

Heterocyclic Antioxidants in Nature: Coumarins

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Abstract: Coumarins represent an important family of naturally occurring and/or synthetic oxygen-containing heterocycles, bearing a characteristic benzopyrone framework. These compounds are widely distributed both in human diet and therapeutics. Among other characteristics, several coumarin derivatives proved to have an interesting antioxidant profile. In the present review, an overview of natural coumarins and their role as antioxidants is presented and discussed.

Introduction:

Coumarins (Figure 1) are an important class of phytochemicals. Coumarins are chemical entities that can be found in several species of the plant's kingdom [1]. Some of these compounds are responsible for the organoleptic properties of the matrix in which they are present. Therefore, coumarins (molecules bearing a benzopyrone framework) represent an important class of naturally occurring and/or synthetic oxygen-containing heterocycles [2].

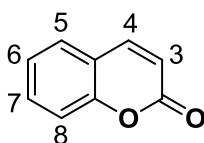


Figure 1 – Coumarin scaffold and numbering of the molecule.

Coumarins, a class of lactones bearing a π - π conjugated electron-rich system composed by a benzene ring fused to α -pyrone one, present specially good charge-transport properties [3]. The simplicity and chemical versatility of this scaffold are the key points for the wide range of applications [4,5, 6].

The coumarin name comes from the French term used to denominate the tonka bean – *coumarou*– the natural source from which the coumarin was firstly isolated in 1820 [5]. This bean has a sweet odor, easy to be recognized as the scent of new-mown. Therefore, since 1882 coumarin has been used in perfumes. Plants produce it as a chemical defense to discourage predation [5].

In the last century, coumarins have been a topic of interest in areas as natural products, organic and medicinal chemistry. Therefore, the extraction, synthesis and biological study of coumarins have become an attractive rapidly developing research area [7]. Moreover, many coumarin and derivatives emerged as medicinal candidates or drugs presenting strong pharmacological activities. In most of the cases, these properties are accomplished of low toxic and low side effect, lower drug resistance, high bioavailability, wide spectrum of action, *etc.* [8,9]. Several efforts have been made centred in the development of coumarin-based anticoagulants, antioxidants, antimicrobials (anti-virals, antifungals and anti-parasitics), anticancer agents, anti-diabetics, analgesics, anti-neurodegenerative and anti-inflammatory agents, based on the properties of this naturally occurring family [10,11,12,13].

Great efforts have been directed towards the extraction and purification of naturally occurring biologically active coumarins from a large variety of plants, animals and microorganisms. Similar efforts have been made towards the artificial synthesis of coumarin derivatives with novel structural features and biological properties [14,15,16]. These molecules have been increasingly attracting special interest as drugs due to their outstanding contributions in the prevention and treatment of several diseases. Over the years, coumarin-based research has become an extremely attractive area [17,18,19].

In this context, an overview of the role of natural coumarins as important antioxidants will be presented and discussed in the current review. During the research on the discovery of natural antioxidants from medicinal plants, some works reported the isolation of coumarin derivatives [20,21,22,23]. Epidemiological studies have correlated the ingestion of coumarin-based compounds in the diet with interesting benefits to health mainly due to their antioxidant activity [24]. The benefits of antioxidants to prevent chronic diseases were also already proven (Figure 2) [25].

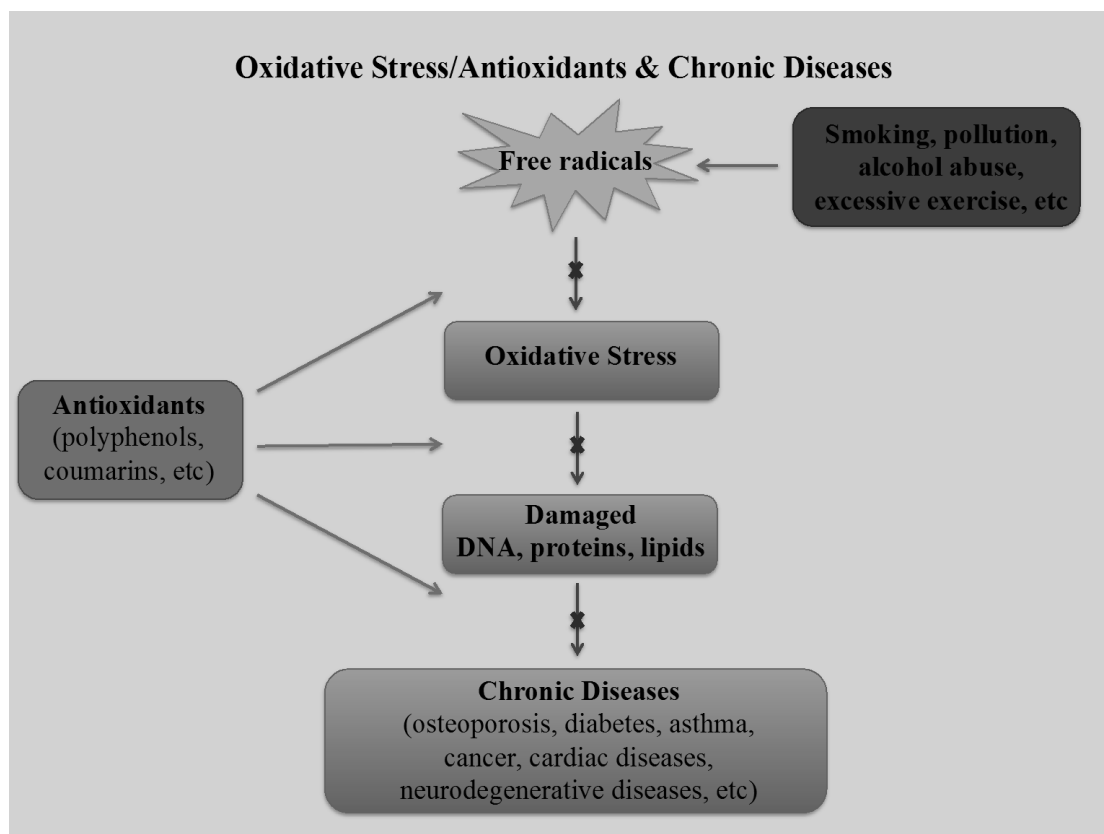


Figure 2 – Benefits of antioxidants in human health.

The most common assays performed to evaluate the antioxidant capacity described in this review are related to different reactive oxygen species (ROS) involved in the oxidative process: superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), nitric oxide (NO^{\bullet}), peroxynitrite ($ONOO^-$), hydroxyl radical (HO^{\bullet}), 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) and 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid radical ($ABTS^{\bullet+}$).

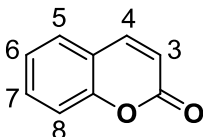
Several reaction mechanisms were studied to investigate the antioxidant profile of coumarins. Among them, hydrogen atom transfer (HAT) and single electron transfer transfer (SET), were highly cited.

The presence of hydroxyl groups in the coumarin scaffold contributes to prevent or minimize several types of oxidative processes [26]. It is recognized that the antioxidant effectiveness of coumarins, especially 4-hydroxycoumarins, is associated to the presence of those hydroxyl groups [27]. These substituents act as potent hydrogen donors contributing to the stability of the generated coumaric radical, due to electron delocalization across the molecule [28,29]. The potential of hydroxycoumarins as anti-inflammatory and antioxidant agents, along with the role of lipoic acid as endogenous thiol antioxidant, prompted the synthesis of hybrid derivatives that display higher OH scavenging activity than α -lipoic acid itself [30].

Simple coumarins as antioxidants:

The coumarin itself, with no substitutions on it, was studied as an antioxidant agent [10]. Also, several naturally occurring simple coumarins were studied, presenting one or more hydroxyl groups on its structure. This is imperative for the presence of this activity. The most relevant examples, described in the last 30 years, are umbelliferone, scopoletin, umbelliferone 3-carboxylic acid, esculetin, 5,8-dihydroxycoumarin, daphnetin and fraxetin (Table 1). Since the experimental results were obtained in different sources, it is difficult to compare their real potential as antioxidants. However, some of the most relevant results are presented in this section.

Table 1 – Chemical structure of the simple coumarins described in this section.

						
Compounds	3	4	5	6	7	8
Umbelliferone	H	H	H	H	OH	H
Scopoletin	H	H	H	OCH ₃	OH	H

Esculetin	H	H	H	OH	OH	H
5,8-Dihydroxycoumarin	H	H	OH	H	H	OH
Daphnetin	H	H	H	H	OH	OH
Fraxetin	H	H	H	OCH ₃	OH	OH
Scoparone	H	H	H	OCH ₃	OCH ₃	H
Crenulatin	H	H	H	CHO	OCH ₃	H
Mansorin A	CH ₃	H	CH(CH ₃) ₂	OCH ₃	H	CH ₃
Mansorin B	CH ₃	H	CH(CH ₃) ₂	OH	H	CH ₃
Umbelliferone 3-carboxylic acid	COOH	H	H	H	OH	H
4,5-Dimethoxy-7-methylcoumarin	H	OCH ₃	OCH ₃	H	CH ₃	H
4,7-Dimethoxy-5-methylcoumarin	H	OCH ₃	CH ₃	H	OCH ₃	H
Isofraxidin	H	H	H	OCH ₃	OH	OCH ₃
Fraxidin	H	H	H	OCH ₃	OCH ₃	OH
(5,7-Dimethoxycoumarin-8-yl)acetaldehyde	H	H	OCH ₃		OCH ₃	CHO

Umbelliferone and scopoletin were deeply studied and described as antioxidant [31]. Both compounds were presented in big amounts in ethyl acetate and methanol bark extracts of *Melicope glabra* [31,32]. These extracts were evaluated for their antioxidant capacities by DPPH scavenging activity and β -carotene bleaching/linoleic acid system. Both extracts exhibited strong inhibition against the DPPH (IC₅₀ values of 24.81 and 13.01 μ g/mL, respectively) and strong antioxidant activity in β -carotene bleaching assay. Both samples were found to have high phenolic content with values of 39 and 44 mg GAE/g (gallic acid equivalents/gram) as indicated by Follin-Ciocalteau's reagent [32].

The antioxidant profile of the isolated umbelliferone was studied: Scavenging of DPPH IC₅₀ >

200 μM , scavenging of OH $\text{IC}_{50} > 5000 \mu\text{M}$, scavenging $\text{O}_2^{\cdot-}$ $\text{IC}_{50} = 26.7 \mu\text{M}$, scavenging HOCl $\text{IC}_{50} > 2000 \mu\text{M}$, scavenging ABTS $\text{IC}_{50} = 0.53 \mu\text{M}$ [24] and scavenging ONOO^- $\text{IC}_{50} = 36.28 \mu\text{M}$ [33]. In the first report of the isolation and antioxidant potential of umbelliferone from *Acacia nilotica*, it was found that its antioxidant effect was dose-dependent up to 100 $\mu\text{g/mL}$ and then leveled off with no further increase in activity [34].

The profile of the isolated scopoletin was also evaluated: Scavenging of DPPH $\text{IC}_{50} > 200 \mu\text{M}$, scavenging of OH $\text{IC}_{50} > 5000 \mu\text{M}$; scavenging $\text{O}_2^{\cdot-}$ $\text{IC}_{50} > 200 \mu\text{M}$, scavenging HOCl $\text{IC}_{50} > 2000 \mu\text{M}$ and scavenging ABTS $\text{IC}_{50} = 1.57 \mu\text{M}$ [24].

Umbelliferone 3-carboxylic acid was extracted from *Angelica decursiva*, as umbelliferone, and was evaluated for its antioxidant profile: Scavenging of DPPH $\text{IC}_{50} = 681.86 \mu\text{M}$; scavenging of IC_{50} ABTS = 11.20 μM ; scavenging NO $\text{IC}_{50} > 500 \mu\text{M}$; scavenging ONOO^- $\text{IC}_{50} = 8.04 \mu\text{M}$ [33].

Some dihydroxycoumarins have also been ascribed as antioxidant agents [35,36]. Esculetin (6,7-dihydroxycoumarin) isolated from *Angelica decursiva*, *Angelica dahurica* and *Fraxinus chinensis* [24,37,38], was studied for its scavenging capacity against different radicals: Scavenging of DPPH $\text{IC}_{50} = 17.2 \mu\text{M}$; scavenging of OH $\text{IC}_{50} = 2783 \mu\text{M}$; scavenging $\text{O}_2^{\cdot-}$ $\text{IC}_{50} = 2.3 \mu\text{M}$; scavenging HOCl $\text{IC}_{50} > 2000 \mu\text{M}$; scavenging ABTS $\text{IC}_{50} = 2.45 \mu\text{M}$ [24]. Esculetin showed one of the strongest antioxidant activities [39] of the tested compounds, showing in particular one of the strongest scavenging activities against DPPH [37].

5,8-Dihydroxycoumarin was isolated and studied from aerial parts of sweet grass (*Hierochloë odorata* L.). A clear linear dependency of radical scavenging capacity for this coumarin in DPPH and ABTS assays was determined. Although the basic principles of these reactions are similar, the ABTS scavenging assay is preferable for its ability to evaluate radical scavenging characteristics of both lipophilic and hydrophilic antioxidants, and the IC_{50} value of 5,8-dihydroxycoumarin in DPPH assay was 0.0185%, while in the ABTS reaction it was remarkably higher, 0.028% [40].

Daphnetin, the 7,8-dihydroxycoumarin, isolated from *Gerbera piloselloides* [41] showed antioxidant activity in the DPPH assay, as much of the other simple hydroxycoumarins, and fraxetin (extracted from *Angelica dahurica*) also showed antioxidant activity by inhibiting lipid peroxidation [42]: Daphnetin – scavenging of DPPH $\text{IC}_{50} = 20.6 \mu\text{M}$, scavenging of OH $\text{IC}_{50} =$

3182 μM , scavenging $\text{O}_2^{\cdot-}$ $\text{IC}_{50} = 3.2 \mu\text{M}$, scavenging HOCl $\text{IC}_{50} > 2000 \mu\text{M}$ and scavenging ABTS $\text{IC}_{50} = 2.15 \mu\text{M}$ [24]; Fraxetin – scavenging of DPPH $\text{IC}_{50} = 44.1 \mu\text{M}$, scavenging of IC_{50} ABTS = 37.4 μM and scavenging of H_2O_2 $\text{IC}_{50} = 40.5 \mu\text{M}$ [39].

Scoparone and crenulatin were isolated from the crude acetone extract of the roots of *Citrus hystrix* in 2013 [43] and the potential of the plant extract as antioxidant was determined: IC_{50} values of 0.21 and 3.7 mg/mL in DPPH and OH assays, respectively [43].

New coumarins mansorin A and mansorin B were isolated from *Mansonia gagei*, but from the extract mansonone N (non coumarinic compound) was the only isolated product that showed radical scavenging properties [44].

Constituents of the root of *Anemone tomentosa* were isolated and evaluated as antioxidants [45]. 4,5-Dimethoxyl-7-methylcoumarin, 4,7-dimethoxy-5-methylcoumarin, isofraxidin and fraxidin [42] were tested for their capacity of scavenging the DPPH, presenting IC_{50} values of 75.16, 78.28, 51.25 and 53.31 μM , respectively [45].

Isofraxidin was also isolated from *Anneslea fragrans* var. *lanceolata*, and its profile as antioxidant was also evaluated: Scavenging of DPPH $\text{IC}_{50} = 51.6 \mu\text{M}$ and scavenging of IC_{50} ABTS = 12 77.3 μM [46].

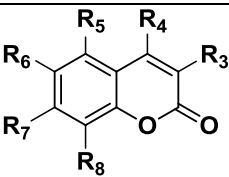
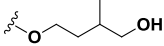
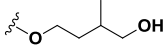
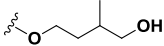
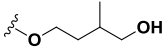





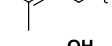
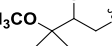
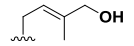
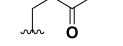
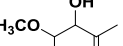
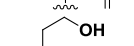
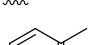
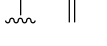
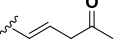
Antioxidant potential and phenolic constituents of *Salvia cedronella* and *Murraya alata* were determined [47,48] Some coumarins are presented in both extracts, but in the first case the antioxidant profile was only evaluated to different fractions of the extract. In the second one, the inhibitory profile of 2-(5,7-dimethoxycoumarin-8-yl)acetaldehyde on lipopolysaccharide-activated (LPS-activated) NO production in RAW 264.7 cells was evaluated: $\text{IC}_{50} = 13.8 \mu\text{M}$ [48].

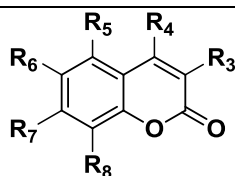
SAR studies on these particular compounds showed that the introduction of short-chain substituents provides more active compounds than the long-chain ones [49]. In general, esculetin showed the strongest quenching capacities against DPPH, OH radicals, followed by daphnetin [24].

Aliphatic, terpenoid and aromatic-containing coumarins as antioxidants:

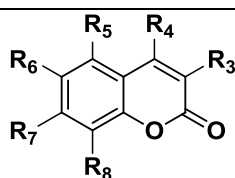
Coumarins bearing terpenoid-like, aromatic, or random aliphatic substituents were also found in different natural sources. Some of them have been isolated and fully characterized, and presented interesting antioxidant properties. The structures of these compounds are summarized in Table 2.

Table 2. Chemical structure of aliphatic, terpenoid and aromatic-containing coumarins described in this section.

<div>  </div>						
Compounds	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
Armenin	H	H	H	H	OH	
Isoarmenin	H	H	H	H		OH
Lacarol	H	H	OH	H	OCH ₃	
Deoxylacarol	H	H	H	H	OCH ₃	
Peroxytamarin	H	H	H		OCH ₃	H
6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin	H	H	H		OCH ₃	H
Suberosin	H	H	H		OCH ₃	H
Tamarin	H	H	H		OCH ₃	H
7-Methoxy-8-(3-methyl-2,3-epoxy-1oxobutyl)chromen-2-one	H	H	H		OCH ₃	H
Osthenol	H	H	H	H	OH	
Osthole	H	H	H	H	OCH ₃	
Yuehgesin B	H	H	H	H	OCH ₃	
Micromarin-F	H	H	H	H	OCH ₃	
Hassanon	H	H	H	H	OCH ₃	
Albiflorin-3	H	H	H	H	OCH ₃	
7-O-Methylphellodenol-B	H	H	H	H	OCH ₃	
(Z)-5,7-Dimethoxy-8-(3-methylbuta-1,3-dien-1-yl)coumarin	H	H	OCH ₃	H	OCH ₃	
Toddalenone	H	H	OCH ₃	H	OCH ₃	



Compounds	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
Mammea B/BD	H	Pr	OH		OH	
Mammea B/BB	H	Pr	OH		OH	
6-Hydroxy-7-[(3,7-dimethylocta-2(<i>E</i>),4(<i>E</i>),7-trien-1-yl)oxy]coumarin	H	H	H	OH		H
Prenyletin	H	H	H	OH		H
Haplopinol	H	H	H	OH		H
6-Hydroxy-7-[(5-hydroxy-3,7-dimethylocta-2(<i>E</i>),6-dien-1-yl)oxy]coumarin	H	H	H	OH		H
6-Hydroxy-7-[(7-hydroxy-3,7-dimethylocta-2(<i>E</i>),5(<i>E</i>)-dien-1-yl)oxy]coumarin	H	H	H	OH		H
Auraptene	H	H	H	H		H
4-Hydroxy-7-methoxy-3-(3,7,11-trimethyl-9-oxododeca-3(<i>E</i>),7(<i>Z</i>)-dien-2-yl)coumarin		OH	H	H	OCH ₃	H
Fukanemarin A		OH	H	H	OH	H
Fukanemarin B		OH	H	H	OCH ₃	H
Glycyrin		H	OCH ₃		OCH ₃	H
Glycycoumarin		H	OCH ₃		OH	H
3-(2,4-Dihydroxyphenyl)-5-hydroxy-7-methoxy-8-(2-methylbut-3-en-2-yl)coumarin		H	OH	H	OCH ₃	
3-(2,4-Dihydroxy-3-(3-methylbut-2-en-1-yl)phenyl)-7-hydroxycoumarin		H	H	H	OH	H
Subcoriacin		H	H		OH	H
3'-Hydroxymelanettin	H		H	OH	OCH ₃	H
Melanettin	H		H	OH	OCH ₃	H
Fraxin	H	H	H	OCH ₃	OH	



Compounds	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
Esculin	H	H	H		OH	H
Ferulsinaic Acid	H	H	H	H		H

Mojarrab et al. studied MeOH extracts of the aerial parts of *Artemisia armeniaca*, and being able to isolate four coumarins (armenin, isoarmenin, lacarol and deoxylacarol). Their structures were determined by spectroscopic means, including UV, MS, 1D- and 2D-NMR. The in vitro free-radical scavenging properties of the extracts and of the isolated compounds were evaluated by the DPPH assay. The free radical scavenging activity in the DPPH assay was calculated as the concentration that caused a 50% reduction in DPPH absorbance at 517 nm (RC₅₀ expressed in mg/mL). The MeOH extract presented a RC₅₀ = 0.0317 mg/mL, whereas from the isolated coumarins, lacarol was found to be the most active (RC₅₀ = 0.0302 mg/mL) [50].

Panthong et al. isolated several coumarins from the acetone crude extract of the roots of *Citrus hystrix*. Their structures were determined and confirmed by analysis of 1D and 2D NMR spectroscopic data. The antioxidant activity using DPPH, OH and O₂^{•-} assays was assessed, but these compounds did not present significant antioxidant activity [43].

Osthole is a coumarin derivative that has been isolated from plants such as *Prangos tschimganica* [51] and *Ostericum koreanum* Maximowicz [52]. Ethyl acetate extracts of *O. koreanum* strongly scavenged O₂^{•-}, H₂O₂, OH and NO with the IC₅₀ being 23.582, 66.310, 170.582 and 60.779 mg/mL, respectively and therefore presenting an antioxidant activity comparable to that of ascorbic acid [52]. It has also been demonstrate that osthole protect neuronal damage induced by significant reduction in the infarct volume, cerebral edema and the neurological deficit scores of rat submitted to middle cerebral artery occlusion (MCAO) due to a possible mechanisms in which its anti-oxidative action and anti-inflammatory property is involved [53]. In addition, osthole markedly inhibited the increased NO levels

induced by intestinal I/R injury, suggesting that osthole exerts therapeutic effects against intestinal I/R injury by attenuating oxidative stress, reducing excessive neutrophil infiltration and modulating NO levels [54].

Some 8-alkylcoumarins from the fruits of *Cnidium monnieri* have been isolated. From the isolated compounds, 7-O-methylphellodenol-B and 7-methoxy-8-(3-methyl-2,3-epoxy-1oxobutyl)chromen-2-one, prove to protect against H₂O₂ induced oxidative stress damage [55]. Some other coumarins isolated from the leaves of *Murraya alata* such as toddalenone showed inhibition of NO production in LPS-induced RAW 264.7 macrophages with IC₅₀ values between 6.0 and 14.5 μ M [48].

From the stem bark extract of *Mammea usambarensis*, two compounds namely mammea B/BB and mammea B/BD were isolated. These compounds showed antioxidant activities in DPPH assay at 4,012 and 2,176 μ mol TE/g, respectively. The potential antioxidant activities of these compounds may have been attributed by the presence of hydroxyl groups, which acts as good hydrogen donors. The lower activities of the isolated compounds compared to the crude extracts may be attributed to the synergistic effect [56].

Torres et al. isolate 8 coumarins from the resinous exudate of *Haplopappus multifolius*. The radical scavenging activity displayed by the extract (IC₅₀ = 45.5 mg/mL) by the DPPH assay confirmed the protective role of the resin towards ROS present in the environment. From the isolated coumarins, only the phenolic coumarins 7-(((2E,4E)-3,7-dimethylocta-2,4,7-trien-1-yl)oxy)-6-hydroxycoumarin, prenyletin, haplopinol, (Z)-6-hydroxy-7-((5-hydroxy-3,7-dimethylocta-2,6-dien-1-yl)oxy)coumarin and 6-hydroxy-7-(((2E,5E)-7-hydroxy-3,7-dimethylocta-2,5-dien-1-yl)oxy)coumarin displayed a moderate anti-oxidant activity (IC₅₀ 247.9, 109.3, 125.9, 250.1 and 251.5 μ M, respectively) [57]. Other terpene-like coumarins such as auraptene, are a natural monoterpene isolated from plants of the genus *Citrus*. Some studies demonstrate that auraptene increased NO production and iNOS phosphorylation in auraptene-treated cells [58]. A 3-terpenoidcoumarin, 4-hydroxy-7-methoxy-3-((3E,7Z)-3,7,11-trimethyl-9-oxododeca-3,7-dien-2-yl)coumarin, isolated from *Ferula pallida* also presented antioxidant properties showing values of inhibition percentage of lipid peroxidation and absorbance change due to scavenging of DPPH of 16.4% and 0.158, respectively [51].

Fukanemarin A and fukanemarin B are sesquiterpene coumarin derivatives isolated from an

80% aqueous methanol extract of the roots of *Ferula fukanensis* (FFE). The FFE and the isolated coumarins inhibited NO production and induced NO synthase (iNOS) gene expression by a murine macrophage-like cell line (RAW 264.7), which was activated by LPS and recombinant mouse interferon- γ (IFN- γ). FFE caused concentration-dependent inhibition of NO production by macrophages during incubation (IC_{50} = 21.9 μ g/mL), whereas fukanemarin A and fukanemarin B presented an inhibitory effect with IC_{50} values of 19.5 and 30.2 μ M, respectively. Therefore these compounds inhibited iNOS mRNA expression in a dose-dependent manner. Inhibition of iNOS by these sesquiterpene coumarin derivatives may be mediated through the expression of these transcription-activating factors, thereby inhibiting iNOS transcription [59,60].

Some 3-arylcoumarins have been isolated from Licorice, the root of *Glycyrrhiza glabra*. Antioxidant studies on these compounds by the Racimat method showed that some of the isolated coumarins glycyrin, glycy coumarin, 3-(2,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-8-(2-methylbut-3-en-2-yl)coumarin and 3-(2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)phenyl)-7-hydroxycoumarin presented good oxidation stability, with induction periods of 4.6, 3.5, 4.0, 8.9 hours, respectively, and protection factor values of 0.9, 0.3, 0.6 and 2.7, respectively [61]. Additional antioxidant studies have been performed on glycy coumarin, which showed EC_{50} values of ABTS scavenging and inhibitory activity on lipid peroxidation of 4.32 and 11.9 μ M, respectively [62], and DPPH scavenging activity SC_{50} of 7.92 μ g/mL [63].

Another 3-arysubstituted coumarin, subcoriacin, isolated from *Eysenhardtia subcoriacea* was demonstrated to improve the endogenous antioxidant system. A significant increase in the activities of the antioxidant enzymes glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase (CAT) was observed. The increase in the activities of the antioxidant enzymes together with a decrease in blood glucose levels in both treatments demonstrate and support the relationship between the hypoglycemic and antioxidant activities displayed by subcoriacin [64].

Two 4-arylcoumarins, 3'-hydroxymelanettin and melanettin, were isolated and characterized from the ethyl acetate-soluble fraction of the heartwood of *Dalbergia odorifera* and it was studied their potential to inhibit the LPS-induced NO production in RAW 264.7 cells. The IC_{50} values for the inhibitory effects of 3'-hydroxymelanettin and melanettin were 45.5 and 53.2 μ M, respectively, indicating that these compounds are potential structural prototypes to treat anti-inflammatory diseases associated with the excess production of NO [65].

Some sugar-containing coumarins such as fraxin or esculin have also been isolated from natural sources. Lee et al. studied some antioxidant properties of fraxin isolated from *Stewartia koreana*, and they found that this compound showed DPPH and $O_2^{\bullet-}$ scavenging activities with IC_{50} values of 40.5 and 48.7 $\mu\text{g/ml}$, respectively [66]. Esculin, isolated from *Fraxinus chinensis* extract (FCE), also presented a modest antioxidant activity. Data of the SC_{50} values for the FCE and esculin via the DPPH method were 50.0 and 133.3 $\mu\text{g/ml}$, respectively [37].

Ferulsinaic acid (FA) is the first member of a new rearranged class of sesquiterpene coumarins from the genus constituted by about 170 species of flowering plants in the family *Apiaceae*. Patients with diabetic nephropathy (DN) have decreased antioxidant enzyme expression and therefore the imbalance between the production of ROS and antioxidant defense mechanisms is believed to be involved [67]. Treatment of diabetic rats with FA significantly decreased the concentrations of the malondialdehyde (MDA) and significantly increased SOD, CAT, GSHPx activities and GSH concentrations in a dose-dependent manner, indicating that FA ameliorates oxidative stress in the diabetic rats [68].

A series of coumarin-chalcone derivatives was study in order to investigate the role of adjacent hydroxyl groups on the antioxidant proprieties. Different antioxidant mechanisms have been investigated, including HAT and SET. Based on the obtained results, the HAT mechanism was proposed as the most important one for the antioxidant protection exerted by this class of compounds [69]. Recently, different reaction mechanisms were investigated for this class of compounds. It was found that the largest contributions to the overall peroxy radical scavenging activity of the studied compounds are the hydrogen transfer in lipid media, and the sequential proton loss hydrogen atom transfer (SPLHAT) in aqueous solution [70].

Ring-fused coumarins as antioxidants:

In the last years, several ring-fused coumarins, in particular pyrano- and furocoumarins, were isolated from natural sources and their antioxidant capacity was studied [71]. Some benzocoumarins and 1,4-dioxanecoumarins were also described. The activities of these ring-fused derivatives are related to the structural diversity of the substituents.

Two new 8-methylbenzo[*h*]coumarins (Figure 3), muralatins A and B, were isolated from the leaves of *Murraya alata*. Their inhibitory effects of the isolates on LPS-activated NO

production in RAW 264.7 cells was evaluated: IC₅₀ muralatin A = 12.4 μ M and IC₅₀ muralatin B = 9.1 μ M [48]. Djalonensone and alternariol, benzo[*c*]coumarins, were isolated from the solid culture of the endophytic fungus *Botryosphaeria dothidea* KJ-1, collected from the stems of white cedar (*Melia azedarach* L.) [72]. These metabolites showed no activity in DPPH assays [72].

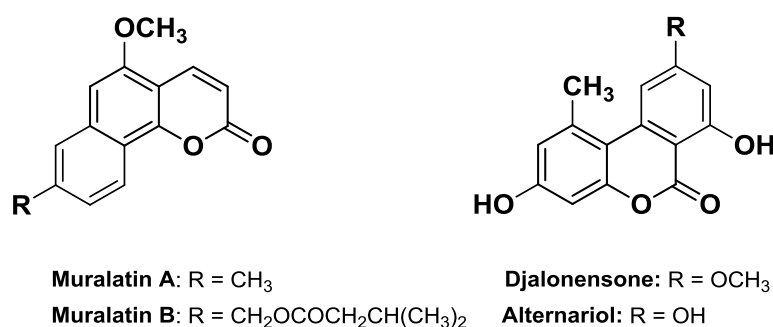


Figure 3 – Examples of natural benzocoumarins.

Different pyranocoumarins (Figure 4) were isolated from the crude acetone extract of the roots of *Citrus hystrix* for the first time in 2013 [43]. The antioxidant profile of hystrixarin, *trans*-decursidinol and khellactone were determined. These compounds showed weaker activity than the crude extract, possibly indicating a synergistic effect in the crude extract; the values were not presented in the original manuscript [43]. Khellactone was also isolated from *Polygonum amplexicaule*, displaying moderated activity in the DPPH assay [73]. Effects of nordentatin, isolated from *Citrus medica* var, on NO production and DPPH scavenging activity were evaluated (IC₅₀ = 10.2 μ M and 53.6 μ M, respectively) [74].

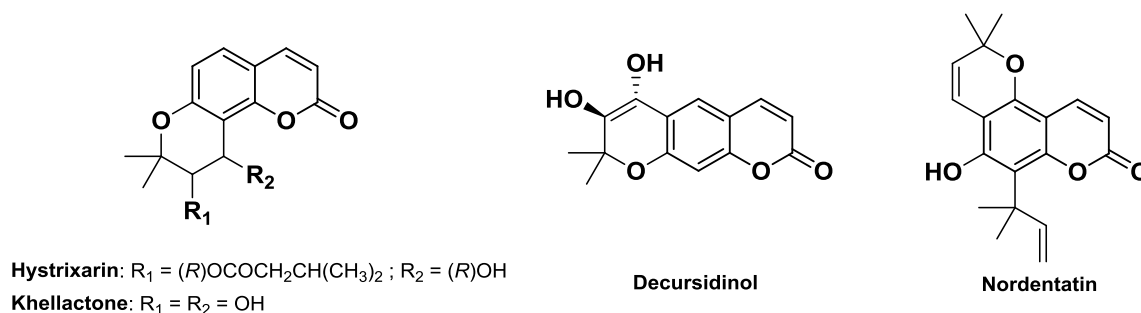
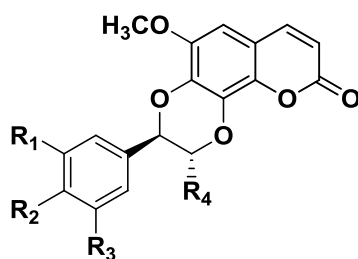


Figure 4 – Examples of natural pyranocoumarins.

Some 1,4-dioxanecoumarins (Figure 5) were isolated from *Acer saccharum* [75]. Cleomiscosin C and one derivative were isolated from *Hibiscus syriacus* and proved to inhibit lipid peroxidation with IC₅₀ values of 0.7 and 1.4 μ g/mL, respectively.



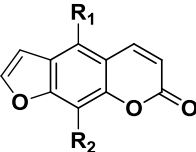
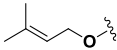
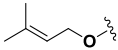
Cleomiscosin C: R₁ = OCH₃ ; R₂ = OH ; R₃ = OCH₃ ; R₄ = CH₂OH

Cleomiscosin C derivative: R₁ = OCH₃ ; R₂ = OH ; R₃ = OCH₃ ; R₄ = CH₃

Figure 5 – Examples of 1,4-dioxanecoumarins.

Furocoumarins, based on the position of the furan-ring, are divided into the psoralen type (furan-ring at position 6,7 and also called linear-type) and the angelicin type (furan-ring at position 7,8 and also called angular-type). Structures of natural linear-furocoumarins and angular-furocoumarins with antioxidant activity are presented in tables 3 and 4, respectively.


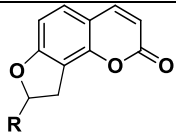

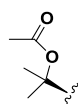
Table 3. Psoralens and dihydropsolarens.

		
Psoralens	R ₁	R ₂
Psoralen	H	H
Bergapten	OCH ₃	H
Xanthotoxol	H	OH
Xanthotoxin	H	OCH ₃
9-Hydroxy-4-methoxypsoralen	OCH ₃	OH
Isopimpinellin	OCH ₃	OCH ₃
Alloisoimperatorin	OH	CH ₂ CH=C(CH ₃) ₂
Imperatorin		H
Cnidilin		OCH ₃

Phellopterin		
Pabulenol		H
Neobyakangelicol		
8-Geranyloxypsoralen	H	
Feroniellin B		H
Heraclenin	H	
Heraclenol	H	
Byakangelicol		
Byakangelicin		
Oxypeucedanin		H
Oxypeucedanin hydrate		H
Dihydropsolarens	R	
Nodakenin		
Nodakenetin		

Table 4. Angelicins and dihydroangelicins.

Angelicins	R ₁	R ₂	R ₃
Angelicin	H	H	H
Isobergapten	OCH ₃	H	H

Pimpinellin	OCH ₃	OCH ₃	H
Sphondin	H	OCH ₃	H
Oroselone	H	H	
			
Dihydroangelicins		R	
Columbianetin			
Columbianetin acetate			

Recently, it was reported the isolation of new furocoumarins from the aerial parts of *Angelica urumiensis* and *Ammi majus* used in traditional medicine [76,77]. The isolated furocoumarins showed to possess moderate antioxidant activity in DPPH scavenging assay.

Psoralen, isolated from *P. tschimganic* and *Cachrys pungens* Jan [78], exhibited 28.8% of inhibition of lipid peroxidation [51]. This compound has no capacity of DPPH scavenging [51].

Bergapten was isolated from Chinese herbs, and its effects on lipid peroxidation were determined: 22.15% in brain and 27.96% in kidney homogenates [79].

Xanthotoxol was isolated from *P. tschimganica* and its inhibition of lipid peroxidation in liposomes (91.7%, IC₅₀ = 50 µM) and absorbance change due to scavenging of DPPH (IC₅₀ = 0.331 µM), were calculated [51]. This compound was also isolated from Chinese herbs, and its effects on lipid peroxidation were determined: 71.31% in brain and 61.94% in kidney homogenates [79].

From the furocoumarin fraction from *Angelica officinalis* L. fruits they were isolated three major coumarins (imperatorin, xanthotoxin, and bergapten) that did not possess anti-radical activity against both DPPH and *N,N*-dimethyl-*p*-phenyldiamine (DMPD), while they exhibited a moderate level of ferric reduction activity potential (FRAP) and phosphomolybdenum-reducing antioxidant power (PRAP). In ferrous ion-chelation test,

imperatorin had better activity than the other two tested coumarins [80]. Imperatorin was also extracted from genus *Casimiroa* (Rutaceae) seeds. To deeply study the activity of *Casimiroa* extracts and phyto-constituents it was determined their antioxidant capacity using the DPPH assay. Both *Casimiroa edulis* and *Casimiroa pubescens* elicited a concentration-dependent antioxidant activity, starting from 20 µg/mL and reaching an effect of 72.0% and 58.4% respectively, at the concentration of 600 µg/mL. Among the isolated compounds imperatorin and 8-geranyloxypsoralen revealed higher antioxidant activity; unexpectedly, the latter exerts an evident activity reaching the 91.9% at 5.0 mM. Among the isolated compounds imperatorin revealed higher antioxidant activity; at the concentration of 5.0 mM, imperatorin showed an action of 73.8%, with an IC₅₀ of 2.25 mM [81].

Xanthotoxin were then isolated from *Cachrys pungens* Jan from Italy, but its profile as antioxidant was not studied by this research group [78]. This compound was also isolated from Chinese herbs, and its effects on lipid peroxidation were determined: 8.36% in brain and 7.31% in kidney homogenates [79].

In vitro antioxidant capacity of roots of *Ostericum koreanum maximowicz* were studied. Oxypeucedanin hydrate was one of the most important constituents of the ethyl acetate extract of the roots. The ethyl acetate fraction of *O. koreanum* roots was subjected to *in vitro* antioxidant activity with different methods for free radical scavenging activities. Among all the tested methods, the ethyl acetate fraction was expressed very active, exhibiting a good Trolox equivalent values and IC₅₀ comparable to that of the common antioxidants: Trolox and ascorbic acid [52].

Of all the compounds tested, isolated from *Angelicae dahuricae*, 9-hydroxy-4-methoxypsoralen, and alloisoimperatorin showed the highest DPPH scavenging/reducing properties. Their IC₅₀ were 6.1 and 9.4 µg/mL, respectively, whereas for the other nine furocoumarins (namely oxypeucedanin hydrate, byakangelicin, pabulenol, neobyakangelicol, byakangelicol, oxypeucedanin and imperatorin, phellotorin) presented IC₅₀ values were higher than 200 µg/mL [82].

Heraclenol was isolated from *P. tschimganica* and its inhibition of lipid peroxidation in liposomes (87.2, IC₅₀ = 75 µM) and absorbance change due to scavenging of DPPH (IC₅₀ = 0.019 µM), were calculated [51].

Effects of some furocoumarins isolated from Chinese Herbs on NO produced from RAW 264.7 cells (including byakangelicol, oxypeucedanin, oxypeucedanin hydrate, xanthotoxin and cnidilin) showed more than 50% inhibition of NO production at 20 mg/mL. Also, some of these furocoumarins showed more inhibition effects of iNOS expression than both simple coumarins [80,83].

Nodakenin and its aglycone nodakenetin, isolated from *Angelica decursiva*, were completely inactive at the concentrations tested in all the antioxidant assays used in this study: Scavenging of DPPH $IC_{50} > 800 \mu M$, scavenging ABTS $IC_{50} > 100 \mu M$, scavenging of NO^{\bullet} $IC_{50} > 500 \mu M$ and scavenging $ONOO^{-}$ $IC_{50} > 50 \mu M$. Nodakenetin exerted weak suppressive effects toward NO production with 20.72% inhibition at the concentration of 125 $\mu g/mL$ [33]. Nodakenin was also presented in the furocoumarin fraction from *Angelica officinalis* L. fruits [80].

Of the angelicin-type furocoumarins, angelicin (the simplest molecule of this series) showed a moderate inhibitory effect (53.6%) on LPS-induced NO generation, in RAW 264.7 cells. Isobergapten, presenting a methoxy group at the position five, proved to display a considerably lower inhibitory activity (8.8%). However, the similar compound sphondin (with methoxy group at position 6) showed a strong inhibitory effect. In addition, pimpinellin, presenting two methoxy groups both 5 and 6 positions, proved to be slightly active than angelicin. Therefore, the presence of a methoxy group at position 6 of the angular furocoumarin skeleton seems to be important to modulate the biological activity, and the addition of a methoxy group at position 5 proved to decrease the activity.

Regarding the psoralen-type furocoumarin series, psoralen (the simplest molecule of this series) proved to have significantly lower activity than angelicin. The activities of xanthotoxin and cnidilin, with a methoxy groups at position 8, showed increased inhibitory effects (57.8 and 53.7%, respectively). In addition, the presence of an additional dimethylallyloxy group at position 5 of the last one, seems to have no significant influence. This data was confirmed by comparing the chemical structure and biological activity of psoralen (27.1%) and isoimperatorin (28.1%). On the other hand, bergapten, presenting a methoxy group at position 5, proved to be inactive. Isopimpinellin (33.0%), with two methoxy groups at positions 5 and 8, presented similar activity when compared to xanthotoxin (57.8%). Oxypeucedanin (53.7%) and oxypeucedanin hydrate (83.2%) have on their structures oxidized prenyloxy groups at position 5. In these molecules the double bond of the

dimethylallyloxy group is replaced by an epoxyl or two hydroxyl groups. This structural modification proved to be significantly important, increasing the activities comparing to the non-oxidative compound (isoimperatorin). However, analyzing the activities of byakangelicin, byakangelicol and phellopterin, only byakangelicol (presenting a dimethylallyloxy group saturated by an epoxyl at position 8) proved to display high activity. On the other hand, heraclenin, compared with byakangelicol, presenting the same moiety at position 8 but lacking a substitution at position 5, proved to be inactive. Based on these results, functional substitutions on the benzene ring can selectively modulate NO production. The process is complex and cannot be simply explained based on hydrophilic or hydrophobic substitutions. In summary, the previous study clearly demonstrates that angelicins are more potent than psoralens. For the angelicins, the methoxy group at position 5 seems to be important for the activity. Regarding the psoralens, methoxy groups at position 8 or the double bond of the dimethylallyloxy group saturated by two hydroxyl groups at position 5 are responsible to the increasing of the activity. Comparing to the other furocoumarins tested, sphondin is the most potent inhibitor of NO production and iNOS expression in LPS-induced RAW 264.7 macrophages [83].

The main conclusion of this study was that sphondin can inhibit the induction of iNOS in LPS-activated murine macrophages. This conclusion was based on three main ideas: (1) The least nitrite accumulated in cell supernatants when sphondin was added simultaneously with LPS; a delayed addition resulted in a decreased effect; (2) Sphondin did not inhibit NO accumulation after LPS stimulation of RAW 264.7 cells, and did not show a direct effect on enzymatic activity of iNOS; (3) Western blot analyses demonstrated markedly reduced levels of iNOS protein in LPS-activated cells treated with sphondin as compared to untreated cells [83].

Angelicin was also isolated from Chinese herbs, and its effects on lipid peroxidation were determined: 16.32% in brain and 15.27% in kidney homogenates [79].

Feroniellin B was isolated from the extract of *Feroniella lucida* roots. The IC₅₀ of the lipid peroxidation inhibition was 52 µM [84].

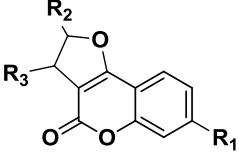
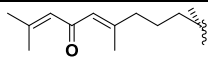
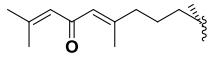
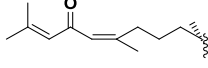
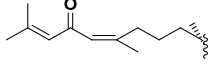
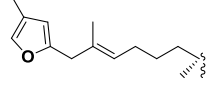
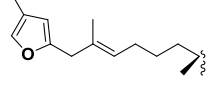
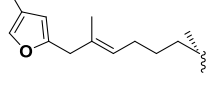
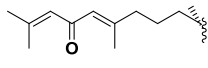
Oroselone isolated from *P. pabularia* and *Cachrys pungens* Jan, exhibited 11.8% of inhibition of lipid peroxidation and 0.043 of capacity of DPPH scavenging [51]. Oroselone was also isolated from Chinese herbs, and its effects on lipid peroxidation were determined:

4.46% in brain and -9.46% in kidney homogenates [79].

Columbianetin was isolated from *Cachrys pungens* Jan [78] and Chinese herbs, and its effects on lipid peroxidation were determined: 15.71% in brain and 6.67% in kidney homogenates [79]. Columbianetin acetate was also isolated from Chinese herbs, and its effects on lipid peroxidation were determined: 4.97% in brain and 4.73% in kidney homogenates [79].

Other angular dihydrofurocoumarins (dihydrofuro[3,2-*c*]coumarins) were isolated from natural sources and studied as antioxidant compounds (Table 5).

Table 5. Dihydrofuro[3,2-*c*]coumarins.

			
Dihydrofuro[3,2- <i>c</i>]coumarins	R ₁	R ₂	R ₃
Fukanefuomarin A	OH		(<i>S</i>)CH ₃
Fukanefuomarin B	OH		(<i>R</i>)CH ₃
Fukanefuomarin C	OH		(<i>S</i>)CH ₃
Fukanefuomarin D	OH		(<i>R</i>)CH ₃
Fukanefuomarin E	OCH ₃	(<i>S</i>)CH ₃	
Fukanefuomarin F	OCH ₃	(<i>S</i>)CH ₃	
Fukanefuomarin G	OCH ₃		(<i>R</i>)CH ₃
2,3-Dihydro-7-hydroxy-2 <i>R</i> ,3 <i>R</i> -dimethyl-2-[4,8-dimethyl-3(<i>E</i>),7-nonadien-6-onyl]furo[3,2- <i>c</i>]coumarin	OH		H

2,3-Dihydro-7-hydroxy-2*R*,3*R*-dimethyl-2-[4,8-dimethyl-3(*E*),7-nonadien-6-onyl]furo[3,2-*c*]coumarin (IC₅₀ = 24.6 μM), fukanefuomarin A (IC₅₀ = 13.0 μM), fukanefuomarin B (IC₅₀ =

16.2 μM), fukanefuromarin C ($\text{IC}_{50} = 11.1 \mu\text{M}$) and fukanefuromarin D ($\text{IC}_{50} = 8.9 \mu\text{M}$), were isolated from a 80% aqueous methanol extract of the roots of *Ferula fukanensis* and had stronger inhibition than quercetin on NO production ($\text{IC}_{50} = 31.2 \mu\text{M}$) [60]. The chloroform and water fractions showed 60% and 33.3% inhibition, respectively, at 30 μM .

Fukanefuromarin E, fukanefuromarin F and fukanefuromarin G were isolated from aqueous methanol extract of the roots of *Ferula fukanensis*. The FFE and the isolated coumarins inhibited NO production and induced iNOS gene expression by a murine macrophage-like cell line (RAW 264.7), which was activated by LPS and recombinant mouse IFN- γ , as described before. FFE caused concentration-dependent inhibition of NO production by macrophages during incubation ($\text{IC}_{50} = 21.9 \mu\text{g/mL}$), whereas fukanefuromarin E, fukanefuromarin F and fukanefuromarin G presented an inhibitory effect with IC_{50} values of 29.0 μM , 30.7 μM and 27.3 μM , respectively. Fukanefuromarin E proved to inhibit iNOS mRNA expression in a dose-dependent manner [59].

A series of polyhydroxycoumarins was studied, and in the experiments, a fundamental requirement for the expression of antioxidant activity appeared clearly to be, together with their redox properties, the ability to interact with biomembranes. Both HAT and SET mechanisms were described as possible for the antioxidant activity, but the electron delocalization stabilization as proposed in this study suggested the HAT mechanism to be a dominant factor for the AOA of coumarin derivatives. In conclusion, the antioxidant activity of coumarins appeared to be dictated not only by their structural features, but also by their location in the membrane [85].

Ellagic acid derivatives as antioxidants:

Ellagic acid (EA, Figure 6) and some of its derivatives natural phenolic antioxidants found in numerous fruits and vegetables such as *Alpinia galangal*, *Alpinia calcarata* [86], *Psidium cattleianum* [87], *Polygonum runcinatum* [88] or *Oenothera paradoxa* [89] among many others. Many studies demonstrate the ability of this compound, of the extracts containing this compound or their derivatives as potent antioxidants presenting scavenging activities for $\text{O}_2^{\bullet-}$, H_2O_2 , HOCl, NO, ONOO^- and singlet oxygen ($^1\text{O}_2$) [87] and also good DPPH and ORAC IC_{50} and FRAP EC_1 [90].

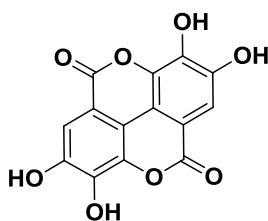


Figure 6. Chemical structure of ellagic acid.

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