


Review

# The Antioxidant Activity of Prenylflavonoids

Clementina M. M. Santos <sup>1,\*</sup> and Artur M. S. Silva <sup>2,\*</sup> 

<sup>1</sup> Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>2</sup> QOPNA & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

\* Correspondence: clemens@ipb.pt (C.M.M.S.); artur.silva@ua.pt (A.M.S.S.)

Received: 3 January 2020; Accepted: 3 February 2020; Published: 6 February 2020



**Abstract:** Prenylated flavonoids combine the flavonoid moiety and the lipophilic prenyl side-chain. A great number of derivatives belonging to the class of chalcones, flavones, flavanones, isoflavones and other complex structures possessing different prenylation patterns have been studied in the past two decades for their potential as antioxidant agents. In this review, current knowledge on the natural occurrence and structural characteristics of both natural and synthetic derivatives was compiled. An exhaustive survey on the methods used to evaluate the antioxidant potential of these prenylflavonoids and the main results obtained were also presented and discussed. Whenever possible, structure-activity relationships were explored.

**Keywords:** ABTS assay; antioxidant; chelation studies; DPPH radical; flavonoids; FRAP; lipid peroxidation; natural products; prenyl; ROS

## 1. Introduction

Flavonoids are oxygen heterocyclic compounds widespread throughout the plant kingdom. This class of secondary metabolites are responsible for the color and aroma of many flowers, fruits, medicinal plants and plant-derived beverages and play an important protective role in plants against different biotic and abiotic stresses. Flavonoids are also known for their nutritional value and positive therapeutic effects on humans and animals [1,2].

The basic structure of a flavonoid consists of a dibenzo- $\gamma$ -pyrone framework and the degree of unsaturation and oxidation of the C ring defines the subclasses of this compounds such as chalcones, flavones, isoflavones and their dehydro derivatives. Different substitution patterns that includes hydroxyl, methyl, methoxyl, prenyl and glycosyl groups can be attached to the flavonoid unit, varying the number, type and position of the substituents [3].

Over the past two decades there have been an increasing number of reports on the isolation of prenylated flavonoids, belonging to the Leguminosae and Moraceae families, with some distribution among others such as Cannabaceae, Euphorbiaceae, Guttiferae, Rutaceae, Umbelliferae, etc. [4,5]. Barron and Ibrahim reviewed more than 700 prenylated flavonoids up to the end of 1994 [5] and Botta et al. compiled it from 1995 till 2004 [6]. Most of the prenylated flavonoids have been identified as chalcones, dihydrochalcones, flavones, flavanones, flavonols and isoflavones, being C-prenyl more common than O-prenyl derivatives. In addition, prenyl side-chains can include variations in the number of carbons, oxidation, dehydration, cyclization or reduction to give a huge array of compounds with an impressive antioxidant potential [4].

Along the manuscript, “prenyl” will be used a general term to identify prenyl/isopentenyl, geranyl and farnesyl side chains as well as furano or dimethylchromano derivatives.

The beneficial effects of flavonoids appear to be related to the various biological and pharmacological activities as anti-inflammatory, antimicrobial, antioxidant, antitumor, estrogenic,

and immunosuppressive properties, among others. According to the literature, prenylation of flavonoids can induce an increase in their bioactivities namely as antimicrobial and anticancer agents, however, a decrease in the bioavailability and plasma absorption is recorded, when compared to related non-prenylated derivatives [7–11].

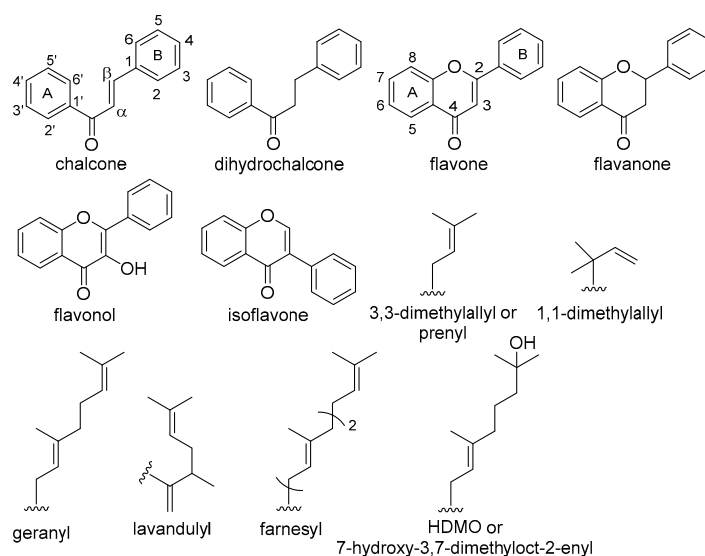
The available information concerning the antioxidant activity of prenylated flavonoids is sparse, appearing some studies in a couple of review papers [7,8]. Taking into account our both interest (organic and medicinal chemistry) for the identification of prenylated flavonoids that has already been tested for their antioxidant activity, a systematic revision of the literature was made using PubMed® and Web of Knowledge® databases. The research was limited to the 21st century, with publications from January 2000 to October 2019, using the terms “prenyl”, “flavonoid”, “prenylated flavonoid” in combination with “antioxidant”, as keywords. After a careful analysis of nearly one hundred of papers, we excluded many of them due to the absence of the antioxidant effects for the target compounds, leaving 59 papers to be included and discussed in the present review.

Herein, it is our propose to organize and summarize the structural and chemical diversity of natural and synthetic flavonoids applied as antioxidants, providing the main in vitro and in vivo methodologies used in this field of research. This information is very useful for organic chemists to know the trends of structure-antioxidant activity relationship, in order to develop efficient routes for the total synthesis of natural derivatives, to develop improved strategies to maximize the synthesis of such natural and synthetic compounds or even in the design and synthesis of novel flavonoids with different prenyl substituents in different positions of the main skeleton. So, the studies here summarized and the promising results obtained highlights the importance of prenylated flavonoids as potential antioxidant agents.

## 2. Natural Occurrence and Structural Variation of Prenylflavonoids with Antioxidant Activity

From the natural prenylflavonoids with antioxidant activity most of them are from Moraceae and Fabaceae families with a limited number of derivatives from Apiaceae, Asteraceae, Cannabaceae, and Euphorbiaceae.

The most common substitution among the flavonoid family with antioxidant properties is represented by the 3,3-dimethylallyl chain. 1,1-Dimethylallyl, geranyl, lavandulyl and farnesyl units can also be present in such structures. They can be found in chalcones, dihydrochalcones, flavones, flavanones, isoflavones, xanthone-type and other complex molecules (Figure 1). As referred before, C-prenylated compounds are more abundant than O-prenylated ones, being most of the O-prenylflavonoids obtained by synthesis.



**Figure 1.** Chemical structures of some flavonoids and main prenylation patterns.

**Chalcone derivatives.** This is the most abundant class of prenylated flavonoids with antioxidant activity, being a great number of the natural derivatives obtained from different extracts of hops [12–17], belonging to the Cannabaceae family. A limited number of derivatives can be obtained from Moraceae [18–20], Fabaceae [21–23] and Asteraceae [24] families. More than half of the chalcones, mainly derivatives of xanthohumol, were prepared by synthesis [12–14,25–29] (compounds 1–44, Table 1). To refer that Popoola et al. identified isoxanthohumol isolated from the aerial part of *Helichrysum teretifolium* as a chalcone derivative 21 [24] while the remaining literature classify isoxanthohumol as a flavanone derivative 102 [12–14].

**Table 1.** Naturally occurring prenylated chalcones with antioxidant activity.

N	Trivial Name	Species	Extract	Ref.
Cannabaceae				
1	xanthohumol	<i>Humulus lupulus</i>	hop	[12–17]
2	xanthogalenol	<i>Humulus lupulus</i>	hop	[12,14]
3	desmethylxanthohumol	<i>Humulus lupulus</i>	hop	[12–14]
4	4′-O-methylxanthohumol	<i>Humulus lupulus</i>	hop	[12–14]
5	5′-prenylxanthohumol	<i>Humulus lupulus</i>	hop	[12–14,16]
6	dehydrocycloxanthohumol	<i>Humulus lupulus</i>	hop	[12–14]
7	dehydrocycloxanthohumol hydrate	<i>Humulus lupulus</i>	hop	[12–14]
8	xanthohumol H	<i>Humulus lupulus</i>	hop	[16]
9	xanthohumol C	<i>Humulus lupulus</i>	hop	[16]
10	1′′,2′′-dihydroxanthohumol C	<i>Humulus lupulus</i>	hop	[16]
11	3′-geranylchalconaringenin	<i>Humulus lupulus</i>	hop	[12–14,16]
12	3′-geranyl-6′-O-methylchalconaringenin	<i>Humulus lupulus</i>	hop	[16]
13	2′-O-methyl-3′-prenylchalconaringenin	<i>Humulus lupulus</i>	hop	[16]
Moraceae				
14	isobavachalcone	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
15	flemichapparin A	<i>Artocarpus scortechinii</i>	leaf and stem bark	[19]
14	isobavachalcone	<i>Dorstenia barteri</i>	twig and leaf	[20]
16	bartericin A	<i>Dorstenia barteri</i>	twig and leaf	[20]
Fabaceae				
17	licochalcone A	<i>Glycyrrhiza inflata</i>	root	[21]
18	kuraridin	<i>Sophora flavescens</i>		[22,23]
19	kuraridinol	<i>Sophora flavescens</i>		[22,23]
Asteraceae				
20	heliteretifolin	<i>Helichrysum teretifolium</i>	aerial part	[24]
21	isoxanthohumol	<i>Helichrysum teretifolium</i>	aerial part	[24]
22	2′,4′,6′-trihydroxy-3′-prenylchalcone	<i>Helichrysum teretifolium</i>	aerial part	[24]
Obtained by synthesis				
1	xanthohumol	synthesis		[16,25–27]
2	xanthogalenol	synthesis		[25]
3	desmethylxanthohumol	synthesis		[25]
4	4′-O-methylxanthohumol	synthesis		[25]
23	4′-O-5′-C-diprenylxanthohumol	synthesis		[12–14]
24	4-O-methylxanthohumol	synthesis		[28]
25	4-O-acetyl xanthohumol	synthesis		[27,28]
26	4,4′-di-O-acetyl xanthohumol	synthesis		[27]
27	4-O-decanoyl xanthohumol	synthesis		[27]
28	4-O-dodecanoyl xanthohumol	synthesis		[27]
29	4,4′-di-O-dodecanoyl xanthohumol	synthesis		[27]
30	4-O-pivaloyl xanthohumol	synthesis		[27]
31	4,4′-di-O-pivaloyl xanthohumol	synthesis		[27]
32	3-hydroxyxanthohumol	synthesis		[25,26]
33	3-methoxyxanthohumol	synthesis		[28]

Table 1. Cont.

N	Trivial Name	Species	Extract	Ref.
34	2,2',4'-trihydroxy-6'-methoxy-3'-prenylchalcone	synthesis		[25]
35	2',3,4'-trihydroxy-6'-methoxy-3'-prenylchalcone	synthesis		[25]
36	2',3,4',5-tetrahydroxy-6'-methoxy-3'-prenylchalcone	synthesis		[25]
37	2',3,4,4',5-pentahydroxy-6'-methoxy-3'-prenylchalcone	synthesis		[25]
38	2',4'-dihydroxy-3,4,6'-trimethoxy-3'-prenylchalcone	synthesis		[25]
39	4,6'-dihydroxy-2',4'-dimethoxy-3'-prenylchalcone	synthesis		[25]
40	3-hydroxyxanthohumol C	synthesis		[28]
41	3-methoxyxanthohumol C	synthesis		[28]
42	3-hydroxyxanthohumol H	synthesis		[28]
43	3-methoxyxanthohumol H	synthesis		[28]
44	(2E,2'E)-1,1'-[methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl)]bis[3-(2,3,4-trihydroxyphenyl)prop-2-en-1-one]	synthesis		[29]

Most of the known derivatives are prenylated on the A-ring (Table 2), except licochalcone A (17) that has an 1,1-dimethylallyl unit at C-5 of B-ring (Figure 2). Among the former ones, 3'-prenyl substitution is the most abundant side attachment, being most of these derivatives also hydroxylated at positions 2' > 4 > 4' and methylated at positions 6' > 4' > 2' (Table 2). Other examples are those two having a 3'-geranyl group (compounds 11 and 12), two 3'-lavandulyl derivatives (compounds 18 and 19), three derivatives of xanthohumol H possessing a 3-hydroxy-3-methylbutyl group at C-5' (compounds 8, 40 and 41) and bartericin A (16), an example of a diprenylated chalcone with a 3-prenyl and a 5'-(2-hydroxy-3-methylbut-3-enyl) moieties (Figure 2). The remaining chalcones exhibit a ring, appearing most of the times as 6,7-(2,2-dimethylchromeno) derivatives. Among them, the O-prenylated chalcone reported as heliteretifolin (20, Figure 2), isolated from a *H. teretifolium* methanolic extract and the chalcone dimer 44 (Figure 3) obtained by synthesis.

In contrast to the chalcones, only two prenylated dihydrochalcones, elastichalcone B (46) isolated from the leaves of *A. elasticus* [30] and tetrahydroxanthohumol (45) obtained by synthesis [12–14], are reported (Figure 3).

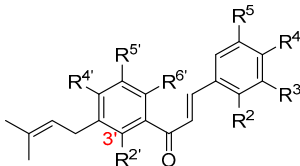
**Flavone derivatives.** The naturally-occurring derivatives were obtained mainly from Moraceae family, belonging to *Artocarpus* [18,19,21,30–36], *Dorstenia* [20,37] and *Cudrania* [38,39] species (compounds 47–76, Table 3 and Figures 4–6). A few examples from Fabaceae [22,40] and Euphorbiaceae [41,42] families were also isolated. Only three analogues were synthesized and were O-prenylated ones (7-O-prenyl- 73, 7-O-geranyl- 74 and 7-O-farnesylbaicalein 75) (Table 3 and Figure 6) [43].

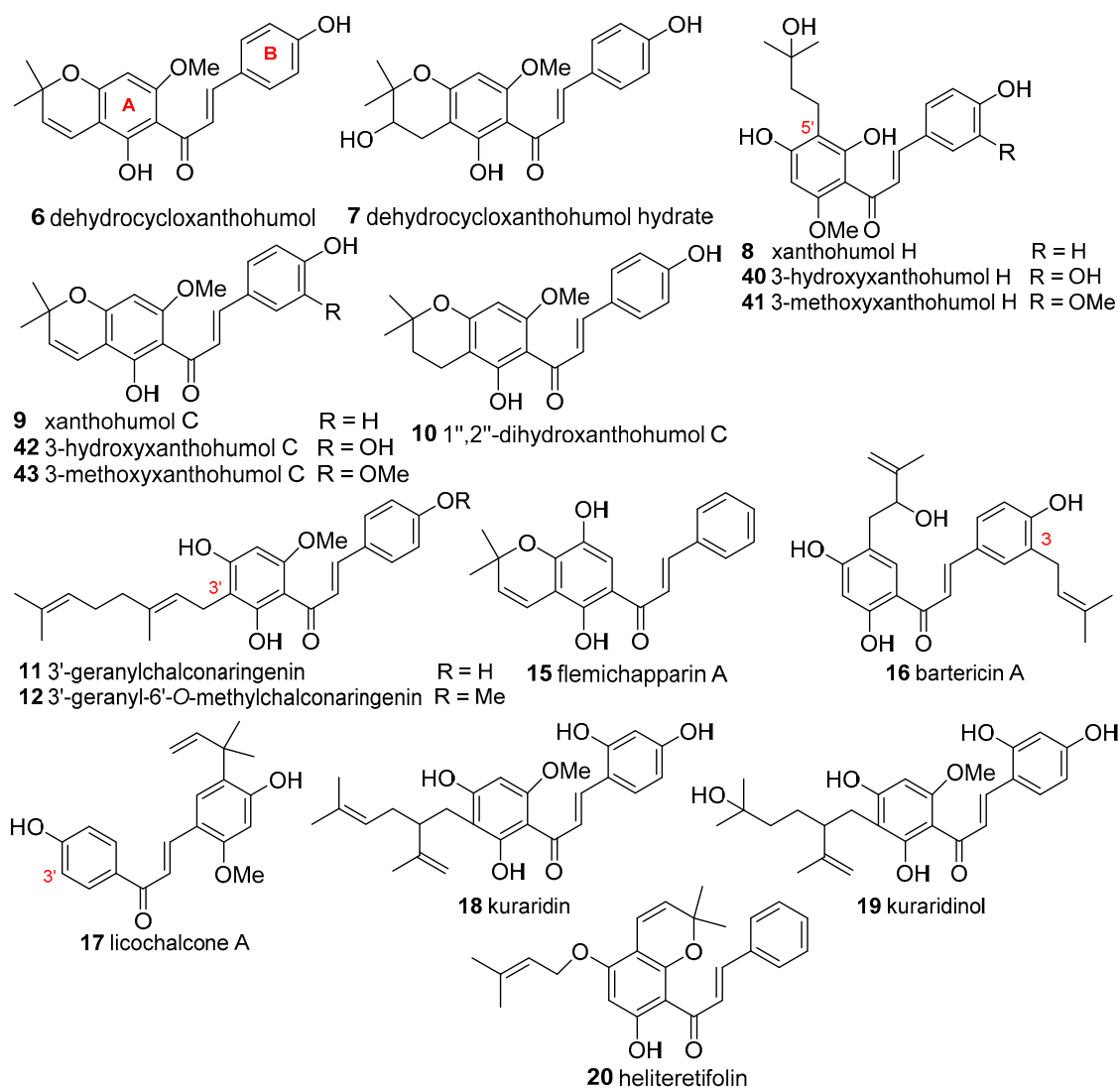
All the natural prenylflavones with antioxidant activity are C-substituted, being most of them mono- (Figure 4) and di-prenylated (Figure 5). There is a single case of a triprenylated derivative, artelastoheterol (57), isolated from *Artocarpus elasticus* (Table 3 and Figure 6) [34].

**Flavanone derivatives.** The compounds belonging to this group are isolated mainly from plants of the Fabaceae family [22,23,40,44–46]. Several derivatives were also isolated from Moraceae [16,20,37–39], Asteraceae [24] and Cannabaceae [12–14] families. A great number of prenylated flavanones were collected from propolis of different origins [47,48] and some of them were obtained by synthesis [12,14,49] (compounds 77–118, Table 4).

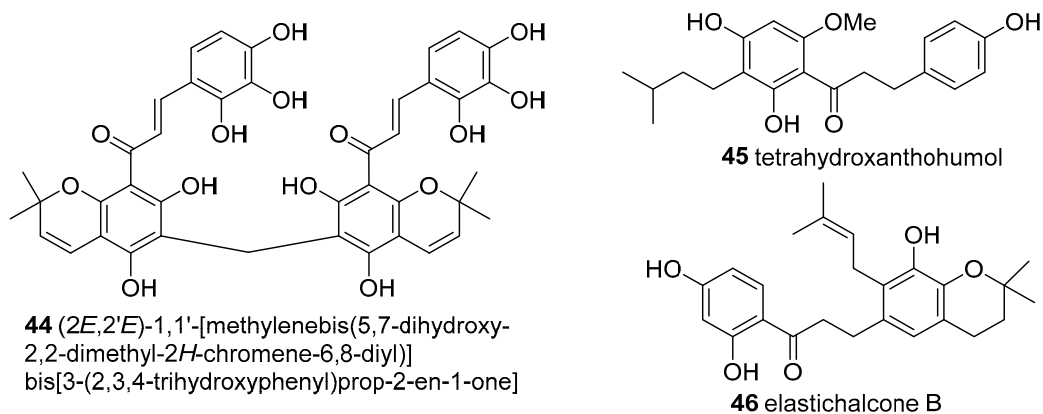
Flavanones represent the second most abundant class of prenylated flavonoids with antioxidant activity. These derivatives can be grouped according to their substitution in the flavanone moiety. Thus, a great number of compounds are monoprenylated in A-ring (Table 5), although one can also find diprenylated in A-ring, monoprenylated in B-ring and prenylated in both A- and B-rings (Figure 7). Some prenylated flavanonols can also be found in Figure 8.

**Table 2.** Chemical structures of 3'-prenylated chalcones 1–5, 13, 14 and 22–39 with antioxidant activity.

N	Comp.	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>2'</sup>	R <sup>4'</sup>	R <sup>5'</sup>	R <sup>6'</sup>
									
1	xanthohumol	H	H	OH	H	OH	OH	H	OMe
2	xanthogalenol	H	H	OH	H	OH	OMe	H	OH
3	desmethylxanthohumol	H	H	OH	H	OH	OH	H	OH
4	4'-O-methylxanthohumol	H	H	OH	H	OH	OMe	H	OMe
5	5'-prenylxanthohumol	H	H	OH	H	OH	OH	prenyl	OMe
13	2'-O-methyl-3'-prenylchalconaringenin	H	H	OH	H	OMe	OH	H	OH
14	isobavachalcone	H	H	OH	H	OH	OMe	H	H
21	isoxanthohumol	H	H	H	H	OH	OMe	H	OH
22	2',4',6'-trihydroxy-3'-prenylchalcone	H	H	H	H	OH	OH	H	OH
23	4'-O-5'-C-diprenylxanthohumol	H	H	OH	H	OH	Oprenyl	prenyl	OMe
24	4-O-methylxanthohumol	H	H	OMe	H	OH	OH	H	OMe
25	4-O-acetyl xanthohumol	H	H	OAc	H	OH	OH	H	OMe
26	4,4'-di-O-acetyl xanthohumol	H	H	OAc	H	OH	OAc	H	OMe
27	4-O-decanoyl xanthohumol	H	H	Odecanoyl	H	OH	OH	H	OMe
28	4-O-dodecanoyl xanthohumol	H	H	Ododecanoyl	H	OH	OH	H	OMe
29	4,4'-di-O-dodecanoyl xanthohumol	H	H	Ododecanoyl	H	OH	Ododecanoyl	H	OMe
30	4-O-pivaloyl xanthohumol	H	H	Opivaloyl	H	OH	OH	H	OMe
31	4,4'-di-O-pivaloyl xanthohumol	H	H	Opivaloyl	H	OH	Opivaloyl	H	OMe
32	3-hydroxyxanthohumol	H	OH	OH	H	OH	OH	H	OMe
33	3-methoxyxanthohumol	H	OMe	OH	H	OH	OH	H	OMe
34	2,2',4'-trihydroxy-6'-methoxy-3'-prenylchalcone	OH	H	H	H	OH	OH	H	OMe
35	2',3,4'-trihydroxy-6'-methoxy-3'-prenylchalcone	H	OH	H	H	OH	OH	H	OMe
36	2',3,4',5-tetrahydroxy-6'-methoxy-3'-prenylchalcone	H	OH	H	OH	OH	OH	H	OMe
37	2',3,4,4',5-pentahydroxy-6'-methoxy-3'-prenylchalcone	H	OH	OH	OH	OH	OH	H	OMe
38	2',4'-dihydroxy-3,4,6'-trimethoxy-3'-prenylchalcone	H	OMe	OMe	H	OH	OH	H	OMe
39	4,6'-dihydroxy-2',4'-dimethoxy-3'-prenylchalcone	H	H	OH	H	OMe	OMe	H	OH



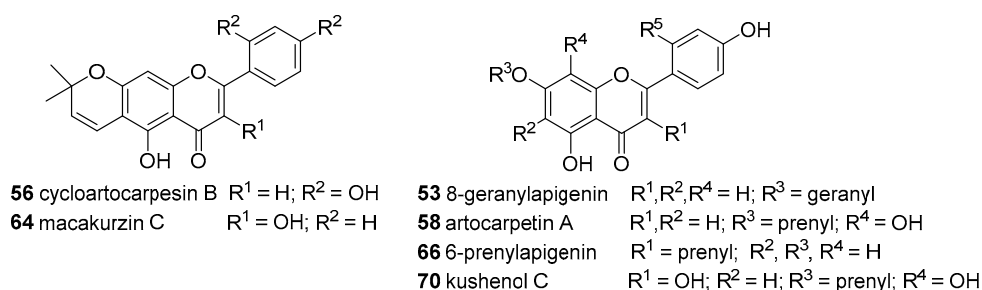
**Figure 2.** Chemical structures of other prenylated chalcones 6–12, 15–20 and 40–43 with antioxidant activity.

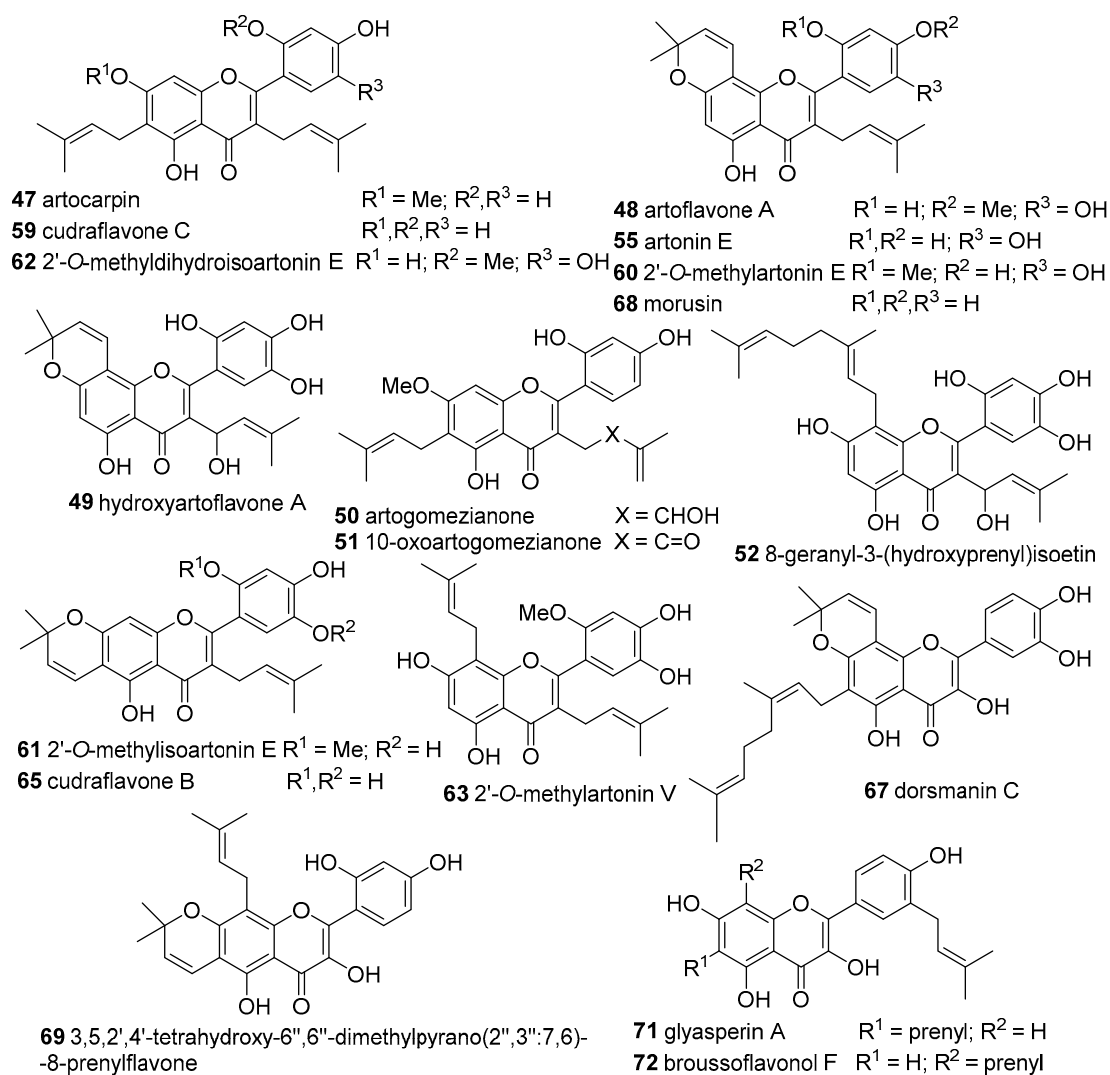


**Figure 3.** Chemical structures of chalcone dimer 44 and dihydrochalcones 45 and 46 with antioxidant activity.

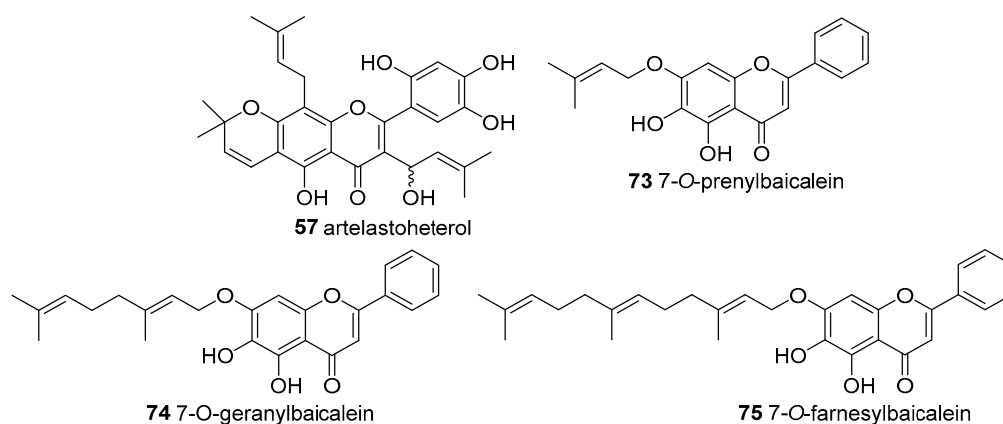
**Table 3.** Natural occurrence of prenylated flavones with antioxidant activity.

N	Trivial Name	Species	Extract	Ref.
Moraceae				
47	artocarpin	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
48	artoflavone A	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
49	hydroxyartoflavone A	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
50	artogomezianone	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
51	10-oxoartogomezianone	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
52	8-geranyl-3-(hydroxyprenyl)isoetin	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
53	8-geranylapiogenin	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
47	artocarpin	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
54	4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(γ,γ-dimethyl)allylflavone	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
55	artoinin E	<i>Artocarpus communis</i>	plant	[21]
48	artoflavone A	<i>Artocarpus communis</i>	cortex of root	[34]
56	cycloartocarpesin B	<i>Artocarpus elasticus</i>	leaf	[30]
57	artelastoheterol	<i>Artocarpus elasticus</i>	root bark	[34]
47	artocarpin	<i>Artocarpus heterophyllus</i>	plant	[31]
58	artocarpetin A	<i>Artocarpus heterophyllus</i>	plant	[31]
49	artocarpin	<i>Artocarpus incisa</i>	heartwood	[32]
47	artocarpin	<i>Artocarpus integer</i>	heartwood	[36]
59	cudraflavone C	<i>Artocarpus integer</i>	heartwood	[36]
55	artoinin E	<i>Artocarpus nobilis</i>	root bark	[33]
60	2'-O-methylartoinin E	<i>Artocarpus nobilis</i>	root bark	[33]
61	2'-O-methylisoartoinin E	<i>Artocarpus nobilis</i>	root bark	[33]
62	2'-O-methyldihydroisoartoinin E	<i>Artocarpus nobilis</i>	root bark	[33]
63	2'-O-methylartoinin V	<i>Artocarpus nobilis</i>	root bark	[33]
47	artocarpin	<i>Artocarpus scortechinii</i>	leaf and stem bark	[19]
55	artoinin E	<i>Artocarpus scortechinii</i>	leaf and stem bark	[19]
54	4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(γ,γ-dimethyl)allylflavone	<i>Artocarpus scortechinii</i>	leaf and stem bark	[19]
64	macakurzin C	<i>Artocarpus scortechinii</i>	leaf and stem bark	[19]
56	cycloartocarpesin B	<i>Cudrania tricuspidata</i>	root bark	[38,39]
65	cudraflavone B	<i>Cudrania tricuspidata</i>	root bark	[38,39]
66	6-prenylapiogenin	<i>Dorstenia kameruniana</i>	twig and leaf	[20]
67	dorsmanin C	<i>Dorstenia mannii</i>	twig and leaf	[20]
68	morusin	<i>Morus alba</i>	root bark	[21]
Fabaceae				
69	3,5,2',4'-tetrahydroxy-6'',6''-dimethylpyrano-(2'',3'':7,6)-8-prenylflavone	<i>Eriosema chinense</i>	root	[40]
70	kushenol C	<i>Sophora flavescens</i>	root	[22]
Euphorbeaceae				
71	glyasperin A	<i>Macaranga gigantea</i>	leaf	[42]
72	broussoflavonol F	<i>Macaranga gigantea</i>	leaf	[42]
71	glyasperin A	<i>Macaranga pruinosa</i>	leaf	[41]
Obtained by synthesis				
73	7-O-prenylbaicalein	synthesis		[16,43]
74	7-O-geranylbaicalein	synthesis		[43]
75	7-O-farnesylbaicalein	synthesis		[43]

**Figure 4.** Chemical structures of mono-C-prenylated flavones 53, 56, 58, 64, 66 and 70 with antioxidant activity.



**Figure 5.** Chemical structures of di-C-prenylated flavones 47–52, 55, 59, 60–63, 65, 67–69, 71 and 72 with antioxidant activity.



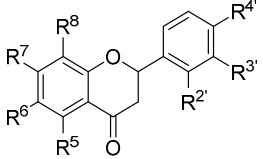
**Figure 6.** Chemical structures of triprenylated flavone 57 and mono-O-prenylated flavones 73–75 with antioxidant activity.



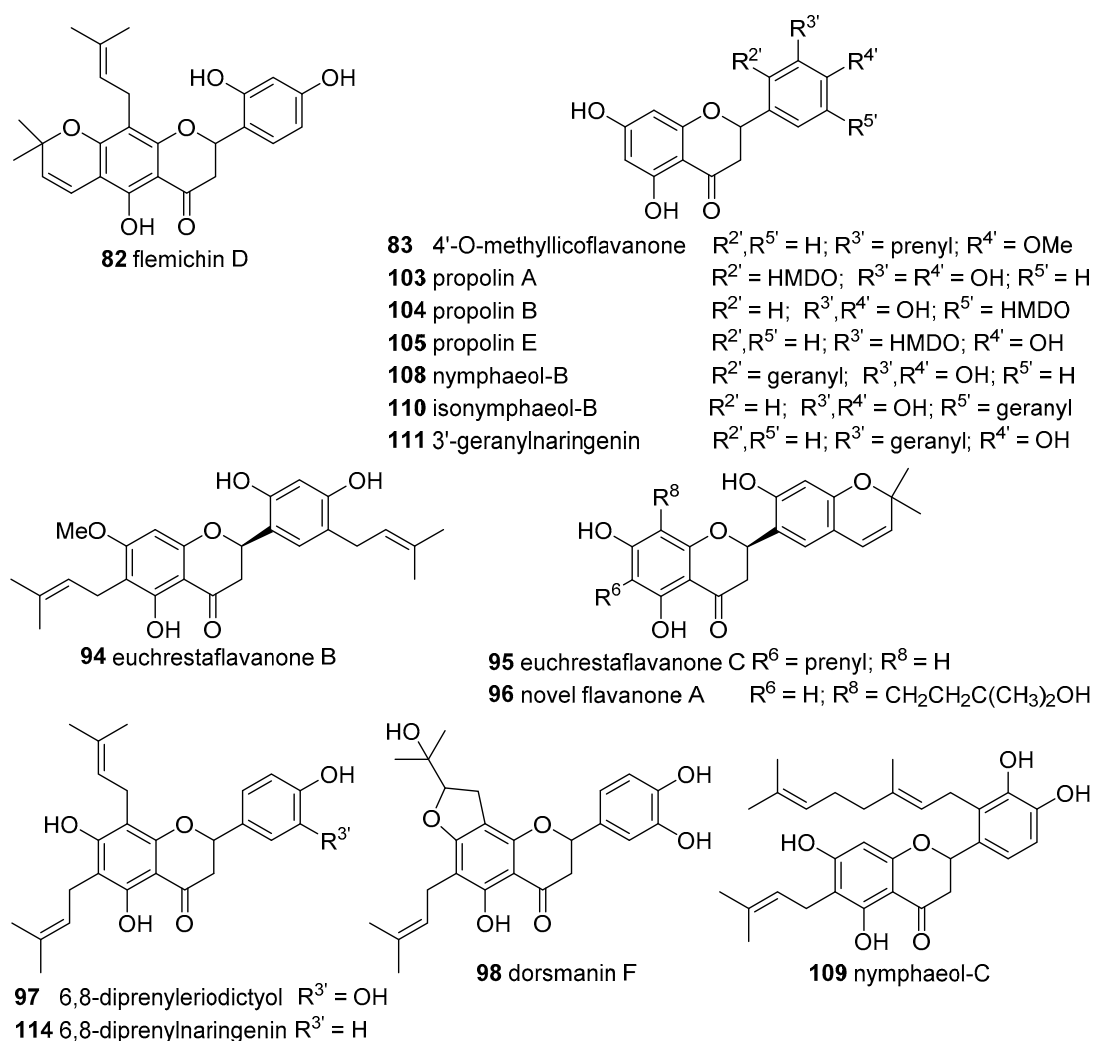
Table 4. Natural occurrence of prenylated flavanones with antioxidant activity.

N	Trivial Name	Species	Extract	Ref.
Fabaceae				
76	floranol	<i>Dioclea grandiflora</i>	root	[44]
77	khonklonin A	<i>Eriosema chinense</i>	root	[40]
78	2'''',3'''-epoxykhonklonin A	<i>Eriosema chinense</i>	root	[40]
79	lupinifolinol	<i>Eriosema chinense</i>	root	[40]
80	3- <i>epi</i> -lupinifolinol	<i>Eriosema chinense</i>	root	[40]
81	2-hydroxylupinifolinol	<i>Eriosema chinense</i>	root	[40]
82	flemichin D	<i>Eriosema chinense</i>	root	[40]
83	4'-O-methyllicoflavanone	<i>Erythrina orientalis</i>	stem bark	[46]
84	5,7-dihydroxy-6-methyl-8-prenylflavanone	<i>Eysenhardtia platycarpa</i>	leaf	[45]
85	5,7-dihydroxy-4'-methoxy-6-methyl-8-prenylflavanone	<i>Eysenhardtia platycarpa</i>	leaf	[45]
86	5,7-dihydroxy-6-prenylflavanone	<i>Eysenhardtia platycarpa</i>	leaf	[45]
87	5-hydroxy-7-methoxy-6-prenylflavanone	<i>Eysenhardtia platycarpa</i>	leaf	[45]
88	5,7-dihydroxy-4'-methoxy-8-prenylflavanone	<i>Eysenhardtia platycarpa</i>	leaf	[45]
89	leachianone	<i>Sophora flavescens</i>	root	[22]
90	kushenol E	<i>Sophora flavescens</i>	root	[22]
91	sophoraflavanone G	<i>Sophora flavescens</i>	root	[22]
92	kurarinone	<i>Sophora flavescens</i>	root	[22]
93	kurarinol	<i>Sophora flavescens</i>	root	[22]
Moraceae				
88	5,7-dihydroxy-4'-methoxy-8-prenylflavanone	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
94	euchrestaflavanone B	<i>Cudrania tricuspidata</i>	root bark	[38,39]
95	euchrestaflavanone C	<i>Cudrania tricuspidata</i>	root bark	[38,39]
96	novel flavanone A	<i>Cudrania tricuspidata</i>	root bark	[38,39]
97	6,8-diprenyleryodictyol	<i>Dorstenia mannii</i>	twig and leaf	[20,37]
98	dorsmanin F	<i>Dorstenia mannii</i>	twig and leaf	[20,37]
Asteraceae				
99	isoglabranin	<i>Helichrysum teretifolium</i>	aerial part	[24]
100	glabranin	<i>Helichrysum teretifolium</i>	aerial part	[24]
101	7-methoxyisoglabranin	<i>Helichrysum teretifolium</i>	aerial part	[24]
Cannabaceae				
102	isoxanthohumol	<i>Humulus lupulus</i>	hop	[12–14]
Obtained from propolis				
103	propolin A	Japanese	ethanol	[47]
104	propolin B	Japanese	ethanol	[47]
105	propolin E	Japanese	ethanol	[47]
106	prokinawan	Japanese	ethanol	[47]
107	nymphaeol A	Japanese	ethanol	[47]
108	nymphaeol B	Japanese	ethanol	[47]
109	nymphaeol C	Japanese	ethanol	[47]
110	isonymphaeol B	Japanese	ethanol	[47]
111	3'-geranylnaringenin	Japanese	ethanol	[47]
103	propolin A	Taiwanese glue	95% ethanol	[48]
104	propolin B	Taiwanese glue	95% ethanol	[48]
Obtained by synthesis				
112	6-prenylnaringenin	synthesis		[12,14]
113	8-prenylnaringenin	synthesis		[12,14]
114	6,8-diprenylnaringenin	synthesis		[12,14]
115	6-geranylnaringenin	synthesis		[12,14]
116	8-geranylnaringenin	synthesis		[12,14]
102	isoxanthohumol	synthesis		[49]
117	4'-O-acylisoxanthohumol	synthesis		[49]
118	7,4'-O-diacetylisoxanthohumol	synthesis		[49]

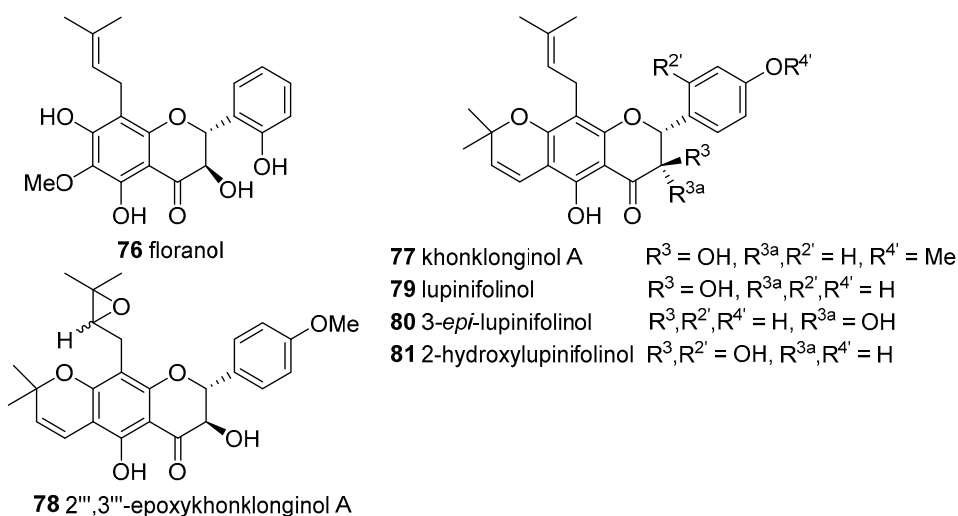
**Table 5.** Chemical structures of flavanones monoprenylated in A-ring **84–89**, **91–93**, **99**, **100–102**, **106**, **107**, **112**, **113** and **115–118** with antioxidant activity.

N	Comp.	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>	R <sup>8</sup>	R <sup>2'</sup>	R <sup>3'</sup>	R <sup>4'</sup>
								
84	5,7-dihydroxy-6-methyl-8-prenylflavanone	OH	Me	OH	prenyl	H	H	H
85	5,7-dihydroxy-4'-methoxy-6-methyl-8-prenylflavanone	OH	Me	OH	prenyl	H	H	OMe
86	5,7-dihydroxy-6-prenylflavanone	OH	prenyl	OH	H	H	H	H
87	5-hydroxy-7-methoxy-6-prenylflavanone	OH	prenyl	OMe	H	H	H	H
88	5,7-dihydroxy-4'-methoxy-8-prenylflavanone	OH	H	OH	prenyl	H	H	OMe
89	leachianone	OH	H	OH	prenyl	OH	H	OH
91	sophoraflavanone G	OH	H	OH	lavandulyl	OH	H	OH
92	kurarinone	OMe	H	OH	lavandulyl	OH	H	OH
93	kurarinol	OMe	H	OH	OHlavandulyl	OH	H	OH
99	isoglabranin	OH	prenyl	OH	H	H	H	H
100	glabranin	OH	H	OH	prenyl	H	H	H
101	7-methoxyisoglabranin	OH	prenyl	OMe	H	H	H	H
102	isoxanthohumol	OMe	H	OH	prenyl	H	H	OH
106	prokinawan	OH	HDMO <sup>1</sup>	OH	H	H	OH	OH
107	nymphaeol A	OH	geranyl	OH	H	H	OH	OH
112	6-prenylnaringenin	OH	prenyl	OH	H	H	H	OH
113	8-prenylnaringenin	OH	H	OH	prenyl	H	H	OH
115	6-geranylnaringenin	OH	geranyl	OH	H	H	H	OH
116	8-geranylnaringenin	OH	H	OH	geranyl	H	H	OH
117	4'-O-acetylisoxanthohumol	OMe	H	OH	prenyl	H	H	OAc
118	7,4'-O-diacetylisoxanthohumol	OMe	H	OAc	prenyl	H	H	OAc

<sup>1</sup> HDMO = 7-hydroxy-3,7-dimethyloct-2-enyl.



**Figure 7.** Chemical structures of flavanones diprenylated in A-ring **97**, **98** and **114**; monoprenylated in B-ring **83**, **103**–**105**, **108**, **110** and **111** and prenylated in A- and B-ring **94**–**96** and **109** with antioxidant activity.



**Figure 8.** Chemical structures of prenylated flavanonols **76**–**81** with antioxidant activity.

**Isoflavone derivatives.** Prenylated isoflavones were isolated only from Fabaceae [46,50,51], Moraceae [52] and Apiaceae [53] families (compounds 119–130, Table 6). The major part of the derivatives are prenylated in A-ring, as 6-prenyl or 8-prenyl, along with some derivatives with a 6,7-(2,2-dimethylchromene)-fused unit. A single example of isoflavone with a prenyl moiety in B-ring is angustone C (130), isolated from *Azorella madreporica*. Erynone (121) isolated from *Erythrina stricta*, represents a complex isoflavanone substituted with several prenyl units (Figure 9).

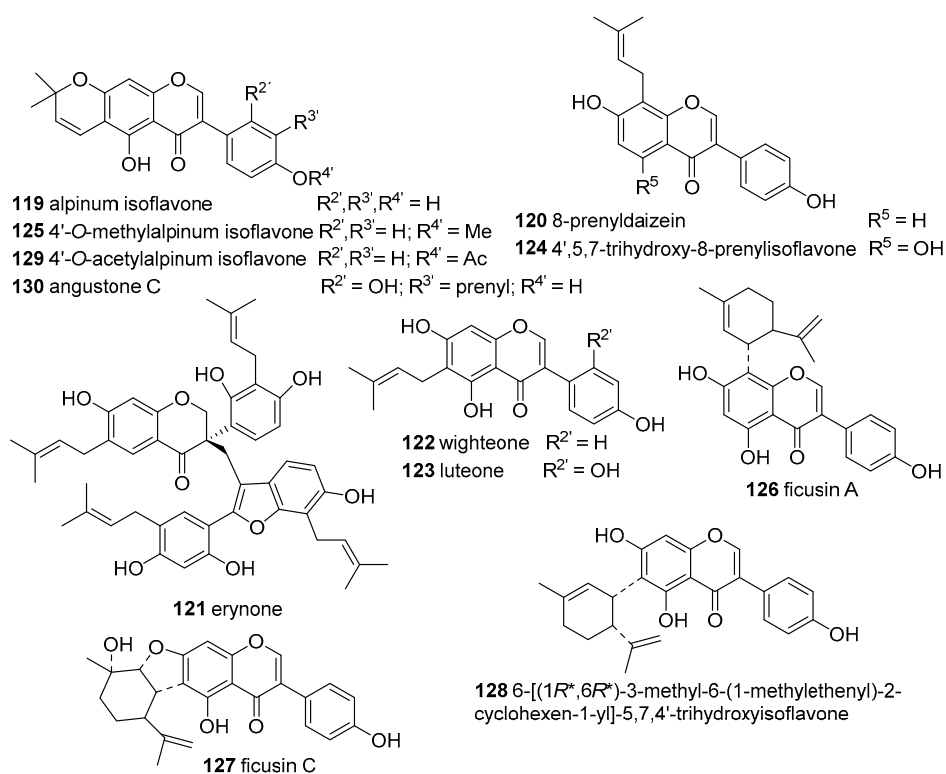
**Table 6.** Natural occurrence of prenylated isoflavones with antioxidant activity.

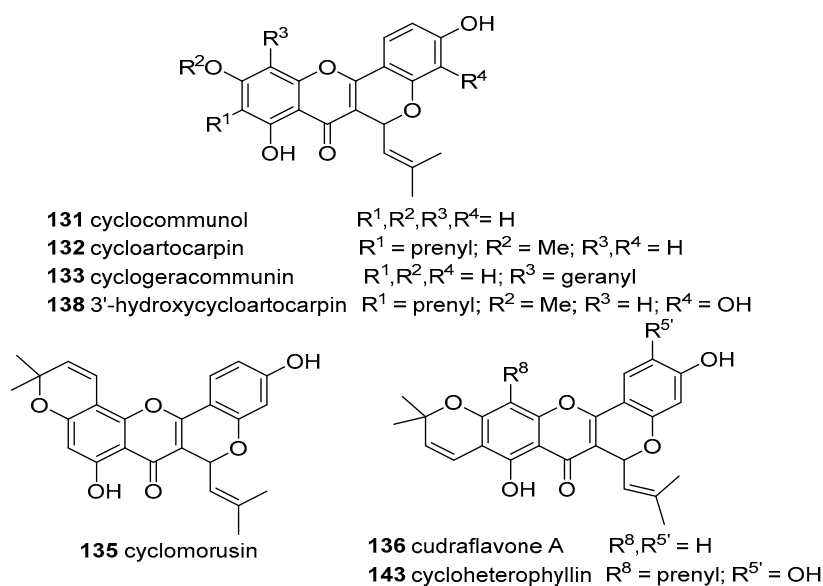
N	Trivial Name	Species	Extract	Ref.
Fabaceae				
119	alpinum isoflavone	<i>Erythrina orientalis</i>	stem bark	[46]
120	8-prenyldaidzein	<i>Erythrina orientalis</i>	stem bark	[46]
119	alpinum isoflavone	<i>Erythrina stricta</i>	stem bark	[50]
121	erynone	<i>Erythrina stricta</i>	stem bark	[50]
122	wighteone	<i>Erythrina stricta</i>	stem bark	[50]
123	luteone	<i>Erythrina stricta</i>	stem bark	[50]
119	alpinum isoflavone	<i>Erythrina variegata</i>	stem bark	[51]
124	4',5,7-trihydroxy-8-prenylisoflavone	<i>Erythrina variegata</i>	stem bark	[51]
Moraceae				
119	alpinum isoflavone	<i>Ficus tikoua</i>	rhizomes	[52]
125	4'-O-methylalpinum isoflavone	<i>Ficus tikoua</i>	rhizomes	[52]
126	ficusin A	<i>Ficus tikoua</i>	rhizomes	[52]
127	ficusin C	<i>Ficus tikoua</i>	rhizomes	[52]
128	6-[(1R*,6R*)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5,7,4'-trihydroxyisoflavone	<i>Ficus tikoua</i>	rhizomes	[52]
Apiaceae				
119	alpinum isoflavone	<i>Azorella madreporica</i>	aerial part	[53]
129	4'-O-acetylalpinum isoflavone	<i>Azorella madreporica</i>	aerial part	[53]
130	angustone C	<i>Azorella madreporica</i>	aerial part	[53]

**Xanthone-type compounds.** All prenylated xanthone-type derivatives were isolated from different species of *Artocarpus*: *A. altalis* [35], *A. anisophyllus* [18], *A. communis* [34], *A. elasticus* [31], *A. heterophyllus* [12], *A. incisa* [32], *A. integer* [36], *A. kemando* [54], *A. nobilis* [33], *A. obtusus* [55], *A. scortechinii* [19], which belongs to Moraceae family (compounds 131–150, Table 7). In this group we can find compounds with at least four-fused rings which structures may result from the cyclization of the prenyl group in the flavone unit to give a pyran ring (Figure 10) or can be xanthone nucleus, saturated or not, possessing other rings attached to the main core, leading to compounds with at most six-fused rings (Figure 11).

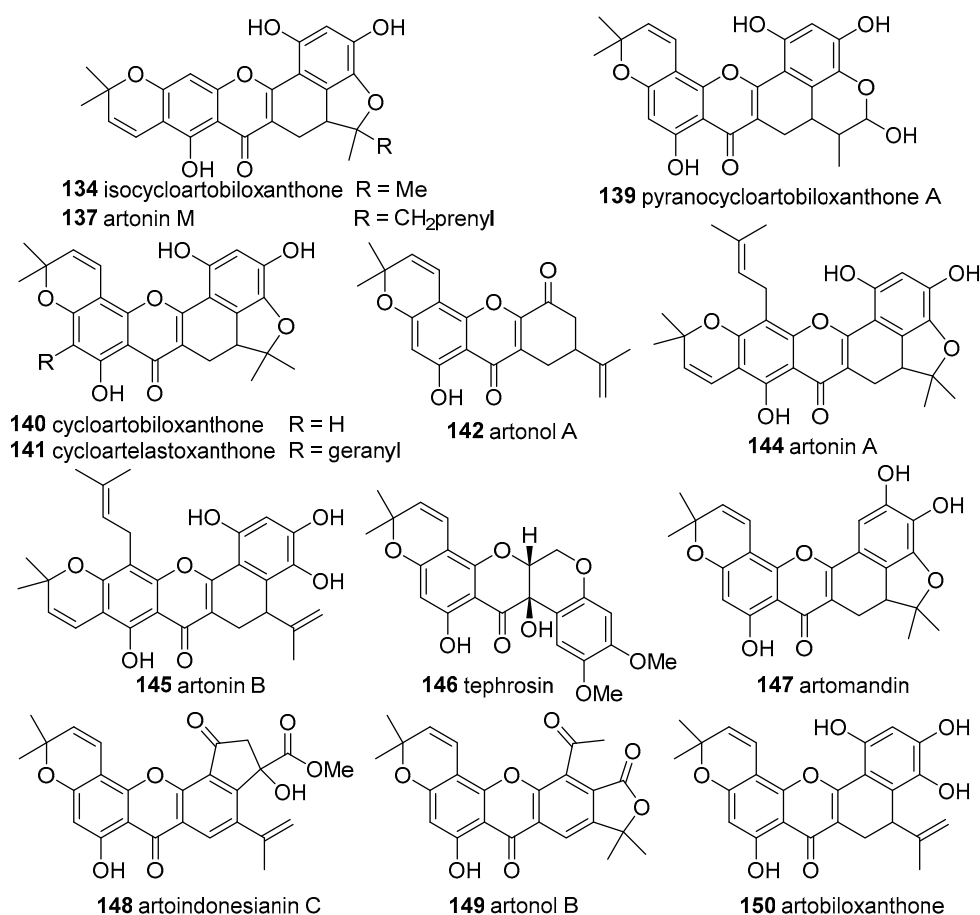
**Table 7.** Natural occurrence of prenylated xanthone-type derivatives with antioxidant activity.

N	Trivial Name	Species	Extract	Ref.
Moraceae				
131	cyclocommunol	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
132	cycloartocarpin	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
133	cyclogeracommunin	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
134	isocycloartobiloxanthone	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
135	cyclomorusin	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
136	cudraflavone A	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
137	artoinin M	<i>Artocarpus altilis</i>	heartwood and cortex	[35]
132	cycloartocarpin	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
138	3'-hydroxycycloartocarpin	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
139	pyranocycloartobiloxanthone A	<i>Artocarpus anisophyllus</i>	heartwood and leaf	[18]
133	cyclogeracommunin	<i>Artocarpus communis</i>	cortex of root	[34]
140	cycloartobiloxanthone	<i>Artocarpus elasticus</i>	root bark	[34]
141	cycloartelastoxanthone	<i>Artocarpus elasticus</i>	root bark	[34]
142	artanol A	<i>Artocarpus elasticus</i>	root bark	[34]
143	cycloheterophyllin	<i>Artocarpus heterophyllus</i>	plant	[31]
144	artoinin A	<i>Artocarpus heterophyllus</i>	plant	[31]
145	artoinin B	<i>Artocarpus heterophyllus</i>	plant	[31]
132	cycloartocarpin	<i>Artocarpus incisa</i>	heartwood	[32]
146	tephrosin	<i>Artocarpus integer</i>	heartwood	[36]
147	artomandin	<i>Artocarpus kemando</i>	stem bark	[54]
148	artoindonesianin C	<i>Artocarpus kemando</i>	stem bark	[54]
149	artanol B	<i>Artocarpus kemando</i>	stem bark	[54]
140	cycloartobiloxanthone	<i>Artocarpus nobilis</i>	root bark	[33]
150	artobiloxanthone	<i>Artocarpus nobilis</i>	root bark	[33]
139	pyranocycloartobiloxanthone A	<i>Artocarpus obtusus</i>	stem bark	[55]
136	cudraflavone A	<i>Artocarpus scorteichinii</i>	leaf and stem bark	[19]
140	cycloartobiloxanthone	<i>Artocarpus scorteichinii</i>	leaf and stem bark	[19]

**Figure 9.** Chemical structures of prenylated isoflavones 119–130 with antioxidant activity.



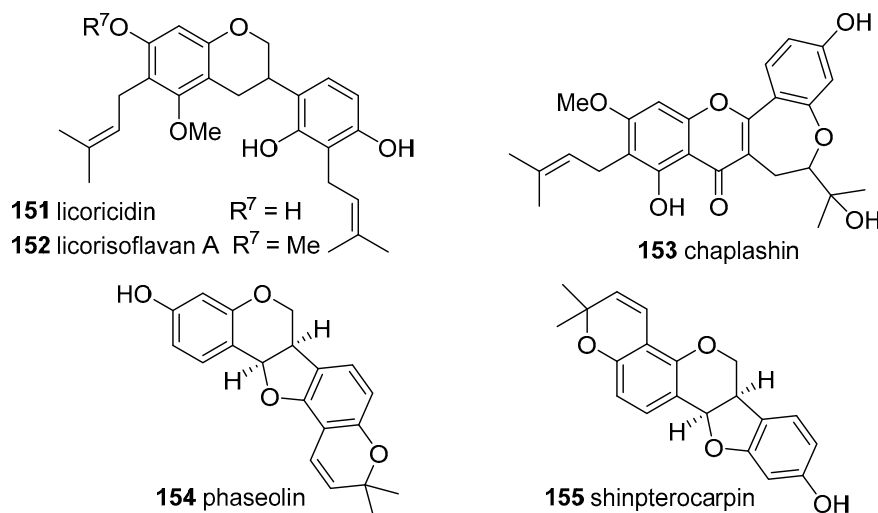
**Figure 10.** Chemical structures of prenylated xanthone-type derivatives **131–133**, **135**, **136**, **138** and **143** with antioxidant activity.



**Figure 11.** Chemical structures of prenylated xanthone-type derivatives **134**, **137**, **139–142** and **144–150** with antioxidant activity.

**Miscellaneous compounds.** Only two examples of isoflavan-type, licoricidin (**151**) and licorisoiflavan A (**152**), were isolated from *Glycyrrhiza uralensis* Fisher [21] (Figure 12). Chaplashin (**153**),

a flavone containing an oxepin ring, was isolated for the first time from the leaves and the heartwoods of *A. anisophyllum* Miq [18]. Two prenylated pterocarpan, phaseollin (154) and shinpterocarpin (155), have been isolated from the stem bark of *Erythrina orientalis* [46] (Figure 12).



**Figure 12.** Chemical structures of prenylated flavonoid-type derivatives 151–155 with antioxidant activity.

### 3. Methods for the Evaluation of the Antioxidant Activity of Prenylflavonoids

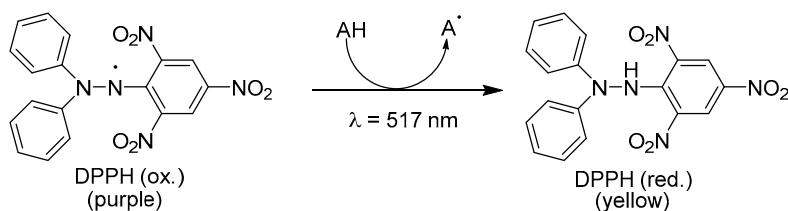
Various methods have been applied to study the antioxidant properties of a wide variety natural and synthetic prenylated flavonoids. For the in vitro methods, the most common ones are those involving electron transfer mechanisms such as DPPH, FRAP and TEAC assays; hydrogen atom transfer mechanisms such as for the inhibition of ROS and RNS scavenging assays and metal chelation studies. In the former case, DPPH radical scavenging method is by far the most frequently used, probably due to its simplicity in terms of time effort, experimental procedure and cheap reagents. Considering the in vivo models, two methods were used to evaluate the antioxidant potential of several prenylated flavonoids that include lipid peroxidation assay and LDL oxidation assay.

#### 3.1. In Vitro Methods

##### 3.1.1. Electron Transfer Mechanisms

##### DPPH Radical Scavenging Activity

DPPH $\cdot$  (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical characterized by an absorption band at about 517 nm. In the presence of an antioxidant molecule (AH), DPPH $\cdot$  trap a hydrogen atom to its reduced hydrazine form with consequent loss of the typical purple colour to a pale yellow one (Scheme 1).



**Scheme 1.** Reaction scheme involved in DPPH radical scavenging activity assay.

The percentage of the DPPH• scavenging is calculated according to the following Equation (1):

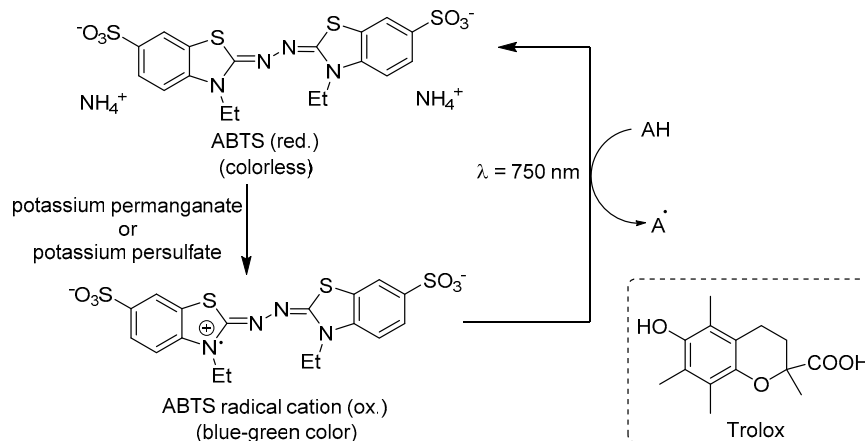
$$\% \text{ inhibition of DPPH radical} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (1)$$

where  $A_{\text{control}}$  is the absorbance of the control (before the reaction take place) and  $A_{\text{sample}}$  is the absorbance after the reaction occurred [56].

The activity is expressed as inhibitory concentration  $IC_{50}$ , that is the amount of antioxidant necessary to decrease by 50% the initial DPPH• concentration. Some disadvantages of this method is the steric accessibility of the radical by large antioxidant molecules, spectrophotometric measurements can be affected by compounds that absorb at the same wavelength of the determination and cannot be applied for measuring the antioxidant capacity of plasma since precipitation of proteins may occur in alcoholic media.

#### ABTS Radical Cation Scavenging Activity

$ABTS^{\bullet+}$  (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) is a stable blue–green chromophore radical cation characterized by an absorption band at about 750 nm which losses its colour in the presence of an antioxidant molecule (Scheme 2).  $ABTS^{\bullet+}$  is generated by reacting a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt. In this assay, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is usually used as antioxidant standard. The obtained results are expressed as Trolox equivalent antioxidant capacity (TEAC) values from the trolox standard curve [56]. This methodology can be applied to measure both hydrophilic and lipophilic antioxidant capacities since the  $ABTS^{\bullet+}$  is soluble in both aqueous and organic solvents and is not affected by ionic strength of the medium.

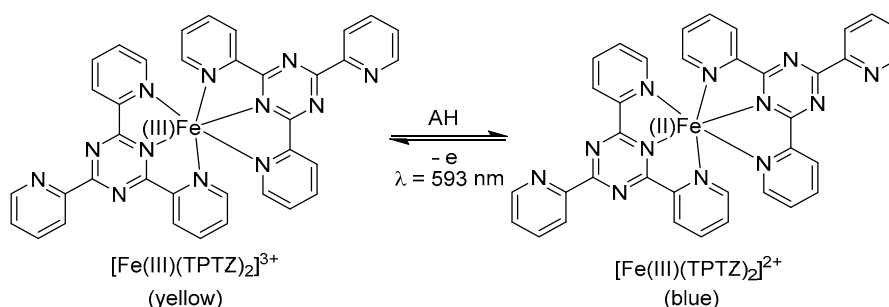


**Scheme 2.** Reaction scheme involved in ABTS radical cation scavenging activity assay.

#### Ferric Reducing Antioxidant Power (FRAP) Method

This method is based on the reduction of the colourless complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the blue-coloured ferrous form at low pH (Scheme 3). FRAP reagent is produced by mixing acetate buffer (pH 3.6), TPTZ solution and  $FeCl_3 \cdot 6H_2O$ . FRAP values are obtained by comparing the absorbance change at 593 nm in reaction mixtures with those containing ferrous ions in known concentration [17,53]. The redox potential of Fe(III) salt (−0.70 V) is comparable to that of  $ABTS^{\bullet+}$  (0.68 V), therefore, the main difference between TEAC assay and the FRAP assay is that the first is carried out at neutral pH and latest under acidic (pH 3.6) conditions. Usually a calibration curve using antioxidant trolox is made and the results are expressed as trolox equivalents per kg (solid food) or per L (beverages) of sample [56].



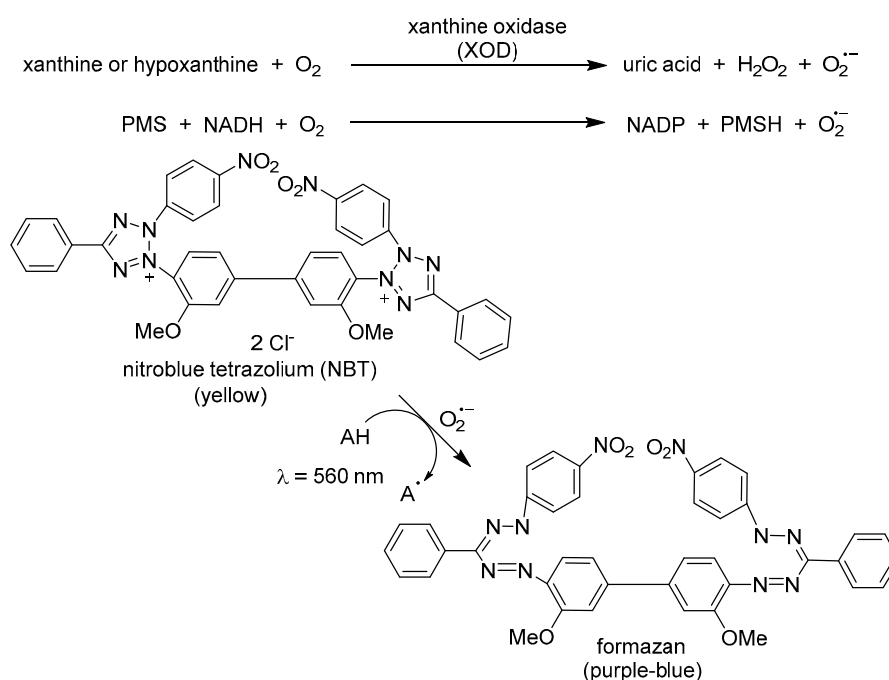


**Scheme 3.** Reaction scheme involved in FRAP assay.

### 3.1.2. Hydrogen Atom Transfer Mechanisms

#### Superoxide Radical Anion Scavenging Activity

Superoxide radical anion ( $\text{O}_2^{\bullet-}$ ) can be generated by two different approaches, using hypoxanthine or xanthine/xanthine oxidase (XOD) system at pH 7.4 [14,26,35] or using a non-enzymatic reaction of phenazine methosulphate (PMS) in the presence of nicotinamide adenine dinucleotide (NADH) [56] (Scheme 4). In both systems, superoxide anion radicals can reduce nitroblue tetrazolium (NBT) into formazan, and the effects are determined spectrophotometrically at 560 nm. Higher is the scavenging potential of the antioxidant molecule, lower is the formation of formazan and consequently, lower is the absorbance [56]. Other detectors can be used, being cytochrome c the second option, which reduction is followed spectrophotometrically at 550 nm [31,32]. The results are typically expressed as inhibitory concentration  $\text{IC}_{50}$  values.



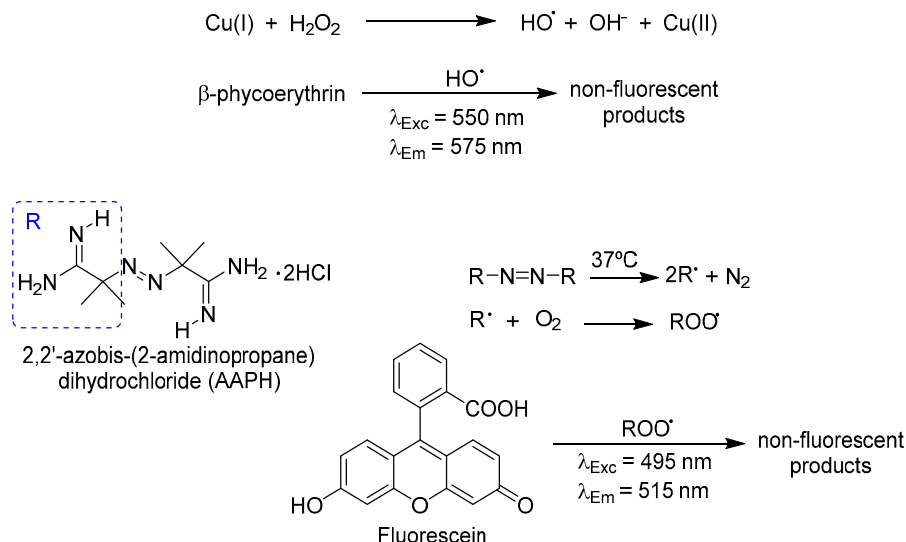
**Scheme 4.** Reaction scheme involved in superoxide radical anion scavenging activity assay.

#### Oxygen Radical Absorbance Capacity (ORAC) Method

The ORAC method can be applied to both hydrophilic and lipophilic environments and for the detection of both hydroxyl and peroxy radicals, formed during lipid oxidation chain reactions (autoxidation) and involving hydrogen atom transfer reactions.

The system  $\text{H}_2\text{O}_2$ - $\text{CuSO}_4$  is generally used as hydroxyl radical generator and  $\beta$ -phycoerythrin used as a redox-sensitive fluorescent indicator protein, which decay in the fluorescence is measured in

the presence of free radical scavengers, using Trolox as standard (Scheme 5). The ORAC value is then calculated from the trolox equivalent and expressed as ORAC units or value by taking the difference of areas-under-the-decay curves between blank and sample and/or standard. Higher the ORAC value, higher the antioxidant potential of the tested compounds [56].



**Scheme 5.** Reaction schemes involved in ORAC assay for the detection of hydroxyl and peroxy radicals.

2,2'-Azobis-(2-amidinopropane)dihydrochloride (AAPH) is the most used peroxy radical generator in hydrophilic systems [24] and as fluorescent probe can be used  $\beta$ -phycoerythrin or more recently fluorescein [16,25,28]. Thus, peroxy radical are formed by thermodecomposition of AAPH, giving an alkyl radical that react with molecular oxygen to give peroxy radical. The decay in fluorescence is recorded and the results of the scavenging activity is expressed as Trolox equivalents [56].

#### Other ROS/RNS Scavenging Activity

Other methodologies can be applied to evaluate the antioxidant potential of a series of natural and synthetic matrix against a series of reactive oxygen and nitrogen species (ROS and RNS). For prenylated flavonoids there are only a couple of papers referring the scavenging activity profile against hydrogen peroxide [31] and peroxyxynitrite anion [22], which led us to describe them in the next section, prior the discussion of the results obtained in such assays.

#### 3.1.3. Metal Chelation

Copper and iron chelation by prenylated flavonoids were determined by the difference in their UV-vis spectra (190–900 nm) produced when this metal ions were incubated with the tested compounds. The results are expressed as the difference in the absorbance or spectral shift of sample in the presence and in the absence of the metal ions [31,44,56].

### 3.2. In Vivo Methods

#### 3.2.1. Lipid Peroxidation Assay

Lipid peroxidation is usually induced by metal ions such as iron and measured by the thiobarbituric acid method, being the levels of peroxides formed expressed as TBARS. Other systems used for prenylated flavonoids were Mb(IV)-induced arachidonic acid peroxidation [32] and metal-ion independent systems using *tert*-butyl hydroperoxide (TBHP)-induced lipid peroxidation in liver microsomes or oxidation of  $\beta$ -carotene/linoleic acid emulsion. One of the limitations of this technique

is the time consuming that depends on the oxidation of a substrate which is influenced by temperature, pressure, matrix, etc., particularly important when a great numbers of samples are involved. [47].

### 3.2.2. LDL Oxidation Assay

Cu(II)-induced low-density lipoprotein (LDL) oxidation is determined by measuring, in an initial stage, the formation of conjugated dienes through the increase of absorbance at 234–250 nm and at the end, the generated amount of lipid peroxides by the TBARS assay at 532 nm, using MDA for the standard curve [12]. 3-Morpholinolinosydnomine (SIN-1), a peroxyxynitrite generator, can also be used to induce LDL oxidation [15].

## 4. Antioxidant Effects of Prenylflavonoids

### 4.1. In Vitro Methods

#### 4.1.1. Electron Transfer Mechanisms

##### DPPH Radical Scavenging Activity

A great number of prenylated flavonoid derivatives were evaluated for their ability to scavenge DPPH•. Ko et al. evaluated DPPH• decolorization induced by xanthone-type cycloheterophyllin (**143**), artonin A (**144**) and artonin B (**145**), isolated from the plant *A. heterophyllum* Lam. The scavenging activity was expressed as the concentration (IC<sub>0.20</sub>) of the test compounds that induced a decrease of 0.20 in absorbance in a 30 min period of time. The results pointed out that **143–145** increased DPPH• decolorization in a concentration-dependent manner with IC<sub>0.20</sub> of 9.6 ± 0.7, 8.4 ± 0.3, and 12.2 ± 0.6 µM, respectively. The positive control α-tocopherol scavenged DPPH• with an IC<sub>0.20</sub> of 11.9 ± 0.2 µM [31].

The flavanones 6,8-diprenyleriodictyol (**97**) and dorsmanin F (**98**) and the flavonol dorsmanin C (**67**) were isolated from the twig and leaf of *D. mannii* samples collected in Central Province of Cameroon. The DPPH• scavenging activity assay was performed in experiments of only 20 min since a plateau at 15 min was reached. The prenylated flavonoids were tested in concentrations of 1, 10 and 100 µM, causing a rapid decrease in the absorbance, dependent of the concentration, and compared with butylated hydroxytoluene (BHT), a common antioxidant used as a food additive. The potency of DPPH• scavenging activity followed the order: **67** > **97** > **98** >> BHT. These results seem to indicate that the C2=C3 double bond and the 3-OH group of the flavonol are important features for the high scavenging potency, when compared with the flavanones [37].

Two flavanones, propolin A (**103**) and propolin B (**104**), were isolated and characterized from Taiwanese propolis glue collected from hives located in the area of Bagwa Shan, Taiwan. Both compounds were tested in concentrations ranging from 3.125 to 25 µg/mL and exhibited strong scavenging effects against DPPH• with IC<sub>50</sub> values of 5.0 and 9.0 µg/mL, respectively [48].

Omisore et al. tested the DPPH• scavenging effects of five flavonoids from *Dorstenia* species: the chalcones isobavachalcone (**14**) and bartericin A (**16**) isolated from *D. barteri*, the flavone 6-prenyl-apigenin (**66**) from *D. kameruniana*, and the flavanones 6,8-diprenyleriodictyol (**97**) and dorsmanin F (**98**) isolated from *D. mannii*. The concentration needed to decrease the remaining DPPH• by 50% (the initial substrate concentration EC<sub>50</sub>) was the parameter used to measure the antioxidant capacity. Thus bartericin A (**16**, EC<sub>50</sub> 47.85 ± 2.15 µg/mL) and 6,8-diprenyleriodictyol (**97**, EC<sub>50</sub> 32.12 ± 1.10 µg/mL) as well as the positive controls quercitrin (EC<sub>50</sub> 28.16 ± 0.84 µg/mL) and ascorbic acid (EC<sub>50</sub> 19.33 ± 0.3 µg/mL) showed high antioxidant capacity (EC<sub>50</sub> < 50 µg/mL), while isobavachalcone (**14**, EC<sub>50</sub> 84.33 ± 0.27 µg/mL), 6-prenylapigenin (**66**, EC<sub>50</sub> 86.43 ± 0.26 µg/mL) and dorsmanin F (**98**, EC<sub>50</sub> 53.89 µg/mL) presented moderate antioxidant capacity (EC<sub>50</sub> > 50 µg/mL). The scavenging effects followed the order: ascorbic acid > quercitrin > 6,8-diprenyleriodictyol (**97**) > bartericin A (**16**) > dorsmanin F (**98**) > isobavachalcone (**14**) > 6-prenylapigenin (**66**) [20]. A detailed overview on the natural occurrence, synthesis, biosynthesis and pharmacological properties of isobavachalcone (**14**) was published by Kuete and Sandjo in 2012 [57].

From the root bark of *Cudrania tricuspidata* (Carr.) Bureau collected in Hyoupchun, Korea it was possible to isolate two flavones—cycloartocarpesin B (56) and cudraflavone B (65)—and three flavanones—euchrestaflavanone B (94), euchrestaflavanone C (95) and a novel flavanone A (96). None of the isolated flavonoids were effective against DPPH•, presenting only 10% scavenging activity at 300 µM concentration [38].

Kumazawa et al. isolated nine flavanones (propolin A (103), propolin B (104), prokinawan (106), propolin E (105), nymphaeol A (107), nymphaeol B (108), nymphaeol C (109), isonymphaeol B (110) and 3'-geranylnaringenin (111)) from propolis collected in Okinawa, Japan. The antioxidant properties against DPPH• at concentrations ranging from 3.125 to 100 µM, after 1 h of incubation were examined. BHT, α-tocopherol, and eriodictyol were tested as positive controls. A strong DPPH• scavenging activity was recorded for compounds 103, 104 and 106–110 with IC<sub>50</sub> values between 5.2 and 10.9 µM, similar to those obtained for the positive controls BHT (IC<sub>50</sub> 16.8 ± 2.7 µM), α-tocopherol (IC<sub>50</sub> 11.4 ± 0.9 µM) and eriodictyol (IC<sub>50</sub> 4.7 ± 0.7 µM). Propolin E (105) and 3'-geranylnaringenin (111) showed IC<sub>50</sub> values of 62.6 ± 2.2 and 64.2 ± 3.5 µM, respectively. The high scavenging effects of these compounds may be related to the catechol unit present in such structures, an important characteristic for the antioxidant activity of flavonoids [47].

Five flavones—artnonin E (55), 2'-O-methylartnonin E (60), 2'-O-methylisoartnonin E (61), 2'-O-methyldihydroisoartnonin E (62) and 2'-O-methylartnonin V (63)—and two xanthone-type compounds—artobiloxanthone (150) and cycloartobiloxanthone (140)—were obtained from the root bark of *A. nobilis* collected in Central Province of Sri Lanka. The authors claimed to have evaluated the DPPH• scavenging potential by a TLC bio-autography method and that all the compounds were strong scavengers, but in fact no data was published in the manuscript [33].

Jung et al. isolated from *Sophora flavescens* collected in Kyeong Buk Province, Korea eight flavonoids: the chalcones kuraridin (18) and kuraridinol (19), the flavonol kushenol C (70) and the flavanones leachianone (89), kushenol E (90), sophoraflavanone G (91), kurarinone (92) and kurarinol (93). The DPPH• decolorization was investigated at 520 nm after 30 min of reaction. The results pointed out that flavanones 89–93 were ineffective scavengers, with percentages of inhibition less than 50 at a concentration of 200 µg/mL. On the other hand, flavonol 70 showed the highest scavenging activity with an IC<sub>50</sub> value of 10.67 ± 0.23 µM, similar to that of the positive control, L-ascorbic acid (IC<sub>50</sub> 8.70 ± 0.22 µM) followed by chalcone 19 and chalcone 18 with IC<sub>50</sub> values of 86.23 ± 2.44 and 111.77 ± 0.72 µM, respectively [22]. Once again, the high scavenging activity of flavonol compared to flavanones can be related to the presence of the C2=C3 double bond and the 3-OH group in the flavonol skeleton. In another study, sophoraflavanone G (91) and kurarinone (92) exhibited IC<sub>50</sub> values of 5.26 and 7.73 µg/mL, respectively, in DPPH• scavenging assay, with no mention to positive control data [23].

Six derivatives were isolated from *Artocarpus* species and their DPPH• scavenging activity investigated. The flavone artoflavone A (48) and the xanthone-type compound cyclogeracommunin (133) were isolated from the cortex of roots of *A. communis* collected at Kaohsiung Hsien, Taiwan [34]. The flavone artelastoheterol (57) and the xanthone-type compounds cycloartobiloxanthone (140), cycloartelastoxanthone (141) and artanol A (142) were isolated from root bark of *A. elasticus* collected at Ping-Tung Hsien, Taiwan [58]. Cyclogeracommunin (133) and artanol A (142) were not able to scavenge DPPH•. Artoflavone A (48), artelastoheterol (57), cycloartobiloxanthone (140) and cycloartelastoxanthone (141) exhibited scavenging activity in a concentration-dependent manner with IC<sub>50</sub> values of 24.2 ± 0.8, 42.2 ± 2.8, 26.8 ± 1.2 and 18.7 ± 2.2 µM, respectively. In addition, the IC<sub>50</sub> values of the positive controls BHT and α-tocopherol were 80.0 ± 10.9 and 18.1 ± 1.5 µM, respectively. From the results we can state that compounds with a 2,2-dimethylpyran ring substituted at C-7 and C-8 of the flavonoid, such as derivatives 48, 140 and 141, enhanced the DPPH• scavenging activity of the prenylated flavonoids [34].

Pyranocycloartobioxanthone A (**139**) was isolated from the stem bark of the endemic and rare *A. obtusus* collected from Sarawak, Malaysia and showed strong DPPH• scavenging activity with an IC<sub>50</sub> value of 2.0 µg/mL. No information was given for the positive control [55].

Rahman et al. isolated two prenylated isoflavones from the stem bark of *E. variegata* collected at Dhaka, Bangladesh and evaluated their DPPH• scavenging potential. 4',5,7-Trihydroxy-8-prenyl isoflavone (**124**) and alpinum isoflavone (**119**) demonstrated high antioxidant activity, having IC<sub>50</sub> values of  $6.42 \pm 1.36$  and  $8.30 \pm 1.41$  µg/mL, respectively, similar to that obtained by the positive control, *tert*-butyl-1-hydroxytoluene (BHT, IC<sub>50</sub> 5.88 µg/mL) [51]. Very recently, a pharmacological overview on alpinum isoflavone (**119**) has been published by Ateba et al. [59].

Three xanthone-type derivatives were isolated from the stem bark of *A. kemando*: artomandin (**147**), artoindonesianin C (**148**) and artonol B (**149**). Although **147** scavenged DPPH• with an IC<sub>50</sub> of 38.0 µg/mL, it was considerably lower effect than the positive control vitamin C (IC<sub>50</sub> 12.2 µg/mL). In addition, artoindonesianin C (**148**) and artonol B (**149**) were weak scavengers, with IC<sub>50</sub> values above 120 µg/mL [54].

5,7-Dihydroxy-6-methyl-8-prenylflavanone (**84**), 5,7-dihydroxy-4'-methoxy-6-methyl-8-prenylflavanone (**85**), 5,7-dihydroxy-6-prenylflavanone (**86**), 5-dihydroxy-7-methoxy-6-prenylflavanone (**87**), and 5,7-dihydroxy-4'-methoxy-8-prenylflavanone (**88**) were isolated from leaves of *E. platycarpa*. The scavenging effects were determined using concentrations of 10, 100 and 1000 µM of each compound and the results showed that the scavenging potential is directly proportional to the concentration of the prenylated flavanones. Moreover, flavanones did not show a remarkable reduction of DPPH• being flavanone **88** the most active, with a  $43.1 \pm 3.9\%$  of reduction for a 1000 µM concentration (the positive control quercetin presented 92.9% of reduction) [45].

Lan et al. isolated 14 derivatives from the heartwood and cortex of *A. altilis* and determined their DPPH scavenging effects. Seven flavones—artocarpin (**47**), artoflavone A (**48**), hydroxyartoflavone A (**49**), artogomezianone (**50**), 10-oxoartogomezianone (**51**), 8-geranyl-3-(hydroxyprenyl)isoetin (**52**), 8-geranylapiogenin (**53**)—and seven xanthone-type compounds—cyclocommunol (**131**), cyclo-artocarpin (**132**), cyclogeracommunin (**133**), isocycloartobioxanthone (**134**), cyclomorusin (**135**), cudraflavone A (**136**) and artonin M (**137**). Most of the tested compounds presented a weak scavenging effect with IC<sub>50</sub> > 300 µM, except for hydroxyartoflavone A (**49**, IC<sub>50</sub>  $20.9 \pm 2.1$  µM) > isocycloartobioxanthone (**134**, IC<sub>50</sub>  $33.9 \pm 1.5$  µM) > artoflavone A (**48** (15), IC<sub>50</sub>  $53.5 \pm 3.1$  µM). Even so, these compounds are weaker scavengers than the positive control, quercetin (IC<sub>50</sub>  $10.2 \pm 1.4$  µM) [35].

From the roots of *E. chinense* collected at Ubonratchathani Province, Thailand, was isolated one flavonol 3,5,2',4'-tetrahydroxy-6'',6''-dimethylpyrano(2'',3'':7,6)-8-(3''',3'''-dimethylallyl)flavone (**69**) and six flavanones—khonklonin A (**77**), 2''',3'''-epoxykhonklonin A (**78**), lupinifolinol (**79**), 3-*epi*-lupinifolinol (**80**), 2-hydroxylupinifolinol (**81**) and flemichin D (**82**)—and their antioxidant activity was investigated using the DPPH• scavenging system. Flavonol **69** was the most active compound showing an IC<sub>50</sub> value of  $35 \pm 1$  µM, similar to the obtained for BHT (IC<sub>50</sub>  $39 \pm 1$  µM). The order of efficacy for the remaining compounds were: **81** ( $252 \pm 1.1$ ) > **82** ( $538 \pm 24$ ) > **80** ( $681 \pm 11$ ) > **79** ( $1768 \pm 210$ ) > **78** ( $2553 \pm 207$ ) > **77** ( $7919 \pm 55$ ) µM [40].

Elastichalcone B (**45**) and cycloartocarpesin B (**56**) 3 isolated from the leaves of *A. elasticus* collected from Ulu Langat, Malaysia exhibited DPPH• scavenging activity with IC<sub>50</sub> values of 11.30 and 11.89 µg/mL, respectively. No data concerning any positive control was given by the authors [30].

Three isoflavones were isolated from the aerial parts of *Azorella madreporica* collected in Chile: alpinum isoflavone (**119**), angustone C (**130**) and 4'-acetylalpinum isoflavone (**129**). These compounds demonstrated modest DPPH• scavenging activity in the following order: **130** (IC<sub>50</sub>  $134.61 \pm 0.67$  µM) > **119** (IC<sub>50</sub>  $160.75 \pm 0.41$  µM) > **129** (IC<sub>50</sub>  $309.87 \pm 0.90$  µM). Quercetin was used as positive control and reached an IC<sub>50</sub> value of  $24.93 \pm 0.03$  µM [53].

The chalcone xanthohumol (**1**) extracted from hop pellets (*Humulus lupulus*) did not show any scavenging effect on DPPH• scavenging system [17]. One chalcone isobavachalcone (**14**), two flavones—4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(γ,γ-dimethyl)allylflavone (**54**)



and artocarpin (**47**)—one flavanone: 5,7-dihydroxy-4'-methoxy-8-prenylflavanone (**88**), three xanthenes—3'-hydroxycycloartocarpin (**138**), pyranocycloartobiloxanthone A (**139**) and cycloartocarpin (**132**)—and the miscellaneous derivative chaplashin (**153**) were isolated for the first time from the leaves and the heartwoods of *A. anisophyllum* Miq collected in Malaysia. Pyranocycloartobiloxanthone A (**139**), artocarpin (**47**) and 3'-hydroxycycloartocarpin (**138**) were the most active DPPH• scavengers with SC<sub>50</sub> (concentration required to produce 50% stimulation) values of 20.2, 140.0 and 152.9 µg/mL, respectively. Isobavachalcone (**14**), flavone **54** and chaplashin (**153**) were poor scavengers with SC<sub>50</sub> values superior to 400 µg/mL and no scavenging activity was recorded for flavanone **88** and cycloartocarpin (**132**). BHA was used as positive control, with a SC<sub>50</sub> value of 17.5 µg/mL [18].

Two prenylated pterocarpan—phaseollin (**154**) and shinpterocarpin (**155**)—the flavanone 4'-O-methyllicoflavanone (**83**) and two isoflavones, alpinum isoflavone (**119**) and 8-prenyldaidzein (**120**) have been isolated from the stem bark of *E. orientalis* collected in East Java, Indonesia. The results pointed out that antioxidant activity against DPPH• scavenging by phaseollin (**154**, IC<sub>50</sub> 241.9 µM) and 8-prenyldaidzein (**120**, IC<sub>50</sub> 174.2 µM) were more effective than the positive control, ascorbic acid (IC<sub>50</sub> 329.0 µM). The remaining compounds **83**, **119** and **155** showed moderate activity with IC<sub>50</sub> values of 648.1, 708.5 and 909.8 µM, respectively [46].

Akter et al. isolated from stem bark of *E. stricta* Roxb., collected in India, four isoflavones—alpinum isoflavone (**119**), erynone (**121**), wightone (**122**) and luteone (**123**)—and assessed their antioxidant activity using dot-dot and DPPH• staining methods. Erynone (**121**) was the most active compound while luteone (**123**) possessed moderate antioxidant activity. Derivatives **122** and **123** were completely inactive against DPPH• scavenging activity. The authors suggested that the high effect of erynone (**121**) is due to its highest number (six) of free phenolic hydroxyl groups and therefore high ability to donate phenolic hydrogens to DPPH•, when compared to the related structures **119**, **122** and **123** [50].

Stompór et al. synthesized isoxanthohumol (**102**) and two acetylated derivatives **117** and **118** and evaluated their DPPH• scavenging properties. Acylation of **102** decreased the antioxidant activity of its analogues in the order: isoxanthohumol (**102**) > 4'-O-acetylisoxanthohumol (**117**) > 7,4'-di-O-acetylisoxanthohumol (**118**). Thus, **102** (EC<sub>50</sub> 7.6 mM) is about 8-fold stronger antioxidant than its monoacyl derivative **117** (EC<sub>50</sub> 59.7 mM) and 10-fold stronger than the diacyl derivative **118** (EC<sub>50</sub> 73.5 mM) due to the presence of two free hydroxyl groups in the molecule [49].

A series of eight chalcones were obtained by synthesis: xanthohumol (**1**), 4-O-acetyl-xanthohumol (**25**), 4,4'-di-O-acetyl-xanthohumol (**26**), 4-O-decanoylxanthohumol (**27**), 4-O-dodecanoylxanthohumol (**28**), 4,4'-di-O-dodecanoylxanthohumol (**29**), 4-O-pivaloylxanthohumol (**30**) and 4,4'-di-O-pivaloylxanthohumol (**31**). All the compounds were tested for their ability to scavenge DPPH• and the results expressed as Trolox equivalents, defined as the concentration of Trolox (µM) having the same activity as 1 g of the tested compound. Thus, the most active compound was **25** (15 µM TEAC/g), a two-fold stronger scavenger than **31** (37 µM TEAC/g) and **1** (38 µM TEAC/g). The values for the remaining compounds **25–30** ranged from 44 to 70 µM TEAC/g [27].

From the leaves and stem barks of *A. scortechinii* King collected at Pahang, Malaysia one chalcone flemichapparin A (**15**), four flavones—artocarpin (**47**), 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(γ,γ-dimethyl)allylflavone (**54**), artonin E (**55**) and macakurzin C (**64**)—and two xanthenes—cudraflavone A (**136**) and cycloartobiloxanthone (**140**)—were isolated and their DPPH• scavenging activity evaluated. It was not possible to determine the IC<sub>50</sub> value of compounds **47**, **54**, **64** and **136** while derivatives **15**, **55** and **140** reached IC<sub>50</sub> values of 131.0, 151.6 and 196.0 µM, respectively. Two positive controls were used in this assay being BHA a stronger scavenger (IC<sub>50</sub> 49.87 µM), better than the analyzed compounds, and BHT considerably weaker, with an IC<sub>50</sub> value of 231.6 µM [19].

Zakaria et al. isolated from heartwood of *A. integer* (Thunb.) Merr., collected in Indonesia, the flavones cudraflavone C (**59**) and artocarpin (**47**) and the xanthone tephrosin (**146**) and subjected them to the DPPH• decoloration method at λ<sub>max</sub> 500 nm. The flavones **59** and **47** were the most active, with IC<sub>50</sub> values of 3.35 µg/mL and 4.70 µg/mL, respectively, while the xanthone **146** was 10-fold weaker with an IC<sub>50</sub> value of 55.58 µg/mL. Ascorbic acid (IC<sub>50</sub> 2.79 µg/mL) was used as positive control [36].

The rhizomes of *Ficus tikoua* collected in Yunnan Province, China, furnished five isoflavones—alpinum isoflavone (**119**), 4'-O-methylalpinum isoflavone (**125**), ficusin A (**126**), ficusin C (**127**) and 6-[(1R\*,6R\*)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5,7,4'-trihydroxyisoflavone (**128**)—and their ability to scavenge DPPH• was investigated. All compounds exhibited moderate antioxidant activity with the EC<sub>50</sub> values of 54.8 ± 9.7, 83.6 ± 12.5, 42.4 ± 6.6, 49.3 ± 7.8 and 43.3 ± 6.9 µM, respectively. Propyl gallate used as positive control presented an EC<sub>50</sub> value of 1.8 ± 0.5 µM [52].

From the leaves of *Macaranga pruinosa* collected in Samarinda, Indonesia the flavone glyasperin A (**71**) was isolated and its antioxidant potential tested using a DPPH• scavenging system. The IC<sub>50</sub> value of **71** (443.0 ± 8.0 µg/mL) was almost twice higher than that of the positive control, kaempferol (IC<sub>50</sub> 238.0 ± 3.3 µg/mL) [41].

From *Macaranga gigantea* (Euphorbiaceae) leaves collected in Indonesia the flavones glyasperin A (**71**) and broussoflavonol F (**72**) were isolated. The antioxidant activity was evaluated by their ability to scavenge DPPH•, showing IC<sub>50</sub> values of 125.10 and 708.54 µM, respectively. In addition, the results pointed out that **71** was twice as active as the positive control ascorbic acid (329.01 µM) [42].

#### ABTS Radical Cation Scavenging Activity (TEAC Method)

Rajendran et al. verified the suppression of the absorbance of ABTS•<sup>+</sup> in a concentration-dependent manner for artocarpin (**47**) and cycloartocarpin (**132**). The results demonstrate that the reaction with these compounds show small inhibitory effects even up to 4 min of reaction, when compared with the reaction of the positive control quercetin which is completed within 1 min. Artocarpin (**47**) has two hydroxyl groups in the B ring at 4' and 6' and registered a TEAC value of 910 µM while cycloartocarpin (**132**) has a fused partially saturated six member heterocyclic ring between rings C and B and had a TEAC value of 690 µM. Meanwhile, the control quercetin possesses a catechol structure in the B ring, a C2=C3 double bond in conjunction a 3-OH and 4-carbonyl groups, allowing resonance stabilization for electron delocalization and therefore, an higher TEAC value (1230 µM) when compared with **47** and **132**. These results demonstrate the importance of electron delocalization across the molecule for stabilization of the arylxyl radical [32].

The two prenylated flavones cycloartocarpesin B (**56**) and cudraflavone B (**65**) and three flavanones—euchrestaflavanone B (**94**), euchrestaflavanone C (**95**) and a novel flavanone A (**96**)—had similar ABTS•<sup>+</sup> scavenging activity (IC<sub>50</sub> 4.2–8.3 µM) to that of quercetin (IC<sub>50</sub> 4.0 µM). In addition, the most active compound **65** had the same TEAC value (expresses the numbers of µmols of Trolox having an antioxidant capacity corresponding to 1.0 µmol of the test substance) than quercetin (TEAC value of 5.0). These results point out the importance of the prenyl group for the antioxidant effect against ABTS system [38].

Wu et al. synthesized three baicalein derivatives **73–75** possessing different prenylated chains at C-7 and evaluated their ABTS•<sup>+</sup> scavenging activity. The order of potency was: parent baicalein (IC<sub>50</sub> 5.5 ± 0.40 µM) > 7-prenylbaicalein (**73**, IC<sub>50</sub> 8.8 ± 0.11 µM) ≈ 7-geranylbaicalein (**74**, IC<sub>50</sub> 8.7 ± 0.28 µM) > 7-farnesylbaicalein (**75**, IC<sub>50</sub> 10.6 ± 0.50 µM). Looking at the results, substitution of 7-hydroxyl group of baicalein by terpenoid groups led to a decrease in the scavenging activity and that the largest farnesyl group lead to the weakest scavenger. Even so, the scavenging activities of baicalein and its derivatives **73–75** were higher than that of Trolox (IC<sub>50</sub> 12.6 ± 0.21 µM), the water-soluble vitamin E analogue with one hydroxyl group [43].

Among eight flavonoids isolated from *S. flavescens*, the chalcones kuraridin (**18**) and kuraridinol (**19**) were the most potent ABTS•<sup>+</sup> scavengers with IC<sub>50</sub> values of 10.45 ± 0.07 and 11.90 ± 2.77 µM, respectively. The remaining compounds (the flavonol kushenol C (**70**) and the flavanones leachianone (**89**), kushenol E (**90**), sophoraflavanone G (**91**), kurarinone (**92**) and kurarinol (**93**)) revealed IC<sub>50</sub> values in the range of 14.08 to 28.84 µM, while the positive controls Trolox and L-ascorbic acid exhibited IC<sub>50</sub> values of 24.57 ± 0.11 and 28.86 ± 0.02 µM, respectively. In addition, **70**, **18** and **19** possessed TEAC values of 1.88, 2.45, and 2.44, respectively, whereas those of the flavanones (**89**, **90**, **91**, **92** and **93**) were 1.28, 1.76, 1.05, 1.56, and 1.39, respectively [22].

Lan et al. isolated 14 flavonoids from *A. altilis*, but only five were evaluated in an ABTS<sup>•+</sup> scavenging assay. Isocycloartobioxanthone (**134**) showed a concentration-dependent scavenging behavior and was the most efficient scavenger, with an IC<sub>50</sub> value of  $7.2 \pm 1.6 \mu\text{M}$ , similar to the positive control quercetin (IC<sub>50</sub>  $7.8 \pm 2.1 \mu\text{M}$ ). The order to potency for the other derivatives was: artogomezianone (**50**, IC<sub>50</sub>  $36.9 \pm 2.3 \mu\text{M}$ ) > 8-geranyl-3-(hydroxyprenyl)isoetin (**52**, IC<sub>50</sub>  $156.9 \pm 5.3 \mu\text{M}$ ) > artocarpin (**47**, IC<sub>50</sub>  $265.1 \pm 4.3 \mu\text{M}$ ). No activity was found for cyclocommunol (**131**, IC<sub>50</sub> >  $500 \mu\text{M}$ ) [35].

Xanthohumol (**1**) had a maximum inhibition of ABTS<sup>•+</sup> of 47% at  $60 \mu\text{M}$  concentration and a TEAC value of  $0.32 \pm 0.09 \mu\text{M}$  [17]. In another study involving xanthohumol (**1**) and seven synthetic ester derivatives, 4-*O*-acetyl xanthohumol (**25**,  $160 \mu\text{M}$  TEAC/g) and 4-*O*-decanoyl xanthohumol (**27**,  $170 \mu\text{M}$  TEAC/g) displayed higher antioxidant effect than xanthohumol (**1**,  $190 \mu\text{M}$  TEAC/g). The other tested compounds showed comparable or weaker ABTS scavenger properties than **1** (> $200 \mu\text{M}$  TEAC/g) [27]. The ABTS radical scavenging profile of the chalcone desmethylxanthohumol (**3**) was expressed as the half-maximal effective concentration (EC<sub>50</sub>), presenting an EC<sub>50</sub> of  $0.54 \pm 0.09 \mu\text{M}$ , slightly less than that of gallic acid ( $0.35 \pm 0.01 \mu\text{M}$ ) [29].

Isoxanthohumol (**21**) and 2',4',6'-trihydroxy-3'-prenylchalcone (**22**) displayed the highest TEAC values ( $4529.01 \pm 2.44$ ;  $4170.66 \pm 6.72 \mu\text{M}$  Trolox equivalents/g, respectively, among the flavonoids isolated from *H. teretifolium*. Isoglabranin (**99**), glabranin (**100**) and 7-methoxyisoglabranin (**101**) presented modest ABTS<sup>•+</sup> scavenging effects and heliteretifolin (**20**) was completely inactive [24].

From the seven flavonoids isolated from *A. scortechinii* King it was only possible to determine the ABTS<sup>•+</sup> scavenging potential of flemichapparin A (**15**, IC<sub>50</sub>  $199.7 \mu\text{M}$ ), artonin E (**55**, IC<sub>50</sub>  $145.0 \mu\text{M}$ ) and cycloartobioxanthone (**140**, IC<sub>50</sub>  $269.0 \mu\text{M}$ ). As in the DPPH<sup>•</sup> assay, the positive control BHA used in this assay was a stronger scavenger than the analyzed compounds (IC<sub>50</sub>  $91.01 \mu\text{M}$ ) [19].

Glyasperin A (**71** (IC<sub>50</sub>  $210.0 \pm 2.7 \mu\text{M}$ ) isolated from *M. pruinosa* leaf showed almost half the antioxidant potential than the positive control kaempferol (IC<sub>50</sub>  $111.0 \pm 1.6 \mu\text{M}$ ) in the ABTS<sup>•+</sup> scavenging assay [41].

#### Ferric Reducing Antioxidant Power (FRAP) Method

A weak FRAP value was observed for the isoflavones alpinum isoflavone (**119**,  $35.55 \pm 1.23 \mu\text{M}$  Trolox equivalents), angustone C (**130**,  $24.38 \pm 1.15 \mu\text{M}$  Trolox equivalents) and 4'-acetylalpinum isoflavone (**129**,  $2.32 \pm 0.01 \mu\text{M}$  Trolox equivalents) isolated from *A. madreporica* when compared with the antioxidant quercetin ( $236.43 \pm 6.2 \mu\text{M}$  Trolox equivalents) in the FRAP assay [53].

Xanthohumol (**1**) was a stronger scavenger in the ABTS<sup>•+</sup> scavenging system than in the FRAP system, with  $0.27 \pm 0.04 \mu\text{M}$  Trolox equivalents in the FRAP assay system, and was completely inactive in the scavenging of DPPH<sup>•</sup> [17].

Similarly to the ABTS assay, isoxanthohumol (**21**) and 2',4',6'-trihydroxy-3'-prenylchalcone (**22**) displayed the highest values ( $619.91 \pm 1.97$ ;  $817.94 \pm 4.26 \mu\text{M}$  ascorbic acid equivalents per mg dry weight, respectively), in the FRAP assay. Isoglabranin (**99**), glabranin (**100**) and 7-methoxyisoglabranin (**101**) presented weak FRAP effects and heliteretifolin (**20**) was completely inactive [24].

The FRAP value of chalcone dimer **44** was calculated from the calibration curve derived from dilutions of a vitamin C standard, measuring the decrease in absorption of the complex at 660 nm. Compound **44** exhibited better activity in the FRAP assay than the positive control, gallic acid ( $648.44$  and  $531.02 \text{ mg/mM}$ , equivalent amounts of vitamin C) [29].

It was only possible to determine the FRAP potential of three of the seven flavonoids isolated from *A. scortechinii* King. Artocarpin (**47**), artonin E (**55**) and cycloartobioxanthone (**140**) exhibited FRAP values of  $0.19 \pm 0.19$ ,  $1.32 \pm 1.17$  and  $2.79 \pm 0.19$  Trolox equivalents, respectively. A closer look into the FRAP values of artocarpin (**47**) and artonin E (**55**) we may question the reliability of such results since the error associated is too high. The two positive controls used in this assay, BHA and HBT, provided values of  $0.60 \pm 0.06$  and  $1.89 \pm 0.02$  Trolox equivalents, respectively [19].



#### 4.1.2. Hydrogen Atom Transfer Mechanisms

##### Superoxide Radical Anion Scavenging Activity

Xanthohumol (**1**) was able to scavenge several reactive oxygen species, including the inhibition of superoxide anion radical ( $O_2^{\bullet-}$ ) production, generated by hypoxanthine/xanthine oxidase system and quantified by reduction of NBT, in a concentration-dependent manner, presenting a  $SC_{50}$  of  $27.7 \pm 4.9 \mu M$ . Furthermore, detection of uric acid proved that the result confirm the mechanism of action [14]. With the same detection technique, Lan et al. evaluated  $O_2^{\bullet-}$  scavenging potential of five flavonoids isolated from *A. altalis*. Artogomezianone (**50**) and artocarpin (**47**) exhibited moderate scavenging activities with  $IC_{50}$  values of  $39.7 \pm 3.3$  and  $94.1 \pm 1.8 \mu M$ , respectively, when compared with the positive control quercetin ( $IC_{50}$   $3.9 \pm 0.4 \mu M$ ). 8-Geranyl-3-(hydroxyprenyl)isoetin (**52**), isocycloartobioxanthone (**134**) and cyclocommunol (**131**) displayed weak inhibitory activities, with  $IC_{50}$  values higher than  $300 \mu M$  [35].

In order to evaluate  $O_2^{\bullet-}$  scavenging activity, Ko et al. generated it using a xanthine/xanthine oxidase system and monitored the reduction of cytochrome c at 550 nm. The results indicated that none of the compounds isolated from *A. heterophyllum* Lam (cycloheterophyllin (**143**), artonin A (**144**) and artonin B (**145**)) inhibit xanthine oxidase activity or scavenge  $O_2^{\bullet-}$  [31]. A weak scavenging activity against  $O_2^{\bullet-}$  was registered by Fukai et al. for licochalcone A (**17**), morusin (**68**), licoricidin (**151**) and licorisoflavan A (**152**) [21]. A similar behavior was reported by Rajendran et al. for artocarpin (**47**) and cycloartocarpin (**132**), when compared to the positive controls, ascorbic acid and Trolox [32].

Four spectrophotometric detectors of  $O_2^{\bullet-}$  were used to study the reaction of xanthohumol (**1**) and 3-hydroxyxanthohumol (**32**) in the xanthine oxidase model: NBT at 560 nm, XTT at 510 nm, hydroxylamine at 540 nm, cytochrome c at 550 nm. Superoxide was also detected by formation of 2-hydroxyethidium formed from hydroethidine by xanthine/xanthine oxidase system and quantified after separation by HPLC. The results indicated that xanthohumol lacked superoxide scavenging activity in contrast to the 3'-hydroxy derivative, when sufficient concentration of NBT and other detectors such as XTT, hydroxylamine, cytochrome c and hydroethidine were used. In addition, xanthohumol (**1**) can moderately generate superoxide via auto-oxidation reaction [26].

Finally, Lin et al. evaluated the direct inhibition of xanthine oxidase enzymatic activity, by reacting the enzyme with the tested compounds and using xanthine as substrate. The reaction was monitored for 5 min at 295 nm. Xanthone-derivative artonol A (**142**,  $IC_{50}$   $43.3 \pm 8.1 \mu M$ ) was twice as active as an inhibitor of xanthine oxidase activity than the flavone cyclogeracommunin (**133**,  $IC_{50}$   $73.3 \pm 19.1 \mu M$ ), although considerably less active than the positive control allopurinol ( $IC_{50}$   $2.0 \pm 0.7 \mu M$ ) [34].

##### Oxygen Radical Absorbance Capacity (ORAC) Method

Ko et al. evaluated the ability of cycloheterophyllin (**143**), artonin A (**144**) and artonin B (**145**) to scavenge hydrophilic and lipophilic peroxy ( $RO_2^{\bullet}$ ) radicals. Thus, **144** and **145** scavenged  $RO_2^{\bullet}$  generated by AAPH in aqueous media more efficiently than the positive control ascorbic acid. Due to the quenching fluorescent intensity of  $\beta$ -phycoerythrin by **143**, its effect in aqueous media was not evaluated. Additionally, none of the tested flavonoids scavenge  $RO_2^{\bullet}$  derived from 2,2'-azobis(2,4-dimethylvaleronitrile) in hexane [31].

Xanthohumol (**1**) and isoxanthohumol (**102**) were tested as scavengers of both hydroxyl ( $HO^{\bullet}$ ) and peroxy ( $RO_2^{\bullet}$ ) radicals. Thus,  $\beta$ -phycoerythrin was used as a redox-sensitive fluorescent indicator protein, 2,2'-azobis-(2-amidinopropane) dihydrochloride as  $RO_2^{\bullet}$  generator and  $H_2O_2$ - $CuSO_4$  system as  $HO^{\bullet}$  generator. The results were expressed as ORAC units where 1 ORAC unit equals the net protection of  $\beta$ -phycoerythrin produced by  $1 \mu M$  Trolox. Xanthohumol (**1**) was almost 9-fold and 3-fold more active than Trolox, in scavenging  $HO^{\bullet}$  and  $RO_2^{\bullet}$ , respectively, at  $1 \mu M$  concentration. Isoxanthohumol (**102**) presented a similar effect in scavenging  $HO^{\bullet}$  as Trolox and was even more active than xanthohumol (**1**) in  $RO_2^{\bullet}$  scavenging, especially at  $5 \mu M$  concentration [14]. Using the same experimental procedure, heliteretifolin (**20**), isoxanthohumol (**21**), 2',4',6'-trihydroxy-3'-prenylchalcone

(22), isoglabranin (99), glabranin (100) and 7-methoxyisoglabranin (101) isolated from *H. terefolium* exhibited a weak scavenging activity in ORAC assay for both  $\text{HO}^\bullet$  and  $\text{RO}_2^\bullet$  [24].

Fluorescein can be used as generator of  $\text{RO}_2^\bullet$  by the application of 2,2'-azobis(2-methyl-propionamide) dihydrochloride as a free radical initiator. Vogel et al. tested eleven chalcones (xanthohumol (1), xanthogalenol (2), desmethylxanthohumol (3), 4'-O-methylxanthohumol (24), 3-hydroxyxanthohumol (32), 2,2',4'-trihydroxy-6'-methoxy-3'-prenylchalcone (34), 2',3,4'-trihydroxy-6'-methoxy-3'-prenylchalcone (35), 2',3,4',5-tetrahydroxy-6'-methoxy-3'-prenylchalcone (36), 2',3,4,4',5-pentahydroxy-6'-methoxy-3'-prenylchalcone (37), 2',4'-dihydroxy-3,4,6'-trimethoxy-3'-prenylchalcone (38), and 4,6'-dihydroxy-2',4'-dimethoxy-3'-prenylchalcone (39)) that revealed potent antioxidant activity from 0.9 to 3.8 Trolox equivalents in a concentration range between 0.1 and 2.0  $\mu\text{M}$ . The most active compounds were 3 and 24 with  $3.8 \pm 0.5$  and  $3.8 \pm 0.4$  Trolox equivalents, respectively [25]. This research group also tested other chalcones (xanthohumol (1), 5'-prenyl-xanthohumol (5), xanthohumol H (8), xanthohumol C (9), 1'',2''-dihydroxanthohumol C (10), 3'-geranylchalconaringenin (11), 3'-geranyl-6'-O-methylchalconaringenin (12) and 2'-O-methyl-3'-prenylchalconaringenin (13)) and the ORAC-fluorescein assay revealed a high activity from 1.7 to 5.2 Trolox equivalents in a concentration range between 0.1 and 1.0  $\mu\text{M}$ . Chalcones 8 and 13 were the most potent scavengers, with 4.8 and 5.2 Trolox equivalents, respectively [16]. Next, additional seven chalcones (4-O-methylxanthohumol (24), 4-O-acetyl-xanthohumol (25), 3-methoxyxanthohumol (33), 3-hydroxyxanthohumol C (40), 3-methoxyxanthohumol C (41), 3-hydroxyxanthohumol H (42) and 3-methoxyxanthohumol H (43)) were evaluated in a concentration range of 0.25 to 1.5  $\mu\text{M}$  and presented ORAC values between 0.6 and 3.9 Trolox equivalents. The most active compounds were 42 and 43 showing  $3.9 \pm 0.5$  and  $3.0 \pm 0.2$  Trolox equivalents, respectively, while 24 and 25 were the less active ones ( $< 2$  Trolox equivalents). The remaining chalcones showed moderate scavenging activity with 2.0 and 2.6 Trolox equivalents [28].

Like in the other radical scavenging assays ( $\text{DPPH}^\bullet$  and  $\text{ABTS}^{\bullet+}$ ), glyasperin A (71,  $2.5 \pm 0.6$   $\mu\text{mol}$  Trolox equivalents/mg) isolated from *M. pruinosa* showed a weaker  $\text{RO}_2^\bullet$  scavenging potential than the positive control kaempferol ( $34.9 \pm 0.6$   $\mu\text{mol}$  Trolox equivalents/mg) [41].

### Hydroxyl Radical Detected by ESR

The ability of the prenylated flavonoids to scavenge  $\text{HO}^\bullet$  in a hydrophilic environment can also be measured by ESR spectroscopy, where  $\text{HO}^\bullet$  radicals generated by a Fenton-type reaction are trapped as DMPO spin adducts, giving rise to the corresponding ESR signals. The intensity of the DMPO-OH spin adduct signal is reduced in the presence of radical scavengers and the results are given as the difference of the respective ESR signal intensities of the sample with and without the flavonoid. Lee et al. isolated five flavonoids from *C. tricuspidata* (cycloartocarpesin B (56), cudraflavone B (65), euchrestaflavanone B (94), euchrestaflavanone C (95) and novel flavanone A (96)) and evaluated their ability to scavenge  $\text{HO}^\bullet$ . Compounds 94, 56 and 65 were more active ( $\text{IC}_{50}$  values of  $34.6 \pm 2.7$   $\mu\text{M}$ ,  $46.9 \pm 6.5$   $\mu\text{M}$  and  $40.6 \pm 2.1$   $\mu\text{M}$ , respectively) than Trolox, with an  $\text{IC}_{50}$  value of  $48.2 \pm 2.3$   $\mu\text{M}$ . Euchrestaflavanone C (95) and novel flavanone A (96) were moderate scavengers with  $\text{IC}_{50}$  values  $52.5 \pm 3.6$   $\mu\text{M}$  and  $75.3 \pm 4.8$   $\mu\text{M}$ , respectively [38].

### Other ROS/RNS Scavenging Activity

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) scavenging capacity can also be related to the antioxidant activity of a matrix. This ROS is generated in vivo, under physiological conditions, by peroxisomes, by several oxidative enzymes and by dismutation of superoxide radical, catalyzed by superoxide dismutase. Ko et al. evaluated the content of hydrogen peroxide indirectly using a catalase-based method. The reaction mixture containing  $\text{H}_2\text{O}_2$  and test compound in phosphate buffer pH 7.4 was incubated at 25 °C for 40 min. Then, catalase was added and  $\text{O}_2$  release was monitored polarographically for 0.8 min. The amount of  $\text{H}_2\text{O}_2$  remaining was calculated using a standard curve made of  $\text{O}_2$  production vs.  $\text{H}_2\text{O}_2$  concentration (0.1–2.0  $\mu\text{M}$ ). At 100  $\mu\text{M}$  concentration, cycloheterophyllin (143), artonin A

(144) and artonin B (145) were not able to react directly with  $\text{H}_2\text{O}_2$  since no significant loss of  $\text{H}_2\text{O}_2$  was observed [31].

The  $\text{ONOO}^-$  scavenging activity reported by Jung et al. was measured by monitoring the  $\text{ONOO}^-$  induced oxidation of non-fluorescent DHR to fluorescent rhodamine 123. The assay was performed at 37 °C, reacting the tested compounds dissolved in 10% DMSO,  $\text{ONOO}^-$  and DHR 123 and the fluorimetric signal detected after a 5 min incubation period. The fluorescence intensity of the oxidized DHR 123 was measured at the excitation and emission wavelengths of 485 nm and 530 nm, respectively, and the results were expressed the percent inhibition of oxidation of DHR 123. L-Penicillamine was used as the positive control. From the eight flavonoids analyzed (the chalcones kuraridin (18) and kuraridinol (19), the flavonol kushenol C (70) and the flavanones leachianone (89), kushenol E (90), sophoraflavanone G (91), kurarinone (92) and kurarinol (93)), the flavonol 70 ( $\text{IC}_{50}$   $0.62 \pm 0.01$   $\mu\text{M}$ ) was the most active scavenger, even better than the positive control, L-penicillamine ( $\text{IC}_{50}$   $2.37 \pm 0.09$   $\mu\text{M}$ ). The chalcones 18 and 19 exhibited  $\text{IC}_{50}$  values of 2.89 and 2.19  $\mu\text{M}$ , respectively, while flavanones 89–93 were less active ones, with  $\text{IC}_{50}$  values of 3.17, 3.35, 7.30, 3.42, and 3.64  $\mu\text{M}$ , respectively [22]. As mentioned before for the  $\text{DPPH}^\bullet$  scavenging activity, the structural features of flavonols may enhance also the  $\text{ONOO}^-$  scavenging potential, when compared to flavanone or chalcone derivatives.

#### 4.1.3. Metal Chelation

Ko et al. reported that cycloheterophyllin (143), artonin A (144) and artonin B (145) had peaks at 310 and 418, 302 and 380, and 295 and 400 nm, respectively. No changes in the absorbance or spectral shift were observed after the addition of  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$  or  $\text{Cu}^{2+}$  solutions to the reaction mixtures containing 143, 144 and 145 [31]. A series of other flavonoids were tested by Miranda et al.: xanthohumol (1), xanthogalenol (2), desmethylxanthohumol (3), isoxanthohumol (102) and 8-prenylnaringenin (113). Spectra (200–600 nm) were recorded after preparation of the mixtures and 10 min later, in the absence and in presence of copper ions. Small variations were observed in the spectra of 1, 102 and 113. The chalcones 2 and 3 developed new maxima around 290 nm over a 10-min period, which was attributed to conversion of the chalcones to their isomeric flavanones rather than chelation of copper ions. The importance of 3',4'-dihydroxy substituents (catechol moieties) on the B-ring for copper or iron chelate formation is known and none of the flavonoids in this study had such a profile [12]. In continuation of their studies, 1, 2 and 3 were also tested for their chelation of iron ions, recording the absorbance between 190 and 600 nm, and no changes were induced by this metal in the UV-vis spectra [13]. Unlike Miranda's work, Dufall et al. tested three prenylated flavonoids bearing a catechol unit in the B-ring: 6,8-diprenyleriodictyol (97), dorsmanin C (67) and dorsmanin F (98). Methanolic solutions of the compounds were mixed with copper solution and their interaction was measured between 200 and 800 nm after 10 s and compared with the flavonoid alone. Interestingly, only 67 showed any interaction with  $\text{Cu}^{2+}$  ions indicated by significant bathochromic shift in major absorbance bands upon addition of equimolar concentrations of  $\text{Cu}^{2+}$  ions. These results led us to infer the importance of the catechol unit for the chelating properties but also of the presence of  $\text{C2}=\text{C3}$  double bond in combination with a 3-OH, the structural characteristics of flavonol 67 absent in flavanones 97 and 98 [37]. Meanwhile, 3,5,7,2'-tetrahydroxy-6-methoxy-8-prenylflavanone, known as floranol (76) exhibits two absorption bands at 297 and 340 nm related to the  $\pi-\pi^*$  transitions of chromophores A and B, respectively, which were significantly changed by both coordination to  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  ions. The authors pointed out that these metals probably bind to a site on A-ring through a bidentate coordination involving the 5-OH and 4-carbonyl groups [44].

#### 4.2. In Vivo Methods

##### 4.2.1. Lipid Peroxidation

Ko et al. studied lipid peroxidation promoted by iron ions in rat brain homogenate and measured by the decrease of absorbance at 532 nm. Tetramethoxypropane was used as a standard, and the results

were expressed as nanomoles of MDA equivalents per milligram of protein of rat brain homogenates. Cycloheterophyllin (**143**), artonin A (**144**) and artonin B (**145**) inhibited Fe(II)-induced lipid peroxidation in a concentration-dependent manner with IC<sub>50</sub> values calculated to be  $0.96 \pm 0.21$ ,  $0.47 \pm 0.24$  and  $0.71 \pm 0.13$   $\mu\text{M}$ , respectively. BHT also inhibited this Fe(II)-induced lipid peroxidation with an IC<sub>50</sub>  $1.33 \pm 0.05$   $\mu\text{M}$ . In contrast, no effect on Fe(II)-induced lipid peroxidation in rat brain homogenate was observed for artocarpin (**47**) and artocarpetin A (**58**) at 100  $\mu\text{M}$  concentration [31].

Low inhibitory activity on Fe(II)-induced microsomal lipid peroxidation was observed for the flavonoids isolated from *H. teretifolium* heliteretifolin (**20**), isoxanthohumol (**21**), 2',4',6'-trihydroxy-3'-prenylchalcone (**22**), isoglabranin (**99**), glabranin (**100**) and 7-methoxyisoglabranin (**101**), with IC<sub>50</sub> values superior to 21  $\mu\text{g/mL}$ , when compared with the positive control, epigallocatechin gallate (IC<sub>50</sub> 0.929  $\mu\text{g/mL}$ ) [24].

Rodriguez et al. evaluated the inhibition of metal-ion iron-dependent and independent (*tert*-butyl hydroperoxide, THB) lipid peroxidation in rat liver microsomes by a series of nine flavonoids (xanthohumol (**1**), xanthogalenol (**2**), desmethylxanthohumol (**3**), 4'-O-methylxanthohumol (**4**), 5'-prenylxanthohumol (**5**), dehydrocycloxanthohumol (**6**), dehydrocycloxanthohumol hydrate (**7**), 3'-geranylchalconaringenin (**11**) and isoxanthohumol (**102**)). For the iron-dependent assays, two different systems were applied to induce lipid peroxidation, FeSO<sub>4</sub>/ascorbic acid and FeCl<sub>3</sub>/ADP/NADPH. The results on the lipid peroxidation induced by iron/ascorbate pointed out that all compounds respond in a concentration-dependent manner. Prenylated derivatives were good inhibitors at 5 and 25  $\mu\text{M}$  concentration and generally more effective than the non-prenylated derivatives. Xanthohumol (**1**), desmethylxanthohumol (**3**) and 5'-prenylxanthohumol (**5**) exhibited IC<sub>50</sub> values of 6.0, 6.0 and 5.0  $\mu\text{M}$ , respectively. In the Fe<sup>3+</sup>-ADP/NADPH-induced lipid peroxidation assay at 5  $\mu\text{M}$  concentration, only **1**, **3**, **5** and **24** were efficient inhibitors; meanwhile at 25  $\mu\text{M}$  concentration all nine prenylated flavonoids were effective in preventing lipid peroxidation, being **1**, **3** and **5** the most active ones. All prenylated flavonoids were able to prevent lipid peroxidation induced by THP at 5 and 25  $\mu\text{M}$  concentration. For the highest concentration, the best inhibitors were **3**, **5** and **11** [13].

Studies on artocarpin (**47**) and cycloartocarpin (**132**) as inhibitors of lipid peroxidation was studied by following Mb(IV) reduction, induced by fatty acid, arachidonic acid. Decrease in the absorbance at 532 nm was recorded and the results were expressed as % inhibition of TBARS. At 100  $\mu\text{M}$  concentration, cycloartocarpin (**132**, 34% inhibition) inhibited the formation of MDA more efficiently than ascorbic acid (27% inhibition) and even better than artocarpin (**47**, 24% inhibition) [32].

The effects of nine prenylated flavonoids (propolin A (**103**), propolin B (**104**), propolin E (**105**), prokinawan (**106**), nymphaeol A (**107**), nymphaeol B (**108**), nymphaeol C (**109**), isonymphaeol B (**110**) and 3'-geranylnaringenin (**111**)) on the oxidation of  $\beta$ -carotene/linoleic acid emulsion was monitored spectrophotometrically by measuring the absorbance at 470 nm over a 60 min period of incubation and the results expressed as IC<sub>50</sub> values. Compounds **103**, **104**, **108**, **109** and **110** (possessing a geranyl group on their B-ring) showed higher activity (IC<sub>50</sub> values ranging from 5.8 to 12.7  $\mu\text{M}$ ) than compounds **106** and **107** (possessing a geranyl group on their A-ring, with IC<sub>50</sub> values of 35.8 and 28.9  $\mu\text{M}$ , respectively), suggesting the importance of the position of the geranyl group in the flavonoid skeleton for the antioxidant activity. In addition, the absence of the catechol unit decreased drastically the antioxidant potential, as observed for derivative **105** (IC<sub>50</sub>  $73.7 \pm 2.3$   $\mu\text{M}$ ) and **110** (IC<sub>50</sub>  $98.3 \pm 3.3$   $\mu\text{M}$ ) [47].

#### 4.2.2. LDL Oxidation

Cycloheterophyllin (**143**), artonin A (**144**) and artonin B (**145**) inhibited Cu(II)-catalyzed oxidation of human LDL, as measured by fluorescence intensity, TBARS formation, conjugated diene formation and electrophoretic mobility in a concentration-dependent manner. Specifically, Cu-induced oxidation of LDL for 12 h led to an increased content of TBARS ( $212.3 \pm 21.4$  nmol/mg protein) when compared with TBARS level in unstimulated LDL ( $1.4 \pm 0.5$  nmol/mg protein), however, in the presence of **143**, **144** and **B 145** at 30  $\mu\text{M}$  concentration, the levels of TBARS formation were drastically reduced ( $2.5 \pm 0.8$ ,  $6.0 \pm 1.5$  and  $4.5 \pm 1.2$  nmol/mg protein, respectively) [31].



After 5 h of incubation, Cu(II)-induced oxidation of LDL was monitored by the formation of conjugated dienes and TBARS and the loss of tryptophan fluorescence by a group of 16 prenylated flavonoids (xanthohumol (**1**), xanthogalenol (**2**), desmethyloxanthohumol (**3**), 4'-O-methyl-xanthohumol (**4**), 5'-prenylxanthohumol (**5**), dehydrocycloxanthohumol (**6**), dehydrocyclo-xanthohumol hydrate (**7**), 3'-geranylchalconaringenin (**11**), 4'-O-5'-C-diprenylxanthohumol (**23**), tetrahydroxanthohumol (**45**), isoxanthohumol (**102**), 6-prenylnaringenin (**112**), 8-prenylnaringenin (**113**), 6,8-diprenylnaringenin (**114**), 6-geranylnaringenin (**115**) and 8-geranylnaringenin (**116**)). At concentrations of 5  $\mu$ M, all the prenylchalcones tested inhibited in some extension the formation of conjugated dienes induced by copper sulfate, while for 25  $\mu$ M concentration complete inhibition of conjugated dienes formation occurred. The prenylchalcones showed higher inhibitory effects in the formation of conjugate dienes and on TBARS formation in LDL than the prenylflavanones, both at 5 and 25  $\mu$ M concentration. Interestingly, at 12.5  $\mu$ M concentration, **1** showed higher inhibition of LDL oxidation than  $\alpha$ -tocopherol, but lower than the flavonol quercetin. Concerning the reduction in tryptophan fluorescence induced by copper, chalcones **1**, **3** and **11** inhibited in higher extension tryptophan oxidation in contrast to flavanones **113** and **102** [12].

LDL oxidation was induced by the peroxynitrite generator, 3-morpholinocydonimine (SIN-1), and measured by the formation of conjugated dienes and TBARS. Conjugated diene formation was monitored by recording the absorbance at 250 nm every 30 min for 8 h and then at 18 and 24 h. After a 24 h incubation at 25 °C, TBARS were measured. The results pointed out that xanthohumol (**1**) inhibited SIN-1-induced oxidation of LDL in a dose-dependent manner in the assay for conjugated diene formation and in the TBARS assay. High inhibitions were also recorded in the TBARS formation for a series of other prenylated chalcones at 5  $\mu$ M, (xanthogalenol (**2**), desmethyloxanthohumol (**3**), 5'-prenylxanthohumol (**5**), dehydrocycloxanthohumol (**6**) and dehydro- cycloxanthohumol hydrate (**7**)), while the highly lipophilic prenylchalcones 3'-geranyl-chalconaringenin (**11**) and 4'-O-5'-C-diprenylxanthohumol (**23**) as well as prenylated flavanones **102** and **112–116** were considerably less active. Generally, chalcones are more potent inhibitors of LDL oxidation than flavanones due to their  $\alpha,\beta$ -unsaturated keto group that can act as a Michael acceptor system for peroxynitrite. The introduction of additional prenyl groups further enhances the nucleophilicity of the 2'-OH group but also increases the compound's lipophilicity and reduces its water solubility, making their oxidation products less effective ROS/RNS scavengers in aqueous medium [15].

The prenylated flavonoids 6,8-diprenylerydiol (**97**), dorsmanin C (**67**) and dorsmanin F (**98**) were effective inhibitors, in a concentration-dependent manner, of Cu(II)-mediated oxidation of LDL with  $IC_{50}$  values < 1  $\mu$ M. In this assay, lipid hydroperoxides were measured by the ferrous oxidation-xylenol orange assay and the results expressed as inhibition of lipid hydroperoxide formation. 6,8-Diprenylerydiol (**97**) exhibited similar inhibitory potential to the positive control quercetin [37].

Five derivatives (cycloartocarpesin B (**56**), cudraflavone B (**65**), euchrestaflavanone B (**94**), euchrestaflavanone C (**95**) and novel flavanone A (**96**)) isolated from *C. tricuspidata* were assessed for their potential as inhibitors of Cu(II)-induced oxidation of LDL. All compounds exhibited modest antioxidant activity against LDL oxidation in TBARS assay with  $IC_{50}$  values ranging from 27.2 to 65.6  $\mu$ M, in comparison with an  $IC_{50}$  of 3.6  $\mu$ M obtained for the positive control probucol [39].

The antioxidant activity of floranol (**76**) isolated from the roots of *Dioclea grandiflora*, was evaluated by the inhibition of Cu(II)-induced oxidation of human LDL by measuring the formation of conjugated dienes. Floranol (**76**) inhibited the LDL oxidation, in a dose-dependent manner. Thus, in the absence of **76** a lag phase of  $33 \pm 1$  min was measured; while in the presence of **76**, lag-phases of  $68 \pm 1$  and  $178 \pm 2$  min were obtained for 3 and 10  $\mu$ M concentration, respectively. Concentrations above 30  $\mu$ M practically prevent LDL oxidation [44].

## 5. Conclusions

A good number of prenylated flavonoids (more than 150 derivatives) have been studied over the past two decades focusing on their antioxidant properties. Most of them were isolated from different

parts of plants belonging to the Moraceae and Fabaceae families, with a limited number of derivatives from the Apiaceae, Asteraceae, Cannabaceae, and Euphorbiaceae. Few O- and C-derivatives obtained by synthesis belong to the chalcone, flavone and flavanone class of compounds. A detailed description of all in vitro and in vivo methodologies used to study the antioxidant effects of natural and synthetic derivatives were made, including reactants, temperature, time and type of detection. DPPH radical scavenging assay is undoubtedly the most frequently used technique, probably due to its simplicity and low cost. From the published results, most of the analyzed prenylated flavonoids exhibited high inhibitory effects and some structure-activity relationships were also described. However, it is not easy to make a comparison between compounds since different methods and positive controls were applied. In conclusion, the importance of prenyl groups for the antioxidant properties of flavonoids in several in vitro and in vivo models is highlighted in this review.

**Funding:** The authors are grateful to FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência, Portugal and FEDER, the Partnership Agreement PT2020, for financial support to CIMO (UID/AGR/00690/2019) and QOPNA (UID/QUI/00062/2019) as well as project POCI-01-0145-FEDER-016385.

**Acknowledgments:** Thanks are due to University of Aveiro and Instituto Politécnico de Bragança for all support.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Panche, A.N.; Diwan, A.D.; Chandra, S.R. Flavonoids: An overview. *J. Nutr. Sci.* **2016**, *5*, e47. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Kumar, S.; Pandey, A.K. Chemistry and biological activities of flavonoids: An overview. *Sci. World J.* **2013**, 162750. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Pietta, P.-G. Flavonoids as antioxidants. *J. Nat. Prod.* **2000**, *63*, 1035–1042. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Yang, X.; Jiang, Y.; Yang, J.; He, J.; Sun, J.; Chen, F.; Zhang, M.; Yang, B. Prenylated flavonoids, promising nutraceuticals with impressive biological activities. *Trends Food Sci. Technol.* **2015**, *44*, 93–104. [\[CrossRef\]](#)
5. Barron, D.; Ibrahim, R.K. Isoprenylated flavonoids—A survey. *Phytochemistry* **1996**, *43*, 921–982. [\[CrossRef\]](#)
6. Botta, B.; Vitali, A.; Menendez, P.; Misiti, D.; Monache, G.D. Prenylated flavonoids: Pharmacology and biotechnology. *Curr. Med. Chem.* **2005**, *12*, 713–739. [\[CrossRef\]](#)
7. Chen, X.; Mukwaya, E.; Wong, M.-S.; Zhang, Y. A systematic review on biological activities of prenylated flavonoids. *Pharm. Biol.* **2014**, *52*, 655–660. [\[CrossRef\]](#)
8. Nešpor, J.; Hanko, V.; Karabín, M.; Jelínek, L.; Dostálek, P. Prenylated flavonoids as valuable biologically active compounds from hops. *Kvasný Prum.* **2017**, *63*, 164–172. [\[CrossRef\]](#)
9. Šmejkal, K. Cytotoxic potential of C-prenylated flavonoids. *Phytochem. Rev.* **2014**, *13*, 245–275. [\[CrossRef\]](#)
10. Venturelli, S.; Burkard, M.; Biendl, M.; Lauer, U.M.; Frank, J.; Busch, C. Prenylated chalcones and flavonoids for the prevention and treatment of cancer. *Nutrition* **2016**, *32*, 1171–1178. [\[CrossRef\]](#)
11. Bartmańska, A.; Tronina, T.; Popłoński, J.; Milczarek, M.; Filip-Psurska, B.; Wietrzyk, J. Highly cancer selective antiproliferative activity of natural prenylated flavonoids. *Molecules* **2018**, *23*, 2922. [\[CrossRef\]](#)
12. Miranda, C.L.; Stevens, J.F.; Ivanov, V.; McCall, M.; Frei, B.; Deinzer, M.L.; Buhler, D.R. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. *J. Agric. Food Chem.* **2000**, *48*, 3876–3884. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Rodriguez, R.J.; Miranda, C.L.; Stevens, J.F.; Deinzer, M.L.; Buhler, D.R. Influence of prenylated and non-prenylated flavonoids on liver microsomal lipid peroxidation and oxidative injury in rat hepatocytes. *Food Chem. Toxicol.* **2001**, *39*, 437–445. [\[CrossRef\]](#)
14. Gerhauser, C.; Alt, A.; Heiss, E.; Gamal-Eldeen, A.; Klimo, K.; Knauf, J.; Neumann, I.; Scherf, H.-R.; Frank, N.; Bartsch, H.; et al. Cancer chemopreventive activity of xanthohumol, a natural product derived from hop. *Mol. Cancer Therap.* **2002**, *1*, 959–969.
15. Stevens, J.F.; Miranda, C.L.; Frei, B.; Buhler, D.R. Inhibition of peroxynitrite-mediated LDL oxidation by prenylated flavonoids: The  $\alpha,\beta$ -unsaturated keto functionality of 2'-hydroxychalcones as a novel antioxidant pharmacophore. *Chem. Res. Toxicol.* **2003**, *16*, 1277–1286. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Vogel, S.; Heilmann, J. Synthesis, cytotoxicity, and antioxidative activity of minor prenylated chalcones from *Humulus lupulus*. *J. Nat. Prod.* **2008**, *71*, 1237–1241. [\[CrossRef\]](#)

17. Zhang, X.-L.; Zhang, Y.-D.; Wang, T.; Guo, H.-Y.; Liu, Q.-M.; Su, H.-X. Evaluation on antioxidant effect of xanthohumol by different antioxidant capacity analytical methods. *J. Chem.* **2014**, *2014*, 249485. [\[CrossRef\]](#)
18. Lathiff, S.M.A.; Jemaon, N.; Abdullah, S.A.; Jamil, S. Flavonoids from *Artocarpus anisophyllus* and their bioactivities. *Nat. Prod. Commun.* **2015**, *10*, 393–396. [\[CrossRef\]](#)
19. Arriffin, N.M.; Jamil, S.; Basar, N.; Khamis, S.; Abdullah, S.A.; Lathiff, S.M.A. Phytochemical studies and antioxidant activities of *Artocarpus scortechnii* King. *Rec. Nat. Prod.* **2017**, *11*, 299–303.
20. Omisore, N.O.A.; Adewunmi, C.O.; Iwalewa, E.O.; Ngadjui, B.T.; Adenowo, T.K.; Abegaz, B.M.; Ojewole, J.A.; Watchueng, J. Antitrichomonal and antioxidant activities of *Dorstenia barteri* and *Dorstenia convexa*. *Braz. J. Med. Biol. Res.* **2005**, *38*, 1087–1094. [\[CrossRef\]](#)
21. Fukai, T.; Satoh, K.; Nomura, T.; Sakagami, H. Antinephritis and radical scavenging activity of prenylflavonoids. *Fitoterapia* **2003**, *74*, 720–724. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Jung, H.A.; Jeong, D.-M.; Chung, H.Y.; Lim, H.A.; Kim, J.Y.; Yoon, N.Y.; Choi, J.S. Re-evaluation of the antioxidant prenylated flavonoids from the roots of *Sophora flavescens*. *Biol. Pharm. Bull.* **2008**, *31*, 908–915. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Piao, X.-L.; Piao, X.S.; Kim, S.W.; Park, J.H.; Kim, H.Y.; Cia, S.-Q. Identification and characterization of antioxidants from *Sophora flavescens*. *Biol. Pharm. Bull.* **2006**, *29*, 1911–1915. [\[CrossRef\]](#)
24. Popoola, O.K.; Marnewick, J.L.; Rautenbach, F.; Ameer, F.; Iwuoha, E.I.; Hussein, A.A. Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from *Helichrysum teretifolium*. *Molecules* **2015**, *20*, 7143–7155. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J. Natural and non-natural prenylated chalcones: Synthesis, cytotoxicity and anti-oxidative activity. *Bioorg. Med. Chem.* **2008**, *16*, 4286–4293. [\[CrossRef\]](#)
26. Schempp, H.; Vogel, S.; Hückelhoven, R.; Heilmann, J. Re-evaluation of superoxide scavenging capacity of xanthohumol. *Free Rad. Res.* **2010**, *44*, 1435–1444. [\[CrossRef\]](#)
27. Żołnierczyk, A.K.; Baczyńska, D.; Potaniec, B.; Kozłowska, J.; Grabarczyk, M.; Woźniak, E.; Anioł, M. Antiproliferative and antioxidant activity of xanthohumol acyl derivatives. *Med. Chem. Res.* **2017**, *26*, 1764–1771. [\[CrossRef\]](#)
28. Vogel, S.; Barbic, M.; Jürgenliemk, G.; Heilmann, J. Synthesis, cytotoxicity, anti-oxidative and anti-inflammatory activity of chalcones and influence of A-ring modifications on the pharmacological effect. *Eur. J. Med. Chem.* **2010**, *45*, 2206–2213. [\[CrossRef\]](#)
29. Teng, Y.; Li, X.; Yang, K.; Li, X.; Zhang, Z.; Wang, L.; Deng, Z.; Song, B.; Yan, Z.; Zhang, Y.; et al. Synthesis and antioxidant evaluation of desmethylxanthohumol analogs and their dimers. *Eur. J. Med. Chem.* **2017**, *125*, 335–345. [\[CrossRef\]](#)
30. Ramli, F.; Rahmani, M.; Kassim, N.K.; Hashim, N.M.; Sukari, M.A.; Akim, A.M.; Go, R. New diprenylated dihydrochalcones from leaves of *Artocarpus elasticus*. *Phytochem. Lett.* **2013**, *6*, 582–585. [\[CrossRef\]](#)
31. Ko, F.N.; Cheng, Z.J.; Lin, C.N.; Teng, C.M. Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Rad. Biol. Med.* **1998**, *25*, 160–168. [\[CrossRef\]](#)
32. Rajendran, M.; Manisankar, P.; Gandhidasan, R.; Murugesan, R. Free radicals scavenging efficiency of a few naturally occurring flavonoids: A comparative study. *J. Agric. Food Chem.* **2004**, *52*, 7389–7394. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Jayasinghe, U.L.B.; Samarakoon, T.B.; Kumarihamy, B.M.M.; Hara, N.; Fujimoto, Y. Four new prenylated flavonoids and xanthones from the root bark of *Artocarpus nobilis*. *Fitoterapia* **2008**, *79*, 37–41. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Lin, K.-W.; Liu, C.-H.; Tu, H.-Y.; Ko, H.-H.; Wei, B.-L. Antioxidant prenylflavonoids from *Artocarpus communis* and *Artocarpus elasticus*. *Food Chem.* **2009**, *115*, 558–562. [\[CrossRef\]](#)
35. Lan, W.-C.; Tzeng, C.-W.; Lin, C.-C.; Yen, F.-L.; Ko, H.-H. Prenylated flavonoids from *Artocarpus altilis*: Antioxidant activities and inhibitory effects on melanin production. *Phytochemistry* **2013**, *89*, 78–88. [\[CrossRef\]](#)
36. Zakaria; Soekamto, N.H.; Syah, Y.M.; Firdaus. Isoflavone from *Artocarpus integer* (Thunb.) Merr. and the bioactivity of antioxidants. *Res. J. Pharm. Biol. Chem. Sci.* **2017**, *8*, 907–912.
37. Dufall, K.G.; Ngadjui, B.T.; Simeon, K.F.; Abegaz, B.M.; Croft, K.D. Antioxidant activity of prenylated flavonoids from the West African medicinal plant *Dorstenia mannii*. *J. Ethnopharmacol.* **2003**, *87*, 67–72. [\[CrossRef\]](#)

38. Lee, B.W.; Lee, J.H.; Gal, S.W.; Moon, Y.H.; Park, K.H. Selective ABTS radical-scavenging activity of prenylated flavonoids from *Cudrania tricuspidata*. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 427–432. [\[CrossRef\]](#)
39. Park, K.H.; Park, Y.-D.; Han, J.-M.; Im, K.-R.; Lee, B.W.; Jeong, Y.; Jeong, T.-S.; Lee, W.S. Anti-atherosclerotic and anti-inflammatory activities of catecholic xanthenes and flavonoids isolated from *Cudrania tricuspidata*. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5580–5583. [\[CrossRef\]](#)
40. Thongnest, S.; Lhinhatrakool, T.; Wetprasit, N.; Sutthivaiyakit, P.; Sutthivaiyakit, S. *Eriosema chinense*: A rich source of antimicrobial and antioxidant flavonoids. *Phytochemistry* **2013**, *96*, 353–359. [\[CrossRef\]](#)
41. Arung, E.T.; Sinamabala, J.R.; Rosamah, E.; Kusuma, I.W.; Kuspradini, H.; Alam, A.E.; Amen, Y.; Tanaka, H.; Satria, D.; Shimizu, K.; et al. Antioxidant and antimelanogenesis activities of glyasperin A from *Macaranga pruinosa* leaves. *Nat. Prod. Commun.* **2019**, *7*, 1–5. [\[CrossRef\]](#)
42. Aminah, N.S.; Kristanti, A.N.; Tanjung, M. Antioxidant activity of flavonoid compounds from the leaves of *Macaranga gigantea*. *J. Chem. Pharm. Res.* **2014**, *6*, 688–692.
43. Wu, J.-Y.; Chung, K.-T.; Liu, Y.-W.; Lu, F.-J.; Tsai, R.-S.; Chen, C.-H.; Chen, C.-H. Synthesis and biological evaluation of novel C(6) modified baicalein derivatives as antioxidative agents. *J. Agric. Food Chem.* **2008**, *56*, 2838–2845. [\[CrossRef\]](#)
44. Botelho, F.V.; Alvarez-Leite, J.I.; Lemos, V.S.; Pimenta, A.M.C.; Calado, H.D.R.; Matencio, T.; Miranda, C.T.; Pereira-Maia, E.C. Physicochemical study of floranol, its copper(II) and iron(III) complexes, and their inhibitory effect on LDL oxidation. *J. Inorg. Biochem.* **2007**, *101*, 935–943. [\[CrossRef\]](#)
45. Domínguez-Villegas, V.; Domínguez-Villegas, V.; García, M.L.; Calpena, A.; Clares-Naveros, B.; Garduño-Ramírez, M.L. Anti-inflammatory, antioxidant and cytotoxicity activities of methanolic extract and prenylated flavanones isolated from leaves of *Eysehadtia platycarpa*. *Nat. Prod. Commun.* **2013**, *8*, 177–180. [\[CrossRef\]](#)
46. Tjahjandarie, T.S.; Tanjung, M. Phenolic compounds from the stem bark of *Erythrina orientalis* and their cytotoxic and antioxidant activities. *Der Pharm. Chem.* **2015**, *7*, 206–211.
47. Kumazawa, S.; Ueda, R.; Hamasaka, T.; Fukumoto, S.; Fujimoto, T.; Nakayama, T. Antioxidant prenylated flavonoids from propolis collected in Okinawa, Japan. *J. Agric. Food Chem.* **2007**, *55*, 7722–7725. [\[CrossRef\]](#)
48. Chen, C.-N.; Wu, C.-L.; Shy, H.-S.; Lin, J.-K. Cytotoxic prenylflavanones from *Taiwanese propolis*. *J. Nat. Prod.* **2003**, *66*, 503–506. [\[CrossRef\]](#)
49. Stompor, M.; Świtalska, M.; Podgórski, R.; Uram, L.; Aebischer, D.; Wietrzyk, J. Synthesis and biological evaluation of 4'-O-acetylisoanthohumol and its analogues as antioxidant and antiproliferative agents. *Acta Biochim. Polonica* **2017**, *64*, 577–583. [\[CrossRef\]](#)
50. Akter, K.; Barnes, E.C.; Loa-Kum-Cheung, W.L.; Yin, P.; Kichu, M.; Brophy, J.J.; Barrow, R.A.; Imchen, I.; Vemulpad, S.R.; Jamie, J.F. Antimicrobial and antioxidant activity and chemical characterisation of *Erythrina stricta* Roxb. (Fabaceae). *J. Ethnopharmacol.* **2016**, *185*, 171–181. [\[CrossRef\]](#)
51. Rahman, M.Z.; Rahman, M.S.; Kaisar, A.; Hossain, A.; Rashid, M.A. Bioactive isoflavones from *Erythrina variegata* L. *Turk. J. Pharm. Sci.* **2010**, *7*, 21–28.
52. Fu, G.; Li, W.; Huang, X.; Zhang, R.; Tian, K.; Hou, S.; Li, Y. Antioxidant and alpha-glucosidase inhibitory activities of isoflavonoids from the rhizomes of *Ficus tikoua* Bur. *Nat. Prod. Res.* **2018**, *32*, 399–405. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Bórquez, J.; Kennelly, E.J.; Simirgiotis, M.J. Activity guided isolation of isoflavones and hyphenated HPLC-PDA-ESI-ToF-MS metabolome profiling of *Azorella madreporica* Clos. from northern Chile. *Food Res. Int.* **2013**, *52*, 288–297. [\[CrossRef\]](#)
54. Ee, G.C.L.; Teo, S.W.; Rahmani, M.; Lim, C.K.; Lim, Y.M.; Go, R. Artomandin, a new xanthone from *Artocarpus kemando* (Moraceae). *Nat. Prod. Res.* **2011**, *25*, 995–1003. [\[CrossRef\]](#)
55. Hashima, N.; Rahman, M.; Sukari, M.A.; Ali, A.M.; Alitheen, N.B.; Go, R.; Ismail, H.B.M. Two new xanthenes from *Artocarpus obtusus*. *J. Asian Nat. Prod. Res.* **2010**, *12*, 106–112. [\[CrossRef\]](#)
56. Alam, M.N.; Bristi, N.J.; Rafiquzzaman, M. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm. J.* **2013**, *21*, 143–152. [\[CrossRef\]](#)
57. Kuete, V.; Sandjo, L.P. Isobavachalcone: An overview. *Chin. J. Integr. Med.* **2012**, *18*, 543–547. [\[CrossRef\]](#)



58. Ko, H.-H.; Lu, Y.-H.; Yang, S.-Z.; Won, S.-J.; Lin, C.-N. Cytotoxic prenylflavonoids from *Artocarpus elasticus*. *J. Nat. Prod.* **2005**, *68*, 1692–1695. [[CrossRef](#)]
59. Ateba, S.B.; Mvondo, M.A.; Djiogue, S.; Zingué, S.; Krenn, L.; Njamien, D. A pharmacological overview of alpinum isoflavone, a natural prenylated isoflavonoid. *Front. Pharmacol.* **2019**, *10*, 952. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).