Molecular profiling of Peru Balsam reveals active ingredients responsible for its pharmaceutical properties

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Peru Balsam, a resinous substance derived from Myroxylon balsamum var. pereirae, has historically been used as a topical ointment for various skin conditions such as scabies, poorly healing wounds, eczema, and haemorrhoids. The ingredients responsible of these properties are not fully elucidated. We investigated the chemical composition of two Peru Balsam samples, one historical and one modern, using gas chromatography/mass spectrometry to identify the active ingredients responsible for its pharmaceutical properties. Both Peru Balsam specimens investigated had similar compositions, showing the stability of the substance. Components identified are effective against scabies, exhibit antimicrobial activity and aid skin penetration. These properties are consistent with historical uses of Peru balsam. Several ingredients are also known allergens. This study, combining chemical information with scientific literature related to pharmaceutical properties of natural substances, represents a breakthrough in the elucidation of active ingredients in Peru Balsam.

Keywords: Peru Balsam; Myroxylon balsamum; Meso- and Southern America; gas chromatography/mass spectrometry; allergy; antimicrobial

1. Introduction

Despite its historical significance and its recognised pharmaceutical properties, little is known about the composition of Peru Balsam and in particular the active ingredients responsible for its pharmaceutical properties. This substance is derived from a tree known as Myroxylon balsamum var. pereirae, which grows in Central America (primarily in El Salvador) and South America (Fig. S1) (Amado and Taylor 2006). The resinous substance is called "Peru Balsam" not because of its geographic provenance but because the balsam was most commonly shipped to Spain via Peru (Estes 1995). It is also known under the names Balsam of Peru, balsam-of-Peru tree, black balsam, Indian balsam, balsam Peru and Peruvian balsam (Khan and Abourashed 2009).

Peru Balsam has been widely used as a pharmaceutical substance for its antiseptic properties (Collier and Nitta 1930). Its properties as a contact allergen have also been reported (de Groot 2019). Although Peru Balsam has been used widely historically and is still used today, surprisingly little is known about its chemical composition (de Groot 2019). Modern attempts to characterise the composition of Peru Balsam have been incomplete and conflicting (Steigenberger 2013; Hausen et al. 1995; Mammerler 2007; Yun et al. 1999; Seo, Park, and Park 2012). This work presents the chemical analysis of 2 exudate specimens of Peru balsam in order to elucidate their chemical composition and identify active ingredients (Fig. S2).

2. Elucidating the molecular composition of Peru Balsam

The analyses by GC/MS of the two specimens investigated in this study provided very similar chromatograms dominated by a peak identified as benzyl-benzoate (#11, Fig. S3 and Tab. S1). Benzoic acid (#4), 3,4-dimethoxycinnamaldehyde (#5), cinnamic acid (#8) and benzyl-cinnamate (#13) are also major compounds in both samples. The peak area ratios of benzoic acid to benzyl benzoate and of cinnamic acid to benzyl cinnamate were higher for the aged sample than for the fresh sample, suggesting a partial hydrolysis of the ester bonds (Tab. S2). Compound (#5) did not match any entry in the library but was identified based on its MS spectrum. The [M-1]+, [M-15]+ and [M-29]+ indicate α-cleavage, which is the predominant fragmentation mode of aldehydes, particularly aromatic aldehydes. The peaks at *m/z*=123 and *m/z*=163 point to a phenylpropanoid structure. The structure deviates from coniferyl aldehyde in only the mass equivalent of CH_2. Therefore, a possible structure is 3,4-dimethoxycinnamaldehyde. This substance may correspond to previously unidentified phenylpropanoids that have been reported in the literature (Steigenberger 2013). Nine molecules were also identified as minor components in both samples (Tab. S1; Fig. S3-S10). These include nerolidol (#9), identified in its un-derivatised form, vanillin (#7), 3,3-dihydroxyprop-2-enal (#1), Coniferyl benzoate (#15) and two fatty acids (namely palmitic acid (#12) and stearic acid (#14)). Finally, 3 compounds could not be identified (#2, #3 and #6). The early elution of these compounds points towards relatively low polarity and/or molecular weight. Our results are in accordance with previous studies for the most abundant components. Benzyl-benzoate (#11) and benzyl-cinnamate (#13) have been identified as constituents of the balsam (Hausen et al. 1995; Yun et al. 1999; Mammerler 2007; Steigenberger 2013). Previous investigations have been conflicting regarding the minor constituents. Over 120 constituents have been named as constituents of Peru Balsam, yet only 20 of these have been identified in more than one analysis (Hausen et al. 1995; Yun et al. 1999; Mammerler 2007; Steigenberger 2013). Some differences can be explained by method of preparation. For example, methyl esters (e.g. methyl benzoate and methyl cinnamate) identified by (Hausen et al. 1995; Yun et al. 1999; Mammerler 2007) may form from benzyl benzoate (#11) and benzyl cinnamate (#13) via transesterification with methanol in certain conditions or under prolonged exposure to methanol. Other differences may be explained by storage condition. The concentration of coniferyl benzoate (#15) has been shown to be declining rapidly with age of the sample (Hausen et al. 1995). Contamination from the rather complex production process of the balsam may also explain some of the variability. The present analysis supports the conclusion that Peru Balsam is not reliably identifiable via minor constituents (Courel et al. 2019).

3. Pharmaceutical properties of molecules constituting Peru Balsam

While the pharmaceutical effectiveness of Peru Balsam as a whole has not been scientifically demonstrated, it is evidenced by the individual pharmaceutical properties of its constituents. Historically, Peru Balsam has been used as a topical ointment against various skin conditions (de Groot 2019). It has been used, for example, to treat scabies (Howes 1949; de Groot 2019). This can be substantiated from its chemical composition. It has been shown that benzyl benzoate (#11), which we identified as the major constituent in Peru Balsam, is an effective topical treatment for scabies (Alberici et al. 2000; Sule and Thacher 2007; Goutam et al. 2016). Peru Balsam has also been used against a wide range of skin conditions, such as poorly healing wounds, decubitus, eczema, pruritus, haemorrhoids and anal pruritus (de Groot 2019). The usage of Peru Balsam for such indications may be linked to several constituents that we identified that demonstrate antifungal and antibacterial activity.

Cinnamic acid (#8) has been shown to be effective across a wide range of bacteria and fungi (Melliou and Chinou 2004). Benzoic acid (#4) is a cytostatic (Nair 2001) and is commonly used as a preservative as well. It exerts antimicrobial activity by the suppression of macro-autophagy (Hazan et al. 2004). The substance also disrupts and permeates cell membranes, altering the pH within the cell and disrupts intracellular processes via interaction with several enzymes involved in metabolic routes such as the Krebs cycle, glycolysis, and oxidative phosphorylation (del Olmo et al. 2017). Nerolidol (#9), present in a small amount in the Peru Balsam samples analysed in this study, is also responsible for a range of effects against skin conditions. It is anti-inflammatory (Fonsêca et al. 2016), anti-bacterial, anti-fungal and is effective against a wide range of parasites as discussed in detail by Chan et al. (2016). The antiulcerogenic effects of the Peru Balsam can be attributed to nerolidol (#9) (de Groot, 2019; Klopell et al. 2007).

Several constituents of Peru Balsam enhance skin penetration. They also amplify the effects of other active ingredients by working synergistically (Pandit et al. 2015). Nerolidol (#9) enhances trans dermal drug delivery (Krishnaiah et al. 2006), a property that has been attributed to its lipophilic (El-Kattan et al. 2001) and amphiphilic properties (Aqil et al. 2007). Nerodlidol (#9) also shows synergetic interactions with other substances regarding antibacterial effects (Van Zyl et al. 2010). Benzoic acid (#4) is absorbed well by the skin (van de Sandt et al. 2004). As described, ingredients present positive effects to the skin but Peru Balsam is also recognised as a contact allergen. Reactions are invoked especially by benzyl benzoate (#11), benzyl cinnamate (#13), benzoic acid (#4) and nerolidol (#9) (Hausen et al. 1995). Benzoic acid (#4) and benzyl cinnamate (#13) are known as weak sensitizers (Larmi et al. 1989; Wilhelm et al. 2001; Kolle et al. 2019).

Several constituents of Peru Balsam also exhibit therapeutic properties via other drug delivery routes. Cinnamic acid (#8) exhibits hepatoprotective properties when administered via an intragastric tube (Fernández-Martínez et al. 2007). Another constituent of Peru Balsam, 3,4-dimethoxycinnamaldehyde (#5), was also tested in the same study but showed no effect. Vanillin (#7) may improve psoriatic skin inflammation (Cheng et al. 2017), inflammatory bowel disease (Wu et al. 2009) via oral delivery and promises to combat oxidative brain injury (Makni et al. 2012) as well as sickle cell disease (Zhang et al. 2004) and relax coronary and basilar arteries (Raffai et al. 2015) when injected. Nerolidol (#9) has been shown to protect kidneys from injury in mice when administered interperitoneally (Zhang et al. 2017). Utilizing Peru Balsam in these ways is likely unsafe not only due to its complex chemical composition that may cause unpredictable effects, but especially because of the toxicity of some of the constituents. The clastogenicity and mild genotoxicity of nerolidol was for example observed when administered orally in mice (Pículo et al. 2011).

4. Conclusion

This study using gas chromatography and mass spectrometry allowed us to elucidate the chemical composition of two specimens of Peru Balsam. The similarity in composition of the two samples shows the stability of the substance over time. Then, we were able to identify the active molecules responsible for some of the pharmaceutical properties of Peru Balsam by combining the chemical information and the scientific literature related to pharmaceutical properties of natural substances. This study represents a breakthrough in the elucidation of active ingredients in Peru Balsam.

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Authors contribution

TD and SW designed the project. JB and MJ provided access to the RPS Peru Balsam specimen. MR, MM and MAV performed the analyses. MR, MM, SW, MAV, ER and TD interpreted the data. All authors contributed to the writing of the article.

References

Alberici F, Pagani L, Ratti G, Viale P. 2000. Ivermectin alone or in combination with benzyl benzoate in the treatment of human immunodeficiency virus-associated scabies. Br J Dermatol. 142:969–972.

Amado A, Taylor JS. 2006. Balsam of Peru or Balsam of El Salvador? Contact Dermatitis. 55:119.

Aqil M, Ahad A, Sultana Y, Ali A. 2007. Status of terpenes as skin penetration enhancers. Drug Discov Today. 12:1061–1067.

Chan W-K, Tan LT-H, Chan K-G, Lee LH, Goh B-H. 2016. Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules. 21:529.

Cheng H-M, Chen F-Y, Li C-C, Lo H-Y, Liao Y-F, Ho T-Y, Hsiang C-Y. 2017. Oral administration of vanillin improves imiquimod-induced psoriatic skin inflammation in mice. Jounal Agric Food Chem. 65:10233–10242.

Collier WA, Nitta Y. 1930. Über die Wirkung ätherischer Öle auf verschiedene Bakterienarten. Zeitschr f Hyg. 111:301–312.

Courel B, Adam P, Schaeffer P. 2019. The potential of triterpenoids as chemotaxonomic tools to identify and differentiate genuine, adulterated and archaeological balsams. Microchem J. 147:411–421.

El-Kattan AF, Asbill CS, Kim N, Michniak BB. 2001. The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. Int J Pharm. 215:229–240.

Estes JW. 1995. The European reception of the first drugs from the new world. Pharm Hist. 37:3–23.

Fernández-Martínez E, Bobadilla RA, Morales-Ríos MS, Muriel P, Pérez-Álvarez VM. 2007. Trans-3-phenyl-2-propenoic acid (cinnamic acid) derivatives: structure-activity relationship as hepatoprotective agents. Med Chem (Los Angeles). 3:475–479.

Fonsêca D V., Salgado PRR, de Carvalho FL, Salvadori MGSS, Penha ARS, Leite FC, Borges CJ, Piuvezam MR, de Morais Pordeus LC, Sousa DP, Almeida RN. 2016. Nerolidol exhibits antinociceptive and anti-inflammatory activity: involvement of the GABAergic system and proinflammatory cytokines. Fundam Clin Pharmacol. 30:14–22.

Goutam J, Kharwar RN, Tiwari VK, Mishra A, Singh S. 2016. Isolation and identification of antibacterial compounds isolated from endophytic fungus emericella qaudrilineata (RS-5). Nat Prod Chem Res. 4:1000205.

de Groot AC. 2019. Myroxylon pereirae resin (Balsam of Peru) - A critical review of the literature and assessment of the significance of positive patch test reactions and the usefulness of restrictive diets. Contact Dermatitis. 80:335–353.

Hausen BM, Simatupang T, Bruhn G, Evers P, Koenig WA. 1995. Identification of new allergenic constituents and proof of evidence for coniferyl benzoate in Balsam of Peru. Am J Contact Dermat. 6:199–208.

Hazan R, Levine A, Abeliovich H. 2004. Benzoic acid, a weak organic acid food preservative, exerts specific effects on intracellular membrane trafficking pathways in Saccharomyces cerevisiae. Appl Environ Microbiol. 70:4449–4457.

Howes FN. 1949. Properties and Uses of Gums and Resins. In: Verdoorn F, editor. Veg Gums Resins. Vol. 37. Waltham, Mass., USA: Chronica Botanica Company; p. 163–165.

Khan IA, Abourashed EA. 2009. Balsam Peru. Leung’s Encycl Common Nat Ingredients Used Food, Drugs, Cosmet. 69–71.

Klopell FC, Lemos M, Sousa JPB, Comunello E, Maistro EL, Bastos JK, de Andrade SF. 2007. Nerolidol, an antiulcer constituent from the essential oil of Baccharis dracunculifolia DC (Asteraceae). Zeitschrift für Naturforsch C. 62:537–542.

Köhler FE. 1897. Wikimedia Commons File:Myroxylon balsamum - Köhler–s Medizinal-Pflanzen-141.jpg. Köhler’s Medizinal-Pflanzen [Internet]. [cited 2019 Aug 29]. Available from: https://commons.wikimedia.org/w/index.php?curid=255474

Kolle SN, Natsch A, Gerberick GF, Landsiedel R. 2019. A review of substances found positive in 1 of 3 in vitro tests for skin sensitization. Regul Toxicol Pharmacol. 106:352–368.

Krishnaiah YSR, Al-Saidan SM, Jayaram B. 2006. Effect of nerodilol, carvone and anethole on the in vitro transdermal delivery of selegiline hydrochloride. Pharmazie. 61:46–53.

Larmi E, Lahti A, Hannuksela M. 1989. Immediate contact reactions to benzoic acid and the sodium salt of pyrrolidone carboxylic acid. Contact Dermatitis. 20:38–40.

Makni M, Chtourou Y, Barkallah M, Fetoui H. 2012. Protective effect of vanillin against carbon tetrachloride (CCl4)-induced oxidative brain injury in rats. Toxicol Ind Health. 28:655–662.

Mammerler V. 2007. Contribution to the analysis and quality control of Peru Balsam. [place unknown]: Universität Wien, Wien, Austria.

Melliou E, Chinou I. 2004. Chemical analysis and antimicrobial activity of greek propolis. Planta Med. 70:515–519.

Nair B. 2001. Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. Int J Toxicol. 20:23–50.

del Olmo A, Calzada J, Nuñez M. 2017. Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: Uses, exposure, and controversy. Crit Rev Food Sci Nutr. 57:3084–3103.

Pandit J, Aqil M, Sultana Y. 2015. Terpenes and Essential Oils as Skin Penetration Enhancers. In: Dragicevic N, Maibach HI, editors. Percutaneous Penetration Enhanc - Chem Methods Penetration Enhanc. Berlin, Heidelberg: Springer; p. 173–193.

Pículo F, Macedo G, de Andrade SF, Luis Maistro E. 2011. In vivo genotoxicity assessment of nerolidol. J Appl Toxicol. 31:633–639.

Raffai G, Khang G, Vanhoutte PM. 2015. Vanillin and vanillin analogs relax porcine coronary and basilar arteries by inhibiting L-type Ca2+ channels. J Pharmacol Exp Ther. 352:14–22.

van de Sandt JJM, van Burgsteden JA, Cage S, Carmichael PL, Dick I, Kenyon S, Korinth G, Larese F, Limasset JC, Maas WJM, et al. 2004. In vitro predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. Regul Toxicol Pharmacol. 39:271–281.

Seo S-M, Park H-M, Park I-K. 2012. Larvicidal activity of Ajowan (Trachyspermum ammi) and Peru Balsam (Myroxylon pereira) oils and blends of their constituents against mosquito, Aedes aegypti, acute toxicity on water flea, Daphnia magna, and aqueous residue. J Agric Food Chem. 60:5909–5914.

Steigenberger G. 2013. The Vigani Cabinet – Analysis of historical resinous materials by gas chromatography - mass spectrometry and infrared spectroscopy. [place unknown]: Technical University Dresden, Dresden, Germany.

Sule HM, Thacher TD. 2007. Comparison of ivermectin and benzyl benzoate lotion for scabies in Nigerian patients. Am J Trop Med Hyg. 76:392–395.

Wilhelm K-P, Böttjer B, Siegers C-P. 2001. Quantitative assessment of primary skin irritants in vitro in a cytotoxicity model: comparison with in vivo human irritation tests. Br J Dermatol. 145:709–715.

Wu S-L, Chen J-C, Li C-C, Lo H-Y, Ho T-Y, Hsiang C-Y. 2009. Vanillin improves and prevents trinitrobenzene sulfonic acid-induced colitis in mice. J Pharmacol Exp Ther. 330:370–376.

Yun G, Baizhan L, Xiaolan Z, Liang S, Jialin C, Mei G, Longgen Z. 1999. Determination of chemical components of essential oil in Peru balsam balmtree extract by GC/MX. Anal Instrum. 3:37–39.

Zhang C, Li X, Lian L, Chen Q, Abdulmalik O, Vassilev V, Lai C, Asakura T. 2004. Anti-sickling effect of MX-1520, a prodrug of vanillin: an in vivo study using rodents. Br J Haematol. 125:788–795.

Zhang L, Sun D, Bao Y, Shi Y, Cui Y, Guo M. 2017. Nerolidol protects against LPS-induced acute kidney injury via inhibiting TLR4/NF-κB signaling. Phyther Res. 31:459–465.

Van Zyl RL, Seatlholo ST, Viljoen AM. 2010. Pharmacological interactions of essential oil constituents on the in vitro growth of Plasmodium falciparum. South African J Bot. 76:662–667.