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Review

Flavonoids from *Artemisia annua* L. as Antioxidants and Their Potential Synergism with Artemisinin against Malaria and Cancer

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Abstract: *Artemisia annua* is currently the only commercial source of the sesquiterpene lactone artemisinin. Since artemisinin was discovered as the active component of *A. annua* in early 1970s, hundreds of papers have focused on the anti-parasitic effects of artemisinin and its semi-synthetic analogs dihydroartemisinin, artemether, arteether, and artesunate. Artemisinin per se has not been used in mainstream clinical practice due to its poor bioavailability when compared to its analogs. In the past decade, the work with artemisinin-based compounds has expanded to their anti-cancer properties. Although artemisinin is a major bioactive component present in the traditional Chinese herbal preparations (tea), leaf flavonoids, also present in the tea, have shown a variety of biological activities and may synergize the effects of artemisinin against malaria and cancer. However, only a few studies have focused on the potential synergistic effects between flavonoids and artemisinin. The resurgent idea that multi-component drug therapy might be better than monotherapy is illustrated by the recent resolution of the World Health Organization to support artemisinin-based combination therapies (ACT), instead of the previously used monotherapy with artemisinins. In this critical review we will discuss

the possibility that artemisinin and its semi-synthetic analogs might become more effective to treat parasitic diseases (such as malaria) and cancer if simultaneously delivered with flavonoids. The flavonoids present in *A. annua* leaves have been linked to suppression of CYP450 enzymes responsible for altering the absorption and metabolism of artemisinin in the body, but also have been linked to a beneficial immunomodulatory activity in subjects afflicted with parasitic and chronic diseases.

Keywords: *Artemisia annua*; artemisinin; flavonoids; antimalarial, anticancer; synergism

1. Introduction

A brief search through PubMed, on March 2010, using the keywords "artemisinin" and "malaria" returned 1,266 hits, while using "*Artemisia annua*" and "flavonoids" returned 12 hits, but "artemisia flavonoids" and "*Artemisia annua* flavonoids" combined with "malaria" returned only four and two hits, respectively. In the same way, "artemisinin" and "cancer" returned 117 hits, "Artemisia flavonoids" and "cancer", 12 hits, and "*Artemisia annua* flavonoids" and "cancer", only one hit. This search meant to establish that in the past 15 years there has been plenty of research on the activity of artemisinin against malaria, followed by less on cancer. However, much less work has focused on the role of flavonoids from *A. annua* against malaria and cancer. Although "flavonoids" and "cancer" returned 8,420 hits, the search for "flavonoids" and "malaria" returned only 68 hits, indicating that the beneficial effects of flavonoids in cancer prevention are well accepted, but not so much for their involvement in the treatment of malaria. While the potential synergistic effect of flavonoids with artemisinin or other anticancer and antimalarial drugs is far from fully explored, it seems worthwhile to investigate biological interactions of flavonoids and artemisinin derivatives in both malaria and cancer.

In the light of recent reports of *Plasmodium*-resistant strains in Cambodia/Vietnam borders and the shortage of artemisinin as a raw material to produce artemisinin-based combination therapies (ACT), there is a pressing need to increase effectiveness and affordability of artemisinin derivatives and ACT. The combined use of flavonoids with artemisinins might increase effectiveness of artemisinins, but the combination of artemisinins with pyrimethamine, sulfadoxine, and lumefantrine, recommended by the World Health Organization in current ACT, would still be needed to circumvent malaria recrudescence issues. It is known that flavonoids chelate metals such as iron and copper as part of their antioxidant effects and that iron chelating therapies have been recommended for malaria patients [1]. Thus, the use of flavonoids in combination with artemisinin might provide a more effective treatment for malaria. In that regard, flavonoids could serve as artemisinin synergists by reacting with iron and converting Fe^{+3} to Fe^{+2} [2], the latter being important in the bioactivity of artemisinin [3], leading to the release of short-lived toxic free radicals that are part of the antimalarial and anticancer mode of action of artemisinin.

Individuals afflicted with malaria and cancer have increased blood free radicals [4,5], possibly aggravating the disease scenario or leading to the generation of the disease in the case of cancer. Thus, it might make sense to combine antioxidant flavonoids, tannins, phenolic acids, and coumarins with

artemisinins to treat malaria and cancer, as well as to prevent the latter. The *A. annua* traditional tea is a rich source of both antioxidant phenolics (mostly flavonoids) and artemisinin [6,7]. Levels of artemisinin are lower in such teas than in current treatments with ACT and use of the tea [7,8], or artemisinin alone [9], leads to recrudescence levels that vary greatly, from as low as 10%, in the case of a seven-day course with artesunate [10] to 46%–80% in non-immune patients in Thailand and China [11]. This varying recrudescence is not only related to the short half-life of artemisinin, but also to the duration of treatment and to the loss in sensitivity to dihydroartemisinin (the active blood metabolite) by different strains of *Plasmodium*, which is remediated by the combination of artemisinin with other antimalarial drugs of longer half-lives and different modes of action [11]. However, the flavonoids could be the reason why the tea "reportedly" treated malaria for hundreds of years and, although recrudescence might have occurred then as well, there was compelling evidence from traditional Chinese herbal medicine to justify the addition of *A. annua* to the selection of Chinese plants screened for malaria in 1969, which eventually led to the discovery of artemisinin [12]. Although we do not recommend substitution of the tea for the WHO-recommended ACT, the tea might still be valuable in remote areas of Africa to delay malaria-induced coma and allow one to get to a hospital and receive proper treatment.

This review will focus on the flavonoids found in *A. annua* (Table 1) wherever they relate to anticancer or antimalarial effects, on their own or in synergism with other natural compounds, with synthetic anticancer and antimalarial drugs, and with artemisinins. However, one should keep an open mind and complete the idea where he/she judges we failed. No review is final and, based on what in currently known, or strictly based on the chemical structure of flavonoid, it is quite hard to predict the full spectrum of their biological activity. If there would be no biological activity or benefit for flavonoids, hydroxylated or methoxylated, glycosylated or not, why would plants go through such an energetically-expensive endeavor to produce so many different kinds of flavonoids?

2. Classification of Plant Phenolics

Phenolic phytochemicals (phenolics) occupy a unique position in the area of natural products due to their ubiquitous distribution throughout the plant kingdom and in products (fruits, vegetables, beverages, herbs, cosmetics and nutraceuticals) consumed by the general population on a regular basis [13]. Phenolics are biosynthesized by plants during normal development and in response to stress conditions such as exposure to UV radiation, pest attack, and wounding [14,15]. Phenolic compounds are known to provide protection against a wide range of diseases such as coronary heart disease, stroke, and certain types of cancers [16,17]. Chemically, phenolics are defined as a class of aromatic organic compounds with at least one hydroxyl group attached directly to a benzene ring [18]. Over 8000 phenolics with wide structural diversity and polarities have been isolated from plants [19].

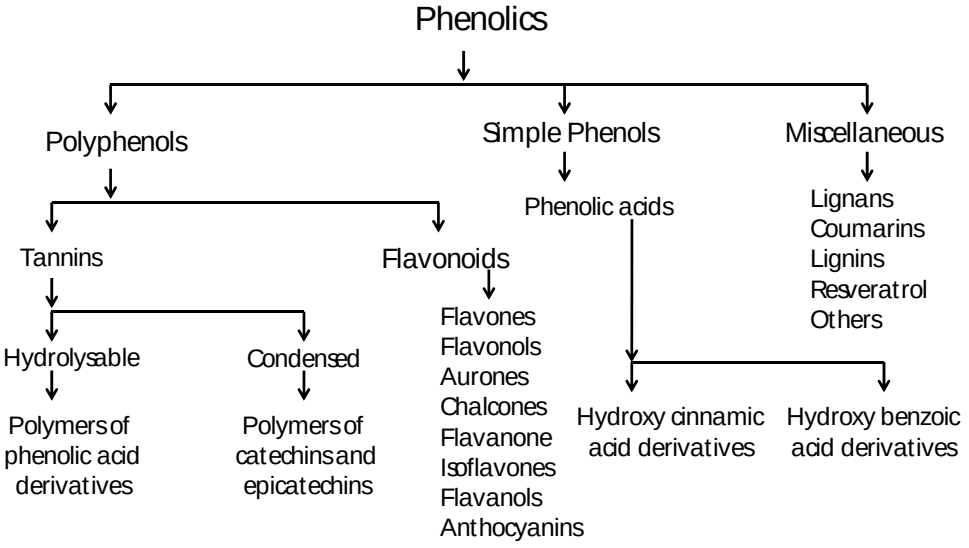
Phenolics can be chemically grouped into three broad categories: polyphenols (tannins and flavonoids), simple phenols (phenolic acids) and a miscellaneous group (Figure 1) [18]. Although we have used this classification, based on chemical structure, for this manuscript, the commonly used byosynthetical classification described in most reviews is also available at http://www.phenol-explorer.eu/compound_classes. Phenolic acids are chemically defined as carboxylic acid derivatives of phenols, whereas no such clear definition for polyphenols is provided in the literature. Rather,

polyphenols are described as a group of chemical substances found in plants, characterized by the presence of more than one phenol unit or building block per molecule. Polyphenols serve as antioxidants as they tend to prevent or neutralize the damaging effects of free radicals. They also give flowers, fruits, and vegetables their color.

Polyphenols can be arranged into two broad classes: tannins and flavonoids. Tannins are astringent, bitter plant polyphenols that either bind or precipitate proteins. Tannins can be further classified chemically into two main groups, hydrolyzable and condensed. Hydrolyzable tannins decompose in water yielding various water-soluble products, such as gallic acid or ellagic acid, protocatechuic acid and sugars. Condensed tannins, also known as proanthocyanidins, are polymers of 2 to 50 (or more) flavonoid units joined by carbon-carbon bonds, which are not cleaved by hydrolysis. Flavonoid is a general name for phytochemicals based on a 15 carbon (C6-C3-C6) skeleton. Over 4,500 different flavonoids have been isolated and identified from plants [20]. Flavonoids can be further divided into multiple groups such as flavones, flavonols, flavanones, dihydroflavonols, chalcones, aurones, isoflavonoids, biflavonoids, *etc.* Flavonoids can occur as free aglycons or as conjugated forms with methoxyl, glycosyl, isoprenyl, prenyl, methylenedioxy, aliphatic acids and other substituents [18,21,22].

Phenolic acids on the other hand can be broadly grouped into two subgroups: hydroxylcinnamic and hydroxylbenzoic acids derivatives (Figure 1). In many cases, the aldehyde analogs such as vanillin are also grouped with phenolic acids. The miscellaneous group comprises all other phenolic compounds not classified into the distinct subgroups above. These include lignans, lignins, coumarins, stilbenes derivatives like resveratrol, and other phenolic compounds [18]. Phenolic compounds can also occur as free aglycons or conjugated with one or more substituents such as methoxyl, glycosyl, prenyl, methylenedioxy, aliphatic acids, *etc.*

Figure 1.General classification of plant phenolics, modified from [18].



Note: The phenolic compounds can occur in free aglycon and conjugated forms with sugars, acids, and other biomolecules.

3. Phenolics from *A. annua*

A few recent reports indicate that *A. annua* is one of the four medicinal plants with the highest ORAC (oxygen radical absorbance capacity) level [23,24]. The total antioxidant capacity (ORAC) of *A. annua* leaves and inflorescences extracts was reported as 1,125 and 1,234 μmoles of Trolox equiv/g, respectively, which is half to two thirds of the ORAC of oregano (the highest reported ORAC for an herb) extracts. The high antioxidant activity of *A. annua* extract is most likely due to its high phenolic content. Over 50 different phenolic compounds belonging to five major groups (flavones, flavonols, coumarins, phenolic acids, and a miscellaneous group have been reported from *A. annua* (Figure 2). The prominent coumarins identified from *A. annua* are coumarin, aesculetin (6,7-dihydroxycoumarin), iso-fraxidin (7-hydroxy-6,8-dimethoxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin), scopolin (7-β-D-glucopyranoside-6-methoxycoumarin), and tomentin (5-hydroxy-6,7-dimethoxy-coumarin). The main components of *A. annua* were recently identified by HPLC-MS as quercetin-glucoside, flaviolin, rhamnetin, chrysoplenol D, and pilloin, although the HPLC-UV data suggested that when detection was done at 335 nm more than 40 components, including chlorogenic acid, were present [25]. The structures of the 11 prominent flavones and 29 flavonols reported from *A. annua* are reported in Table 1 [25,26]. A highly specific feature of *A. annua* is the presence of significant quantities of structurally diverse polymethoxylated flavonoids [27,28]. In addition, other phenolic compounds such as 2,4-dihydroxy-6-methoxy-acetophenone, 5-nonadecyl-3-O-methyletherresorcinol, 2,2,6-trihydroxychromene, and 2,2-dihydroxy-6-methoxychromene have also been isolated from *A. annua* [26].

Figure 2. Major phenolics from *Artemisia annua*, with the great majority being flavones or flavonols.

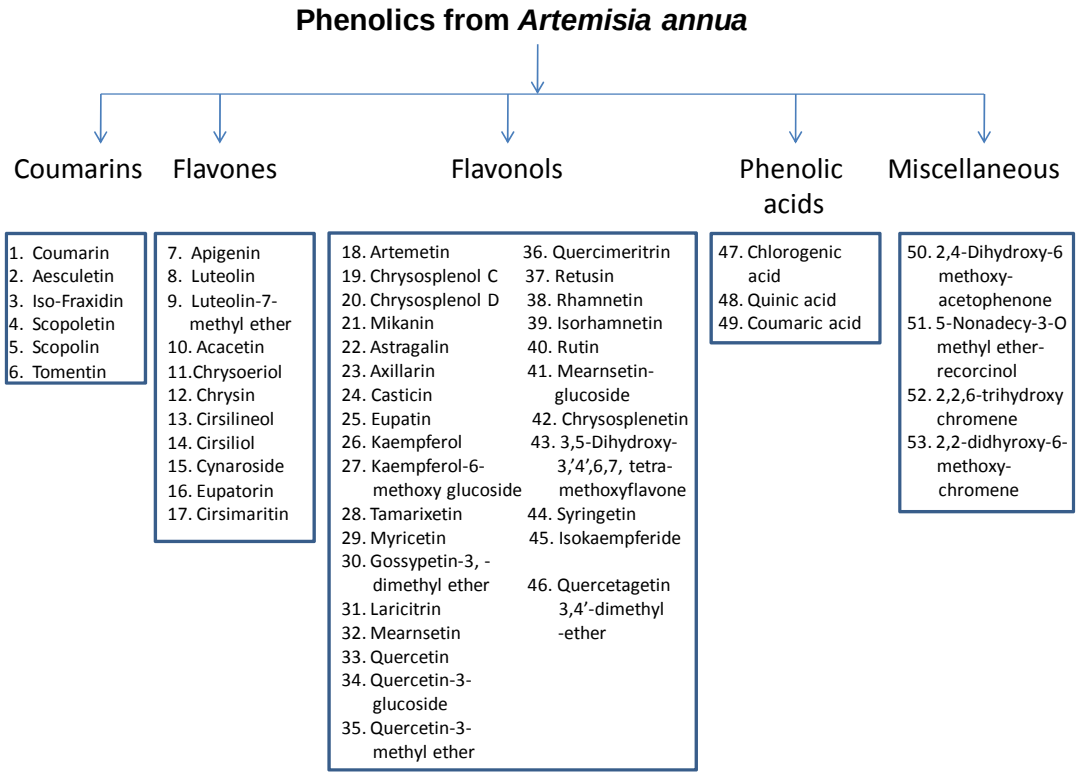
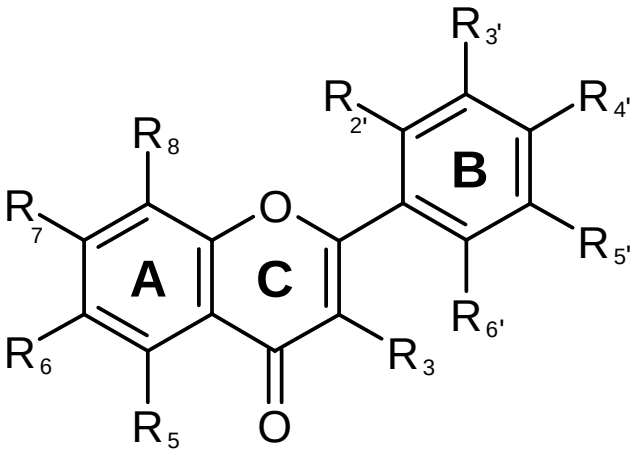


Table 1. Major phenolics reported from *Artemisia annua* and the general structure of flavonoids. The number refers to the number given to each compound in Figure 2. Compounds with a 3-OH group attached to the 2,3-double bond, and adjacent to the 4-carbonyl group in the C ring are predicted to have major antioxidant activity [29]. Substituents (R) are numbered according to the ring position (A, C, and B).



Structure	Phenolic type	Ring and substituent position									
No.	**Flavones**	C(R_3)	A(R_5)	A(R_6)	A(R_7)	A(R_8)	B($R_{2'}$)	B($R_{3'}$)	B($R_{4'}$)	B($R_{5'}$)	B($R_{6'}$)
7	Apigenin	H	OH	H	OH	H	H	H	OH	H	H
8	Luteolin (5,7,3',4'-Tetrahydroxy flavone)	H	OH	H	OH	H	H	OH	OH	H	H
9	Luteolin-7-methylether	H	OH	H	OCH_3	H	H	OH	OH	H	H
10	Acacetin (apigenin-4'-methyl ether) or 5,7-dihydroxy-4-methoxy flavone	H	OH	H	OH	H	H	H	OCH_3	H	H
11	Chrysoeriol (Lutoelin-3'-methyl ether) or5,7,4'-Trihydroxy-3'-methoxy flavone	H	OH	H	OH	H	H	OCH_3	OH	H	H
12	Chrysin (5,7-Dihydroxy flavone)	H	OH	H	OH	H	H	H	H	H	H
13	Cirsilineol (6-Hydroxyluteolin-6,7,3'-trimethyl ether or 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, Fastigenin, Anisomelin, Eupatrin)	H	OH	OCH_3	OCH_3	H	OH	OCH_3	OH	H	H
15	Cynaroside (Luteolin-7-glucoside or 5,7,3',4'-Tetrahydroxyflavone-7-glucoside or Glucoluteolin or Luteoloside or Cinaroside)	H	OH	H	OGlu	H	H	OH	OH	H	H
16	Eupatorin (6-Hydroxyluteolin-6,7,4'-trimethyl ether or 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone)	H	OH	OCH_3	OCH_3	H	H	OH	OCH_3	H	H
17	Cirsimaritin (Scutellarin-6,7-dimethyl ether or 6-Hydroxyapigenin-6,7-dimethyl ether or 5,4'-Dihydroxy-6,7-Dimethoxyflavone or Scorphulein or Cirsumaritin or Cirsitakaogenin)	H	OH	OCH_3	OCH_3	H	H	H	OH	H	H
18	Artemetin	OCH3	OH	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H	H
19	Chrysosplenol-C	OCH3	OH	OH	OCH_3	H	H	OCH_3	OH	H	H

Table 1. *Cont.*

Structure	Phenolic Type	Ring and subsituent position									
No.	**Flavonol**	C(R_3)	A(R_5)	A(R_6)	A(R_7)	A(R_8)	B($R_{2'}$)	B($R_{3'}$)	B($R_{4'}$)	B($R_{5'}$)	B($R_{6'}$)
20	Chrysosplenol-D	OCH_3	OH	OCH_3	OCH_3	H	H	OH	OH	H	H
21	Mikanin	OH	OH	OCH_3	OCH_3	H	H	H	OCH_3	H	H
22	Astragalin (Kaempferol-3-α-D-glucoside)	O-glu	OH	H	OH	H	H	H	OH	H	H
23	Axillarin (5,7,3',4'-Tetrahydroxy-3,6-dimethoxyflavone or quercetagetin -3,6- dimethyl ether)	OCH_3	OH	OCH_3	OH	H	H	OH	OH	H	H
24	Casticin (5,3'-dihydroxy-3,6,7,4'-tetramethyl ether flavone or Quercetagetin -3,6-7,4'-tetramethyl ether)	OCH_3	OH	OCH_3	OCH_3	H	H	OH	OCH_3	H	H
25	Eupatin (3,5,3'-Trihydroxy-6,7,4'-trimethoxyflavone or Quercetagetin -3,6- dimethyl ether)	OH	OH	OCH_3	OCH_3	H	H	OH	OCH_3	H	H
26	Kaempferol (3,5,7,4'-Tetrahydroxy flavone)	OH	OH	H	OH	H	H	H	OH	H	H
27	Kaempferol-6-methox-3-O-β-D-glucoside	OGlu	OH	OCH_3	OH	H	H	H	OH	H	H
28	Tamarixetin	OH	OH	H	OH	H	H	OH	OCH_3	H	H
29	Myricetin (3,5,7,3',4',5'-Hexahydroxy flavone)	OH	OH	H	OH	H	H	OH	OH	OH	H
30	Gossypetin- 3,8-dimethylether	OCH_3	OH	H	OH	OH	H	OH	OCH_3	H	H
31	Laricitrin (3,5,7,3',4', -Pentahydroxy 5'-methoxyflavone)	OH	OH	H	OH	H	H	OH	OH	OCH_3	H
32	Mearnsetin (3,5,7,3',5', -Pentahydroxy 4'-methoxyflavone or Myricetin-4-methyl ether)	OH	OH	H	OH	H	H	OH	OCH_3	OH	H
33	Quercetin (3,5,7,3',4'-Pentahydroxy flavone)	OH	OH	H	OH	H	H	OH	OH	H	H
34	Quercetin-3'- O-β-D-glucoside	OH	OH	H	OH	H	H	O-Glu	OH	H	H
35	Quercetin-3- methylether	OCH_3	OH	H	OH	H	H	OH	OH	H	H
36	Quercimeritrin (Quercetin-7-glucoside)	OH	OH	H	O-Glu	H	H	OH	OH	H	H
37	Retusin (5-Hydroxy-3,7,3'4'-tetramethoxy flavone or Quercetin3,7,3',4'-tetramethylether)	OCH_3	OH	H	OCH_3	H	H	OCH_3	OCH_3	H	H
38	Rhamnetin (Quercetin-7-methylether or 3,5,7,3'-Tetrahydroxy-4'-methoxy flavone)	OH	OH	H	OCH_3	H	H	OH	OH	H	H
39	Isorhamnetin (Quercetin-3'-methylether or 3,5,7,4'-Tetrahydroxy-3'-methoxy flavone)	OH	OH	H	OH	H	H	OCH_3	OH	H	H
40	Rutin (Quercetin-3-rutinoside)	O-Diglyc.	OH	H	OH	H	H	OH	OH	H	H
41	Mearncetin glucoside	OH	OH	H	OGlu	H	H	OH	OCH_3	OH	H
42	Chrysosplenetin (5,4'-Dihydroxy-3,6,7,3'-tetramethoxy flavone or Quercetagetin-3,6-7,3'-tetramethyl ether)	OCH_3	OH	OCH_3	OCH_3	H	H	OCH_3	OH	H	H
43	3,5-Dihydroxy-3',4',6,7,-Tetramethoxyflavone	OH	OH	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H	H
44	Syringetin (Myricetin-3',5'-dimethyl ether)	OH	OH	H	OH	H	H	OCH_3	OH	OCH_3	H
45	Isokaempferide (5,7,4'-Trihydroxy-3-methoxyflavone or Kaemferol-3-methyl ether)	OCH_3	OH	H	OH	H	H	H	OH	H	H
46	Quercetagetin 3,4'-dimethyl ether	OCH_3	OH	OH	OH	H	H	OH	OCH_3	H	H