + | - | - | - | + | + | + | - | - |
| | hE089b | - | + | + | - | - | - | - | - | + | - | - |
| *W. elongata* | mwE090 | - | - | - | - | - | - | - | - | + | - | - |
| | eE090 | - | - | - | - | - | - | + | + | + | - | - |
| | hE090 | - | - | - | - | - | - | - | - | - | - | - |
| *C. patens* | mwE091 | - | - | - | - | - | - | - | - | + | - | - |
| | eE091 | + | - | + | - | - | - | + | + | + | + | + |
| | hE091 | - | - | - | - | - | - | + | - | + | - | - |
| *A. boonei* | mwE092a | - | - | - | - | - | - | - | - | + | - | - |
| | eE092a | - | - | - | - | - | - | - | - | - | - | - |
| | hE092a | - | - | + | - | - | - | - | - | - | - | - |
| | mwE092b | - | - | - | - | - | - | - | + | + | - | - |
| | eE092b | + | - | + | - | - | - | + | + | + | - | - |
| | hE092b | + | + | - | - | - | - | + | + | + | - | + |
| *F. exasperata* | mwE093a | - | - | - | - | - | - | - | - | + | - | - |
| | eE093a | - | - | - | - | - | - | + | + | + | - | - |
| | hE093a | - | - | - | - | - | - | + | - | - | - | - |
| | mwE093b | - | - | - | - | - | - | - | - | + | - | - |
| | eE093b | - | - | - | - | - | - | - | - | - | - | - |
| | hE093b | - | - | - | - | - | - | - | + | + | - | - |
| *M. leucantha* | mwE094 | - | - | - | - | - | - | - | - | + | - | - |
| | eE094 | - | - | - | - | - | - | - | + | + | - | - |
| | hE094 | - | - | + | - | - | - | - | - | + | - | - |
| *D. dewevrei* | mwE095 | - | - | + | - | - | - | - | + | + | + | - |
| | eE095 | - | - | - | - | - | - | - | + | + | - | - |
| | hE095 | - | - | - | - | - | - | - | - | + | - | - |
| *C. alexandri* | mwE096 | + | - | + | - | - | - | - | - | + | + | - |
| | eE096 | - | - | - | - | - | - | - | + | + | - | - |
| | hE096 | - | - | - | - | - | - | - | + | + | - | - |
| *F. variifolia* | mwE097 | - | - | - | - | - | - | - | - | - | - | - |
| | eE097 | - | - | - | - | - | - | + | + | + | - | - |
| | hE097 | - | - | - | - | - | - | - | + | + | - | - |
| *S. guineense* | mwE098a | + | + | + | + | - | + | - | + | + | + | - |
| | eE098a | - | + | + | + | - | - | - | + | + | + | - |
| | hE098a | - | - | - | - | - | - | - | - | + | - | - |
| | mwE098b | + | + | + | + | - | - | - | + | + | + | - |
| | eE098b | - | + | + | + | - | - | - | - | + | + | - |
| | hE098b | - | - | + | + | - | - | - | + | + | + | - |
| *A. polystachius* | mwE099 | - | - | - | - | - | - | - | - | - | - | - |
| | eE099 | - | - | - | - | - | - | + | + | + | - | - |
| | hE099 | + | - | + | - | - | - | + | + | + | - | - |

\+ : samples with ≥40% Inhibition

\- : samples with <40% Inhibition

**SM TABLE 4:** $IC_{50}$ and MIC values with standard deviations obtained from *in vitro* dose response assays

| | Gram | + | | | | + | | | | - | | | | - | | | | - | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Scientific name** | **Extract ID** | ***S. aureus* DSM 1104** | | | | ***S. aureus* DSM 18827** | | | | ***A. baumannii* DSM 102929** | | | | ***E. cloacae* DSM 30054** | | | | ***P. aeruginosa* DSM 1117** | | | |
| | | **$IC_{50}$** | s | **MIC** | s | **$IC_{50}$** | s | **$IC_{50}$** | s | **$IC_{50}$** | s | **MIC** | s | **$IC_{50}$** | s | **MIC** | s | **$IC_{50}$** | s | **MIC** | s |
| *C. parasitica* | eE087 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE087 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *K. anthotheca* | mwE088 | - | - | - | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | eE088 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE088 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *S. myrtina* | mwE089a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE089a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE089a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE089b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE089b | - | - | - | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | hE089b | - | - | - | - | >256 | - | >256 | - | 256 | 0.9 | >256 | - | - | - | - | - | - | - | - | - |
| *W. elongata* | mwE090 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE090 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *C. patens* | mwE091 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE091 | 128 | 0.9 | 256 | 0.4 | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | hE091 | - | - | - | - | - | - | - | - | | | | | - | - | - | - | - | - | - | - |
| *A. boonei* | mwE092a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE092a | - | - | - | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | mwE092b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE092b | 32 | 4.3 | 128 | 0.1 | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | hE092b | 16 | 9.5 | 32 | 0.1 | 32 | 2.5 | >256 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *F. exasperata* | mwE093a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE093a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE093a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE093b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE093b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *M. leucantha* | mwE094 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | eE094 | - | - | - | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | hE094 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *D. dewevrei* | mwE095 | - | - | - | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | eE095 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE095 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *C. alexandri* | mwE096 | >256 | - | >256 | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |
| | eE096 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE096 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *F. variifolia* | eE097 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE097 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *S. guineense* | mwE098a | >256 | - | >256 | - | >256 | - | >256 | - | 64 | 1.1 | >256 | - | 128 | 0.6 | >256 | - | 64 | 1.9 | >256 | - |
| | eE098a | - | - | - | - | >256 | - | >256 | - | 128 | 0.7 | >256 | - | 256 | 4.2 | >256 | - | - | - | - | - |
| | hE098a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE098b | 128 | 1.1 | >256 | - | >256 | - | >256 | - | 128 | 0.4 | >256 | - | 128 | 0.2 | >256 | - | - | - | - | - |
| | eE098b | - | - | - | - | >256 | - | >256 | - | >256 | - | >256 | - | 256 | 2.6 | >256 | | - | - | - | - |
| | hE098b | - | - | - | - | >256 | - | >256 | - | >256 | - | >256 | - | >256 | - | >256 | - | - | - | - | - |
| *A. polystachius* | eE099 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | hE099 | 256 | 4.4 | >256 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Vancomycin** | | <1 | - | 2 | 0 | <1 | - | 1 | 1.8 | >64 | - | >64 | - | >64 | - | >64 | - | >64 | - | >64 | - | >64 | - |
| **Gentamicin** | | 0.125 | | 0.25 | | 0.5 | 0.9 | 1 | 0.1 | >64 | - | >64 | - | n.t. | | | | n.t. | | | | | |
| **Ciprofloxacin** | | n.t. | | | | n.t. | | | | >64 | - | >64 | - | n.t. | | | | n.t. | | | | | |
| **Tetracyclin** | | n.t. | | | | n.t. | | | | 1 | 9.4 | 2 | 1 | n.t. | | | | n.t. | | | | | |
| **Chloramphenicol** | | 2 | 0.6 | 8 | 0.3 | 4 | 1.1 | 16 | 0.8 | 32 | 1.3 | 64 | 0.9 | 2 | 1.7 | 4 | 8.1 | 32 | 0.3 | >64 | - | | |

**NB:** Only extracts showing growth inhibition ≥40% in the library screen at 256 µg/mL are listed. $IC_{50}$ and MIC values are expressed as concentration (µg/mL).
**s**: standard deviation. **n.t:** not tested

## **SM TABLE 4:** Continued

| | **Gram** | + | | | | - | | | | - | | | | - | | | | - | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Scientific name** | **Extract ID** | ***E. faecium* DSM 13590** | | | | ***E. coli* DSM 498** | | | | ***E. coli* DSM 1576** | | | | ***S. maltophilia* DSM 50170** | | | | ***S. enterica* subsp. *enterica* *DSM 11320*** | | | |
| | | **$IC_{50}$** | **s** | **MIC** | **s** | **$IC_{50}$** | **s** | **MIC** | **s** | **$IC_{50}$** | **s** | **MIC** | **s** | **$IC_{50}$** | **s** | **MIC** | **s** | **$IC_{50}$** | **s** | **MIC** | **s** |
| *C. parasitica* | eE087 | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | hE087 | - | - | - | - | >256 | - | >256 | - | 128 | 0.5 | >256 | - | - | - | - | - | - | - | - | - |
| *K. anthotheca* | mwE088 | 16 | 3.7 | 32 | 2.3 | >256 | - | >256 | - | 16 | 1.4 | 256 | 0.3 | 64 | 4.4 | >256 | - | - | - | - | - |
| | eE088 | 64 | 1.8 | 64 | 1.8 | >256 | - | >256 | - | 64 | 0.9 | >256 | - | - | - | - | - | - | - | - | - |
| | hE088 | 64 | 8.4 | 128 | 5 | - | - | - | - | 64 | 5.7 | >256 | - | - | - | - | - | 256 | 5.5 | >256 | - |
| *S. myrtina* | mwE089a | - | - | - | - | >256 | - | >256 | - | 128 | 2.8 | >256 | - | - | - | - | - | - | - | - | - |
| | eE089a | 64 | 2.1 | >256 | - | >256 | - | >256 | - | 256 | 2.1 | >256 | - | - | - | - | - | - | - | - | - |
| | hE089a | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE089b | - | - | - | - | - | - | - | - | 256 | 0.2 | >256 | - | - | - | - | - | - | - | - | - |
| | eE089b | 128 | 5.8 | >256 | - | >256 | - | >256 | - | 256 | 0.8 | >256 | - | - | - | - | - | - | - | - | - |
| | hE089b | - | - | - | - | - | - | - | - | 256 | 1.3 | >256 | - | - | - | - | - | - | - | - | - |
| *W. elongata* | mwE090 | - | - | - | - | - | - | - | - | 128 | 0.3 | >256 | - | - | - | - | - | - | - | - | - |
| | eE090 | 64 | 1.3 | 128 | 3.4 | >256 | - | >256 | - | 256 | 1.4 | >256 | - | - | - | - | - | - | - | - | - |
| *C. patens* | mwE091 | - | - | - | - | - | - | - | - | 128 | 2.2 | >256 | - | - | - | - | - | - | - | - | - |
| | eE091 | 64 | 0.9 | 64 | 0.9 | >256 | - | >256 | - | 128 | 18.1 | >256 | - | 256 | 0.7 | >256 | - | >256 | - | >256 | - |
| | hE091 | 64 | 2.2 | >256 | - | - | - | - | - | 128 | 2.2 | >256 | - | - | - | - | - | - | - | - | - |
| *A. boonei* | mwE092a | - | - | - | - | - | - | - | - | 128 | 1.3 | >256 | - | - | - | - | - | - | - | - | - |
| | hE092a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE092b | - | - | - | - | 256 | 2.1 | >256 | - | 32 | 3.1 | >256 | - | - | - | - | - | - | - | - | - |
| | eE092b | 16 | 1.2 | 64 | 1.6 | >256 | - | >256 | - | 128 | 8.9 | >256 | - | - | - | - | - | - | - | - | - |
| | hE092b | 16 | 3.1 | >256 | - | >256 | - | >256 | - | 256 | 0.4 | >256 | - | - | - | - | - | 256 | 5.3 | 256 | 5.3 |
| *F. exasperata* | mwE093a | - | - | - | - | - | - | - | - | 128 | 1.4 | >256 | - | - | - | - | - | - | - | - | - |
| | eE093a | 32 | 0.1 | 128 | 7.5 | >256 | - | >256 | - | 128 | 3.0 | >256 | - | - | - | - | - | - | - | - | - |
| | hE093a | 32 | 4.1 | >256 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | mwE093b | - | - | - | - | - | - | - | - | 256 | 1.0 | >256 | - | - | - | - | - | - | - | - | - |
| | hE093b | - | - | - | - | >256 | - | >256 | - | 256 | 2.2 | >256 | - | - | - | - | - | - | - | - | - |
| *M. leucantha* | mwE094 | - | - | - | - | - | - | - | - | 128 | 5.0 | - | - | - | - | - | - | - | - | - | - |
| | eE094 | - | - | - | - | >256 | - | >256 | - | 256 | 3.3 | >256 | - | - | - | - | - | - | - | - | - |
| | hE094 | - | - | - | - | - | - | - | - | 256 | 1.4 | >256 | - | - | - | - | - | - | - | - | - |
| *D. dewevrei* | mwE095 | - | - | - | - | >256 | - | >256 | - | 256 | 2.1 | >256 | - | 256 | 0.8 | >256 | - | - | - | - | - |
| | eE095 | - | - | - | - | >256 | - | >256 | - | 256 | 3.4 | >256 | - | - | - | - | - | - | - | - | - |
| | hE095 | - | - | - | - | - | - | - | - | >256 | - | >256 | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. alexandri* | mwE096 | - | - | - | - | - | - | - | - | 256 | 0.6 | >256 | - | 256 | 2.2 | >256 | - | - | - | - | - |
| | eE096 | - | - | - | - | >256 | - | >256 | - | 256 | 3.9 | >256 | - | - | - | - | - | - | - | - | - |
| | hE096 | - | - | - | - | >256 | - | >256 | - | 256 | 1.9 | >256 | - | - | - | - | - | - | - | - | - |
| *F. variifolia* | eE097 | 64 | 2.5 | >256 | - | >256 | - | >256 | - | 256 | 0.4 | >256 | - | - | - | - | - | - | - | - | - |
| | hE097 | - | - | - | - | >256 | - | >256 | - | 256 | 0.6 | >256 | - | - | - | - | - | - | - | - | - |
| *S. guineense* | mwE098a | - | - | - | - | >256 | - | >256 | - | 64 | 2.7 | >256 | - | 32 | 0.3 | 256 | 1.0 | - | - | - | - |
| | eE098a | - | - | - | - | >256 | - | >256 | - | 128 | 4.7 | >256 | - | 64 | 0.8 | 256 | 4.1 | - | - | - | - |
| | hE098a | - | - | - | - | - | - | - | - | 128 | 1.4 | >256 | - | - | - | - | - | - | - | - | - |
| | mwE098b | - | - | - | - | 128 | 0.2 | >256 | - | 32 | 5.1 | 128 | 0.7 | 32 | 0.5 | >256 | - | - | - | - | - |
| | eE098b | - | - | - | - | >256 | - | >256 | - | 128 | 2.6 | 256 | 2 | 128 | 2.7 | >256 | - | - | - | - | - |
| | hE098b | - | - | - | - | - | - | - | - | 256 | 1 | >256 | - | 128 | 0.8 | >256 | - | - | - | - | - |
| *A. polystachius* | eE099 | 128 | 7.5 | 256 | 7.1 | >256 | - | >256 | - | 256 | 1.5 | >256 | - | - | - | - | - | - | - | - | - |
| | hE099 | 32 | 7.6 | 128 | 7.9 | >256 | - | >256 | - | 256 | 1.0 | >256 | - | - | - | - | - | - | - | - | - |
| **Vancomycin** | | >64 | - | >64 | - | n.t. | | | | n.t. | | | | n.t. | | | | n.t. | | | |
| **Gentamicin** | | 8 | 2.3 | >64 | - | <1 | - | 1 | 3.9 | n.t. | | | | 8 | 0.3 | 8 | 0.3 | n.t. | | | |
| **Ciprofloxacin** | | n.t. | | | | n.t. | | | | n.t. | | | | n.t. | | | | n.t. | | | |
| **Tetracyclin** | | n.t. | | | | <1 | - | 2 | 0.5 | n.t. | | | | n.t. | | | | 1 | 1.4 | 2 | 0.4 |
| **Chloramphenicol** | | - | 2 | 4 | 0.8 | n.t. | | | | <1 | - | 4 | 0.1 | n.t. | | | | 4 | 6.8 | 8 | 5.5 |

**NB:** Only extracts showing growth inhibition ≥40% in the library screen at 256 µg/mL are listed. $IC_{50}$ and MIC values are expressed as concentration (µg/mL).
**s**: standard deviation. **n.t:** not tested

**SM TABLE 5:** Results from the COX-2 inhibition library screen at descending concentrations

| | | | | COX-2 Inhibition ≥50 % | | |
|---|---|---|---|---|---|---|
| **Extract ID** | **Plant Species** | **Plant Part** | **Type of Extract** | **50 µg/ml** | **10 µg/ml** | **5 µg/ml** |
| mwE087 | *C. parasitica* | whole plant | methanol/water (9:1, *v/v*) | + | + | + |
| eE087 | | | ethyl acetate | + | + | + |
| hE087 | | | *n*-hexane | + | + | + |
| mwE088 | *K. anthotheca* | stem bark and resin | methanol/water (9:1, *v/v*) | + | + | + |
| eE088 | | | ethyl acetate | + | + | + |
| hE088 | | | *n*-hexane | + | + | + |
| mwE089a | *S. myrtina* | strips of stem bark (refuse) | methanol/water (9:1, *v/v*) | - | - | - |
| eE089a | | | ethyl acetate | + | - | - |
| hE089a | | | *n*-hexane | + | + | + |
| mwE089b | | stem bark | methanol/water (9:1, *v/v*) | + | - | - |
| eE089b | | | ethyl acetate | + | + | - |
| hE089b | | | *n*-hexane | - | - | - |
| mwE090 | *W. elongata* | leaves | methanol/water (9:1, *v/v*) | + | - | - |
| eE090 | | | ethyl acetate | + | - | - |
| hE090 | | | *n*-hexane | + | - | - |
| mwE091 | *C. patens* | dead wood | methanol/water (9:1, *v/v*) | + | - | - |
| eE091 | | | ethyl acetate | + | - | - |
| hE091 | | | *n*-hexane | - | - | - |
| mwE092a | *A. boonei* | stem bark | methanol/water (9:1, *v/v*) | - | - | - |
| eE092a | | | ethyl acetate | - | - | - |
| hE092a | | | *n*-hexane | + | - | - |
| mwE092b | | dead wood | methanol/water (9:1, *v/v*) | + | - | - |
| eE092b | | | ethyl acetate | + | - | - |
| hE092b | | | *n*-hexane | + | + | + |
| mwE093a | *F. exasperata* | stem bark | methanol/water (9:1, *v/v*) | + | - | - |
| eE093a | | | ethyl acetate | + | + | + |
| hE093a | | | *n*-hexane | + | + | + |
| mwE093b | | leaves | methanol/water (9:1, *v/v*) | + | - | - |
| eE093b | | | ethyl acetate | + | + | - |
| hE093b | | | *n*-hexane | + | + | - |
| mwE094 | *M. leucantha* | pith | methanol/water (9:1, *v/v*) | + | - | - |
| eE094 | | | ethyl acetate | + | - | - |
| hE094 | | | *n*-hexane | + | + | + |
| mwE095 | *D. dewevrei* | stem bark | methanol/water (9:1, *v/v*) | + | - | - |
| eE095 | | | ethyl acetate | + | - | - |
| hE095 | | | *n*-hexane | + | - | - |
| mwE096 | *C. alexandri* | stem bark | methanol/water (9:1, *v/v*) | - | - | - |
| eE096 | | | ethyl acetate | - | - | - |
| hE096 | | | *n*-hexane | + | + | + |
| mwE097 | *F. variifolia* | stem bark | methanol/water (9:1, *v/v*) | + | - | - |
| eE097 | | | ethyl acetate | + | + | + |
| hE097 | | | *n*-hexane | + | + | + |
| mwE098a | *S. guineense* | stem bark | methanol/water (9:1, *v/v*) | + | - | - |
| eE098a | | | ethyl acetate | + | - | - |
| hE098a | | | *n*-hexane | + | + | + |
| mwE098b | | leaves | methanol/water (9:1, *v/v*) | - | - | - |
| eE098b | | | ethyl acetate | + | - | - |
| hE098b | | | *n*-hexane | + | - | - |
| mwE099 | *A. polystachyus* | pith | methanol/water (9:1, *v/v*) | + | - | - |
| eE099 | | | ethyl acetate | + | + | + |
| hE099 | | | *n*-hexane | + | + | + |

**SM TABLE 6**: Summary of COX-2 and antibacterial results across species

| | | | LIBRARY SCREEN ACTIVITY: | | | | |
|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant Part** | **Extract ID** | **COX-2 [50µg/mL]** | **Antibacterial Library Screen** | **Number of active extracts on antibacterial library screen** | **Number of strains that extracts were active against on library screen** | **Number of strains each extract was active against on dose-response assays (≤256µg/mL)** |
| *C. parasitica* | whole plant | mwE087 | + | - | 2 | 0 | 0 |
| | | eE087 | + | + | | 1 | nt |
| | | hE087 | + | + | | 2 | 1 |
| *K. anthotheca* | bark | mwE088 | + | + | 3 | 5 | 3 |
| | | eE088 | + | + | | 3 | 2 |
| | | hE088 | + | + | | 3 | 3 |
| *S. myrtina* | dead wood | mwE089a | - | + | 6 | 2 | 1 |
| | | eE089a | + | + | | 3 | 2 |
| | | hE089a | + | + | | 1 | 0 |
| | stripped wood | mwE089b | + | + | | 1 | 1 |
| | | eE089b | + | + | | 4 | 2 |
| | | hE089b | - | + | | 3 | 2 |
| *W. elongata* | leaves | mwE090 | + | + | 2 | 1 | 1 |
| | | eE090 | + | + | | 3 | 2 |
| | | hE090 | + | - | | 0 | nt |
| *C. patens* | dead wood | mwE091 | + | + | 3 | 1 | 1 |
| | | eE091 | + | + | | 7 | 4 |
| | | hE091 | - | + | | 2 | 2 |
| *A. boonei* | bark | mwE092a | - | + | 5 | 1 | 1 |
| | | eE092a | - | - | | 0 | nt |
| | | hE092a | + | + | | 1 | 0 |
| | dead wood | mwE092b | + | + | | 2 | 2 |
| | | eE092b | + | + | | 5 | 3 |
| | | hE092b | + | + | | 6 | 5 |
| *F. exasperata* | bark | mwE093a | + | + | 5 | 1 | 1 |
| | | eE093a | + | + | | 3 | 2 |
| | | hE093a | + | + | | 1 | 1 |
| | leaves | mwE093b | + | + | | 1 | 1 |
| | | eE093b | + | - | | 0 | nt |
| | | hE093b | + | + | | 2 | 1 |
| *M. leucantha* | pith | mwE094 | + | + | 3 | 1 | 1 |
| | | eE094 | + | + | | 2 | 1 |
| | | hE094 | + | + | | 2 | 1 |
| *D. dewevrei* | bark | mwE095 | + | + | 3 | 4 | 2 |
| | | eE095 | + | + | | 2 | 1 |
| | | hE095 | + | + | | 1 | 0 |
| *C. alexandri* | bark | mwE096 | - | + | 3 | 4 | 2 |
| | | eE096 | - | + | | 2 | 1 |
| | | hE096 | + | + | | 2 | 1 |
| *F. variifolia* | bark | mwE097 | + | - | 2 | 0 | nt |
| | | eE097 | + | + | | 3 | 2 |
| | | hE097 | + | + | | 2 | 1 |
| *S. guineense* | bark | mwE098a | + | + | 6 | 8 | 5 |
| | | eE098a | + | + | | 6 | 4 |
| | | hE098a | + | + | | 1 | 1 |
| | leaves | mwE098b | - | + | | 7 | 6 |
| | | eE098b | + | + | | 5 | 3 |
| | | hE098b | + | + | | 5 | 2 |
| *A. polystachius* | pith | mwE099 | + | - | 2 | 0 | nt |
| | | eE099 | + | + | | 3 | 2 |
| | | hE099 | + | + | | 5 | 3 |
| | | **Total** | **43** | **45** | | | |

# Appendix H References:

Schultz, F., Anywar, G., Tang, H., Chassagne, F., Lyles, J. T., Garbe, L.-A., & Quave, C. L. (2020). Targeting ESKAPE pathogens with anti-infective medicinal plants from the Greater Mpigi region in Uganda. *Scientific Reports*, *10*(1), 11935. https://doi.org/10.1038/s41598-020-67572-8

# APPENDIX F:
# Supplementary Materials Chapter 6:
## Fallback Food Hypothesis Fails to Explain the Value of Bark in the Diet of Chimpanzees of the Budongo Forest

### Supplementary Information:

#### Data Collection

***Long-Term Site Data***

The site's long-term behavioral dataset includes all bark ingestion events recorded during focal follows by field staff. Data collection occurs in Sonso between 07:00-16:30, and in Waibira between 06:30-17:00 daily. Standardized focal feeding behaviors were recorded in Sonso from 2008 and in Waibira from 2015. From 2007-2008, data were collected as party composition scans in fifteen-minute intervals. Bark stripping events were recorded when they involved a focal individual, as well as party composition at time of scan, nearest neighbor to the focal, tree species being ingested, dbh, and crown size of the stripped tree. In cases when bark ingestion events did not include block number, party composition scans (taken at 15-minute intervals throughout focal follows) were cross-checked. The focal individual's location from the scan closest to the time of the bark ingestion event was then matched to the relevant observation, and the block number was aggregated with focal data. To eliminate redundant observations, if two or more consecutive bark ingestion events occurred on the same day, by the same individual, in the same block, *and* from the same species, only the first event was kept. If multiple individuals consumed the same tree at the same time, individual bouts were counted as separate events. Events were only extracted from the long-term data for this study if bark was the plant part associated with ingestion. Bark stripping events from 2020-2021 are likely underreported, as data collection was interrupted throughout this year due to the Covid-19 pandemic.

There are two species with possible biased representation in the long-term data: *Khaya anthotheca,* which is commonly exploited for a combination of the resin and bark is likely underrepresented as a single plant part is typically recorded for each feeding event, and our data excludes cases when the plant part consumed was recorded as "resin." *Cleistopholis patens*, on the other hand, is likely over-represented in the dataset. While the living bark of *C. patens* is occasionally consumed, the

dead wood of this species is also a food item in both communities. It is impossible to ascertain from the long-term data whether cases of bark ingestion of *C. patens* are accidental conflations of dead wood and bark. To avoid introducing our own biases, we chose not to remove either species in our analysis, however, the frequency of bark ingestion for these two trees should be cautiously interpreted.

Long-term weather data (rainfall and temperature) were also used from the Budongo Conservation Field Station, which is measured every morning by a member of the field staff (GM) from a station at base camp. Ripe fruit abundance was calculated based on a Fruit Availability Index calculated from long-term site data. Data from two to 30 trees across 18 fruit-bearing feeding species, known to be of importance for Budongo chimpanzees were collected each month. Ripe fruit availability was scored as 0 (absent) or 1 (present) in earlier years, and on a scale from 0-4 in later years. Data on the 0-4 scale were converted to 0 or 1 (all non-zero scores) to be able to combine datasets across years. Fruit Availability Indices used in this study were calculated for the years of 2013-2019 using the formula: FAI = 100 * ($\sum$ (p * f) / $\sum$ (p * 4)), in which FAI is the fruit availability index percentage, p is the basal area of the tree in $cm^2$ and f is the abundance of ripe fruit (Hockings et al., 2010; Takemoto, 2004).

***Long-Term Site Data Manipulation***

To assess the spatial distribution of bark stripping events some data manipulation was required. The BCFS grid system is delineated into lines and blocks, where each block sits between four adjacent trail segments (Badihi et al., 2022). Sonso blocks are consistently 100 x 100m, however, Waibira blocks can vary in size (200 x 500m, 200 x 200m, and 100 x 200m). For uniformity between and throughout datasets over time, we created a new grid system for this study following the methods of Badihi et al. (2022), in which all blocks were recreated following the original Waibira trail system dimensions of 200 x 500m. Smaller blocks were combined to form these larger standardized blocks, allowing us to control for uneven sampling (**SM Figure 1**).

***Behavioral Data Collection During the Study Period***

Criteria for focal selection included 1.) whether parasite load or health state of the individual was known, 2.) whether the individual was or had been exhibiting sickness or unusual feeding behaviors, and 3.) whether the individual was part of a dependent dyad (mother-infant/adopter-

orphan). If no available individual met these criteria, a focal was selected at random. All feeding data were recorded for focal individuals, and *ad libitum* events were recorded for events of interest, including bark stripping events. Group feeding events (events which involved >1 individual) were counted as one event.

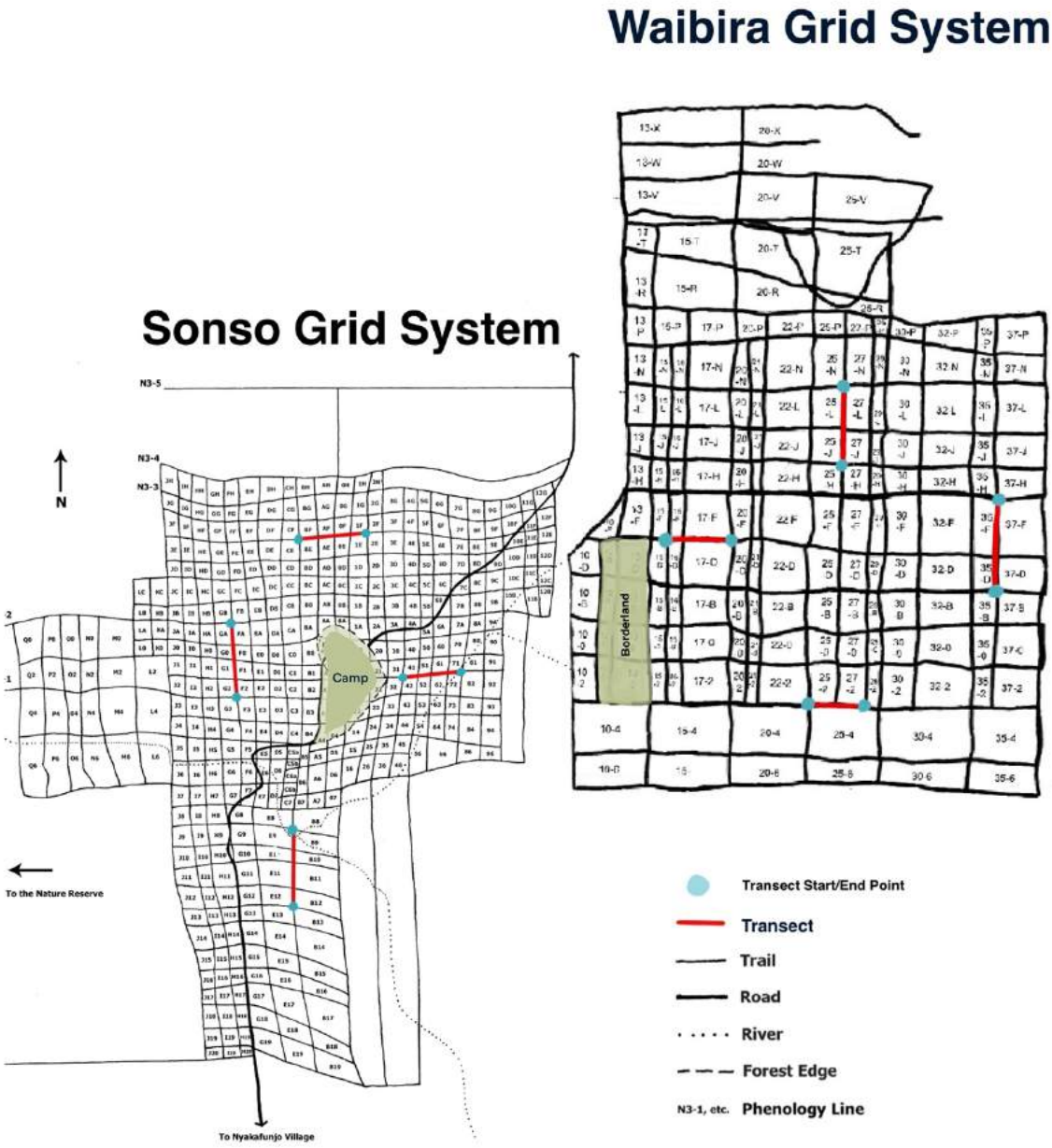
***Forest Transects***

All trees >12cm dbh (diameter at breast height) were measured, as only a few cases of bark stripping from a singular species had been observed on trees smaller than this dbh in the long-term data, and none were observed during the study period. All living trees that met this criterium were measured for dbh and the species identified by Moses Businge, director of phenology at BCFS. When trees showed evidence of bark stripping (fresh wounds, teeth marks, wadges, or characteristic signs of regrowth) we recorded the GPS location, the number of strips, measurement of longest strip, and stage(s) of bark healing from past strips on a 1-4 scale using the metric established by Lapuente et al. (2020). Trees were scanned using 3D Scanner on iPad for *post hoc* measurements. When trees could not be identified dbh was still taken if possible. As only trunks were assessed for signs of bark stripping, stripped species in this dataset are biased toward trees used in terrestrial bark stripping events.

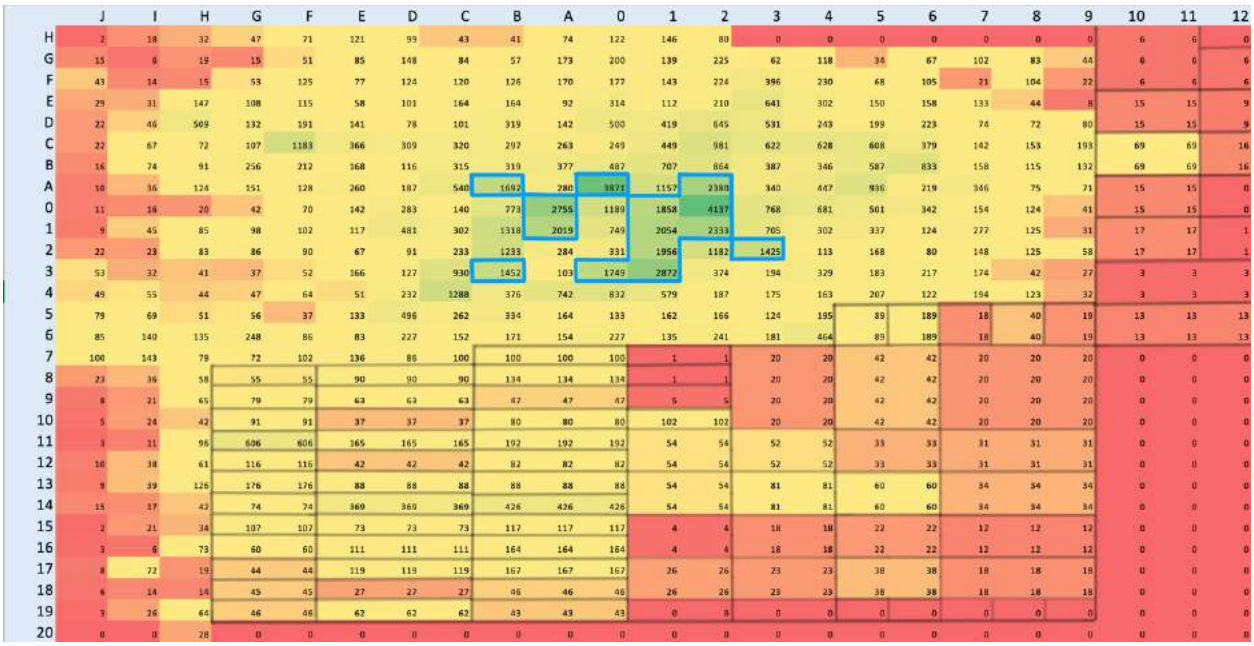
A few species-specific factors may also bias tree representation in the long-term data. *Eucalpytus sp.*, present in the long-term data as a tree used for bark stripping, is an introduced species to Budongo. Only one tree of this species has thus far been identified, located in the Sonso home range (block 1,1). *B. papyrifera* is another introduced species, which grows in high concentrations near the center of the Sonso home range (blocks 2,0 and 2,1) as well as around basecamp (block 0,0). *D. kirkii*, which is stripped at high frequencies in Waibira, has only been reported in a few (relatively high elevation) open areas in Waibira. Due to ecological preference for forest-edge habitats and bushland on rocky slopes (POWO, 2023), *D. kirkii* may be unviable in the Sonso home range.

## Supplementary Figures:


***SM Figure 1:*** *[Red] Sonso grid-system [Blue] Waibira grid-system*




***SM Figure 2:*** *Map of forest transects conducted during the study period*

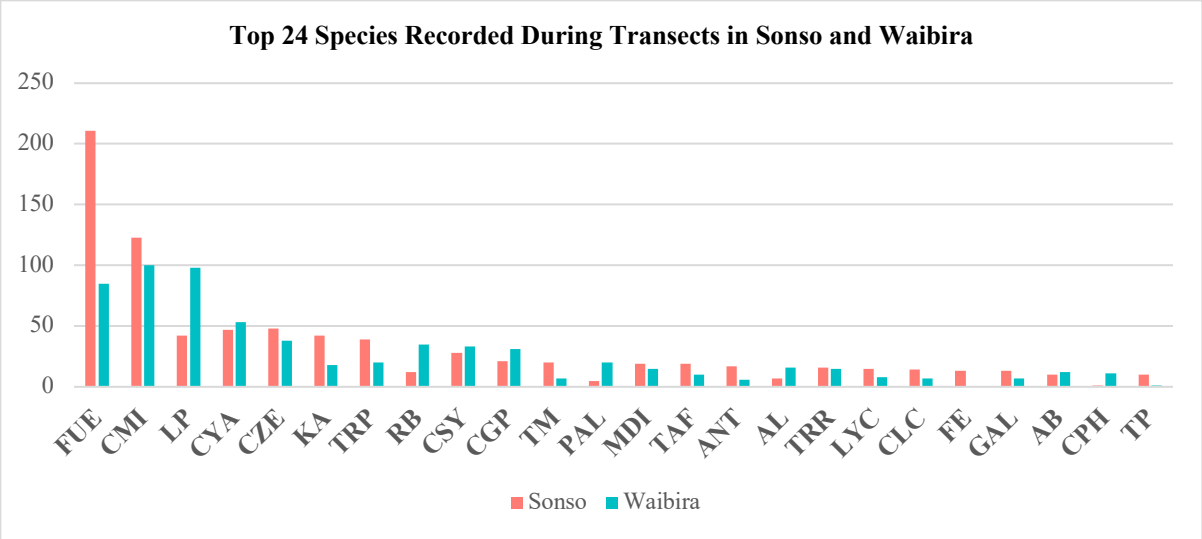


| | J | I | H | G | F | E | D | C | B | A | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H | 2 | 18 | 32 | 47 | 71 | 121 | 99 | 43 | 41 | 74 | 122 | 146 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 0 |
| G | 15 | 6 | 19 | 15 | 51 | 85 | 148 | 84 | 57 | 173 | 200 | 139 | 225 | 62 | 118 | 34 | 67 | 102 | 83 | 44 | 6 | 6 | 6 |
| F | 43 | 14 | 15 | 53 | 125 | 77 | 124 | 120 | 126 | 170 | 177 | 143 | 224 | 396 | 230 | 68 | 105 | 21 | 104 | 22 | 6 | 6 | 6 |
| E | 29 | 31 | 147 | 108 | 115 | 58 | 101 | 164 | 164 | 92 | 314 | 112 | 210 | 641 | 302 | 150 | 158 | 133 | 44 | 8 | 15 | 15 | 9 |
| D | 22 | 46 | 509 | 132 | 191 | 141 | 78 | 101 | 319 | 142 | 500 | 419 | 645 | 531 | 243 | 199 | 223 | 74 | 72 | 80 | 15 | 15 | 9 |
| C | 22 | 67 | 72 | 107 | 1183 | 366 | 309 | 320 | 297 | 263 | 249 | 449 | 981 | 622 | 628 | 608 | 379 | 142 | 153 | 193 | 69 | 69 | 16 |
| B | 16 | 74 | 91 | 256 | 212 | 168 | 116 | 315 | 319 | 377 | 487 | 707 | 864 | 387 | 346 | 587 | 833 | 158 | 115 | 132 | 69 | 69 | 16 |
| A | 10 | 36 | 124 | 151 | 128 | 260 | 187 | 540 | 1602 | 280 | 3823 | 1157 | 2380 | 340 | 447 | 936 | 219 | 346 | 75 | 71 | 15 | 15 | 0 |
| 0 | 11 | 16 | 20 | 42 | 70 | 142 | 283 | 140 | 773 | 2755 | 1189 | 1858 | 4137 | 768 | 681 | 501 | 342 | 154 | 124 | 41 | 15 | 15 | 0 |
| 1 | 9 | 45 | 85 | 98 | 102 | 117 | 481 | 302 | 1318 | 2019 | 749 | 2054 | 2333 | 705 | 302 | 337 | 124 | 277 | 125 | 31 | 17 | 17 | 1 |
| 2 | 22 | 23 | 83 | 86 | 90 | 67 | 91 | 233 | 1233 | 284 | 331 | 1956 | 1182 | 1425 | 113 | 168 | 80 | 148 | 125 | 58 | 17 | 17 | 1 |
| 3 | 53 | 32 | 41 | 37 | 52 | 166 | 127 | 930 | 1452 | 103 | 1749 | 2872 | 374 | 194 | 329 | 183 | 217 | 174 | 42 | 27 | 3 | 3 | 3 |
| 4 | 49 | 55 | 44 | 47 | 64 | 51 | 232 | 1288 | 376 | 742 | 832 | 579 | 187 | 175 | 163 | 207 | 122 | 194 | 123 | 32 | 3 | 3 | 3 |
| 5 | 79 | 69 | 51 | 56 | 37 | 133 | 496 | 262 | 334 | 164 | 133 | 162 | 166 | 124 | 195 | 89 | 189 | 18 | 40 | 19 | 13 | 13 | 13 |
| 6 | 85 | 140 | 135 | 248 | 86 | 83 | 227 | 152 | 171 | 154 | 227 | 135 | 241 | 181 | 464 | 89 | 189 | 18 | 40 | 19 | 13 | 13 | 13 |
| 7 | 100 | 143 | 79 | 72 | 102 | 136 | 86 | 100 | 100 | 100 | 100 | 1 | 1 | 20 | 20 | 42 | 42 | 20 | 20 | 20 | 0 | 0 | 0 |
| 8 | 23 | 36 | 58 | 55 | 55 | 90 | 90 | 90 | 134 | 134 | 134 | 1 | 1 | 20 | 20 | 42 | 42 | 20 | 20 | 20 | 0 | 0 | 0 |
| 9 | 8 | 21 | 65 | 79 | 79 | 63 | 63 | 63 | 47 | 47 | 47 | 5 | 5 | 20 | 20 | 42 | 42 | 20 | 20 | 20 | 0 | 0 | 0 |
| 10 | 5 | 24 | 42 | 91 | 91 | 37 | 37 | 37 | 80 | 80 | 80 | 102 | 102 | 20 | 20 | 42 | 42 | 20 | 20 | 20 | 0 | 0 | 0 |
| 11 | 3 | 31 | 96 | 606 | 606 | 165 | 165 | 165 | 192 | 192 | 192 | 54 | 54 | 52 | 52 | 33 | 33 | 31 | 31 | 31 | 0 | 0 | 0 |
| 12 | 10 | 38 | 61 | 116 | 116 | 42 | 42 | 42 | 82 | 82 | 82 | 54 | 54 | 52 | 52 | 33 | 33 | 31 | 31 | 31 | 0 | 0 | 0 |
| 13 | 9 | 39 | 126 | 176 | 176 | 88 | 88 | 88 | 88 | 88 | 88 | 54 | 54 | 81 | 81 | 60 | 60 | 34 | 34 | 34 | 0 | 0 | 0 |
| 14 | 15 | 17 | 42 | 74 | 74 | 369 | 369 | 369 | 426 | 426 | 426 | 54 | 54 | 81 | 81 | 60 | 60 | 34 | 34 | 34 | 0 | 0 | 0 |
| 15 | 2 | 21 | 34 | 107 | 107 | 73 | 73 | 73 | 117 | 117 | 117 | 4 | 4 | 18 | 18 | 22 | 22 | 12 | 12 | 12 | 0 | 0 | 0 |
| 16 | 3 | 6 | 73 | 60 | 60 | 111 | 111 | 111 | 164 | 164 | 164 | 4 | 4 | 18 | 18 | 22 | 22 | 12 | 12 | 12 | 0 | 0 | 0 |
| 17 | 8 | 72 | 19 | 44 | 44 | 119 | 119 | 119 | 167 | 167 | 167 | 26 | 26 | 23 | 23 | 38 | 38 | 18 | 18 | 18 | 0 | 0 | 0 |
| 18 | 6 | 14 | 14 | 45 | 45 | 27 | 27 | 27 | 46 | 46 | 46 | 26 | 26 | 23 | 23 | 38 | 38 | 18 | 18 | 18 | 0 | 0 | 0 |
| 19 | 3 | 26 | 64 | 46 | 46 | 62 | 62 | 62 | 43 | 43 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

***SM Figure 3:*** *Sonso core areas (outlined in blue) according to party composition data*
**NB:** Blocks outlined in gray are those which comprise of multiple 100x100m blocks

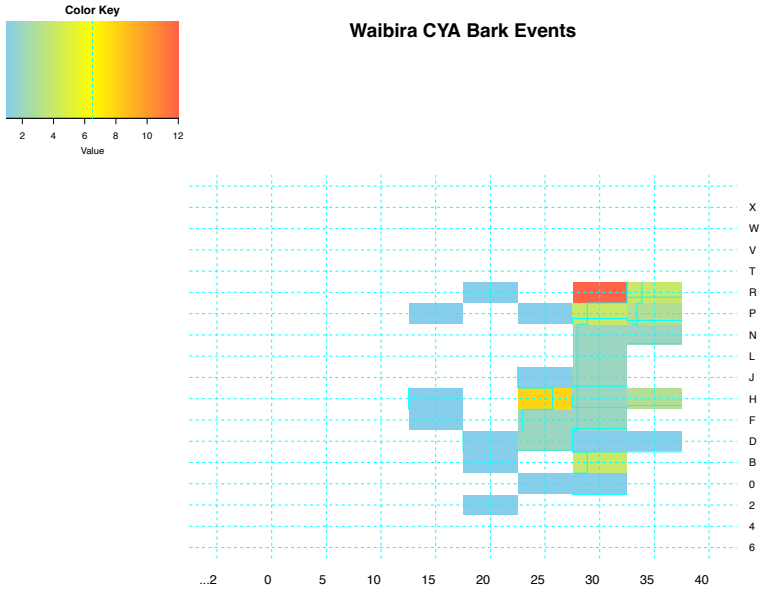
| | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
|---|---|---|---|---|---|---|---|
| X | 0 | 0 | 1 | 12 | 7 | 0 | 0 |
| V | 0 | 0 | 3 | 104 | 2 | 0 | 0 |
| T | 13 | 17 | 17 | 262 | 96 | 27 | 0 |
| R | 90 | 204 | 261 | 549 | 1353 | 678 | 41 |
| P | 54 | 175 | 104 | 284 | 540 | 381 | 14 |
| N | 469 | 476 | 726 | 421 | 790 | 394 | 16 |
| L | 147 | 292 | 212 | 590 | 515 | 229 | 33 |
| J | 83 | 457 | 175 | 271 | 518 | 336 | 15 |
| H | 72 | 216 | 193 | 722 | 476 | 62 | 3 |
| F | 77 | 724 | 517 | 736 | 621 | 72 | 8 |
| D | 116 | 358 | 557 | 549 | 383 | 79 | 6 |
| B | 74 | 240 | 567 | 373 | 212 | 90 | 4 |
| 0 | 59 | 138 | 850 | 293 | 51 | 11 | 0 |
| 2 | 12 | 88 | 163 | 177 | 146 | 22 | 0 |
| 4 | 1 | 133 | 152 | 130 | 46 | 12 | 0 |
| 6 | 0 | 47 | 25 | 56 | 10 | 10 | 0 |

***SM Figure 4****: Waibira core areas (outlined in blue) according to party composition data*




***SM Figure 5:*** *Top 24 species recorded across transects in Sonso and Waibira core areas*

***NB: FUE*** *(Funtumia elastica);* ***CMI*** *(Celtis mildbraedii);* ***LP*** *(Lasiodiscus pervillei);* ***CYA*** *(Cynometra alexandri);* ***CZE*** *(Celtis zenkeri);* ***KA*** *(Khaya anthotheca);* ***TRP*** *(Trichilia prieuriana);* ***RB*** *(Rinorea beniensis);* ***CSY*** *(Croton sylvaticus);* ***CGP*** *(Celtis gomphophyla);* ***TM*** *(Trilepisium madagascariense);* ***PAL*** *(Pouteria altissima);* ***MDI*** *(Margaritaria discoidea);* ***TAF*** *(Tapura fischeri);* ***ANT*** *(Antiaris toxicaria);* ***AL*** *(Alchornea laxifora);* ***TRR*** *(Trichilia rubescens);* ***LYC*** *(Lychnodiscus cerospermus);* ***CLC*** *(Caloncoba crepiniana);* ***FE*** *(Ficus exasperata);* ***GAL*** *(Gambeya albida);* ***AB*** *(Alstonia boonei);* ***CPH*** *(Celtis philppensis);* ***TP*** *(Tabernaemontana pachysiphon)*




***SM Figure 6:*** *Heat map of C. alexandrii distribution in the Waibira home range*


***SM Figure 7:*** *Distribution of A. boonei across Sonso and Waibira forest transects*

## Supplementary Tables:

**SM TABLE 1:** Measurements from unequivocally exploited trees for bark ingestion reported along transects

| Community | Species | Tree dbh (cm) | Number of strips per tree | Strip length (cm) | Total |
|---|---|---|---|---|---|
| **Sonso** | *Ficus exasperata* | 28.03 | 5 | 30 | |
| | | 29.62 | 6 | 75 | |
| | | 35.00 | 13 | 85 | |
| | | 38.22 | 5 | 36 | |
| | | 46.50 | 8 | 129 | |
| | | 50.96 | 12 | 90 | |
| | | 65.00 | 17 | 155 | |
| | | 85.99 | 3 | 45 | |
| | *Ficus sur* | 20.06 | not measured | not measured | |
| | *Ficus variifolia* | 70.06 | 7 | 125 | |
| Sonso Total | | | | | 10 |
| **Waibira** | *Alstonia boonei* | 76.43 | 2 | 10 | |
| | | 101.91 | 2 | 30 | |
| | *Albizia glabberima* | 95.54 | 5 | not measured | |
| | *Albizia zygia* | 46.18 | 1 | 13 | |
| | *Celtis gomphophyla* | 31.53 | 3 | 96 | |
| | | 43.00 | 3 | 35 | |
| | | 47.77 | 1 | 42 | |
| | | 53.34 | 3 | 18 | |
| | | 54.00 | 2 | 47 | |
| | *Caloncoba crepiniana* | 39.50 | 2 | 20 | |
| | *Celtis mildbraedii* | 42.68 | 5 | 45 | |
| | *Ficus mucuso* | 95.54 | 3 | 120 | |
| | *Ficus sur* | 50.00 | 3 | 82 | |
| | | 89.40 | 1 | 40 | |
| | *Pseudospondias microcarpa* | 12.42 | 1 | 25 | |
| Waibira Total | | | | | 15 |

**SM TABLE 2:** Comparing bark strip lengths across species and technique (all indirect evidence)

| Species | Technique(s) | n | Mean strip length (cm) |
|---|---|---|---|
| *Albizia glabberima* | Trunk bite | 1 | 13.0 |
| *Alstonia boonei* | Trunk bite | 7 | 15.8 |
| *Albizia zygia* | Buttress bite | 1 | 13.0 |
| *Celtis gomphophyla* | Buttress bite; Buttress strip (vertical scrape) | 7 | 49.2 |
| *Celtis mildbraedii* | Buttress bite; Trunk bite | 3 | 34.0 |
| *Celtis zenkeri* | Buttress bite | 1 | 78.0 |
| *Ficus exasperata* | Buttress strip (horizontal bite); Buttress strip (cross-hatch); Buttress strip (vertical scrape) | 12 | 79.6 |
| *Ficus mucuso* | Buttress strip (vertical scrape); Buttress strip (horizontal bite | 4 | 146.3 |
| *Ficus sur* | Buttress strip (horizontal bite); Buttress strip (cross-hatch) | 4 | 69.8 |
| *Ficus variifolia* | Buttress strip (cross-hatch) | 1 | 125.0 |
| *Pseudospondias microcarpa* | Trunk bite | 1 | 25.0 |
| *Ficus saussureana* | Buttress strip (horizontal bite); Buttress strip (vertical scrape) | 2 | 60.5 |
| *Ficus polita* | Buttress strip (vertical scrape) | 1 | 87.0 |
| *Ficus natalensis* | Buttress strip (cross hatch) | 1 | 110.0 |

**SM TABLE 3:** Off-Grid Bark Ingestion Events by Species in Long-Term Site Data

| Species | Sonso | Waibira |
|---|---|---|
| *Alstonia boonei* | 8 | |
| Unidentified Climbers | 1 | |
| *Celtis mildbraedii* | 1 | |
| *Cordia millenii* | 1 | 1 |
| *Cleistopholis patens* | 9 | |
| *Cynometra alexandri* | 4 | |
| *Desplatsia dewevrei* | 1 | |
| *Ficus exasperata* | 1 | |
| *Ficus sur* | 1 | |
| *Ficus saussureana* | | 1 |
| *Khaya anthotheca* | 1 | |
| *Lasiodiscus pervillei* | 1 | |
| *Raphia farinifera* | 2 | |
| Unknown tree species | 4 | |
| **Total** | **35** | **1** |

## Supplementary Videos:

All videos are accessible on Google Drive by following the below link:
https://drive.google.com/drive/folders/1FUD6NJW_Ca_Np74U-aF0OHNGtQApqeaD?usp=drive_link

**SM Video 1:** MB stripping AB bark during meat eating event

# Appendix F References:

Badihi, G., Bodden, K., Zuberbühler, K., Samuni, L., & Hobaiter, C. (2022). Flexibility in the social structure of male chimpanzees (Pan troglodytes schweinfurthii) in the Budongo Forest, Uganda. *Royal Society Open Science*, *9*(9), 220904. https://doi.org/10.1098/rsos.220904

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Lapuente, J., Arandjelovic, M., Kühl, H., Dieguez, P., Boesch, C., & Linsenmair, K. E. (2020). Sustainable Peeling of Kapok Tree (Ceiba pentandra) Bark by the Chimpanzees (Pan troglodytes verus) of Comoé National Park, Ivory Coast. *International Journal of Primatology*. https://doi.org/10.1007/s10764-020-00152-9

Takemoto, H. (2004). Seasonal change in terrestriality of chimpanzees in relation to microclimate in the tropical forest. *American Journal of Physical Anthropology*, *124*(1), 81–92. https://doi.org/https://doi.org/10.1002/ajpa.10342

the Royal Botanic Gardens, K. (2023). POWO. Retrieved July 4, 2023, from http://www.plantsoftheworldonline.org

# APPENDIX G:
# Images of Bark Ingestion From Study Period

## *Arboreal Bark Stripping*


***Figure 6:*** *Direct evidence of arboreal bark stripping:* ***a.)*** *Branch peeling FSU* ***b.)*** *Trunk scraping DOK* ***c.)*** *Bark and resin eating KA* ***d.)*** *Branch peeling CYA* ***e.)*** *Branch peeling CSY*

## *Arboreal Bark Stripping: Indirect Evidence*


***Figure 7:*** *Indirect evidence of bark stripping from arboreal bark stripping events:* ***a)****: SD bark wadge* ***b)****: FSS bark wadge* ***c):*** *DOK bark wadge* ***d):*** *FSU bark wadge* ***e.)*** *FSU bark detached strip* ***f.)*** *CYA detached strip* ***g.)*** *CZE detached strip* ***h.)*** *KA bark piece*

## *Terrestrial Bark Stripping*


***Figure 8:*** *Direct evidence of terrestrial bark stripping:* ***a.)*** *Trunk biting SZG* ***b.)*** *Buttress biting CMI* ***c.)*** *Trunk stripping with vertical scraping (and licking) FE* ***d.)*** *Trunk biting SCU* ***e.)*** *Trunk biting AB*

## Terrestrial Bark Stripping: Indirect Evidence (Waibira)

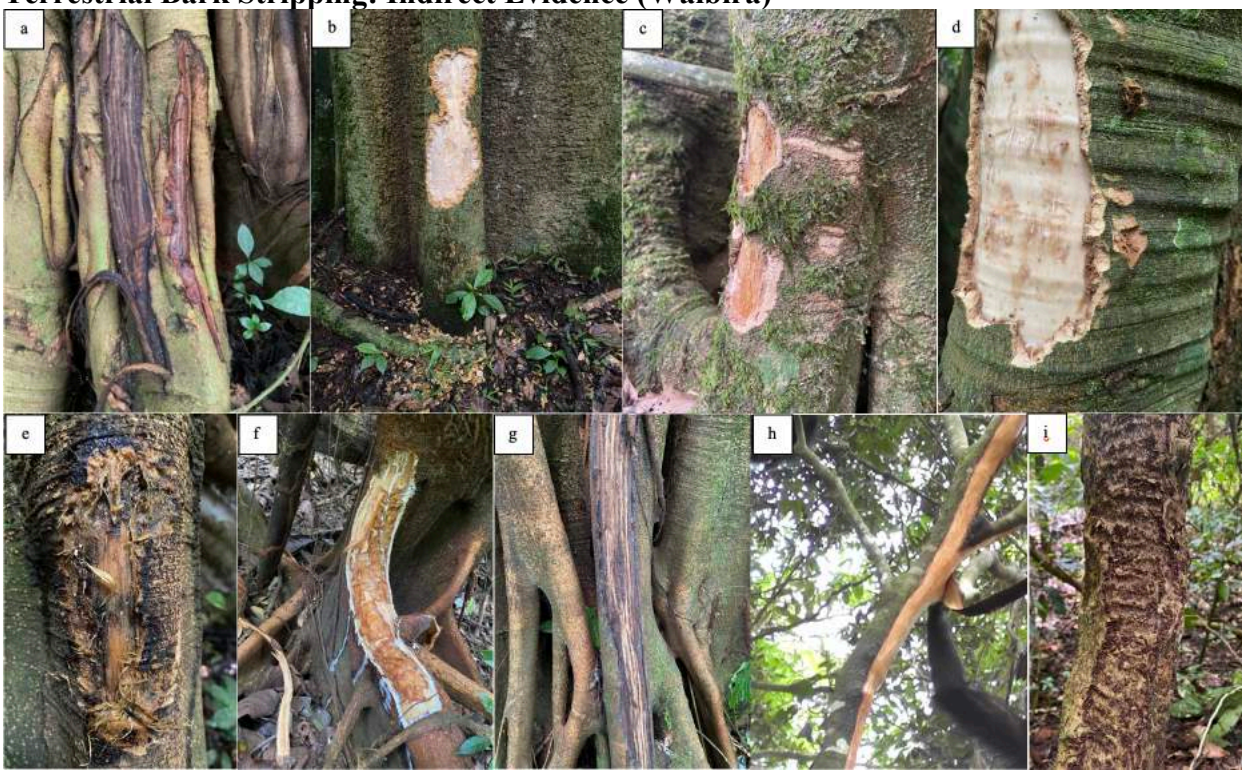


***Figure 9:*** *Indirect evidence of bark stripping in Waibira:* ***a.)*** *Buttress stripping with vert. scraping FM* ***b.)*** *Trunk biting AB* ***c.)*** *Buttress biting CGP* ***d.)*** *Trunk biting CMI* ***e.)*** *Buttress biting AZ* ***f.)*** *Buttress stripping with hor. biting FSS* ***g.)*** *Buttress stripping with vert. scraping FSS* ***h.)*** *Branch peeling CYA* ***i.)*** *Trunk biting SCU* ***j.)*** *Buttress biting CZE* ***k.)*** *Trunk biting DOK* ***l.)*** *Buttress stripping with hor. biting FE* ***m.)*** *Buttress stripping with cross-hatching FN* ***n.)*** *Trunk peeling with vert. scraping FPO* ***o.)*** *Branch peeling CSY* ***p.)*** *Buttress peeling with hor. biting FSU* ***q.)*** *Branch peeling FSU*

## *Terrestrial Bark Stripping: Indirect Evidence (Sonso)*


***Figure 10:*** *Indirect evidence of bark stripping in Sonso* ***a.)*** *Trunk stripping with random biting SZG* ***b.)*** *Buttress stripping with hor. biting FE* ***c.)*** *Buttress stripping with cross-hatching FE* ***d.)*** *Buttress biting CZE* ***e.)*** *Trunk biting AB* ***f.)*** *Buttress biting AZ* ***g.)*** *Buttress stripping with hor. biting FM* ***h.)*** *Buttress stripping with gen. stripping FVR*

# APPENDIX H:
# Natural History Illustrations from Budongo


***Top row**: (left) Ficus polita (right) Broussnetia papyrifera*
***Bottom row**: (left) Aframomum sp. (right) Cynometra alexandri*


***Top row**: (left) Cola gigantea (right) Ficus mucuso*
***Bottom row**: (left) Gambeya perpulchra (right) Ficus exasperata (unripe)*


***Top row**: (left) Caloncoba crepiniana (right) Myranthus holstii*
***Bottom row**: (left) Scutia myrtina (right) Marantochloa leucantha*


***Top row**: (left) Alstonia boonei (right) Christella parasitica*
***Bottom row**: (left) Ficus saussureana (right) Acanthus polystachyus*


***Top row**: (left) Rainy season mushrooms (right) Praying mantis eating a fly*
***Bottom row**: (left) Chimpanzee skull found in Waibira (right) Common house gecko*