Flavonoids and Related Compounds in Parasitic Disease Control

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Abstract: Flavonoids are natural plant compounds increasingly used in therapeutic applications. Their large spectrum of activities depends on their structures and cellular targets. Most recent research shows they are promising drugs for controlling human and animal parasitic diseases. Their multiple effects make it difficult to understand their modes of action, but some of them have been elucidated. This review also deals with their toxicity in mammals.

Key Words: Flavonoids, parasites, drug, control, protozoans, helminths.

1. INTRODUCTION

Parasitic diseases caused by protozoans, helminths and ectoparasites occur throughout the world. These organisms pose a major public health problem because of the diseases and deaths associated with the parasitism of humans and animals. Treatments have been developed but are increasingly limited by the spread of chemical resistance, which in some cases may protect the parasite against several chemical groups simultaneously.

There is therefore a continuous search for new treatments. This search must take into account the increasingly strict requirements in terms of compound toxicity and the persistence of residues in treated individuals and in the environment. Research is thus tending to turn toward the selection of "natural" substances. Flavonoids are good candidate molecules as they have relatively low levels of toxicity. They have been shown to be active against cancers [1, 2] but have many other possible applications, including the treatment of parasitic diseases.

This review provides a description of current knowledge concerning the activity of flavonoids against parasites, the modes of action of these compounds as a function of chemical structure and their cellular targets. It also discusses the possible use of these compounds to prevent parasitic diseases.

2. BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF FLAVONOIDS

2.1. Biochemical Properties

Flavonoids are polyphenolic compounds constituting a major class of secondary metabolites in higher plants. Approximately 5,000 flavonoids have been identified, mostly in plants, in flowers, fruits and, in some cases, leaves. Many flavonoids act as pigments, and these compounds are responsible for the coloration of flowers and fruits in many angiosperm families. All flavonoids absorb in the ultraviolet part of the spectrum, whereas only some absorb visible light. For example, the chalcones and aurones are yellow, whereas anthocyanosides may be red, blue or violet. All the isoflavonoids are colourless. Some flavonoids are found in animals (chrysine, quercetin, and galangin in propolis, [3, 4]).

Flavonoids form a diverse group of aromatic molecules with a common biosynthetic origin: they are all derived from phenylalanine and malonyl-coenzyme A. The first step in flavonoid biosynthesis is catalyzed by chalcone synthase, using malonyl-coenzyme A and 4-coumaroyl-coenzyme A as substrates, and generating hydroxychalcone, the precursor of all flavonoids. A large number of different enzymes are responsible for the diversity of these compounds.

Flavonoids are C_6-C_3-C_6 compounds with various substitutions. They are classified according to the C3 fragment and the type of heterocyclic ring [5, 6]. Three groups with a second aromatic ring B in position 2 have been defined: a) chromone derivatives (flavones, flavonols, flavanones Fig. **1A**, **B**, **C**), b) chroman derivatives (anthocyanidins and catechins, Fig. **1D**, **1E**), c) flavonoids with an open propane chain or a five-membered ring (chalcones, Fig. **1F**) or with an oxygen bridge involving the central carbon atom (C_2) of the 3C – chain, i.e. a furane ring (aurones, Fig. **1G**).

In addition to flavonoids themselves, two other groups with either a second aromatic ring B in position 3 — isoflavonoids (Fig. **1H**) — or in position 4 (4-phenyl-coumarin) —chromane derivative neoflavonoids (Fig. **1I**) — have been identified. Isoflavonoids and neoflavonoids may be considered to be abnormal flavonoids. Unlike most other flavonoids, isoflavonoids have a rather limited taxonomic distribution, being mostly restricted to the Leguminosae. Chalcones and flavanones are good precursors for efficient isoflavonoid production. Common isoflavonoids include genistein, daidzein and biochanin A.

The flavonoids are further divided into subclasses based on the oxidation state and functional groups of the C ring. Flavones and flavonols are γ-pirones, and flavanones and flavanols are their hydroderivatives. Flavonols and flavones are the most widely distributed, and include quercetin, kaempferol, myricetin, chrysin and apigenin.

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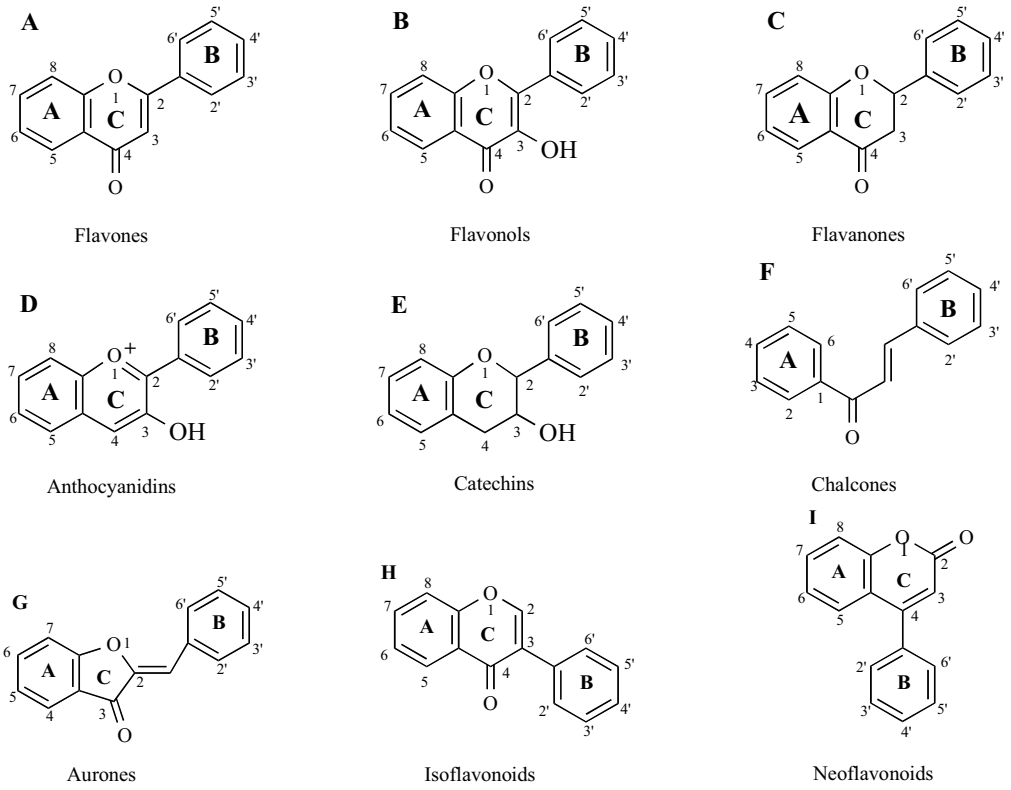

Fig. (1). Chemical structure of the main groups of flavonoids.

Flavonoids may be aglycones, glycosides or methylated derivatives. Flavonoids are often found in plants as glycosides (one or two hydroxyls are glycosylated). Most of the flavonoids present in food are bound to sugars, as β-glycosides. The binding of flavonoids to sugars renders them soluble in water. However, they cannot be absorbed without hydrolysis of the β-glycoside bonds by microorganisms in the gut, as no enzyme capable of splitting these bonds is produced by the gut. However, some flavonoids are poorly metabolized by the gut microflora and are poorly absorbed (e.g. quercetin, rutin) [7, 8, 9]. Some metabolites (e.g. equol) may be more active than the parent compound [10].

Many flavonoids are polymerized into large molecules, tannins, either by the plants themselves or as a result of food processing [11].

2.2. Biological Properties

As a consequence of their biochemical properties, flavonoids influence many biological functions [12, 6]. They act as enzyme inhibitors, precursors of toxic substances, metal-chelating agents and reducing agents. They are involved in energy transfer, morphogenesis, respiration, photosynthesis and gene expression within cells, and have been shown to contribute to behavior and sex determination in individuals. This broad range of biological properties results in flavonoids having diverse pharmacological activities in humans and animals, as analgesic, antiallergic, antiangina, anticancer, antidiabetic, antidiarreal, antihepatotoxic, antiosteoporotic, antispasmodic, antiulcer and vascular protection agents [6]. In plants, they are involved in photosensitisation and their ability to absorb UV radiation protects plant tissues against its harmful effects.

Many flavonoids have antioxidant and antiproliferative properties. The efficacy of flavonoids as antioxidants is related to their ability to quench oxygen-based free radicals. Myricetin is one of the antioxidants found in *Ginkgo biloba* and helps to prevent free radical damage to nerve cells. Proanthocyanidins (also known as pycnogenols) are polyphenolic tannins found in pine bark, tea, peanut, cranberries and grape. Their antioxidant potency — particularly that of the tannins found in grape seeds — is 20 times greater than that of vitamin E. Tannins have been used in traditional medicine to treat lipid peroxidation and oxidative stress, but are now also widely used in cosmetic applications. Some flavonoids, such as quercetin, rutin, curcumin, silymarin and green tea polyphenols, have anti-inflammatory effect, possibly due to their ability to prevent cyclooxygenase and lipoxygenase from acting on arachidonic acid in cell membranes to form inflammatory substances, such as prostaglandins, leukotrienes and nitric oxide [6]. Nobiletin, which is found in citrus fruits, has such anti-inflammatory effects. Quercetin may limit allergy-related problems, such as hay fever, asthma and eczema, by inhibiting calcium channels in the mast cell membrane, thereby preventing histamine release and preventing mast cell degranulation. This flavonoid also decreases the synthesis of pro-inflammatory prostaglandins. Quercetin is structurally related to the anti-allergic drug disodium chromoglycate. It can be synthesized by intestinal bacteria from rutin. Anthocyanidins, found in the skins of some berries, including bilberries in particular, have benefi-

cial effects on eyesight and the circulation. Rutin, which is found in buckwheat, can be used to treat high blood pressure, bruising and bleeding under the skin, including the redness caused by irradiation. Hesperidin, which is found in citrus peel, can help to strengthen fragile capillaries. The isoflavones, genistein and daidzein, are phytoestrogens. They are much weaker than steroid estrogens, but nonetheless display significant interactions within the body. Nobiletin, which is found in citrus fruits, is useful for detoxification and anthocyanidins have some antibacterial effects.

2.3. Effects on Parasites

Most studies on the effects of flavonoids on parasites have focused on protozoans. Flavonoids have been used to treat protozoan diseases in humans, in the traditional medicine practices of American, African and Asian countries. Many of the major flavonoid groups and their derivatives have been shown to display antiparasite activity. In the laboratory, studies of the activity of flavonoids against protozoans have focused on the most common and pathogenic parasites, such as *Plasmodium* (*P. falciparum*), *Trypanosoma* (*T. brucei brucei, T. brucei gambiens* and *T. cruzi*), *Leishmania* (*L. donovani*), *Cryptosporidium* (*C. parvum*) and *Toxoplasma* (*T. gondii*).

Less is known about the effect of flavonoids on helminths and ectoparasites, although plant extracts have been shown to have activity against these parasites. Many tannin-rich plants have been shown to reduce helminth infections in livestock. Nevertheless, scientific evidence confirming efficacy against parasites is limited, and the active compounds have not been fully identified [13].

3. EFFECTS OF FLAVONOIDS ON PROTOZOANS

Most of the tests on protozoans have been carried out *in vitro* on parasite cultures. Flavonoids have many cellular targets and their modes of action vary according to the accessibility of these targets as a function of parasite development. Some flavonoids act on host-parasite interactions, whereas others disturb protozoan development or metabolism. Interestingly, some flavonoids limit the resistance of protozoans to other drugs. Several studies have also established relationships between the activity, chemical structure and mode of action of flavonoids.

3.1. Antiprotozoan (Antiproliferative) Activity of Flavonoids

Various crude plant extracts, plant fractions or purified flavonoids exhibit antiprotozooan activity (Table **1**). Their structures are given in Fig. **2** (**A-H**).

Extracts of wild plants can be more active than those of cultivated plants [14]. High levels of anti-protozoan activity have been observed *in vitro* [15] or *in vivo*, reducing parasitemia and mouse mortality [14]. Some extracts are also active *in vitro* against chloroquine- or mefloquine-resistant *P. falciparum* [14].

The mode of action of the antiprotozoan flavonoids is not fully elucidated. Some of them possess other biological properties such as antibacterial, antiviral and antiproliferative effects on lymphocytes of the polyphenolic-rich extract of the husk fibers of *Cocos nucifera* (Palmae) that displays activity against *Leishmania amazonensis* [16]. Eight alkoxylated and hydroxylated chalcones inhibited the sorbitol-induced lysis of parasitized erythrocytes to a significant extent at concentrations close to their anti plasmodial IC_{50} [17]. Licochalcone A has been identified as a potent membrane-active agent that transforms normal erythrocytes into echinocytes whilst inhibiting the growth of *P. falciparum*, this effect being an apparently indirect consequence of host cell damage [18]. Youn *et al*., (2003-2004) were able to reduce the replication of *Toxoplasma gondii* and *Neospora caninum* by treating parasites *in vitro* with alcoholic extracts of several plants from South Korea [19, 20]. Weiss *et al*. (1998) highlighted the role of heat shock proteins (HSPs) in inducing the development of bradyzoites, the latent stage of *T. gondii* [21]. Quercetin, which inhibits the synthesis of several HSPs (HSP90, HSP70 and HSP27), prevents the induction of bradyzoites *in vitro*. Several hydrolysable tannins prevent *L. donovani* amastigotes from surviving within cells, but have no effect on promastigote forms [22, 23] or are toxic to mammalian macrophages [22].

3.2. Interactions Between Host Cells and Protozoans

Flavonoids may affect the interaction between the host cell and parasite development.

In a study on *T. gondii*, genistein, a protein kinase inhibitor, was found to inhibit the attachment of tachyzoites to macrophages and their penetration into these cells. Genistein inhibited macrophage penetration by 50 %. Confocal microscopy revealed the presence of phosphoproteins in macrophages during interaction with the parasites, suggesting that protein phosphorylation is a key event in the *T. gondii*-host cell interaction [24].

The adhesion of *P. falciparum*-infected erythrocytes to host endothelial cells is a crucial process in parasitic infection. Several endothelial receptors have been identified as involved in this interaction. One such receptor is intercellular adhesion molecule 1 (ICAM-1), a member of the immunoglobulin family of proteins present on the vascular endothelium. Part of the ICAM-1 binding site used by *P. falciparum*-infected erythrocytes (the DE loop) was used to screen a library of compounds for similar structures (based on the crystal structure of human ICAM-1). Thirty-six compounds were identified as having structures similar to that of the DE loop of human ICAM-1, and their inhibition of *P. falciparum*-infected erythrocyte adhesion to ICAM-1 was tested. A flavonoid, (+)-epigalloyl-catechin-gallate (Fig. **2I**), was found to inhibit adhesion in a dose-dependent manner. This provided the first instance of mimeotope-based inhibition of cytoadhesion for *P. falciparum* [25].

Protozoans produce peptides similar to the vertebrate epidermal growth factor (EGF). These EGF-like peptides bind to the EGF receptor *in vivo* and take part in host-parasite interactions. A series of 52 isoflavone analogs of these peptides (dihydroxyisoflavone and trihydroxydeoxybenzoine genistein derivatives), inhibiting activation of the EGF receptor, were studied *in vitro* and *in vivo* to identify new development inhibitors for three parasites: *Sarcocystis neurona, Neospora caninum* and *Cryptosporidium parvum*.

Table 1.

Plants	Flavonoids	Parasites	IC50	EC50	Authors
-	naringenin	*Cryptosporidium parvum*	-	15 µM	Mead and McNair, 2006
-	genistein	*C. parvum*	-	25 µM	Mead and McNair, 2006
-	quercetin	*Encephalitozoon intestinalis*	-	15 µM	Mead and McNair, 2006
-	apigenin	*E. intestinalis*	-	50 µM	Mead and McNair, 2006
Cocos nucifera (Palmae)	-	*Leishmania amazonensis*		10 µg/mL	Mendonca-Filho *et al.*, 2004
(husk fibers)					
	hydrolysable tannins	*L. donovani* amastigotes	-	-	Kiderlen *et al.*, 2001; Kolodziej *et al.*, 2001
-	chalcones (alkoxylated and hydroxylated)	*Plasmodium*	< 20 µM	10 µM	Go *et al.*, 2004
-	dehydrosilybin	*P. falciparum*			Grael *et al*., 2005
	8-(1,1)-DMA-kaempferide				
-	Licochalcone A	*P. falciparum*			Ziegler *et al.*, 2004
Satureja parviflora	eriodictyol, luteolin	*P. falciparum, T. brucei rhodesiense*	25 µM	22.5 µM	van Baren *et al*., 2006
Bidens pilosa	quercetin-3,3-dimethoxy-7-0-rhamnoglucopyranose	*P. berghei, P. falciparum*	-	-	Andrade-Neto *et al*., 2004
Sophora flavescens	-	*Toxoplasma gondii, Neospora caninum*	-	-	Youn *et al.,* 2003, 2004
Sinomenium acutum	-	*T. gondii, N. caninum*	-	-	Youn *et al.,* 2003, 2004
Pulsatilla koreana,	-	*T. gondii, N. caninum*	-	-	Youn *et al.,* 2003, 2004
Ulmus macrocarpa	-	*T. gondii, N. caninum*	-	-	Youn *et al.,* 2003, 2004
Torilis japonica	-	*T. gondii, N. caninum*	-	-	Youn *et al.,* 2003, 2004
-	quercetin	*T. gondii*	-	-	Weiss *et al.*, 1998
Conchocarphus heterophyllus	-	*Trypanosoma*	-	-	Ambrozin *et al.*, 2004
Trichilia ramalhoi	-	*Trypanosoma*	-	-	Ambrozin *et al.*, 2004
Galipea carinata	-	*Trypanosoma*	-	-	Ambrozin *et al.*, 2004
Combretum molle	punicalagin	*Trypanosoma*	-	-	Asres *et al.*, 2001
Lychnophora pohlii	luteolin, vicenin-2	*Trypanosoma cruzi* (trypomastigotes)			Grael *et al.,* 2005

At concentrations of about 15 to 40 µM, 25 analogs inhibited the development of one parasite or of all three by at least 95 % *in vitro*. Mongolian gerbils orally infected with *C. parvum* and treated with 200 or 400 mg of isoflavone analogs had 91 % fewer oocysts than untreated gerbils. This level of inhibition is significantly higher than that observed with reference substances (nitazoxanide, 200 mg/kg/day, paromomycin, 100 mg/kg/day). Cytotoxicity in host cells was evaluated on cell lines in culture (monocytes and human HCT8 epithelial cells) and was shown to be null or moderate. These isoflavone analogs could thus be used as anticoccidial agents [26].

Oxidative damage to erythrocytes has been implicated in the decrease in erythrocyte survival leading to anemia in patients with leishmaniasis. Quercetin**,** which has well documented protective effects against membrane lipoperoxidative damage, inhibits oxidation of the proteins and lipids of red cell membranes. Quercetin also strongly decreases parasite load in the spleen. Combination of this compound with stibanate, which is used to treat leishmaniasis, strengthens this effect [27].

3.3. Effects of Flavonoids on Protozoan Protein Kinases

Protein kinases (PKs) may play key roles in *T. gondii* proliferation, differentiation and invasion. Mitogen-Activated Protein (MAP) kinases has been found in *T. gondii* lysates. The treatment of tachyzoites with genistein before they come

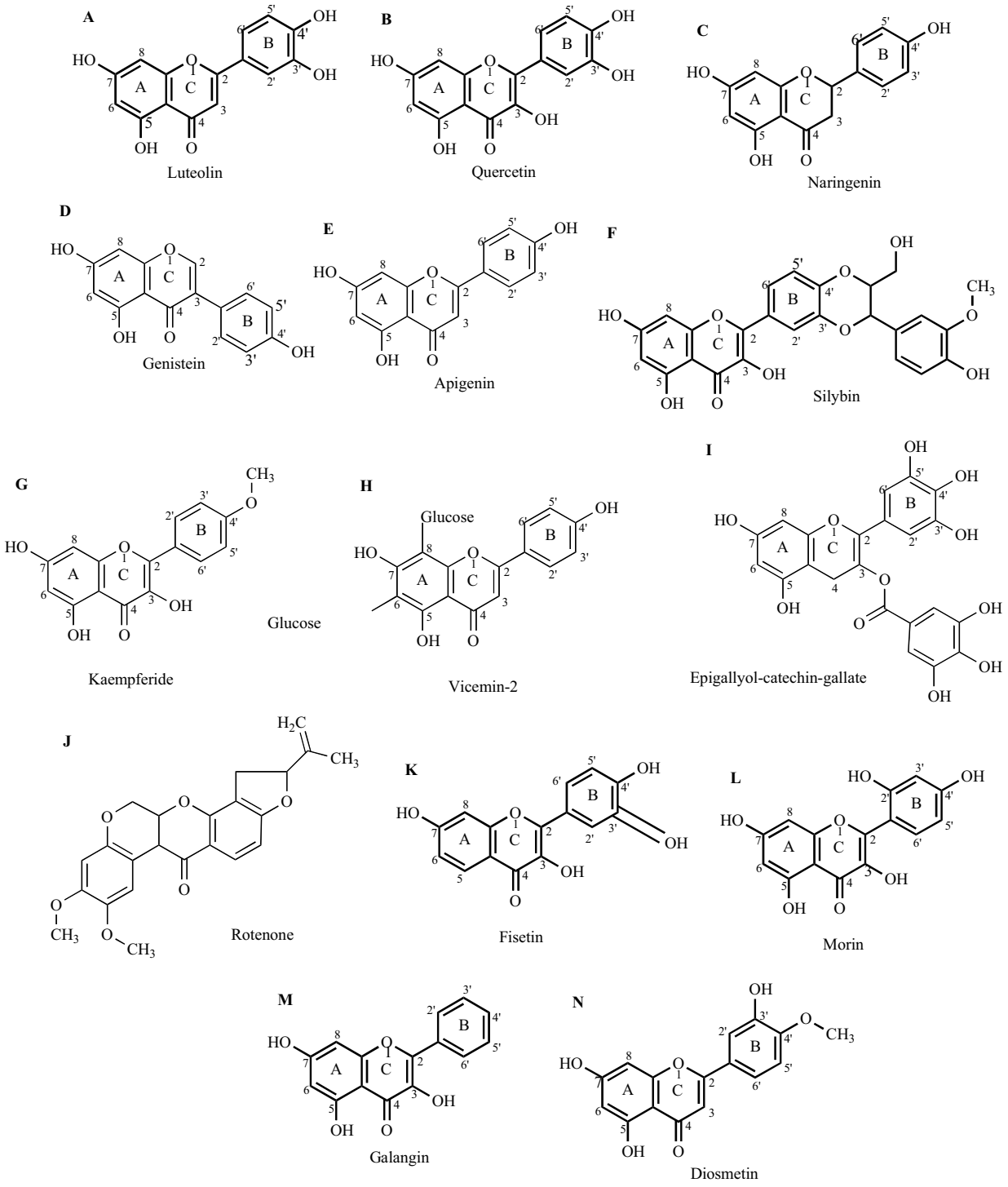

Fig. (2). Primary structure of flavonoids with antiparasitic activity.

into contact with cultured cells significantly decreases their infectivity, by 38 %. This effect is thought to be due to genistein interfering with MAP kinase activation pathways [28].

The uptake of *Leishmania donovani* by macrophages depends on protein tyrosine kinases (PTKs) and is significantly decreased by PTK inhibitors, such as genistein. However, the effects of genistein are limited to the uptake of promastigotes by macrophages, and this compound does not interfere with intracellular replication of the parasite. Published results suggest that the PTK-mediated signal is not related to the ultimate virulence mechanism associated with the intracellular replication of this pathogen [29].

Trypanosoma has kinomes, which contain large numbers of PKs and phosphatases. The PKs of *Trypanosoma* have therefore been evaluated as possible targets for treatment. The inhibitors of some PKs have been found to inhibit proliferation in these protozoans. Three have been shown to affect the growth of epimastigotes and amastigotes of *Trypanosoma cruzi*: staurosporine (which inhibits serine/threonine kinases), genistein (which inhibits tyrosine kinases), and wortmannin (which inhibits 3' phosphatidyl-inositol kinases). Staurosporine is the most effective (IC_{50} 6.43 mM). These PK inhibitors, among which flavonoids, also affect the ultrastructure of the epimastigotes, causing: (i) abnormal nuclear chromatin condensation; (ii) changes in the membrane architecture of the flagella; (iii) aborted cell division and (iv) the formation of autophagosomes. These drugs had no effect on the division of intracellular amastigotes or their differentiation into trypanomastigotes. These results may open up new possibilities for the treatment of Chagas disease [30].

3.4. Effects of Flavonoids on DNA Replication and the Induction of Apoptosis

In *Leishmania* (*L. donovani)*, as in vertebrate cells, topoisomerases affect the topology and organization of intracellular DNA. These enzymes are potentially useful as targets for the development of new antiprotozoan drugs. The bi-subunit of topoisomerase I of parasites of the kinetoplastid family (*Trypanosoma* and *Leishmania*) was recently identified as a potential target for drug development. Baicalein, luteolin and quercetin have been shown to inhibit the growth of promastigotes and amastigotes of *L donovani* by inhibiting the cell cycle and inducing cell apoptosis [31]. For example, protein kinase C (PKC) is an important constituent of the signaling pathways involved in apoptosis. Recent results indicate that the inhibition of PKC by withaferin A is a key event in the induction of apoptosis and that stabilization of the topoisomerase I–DNA complex is required to amplify apoptosis [32]. These topoisomerase I inhibitors are aromatic flavones that can bind DNA by intercalation, due to the presence of a double bond between carbons 2 and 3 of the C-ring [33, 34]. Quercetin has been shown to be highly potent but was only slightly more selective for parasite topoisomerase I than for host cell topoisomerase I [35].

These compounds also affect DNA cleavage by topoisomerase II, forming a stable complex associated with the observed cytotoxic effects on parasites *in vitro* [31].

3.5. Some Flavonoids Affect Protozoan Metabolism

In protozoans, lipid metabolism — including the synthesis pathways for phospholipids, glycosphingolipids and fatty acids and lipid degradation pathways — provides particularly interesting targets for the production of new therapeutic molecules, directed against *P. falciparum* in particular [36]. A flavonoid glycoside, luteolin-7-O-glucoside, has been shown to inhibit the FabI enzyme (enoyl-ACP-reductase). Studies of a library of derivatives of this flavonoid showed these compounds to be directed against three significant enzymes involved in the vital biosynthesis of fatty acids in *P. falciparum:* FabI, FabG (β-ketoacyl-ACP-reductase) and FabZ (β-hydroxacyl-ACP-dehydratase) [37]. Flavones with activity against enzymes involved in lipid metabolism in *P. falciparum* were found to inhibit the tested enzymes moderately (IC_{50} 10-100 μM) for simple hydroxylation patterns, such as for 6-hydroxyflavone. The more complex flavonoids, such as quercetin, had strong activity against each of the three enzymes tested (IC_{50} 0.5-8 μM). Isoflavonoids (genistein) display moderate but selective activity against FabZ. The most active compounds were found to be catechin derivatives (catechin-gallate), which strongly inhibited all three enzymes tested, with IC_{50} values of 0.2 to 1.1 μM [37].

Gallocatechin gallate and epigallocatechin gallate from green tea (*Camellia sinensis)* leaves strongly inhibit trypomastigotes of *T. cruzi* (IC_{50} 0.12 and 0.53 pM, respectively) and decrease the number of amastigotes in infected cells (IC_{50} 100 nM). This effect was attributed to inhibition of arginine kinase, a key enzyme in the energy metabolism of the parasite [38].

Several studies have highlighted the strong anti-malarial activity of chalcones. Multiple mechanisms for this activity have been identified, one of the most important of which seems to be enzyme inhibition [39]. The inhibition, by chalcones, of β-hematin formation in *Plasmodium* provides a useful example. This parasite ingests and degrades the hemoglobin of the host to obtain the amino acids essential to its survival. β-hematin (ferriprotoporphyrin IX) is one of the products of this degradation. This oxidized heme binds to a specific receptor of the parasite ("the heme-binding protein") to form the malaria pigment or hemozoin, an insoluble and inert compound. The mechanism of heme detoxication remains unknown, but is thought to involve a heme polymerase or histidine-rich protease II (HRPII), as shown by Touze *et al*. (2002) [40]. This degradation is inhibited by chalcones and chalcone derivates (sulfamide chalcones), leading to lysis of the parasite [41].

Several aurones are active against *Leishmania* and present only low levels of toxicity to host macrophages [42]. Aurones have been shown to inhibit the parasite mitochondrial fumarate reductase and to block parasite development [43, 44].

Rotenone (Fig. **2J**) is an isoflavonoid with insecticidal properties. It is not used to treat parasite infections, but is nonetheless active against several parasites of humans and animals. Its action against parasites has been linked to site I-specific inhibitor of NAD(P)H oxidase activity or cytochrome reductase activity. However, its activity depends on the parasite: rotenone is active against *Plasmodium falciparum* and *P. berghei* [45], but inactive against *P. yoelii* [46].

3.6. Reversal of Multidrug Resistance by Flavonoids

The multidrug resistance (MDR) system limits the efficacy of chemical treatments in a number of diseases, including several caused by parasites. The development and spread of drug resistance in protozoans (*P. falciparum, L. donovani* and *Toxoplasma gondii)* constitutes a serious, growing public health problem. In particular, the failure of treatment with quinolines, hydroxynaphthoquinones, lactones, sesquiterpenes, antifolates and sulfamides is associated with higher rates of morbidity and mortality in humans infected with *P. falciparum*. This multidrug resistance has been associated with a decrease in drug accumulation within

the parasitic vacuole. In this system, intramembrane proteins, such as P-glycoprotein (Pgp), pump the drugs out of the vacuole, thereby accelerating their efflux and decreasing their efficacy. Two genes encoding such pumps have been identified in *P. falciparum*: the *Pfmdr1* gene, which encodes Pgh-1, a P-glycoprotein ortholog, and the *Pfcrt* gene, which encodes PfCRT (an anion channel). A mutation in the gene encoding PfCRT has been found in isolates with multiple drug resistance. This resistance can be overcome using inhibitors of PfCRT (verapamil). The search for other less toxic inhibitors led to the identification of several flavonoids as potential candidates. These flavonoids belong to the flavone, flavanol and biflavonoid groups [47]. All flavone derivatives strongly inhibit these proteins in human cells [48].

4. EFFECTS OF FLAVONOIDS ON HELMINTHS

The natives of India have traditionally used the edible tuber of *Flemingia vestita* to treat helminth infections. Several isoflavones present in the alcoholic crude extract of this tuber have been shown to have vermifugal/vermicidal effect against cestodes and trematodes [49].

Genstein is one of the putative active compounds identified. It causes rapid muscular contraction followed by flaccid paralysis and alterations in the tegument of parasites. These effects have been observed in *Raillietina echinobothrida*, the cestode of domestic fowl [50] and *Fasciolopsis buski*, the pig intestine fluke. A dichloromethane extract of *Millettia thonningii* and a derived pure mixture of two isoflavonoids, alpinuisoflavone and dimethylalpinuisoflavone, have also been shown to kill schistosomes at two stages of development, cercariae and adults, *in vitro* [51].

The effects of these compounds have been attributed to changes in the activities of several enzymes and/or metabolic processes. Paralysis and putative vermifugal activity in trematodes have been attributed to an increase in nitric oxide (NO) production [52]. It has been suggested that NO acts as neurotransmitter at the neuromuscular junction [53] and causes myoinhibition [54] in helminth parasites. Kar *et al*. (2002) observed increases in neuronal nitric oxide synthase (nNOS) activity and NO release in worms treated *in vitro* with 1.85 mM pure genistein. This flavonoid inhibits non-specific esterases and cholinesterases in helminths, leading to neuromuscular disorganization [52].

In vertebrate cells, high NO concentrations are also known to cause oxidative stress and DNA damage, and to disrupt energy metabolism, calcium homeostasis and mitochondrial function [55]. In the cestode *R. echinobothrida*, genistein treatment has been shown to increase calcium efflux significantly [49, 50], with consequences similar to those observed in the adult liver fluke *Opisthorchis viverrini* after praziquantel treatment and attributed to calcium efflux: depolymerization of the microtrabecular network, tegumental disorganization and a breakdown of myofilaments [56].

Kar *et al*. (2004) showed that the treatment of *Fasciolopsis buski* with genistein markedly decreased the levels of several free amino acids (arginine, ornithine, tyrosine, leucine, isoleucine, valine, alanine, glycine, proline, serine, threonine, and taurine) and increased levels of glutamic acid, glutamine, phosphoserine, citrulline and gamma-aminobutyric acid (GABA) [57]. Citrulline and GABA have been implicated in changes in nitric oxide release resulting in neurological alterations.

The anthelmintic activity of genistein also involves changes in the glucose/glycogen metabolism of *R. echinobothrida* [50]. Genistein has also been used as a tyrosine kinase inhibitor in signal transduction analyses [58], with an effect observed in 24 h old schistosomula but only a partial effect observed in adult worms.

Condensed tannins (CTs) also have anthelmintic properties. CTs are polymers of flavan-3-ol units with a considerable range of structural variation. Their biological properties depend on the nature of the monomers, the degree of polymerization and inter-flavonoid linkages. Few studies have investigated the relationships between the structure and activity of flavan-3-ols and their derivatives. Nevertheless, Molan *et al*. (2003) investigated the biological effects of monomeric flavan-3-ols on the eggs and infective larvae of *Trichostrongylus colubriformis* [59]. They observed inhibitory effects on both hatching and larval development and showed that the addition of a galloyl group increased activity, with flavan-3-ol gallates (gallocatechin and epigallocatechin) more active than the procyanidins catechin and epicatechin. They also observed a correlation between the inhibition of egg hatching and hydroxylation of the B-ring, with the tri-hydroxylated form being the most active. Brunet *et al*. (2006) reported similar effects on the exsheathment of infective larvae [60].

Rotenone interacts with mitochondrial enzymes in helminths, as in protozoans. Its activity again depends on the parasite concerned. Rotenone is active against *Setaria cervi* and *Strongyloides ratti* [61-64] and inactive against *Setaria digitata* [61].

5. EFFECTS OF FLAVONOIDS ON OTHER PARASITES

Six naturally occurring rotenoid esters can be isolated from the root of *Derris eliptica,* a plant from Southeast Asia, or from *Lonchocarpus utilis* or *L. urucu* found in South America. Rotenone is the most potent. Rotenone has been used as a topical treatment for head lice, scabies, and other ectoparasites.

Rotenone has been proposed as a treatment for the honeybee mite *Varroa destructor* [65], the Atlantic salmon Plathyhelminth *Gyrodactylus salaris* [66], cattle hypodermosis [67] and the sheep louse *Bovicola ovis* [68].

6. STRUCTURE-ACTIVITY RELATIONSHIPS IN FLAVONOIDS

Comparisons between flavonoids have revealed relationships between structure and antiparasitic activity. Various substituents have been identified as playing a significant role, depending on their nature, position and the chemical form of the compounds.

6.1. Nature of Substituents

There is evidence to suggest that efficacy against the target depends on the nature of the radical added to the basic structure. Several examples illustrate this point (Fig. **3**).

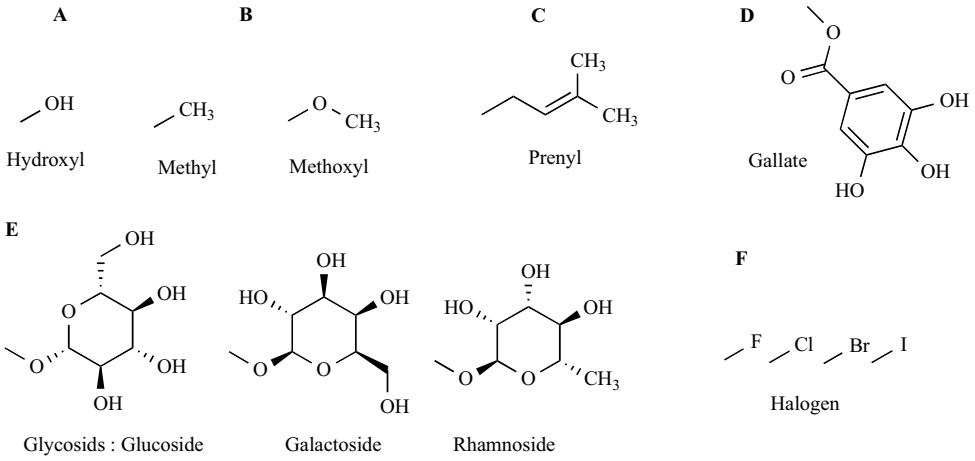

Fig. (3). Main substituents for flavonoids.

6.1.1. Hydroxyl Substituents

The hydroxylation pattern within a flavonoid structure (Fig. **3A**) has a major effect on activity. Flavones without hydroxyl groups have little or no trypanocidal effect. The insertion of a single hydroxyl group in the benzo-γ-chromone portion of the flavone structure has no marked effect, whereas the addition of two such functional groups significantly enhances trypanocidal activity. 7,8-dihydroxyflavone (IC_{50} 0.267 μM) is the most effective compound *in vitro* against *T. brucei rhodesiense,* whereas 7-hydroxyflavone (IC_{50} 28 μM) and flavone (IC_{50} 29 μM) are not very effective. In the flavon-3-ol (flavonol) series, the simplest flavonol, 3-hydroxyflavone (IC_{50} 3 μM) is seven times more potent against *Leishmania* than flavone (IC_{50} 22.5 μM). The number of hydroxyl groups and the pattern of hydroxylation on ring B affect activity, but no clear structure-activity relationship has been established. The most potent combination seems to be that of fisetin (Fig. **2K**) (IC_{50} 2.1 μM), which has four hydroxyl groups at C-3, C-7, C-3', and C-4'. In some cases, stronger activity against *Leishmania* has been associated with the presence of a catechol moiety in ring B. For example, quercetin with a catechol functional group (IC_{50} 3.3 μM) is almost three times more active than kaempferol, with a p-hydroxyphenyl ring (IC_{50} 10.1 μM), and morin (Fig. **2L**), with two meta-positioned hydroxyl groups at C-2' and C-4' (IC_{50} 9.3 μM). However, kaempferol is less active than galangin (Fig. **2M**) (IC_{50} 5.5 μM), which bears an unsubstituted B ring [69].

6.1.2. Methyl or Methoxyl Substituents

The replacement of the hydroxyl groups on the benzochromone skeleton or on the side chain by methoxyl groups (Fig. **3B**) decreases activity by a factor of at least two. For example, the IC_{50} of apigenin is 7 μM, whereas that of genkwanin (7-methoxyapigenin) is 33 μM. Moreover, larger numbers of methoxyl functions seem to be associated with lower levels of activity against *Leishmania,* as shown for luteolin (IC_{50} 2.8 μM), diosmetin (Fig. **2N**) (IC_{50} 23.6 μM) and luteolin-tetramethylether (IC_{50} > 70 μM). Several different trends are associated with methylation of the hydroxyl groups on the flavone structure. In most cases, methylation greatly decreases trypanocidal activity [69]. This situation is clearly illustrated by two pairs of molecules: 7,8-dihydroxy-flavone and 7,8-dimethoxyflavone (IC_{50} 0.267 and 21.6 μM, respectively) and 7,8,3',4'-tetrahydroxyflavone and 7,8,3',4'-tetramethoxyflavone (IC_{50} 1.75 and 196 μM, respectively). In some cases, methylation has a less significant effect on flavone activity. This is the case for apigenin and genkwanin (IC_{50} 18.9 and 28.1 μM, respectively), scutellarein, ladanein and cirsimaritin (IC_{50} 16, 8, and 10.5 μM, respectively) and for luteolin, diosmetin and luteolin tetramethylether (IC_{50} 12.9, 20.3, and 10.3 μM, respectively). A different pattern is observed for chrysin (IC_{50} 20.8 μM) and its methoxylated derivatives. Tectochrysin, the monomethoxy derivative, is almost inactive (IC_{50} 321 μM), whereas chrysin dimethylether has a similar level of trypanocidal activity (IC_{50} 29.5 μM) to chrysin [69].

6.1.3. Prenyl Substituents

Chalcones have antiplasmodial properties. The *in vitro* activity of xanthohumol, a prenylated chalcone (Fig. **3C**), and seven derivatives was evaluated against chloroquine-sensitive or multiresistant strains [70]. The effect of these compounds on glutathione (GSH)-dependent hemin degradation was also analyzed. Four compounds were active, with IC_{50} values below 25 μM for at least one of the two strains. Xanthohumol was the most active compound tested. Three of the compounds tested interfered with the hemin-degradation process, an effect potentially contributing to their antiplasmodial activity. Nevertheless, as one compound inhibited *P. falciparum* development without affecting hemin degradation, other modes of action must also operate.

6.1.4. Gallate Substituents

Several polyphenols with gallate substituents (Fig. **3D**) have been shown to have antiparasitic properties. The flavonol arabinoside, 5-galloylquercetin-3-O-alpha-L-arabinofuranoside, has weak activity against chloroquine-resistant strains of *P. falciparum* (IC_{50} 14.5 μM) and is not cytotoxic to mammalian cells [71]. Another polyphenol, (-)-epigallocatechin-3-gallate, was found to have a significant effect against fatal scuticociliatosis in farmed turbot, which is caused by the histiophagous ciliate *Philasterides dicentrachi* [72].

In catechins with a flavan-3-ol structure, the gallate substitution is essential for inhibitory activity. All C3-galloyl ester derivatives strongly inhibited all three enzymes tested. Interestingly, luteolin and (-)-catechin gallate are active against both susceptible and resistant *P. falciparum.* The stereochemistry at C2 and the hydroxylation pattern of ring B have no major effect [69].

Galloyl moieties increase the toxicity to mammalian macrophages of hydrolysable tannins active against *L. donovani* amastigotes, this toxicity being attributed to tumor necrosis factor (TNF) induction [22].

6.1.5. Glycoside Substituents

Sugar groups decrease lipophilicity and activity against *Leishmania*, particularly at the intracellular amastigote stage [44]. The attachment of one or more sugar units at the C-5 or C-7 position of the flavone skeleton (Fig. **3E**) may decrease potency against *Leishmania* slightly or markedly, as observed for apigenin and luteolin. The glycosylation of hydroxyl groups often dramatically reduces activity. Nevertheless, one common flavonoid glycoside, luteolin-7-O-glucoside, has antimalarial activity as it inhibits the FabI enzyme in P. falciparum with a potency similar to that for the non glycosylated form (IC_{50} 2.5 μM and 2.8 μM, respectively; [37]. An isoflavone, puerarin, the 8-C-glucosyl derivative of daidzen, has amoebicidal and amoebostatic activity *in vitro* against pathogenic *Acanthamoeba* spp. [73]. The use of these new molecules should make it possible to limit parasite development and drug resistance.

6.1.6. Halogen Substituents

Boeck *et al*. (2006) tested analogs of the natural chalcone 2'-hydroxy-4',6'-dimethoxychalcone derived from xanthoxyline present in the leaves and stems of *Sebastian chottia* [74]. Derivatives containing fluorine or bromine groups (Fig. **3F**) were more selective than the natural chalcone.

6.1.7. Sulfonamide Derivatives

Chalcones and sulfamide chalcones (Fig. **4A**) inhibit β-hematin formation in *P. falciparum,* leading to parasite lysis. These results suggest that efficacy against the target depends on the nature of the radical added to the basic chalcone structure [39]. In one study, a series of sulfonamide derivatives was synthesized and assessed for *P. falciparum* inhibition *in vitro* [41]. Inhibition was minimal with the aromatic ring chalcone moiety and greatest if the aromatic nucleus was sulfonamized. The 3,4,5-trimethoxyl and 3-pyridinyl substitutions were the most effective. The most active compound was 1[4' – N (2",5"-dichlorophenyl) sulfonyl-amidephenyl]-3-(4-methylphenyl)-2-propen-1-one, which was effective at a dose of 1 μM.

6.1.8. Phenylurenyl Derivatives

Phenylurenyl derivatives of chalcones (Fig. **4B**) have also been synthesized and assessed for their ability to inhibit the *P. falciparum* cysteine protease falcipain-2, which hydrolyzes globin [75]. The most active derivative was 1-[3'-*NR*-(*NR*'-phenylurenyl)phenyl]-3(3,4,5-trimethoxyphenyl)-2-propen-1-one, which was effective at a dose of 1.76 μM [75].

6.1.9. Chromenylated Derivatives

Another new group of chalcones, chromenochalcones (Fig. **4C**), has been tested *in vitro* for activity against the extracellular promastigotes or intracellular amastigotes of *Leishmania donovani*. The most effective compound was a chromenochalcone with a pyridine ring A, which gave 96 % inhibition of promastigotes (IC_{50} 25 μM) and 96 % inhibition of amastigotes when used at a concentration of 25 μM [76].

6.2. Positions of Substituents

The presence of a carbonyl group at position 4, a double bond between carbon atoms 2 and 3, or a hydroxyl group in position 3 of the C ring appears to be required for optimal antioxidant activity [77].

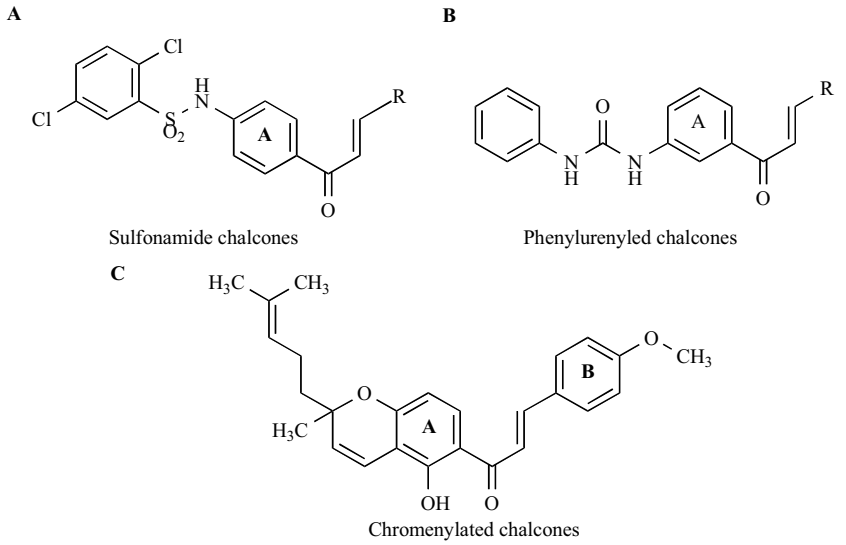

Fig. (4). Derivatives of chalcones.

Nevertheless, the presence of a 3',4',5'-trihydroxyphenyl (pyrogallol) moiety in ring B of a flavonol increases its selectivity for the FabI enzyme, whereas methylation of any of the phenolic hydroxyl groups leads to a loss of inhibitory effect. Isoflavonoids have moderate but selective activity against FabZ in *P. falciparum* [36, 37].

Flavonoids isolated from *Chromolaena hirsute* (Asteraceae) are active against trypomastigote forms of *Trypanosoma cruzi* (6,7,3',4'-tetramethoxyluteollin, 3,6,3'-trimethoxyquercetagetin, 3,6-dimethoxyquercetagetin, 3,6,7-trimethoxyquercetagenin, quercetin and quercetin-3-O -L-rhamnoside). Nevertheless, only those with dihydroxy groups in the B-ring, specifically at positions C-3' and C-4' (3, 6,7-trimethoxyquercetagenin, quercetin and quercetin-3-O -L-rhamnoside), were active against promastigote forms of *Leishmania amazonensis,* whereas those with a methoxy group at the C-3' position were inactive. These molecules had strong antiproliferative effects [78].

The planar nature of flavonoids is important for their activity, as all active flavonoids have a planar structure. Several lines of evidence confirm this hypothesis. Loss of the double bond between C-2 and C-3 abolished activity. Chrysin dimethylether is active whereas ladanein is inactive against *T. cruzi* and *L. donovani*. The catechin without a galloyl group at C-3 also lacks this double bond and deviates from the planar conformation required for binding to targets [69]. The insertion of two hydroxyl functional groups significantly increases leishmanicidal efficiency. Hydroxyl groups in positions C-5, C-7, and C-8 were particularly efficient for inhibiting parasite development [69].

Almost all substitutions at the *para* position of B ring of chalcones reduced both activity against *Leishmania* and macrophage toxicity (e.g. 4-methoxy-2'-hydroxy-4',6'-dimethoxychalcone). The phenyl vinyl structure was identified as important for activity against *Leishmania*. However, modified chalcones with an nitro (NO_2), bromo (Br) or chloro (Cl) substituent in the *meta* position of the B ring should be investigated with a view to developing compounds highly selective against *Leishmania* (4-bromo-2'-hydroxy-4',6'-dimethoxychalcone). These findings indicate a significant increase in the activity of the natural chalcone DMC (2',6'-dihydroxy-4'-methoxychalcone) and of related compounds with substitutions in the B ring against *Leishmania* [74].

Substitutions on ring B of the chalcone template increase activity more strongly than substitutions on ring A, the strongest effects being observed with methoxy or dimethoxy groups. Lipophilicity does not seem to be determinant for activity. These substituted chalcones seem to display selective toxicity against *Plasmodium* species, making them potentially useful for the treatment of malaria [17].

6.3. Chemical Form

The chemical form, such as whether the molecule is dimeric or isomeric, may also have a significant effect on drug efficacy and cytotoxicity. The results obtained by Mbwambo *et al.*, (2006) illustrate these relationships [79]. These authors characterized and compared a new biflavonoid, *ent*-naringeninyl-(I-3alpha,II-8)-4'-*O*-methylnaringenin, two known biflavonoids and five xanthones isolated from the root of *Garcinia livingstonei* from Tanzania. The three monomeric trioxygenated prenylated xanthones were inactive or displayed only weak activity against *L. infantum* and *P. falciparum,* whereas they were highly active against *Trypanosoma*, especially *T. brucei* (IC_{50} 0.87 μM). They displayed no cytotoxicity in the concentration range tested. The differences in activity and cytotoxicity observed with dimeric xanthones were mostly attributable to the isomeric form rather than to dimerization. The biflavonoids (+)-volkensiflavone and (+)-morelloflavone were found to be almost inactive. The new biflavonoid was active only against *P. falciparum*. A marked difference was observed between garcilivins A and C, which are diastereoisomers. Garcilivin A displayed nonselective activity against all parasites tested whereas garcilivin C was active only against *T. brucei*. Garcivilin A was also 25 times more cytotoxic than garcivilin C [79]).

Chalcones can also exist in two isomeric forms, Z or E (Fig. **5**) [80]. Efforts have been made to identify the most active form of chalcones against malaria, based on the successive locking of isomers. The Z-locked isomer is almost inactive, whereas the E-locked isomer had a similar level of activity to the parent chalcone. The E isomer is the active form of the molecule. Isomer E chalcones could therefore potentially be used to treat malaria [80].

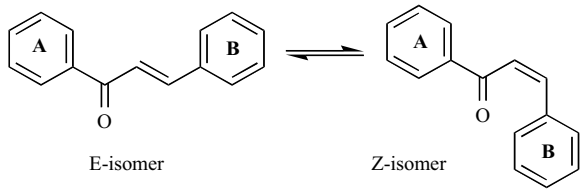

Fig. (5). Chemical form of flavonoids.

7. BIOAVAILABILITY AND TOXICITY FOR MAMMALS

Flavonoids are common components of the human diet. Daily intake has been estimated at 20 mg to 1 g. Although generally considered safe and often beneficial, some adverse cellular effects of flavonoids have been identified. Information on bioavailability is crucial for investigating the health effects of flavonoids, either in the prevention and treatment of many diseases or in prevention of toxicity. The bioavailability depends not only on the intake but also on several other factors such as intestinal absorption, metabolism and excretion.

Although the distribution of flavonoids is widespread in many plants, their average daily intake greatly vary around the world with large differences between Western and Eastern populations. As an example, the average intake of isoflavones in Western populations is very low (< 1 mg/day, [81]) while the Japanese people consume soy products containing 30-50 mg of isoflavonoids daily [82]. The isoflavonoid plasma level has been shown to be 7-110 times higher in Japanese men than in Finnish men.

Absorption and bioavailability have also been shown to differ greatly from one flavonoid to another. The most abundant flavonoids in the diet are not those leading to the high-

est concentrations in the target tissues. The metabolism can result in urinary excretions ranging from 0.3 % to 43 % of the ingested dose according to the flavonoid [83]. The authors also observed that the plasma kinetics differ among polyphenols. Gallic acid is far better absorbed than the other polyphenols while anthocyanains are very poorly absorbed. The substituents of the molecule also plays a significant role : galloylation of the epigallocatechin markedly reduced its absorption. The time for maximal plasma concentration is longer for compounds absorbed after release of aglycones or hydrolyse by gut microflora [83]. The metabolism of flavonoids is thus dependent on the different composition of intestinal microflora. Extensive variability is observed in the absorption that have been attributed to several factors such as food matrix, background diet, inter-individual variations, levels of metabolizing enzymes or transporters. A high fiber diet may promote the growth and activity of microorganisms responsible for the enzymatic hydrolysis of flavonoids and the production of active metabolites while antibiotics destroying microflora stop the process of bioconversion [81]. When flavonoids exist as glycosides, the glycosidic bounds are hydrolyzed by glucosidases of intestinal bacteria. Nutriments stimulating the growth of bifidobacteria in the intestine have been shown to affect the bioavailability of phytooestrogen glycosides and therefore improve their absorption in the gut [82].

Many flavonoids possess several more or less toxic effects. They can produce reactive oxygen species (ROS) and can act as "pro-oxidants" in the presence of redox-active metals, such as copper or iron, and oxygen. They autooxidize to produce ROS that can damage DNA, lipids and other biological molecules, at least *in vitro*. However, *in vivo*, these metal ions are sequestered in forms unable to catalyze free radical reactions. The effects observed depend on chemical structure. Flavonols with pyrogallol or catechol B rings produce ROS. Catechins induce hydrogen peroxide (H_2O_2) generation. Following peroxidase-catalyzed oxidation, phenol-B-ring flavonoids (apigenin, naringenin) produce phenoxyl radicals, which catalyze GSH or NADH cooxidation and generate ROS [84, 85]. Nevertheless, there is equilibrium between the antioxidant properties and prooxidant properties of these molecules.

Mitochondrial toxicity due to the collapse of membrane potential has been demonstrated for some flavonoids or plant extracts rich in flavonoids such as curcumin, baicalin and capsaicin. Flavonoids can also act on phase I and phase II metabolizing enzymes, interact with cellular efflux pumps and cause drug interactions (inhibition or potentialization).

In vivo, some flavonoids have been shown to cause anemia, thrombocytopenia, liver failure, nervous damage and embryo toxicity. Nevertheless, potential safety issues arise only if extremely large doses of flavonoids or isoflavones are consumed daily [86].

Rotenone is highly irritating to the eyes (conjunctivitis), the skin (dermatitis), and the upper respiratory tract (rhinitis) and throat (pharyngitis). Acute poisoning causes an anesthetic-like effect, with initial respiratory stimulation followed by respiratory depression, ataxia, convulsions, and death due to respiratory arrest [87]. Rotenone blocks electron transport in mitochondria by inhibiting oxidation linked to $NADH_2$ [88, 89]. Crystalline rotenone has an acute oral lethal dose (LD_{50}) of 60, 132 and 3000 mg/kg for guinea pigs, rats, and rabbits, respectively [90]. It is unstable in light and heat and almost all toxicity is lost after two to three days of storage in the summer. It is very toxic to fish, and has been used over centuries by indigenous peoples to paralyze fish for capture and consumption.

8. CONDITIONS OF USE AND VALUE OF FLAVONOIDS AS ANTIPARASITIC DRUGS

The natural extraction or chemical synthesis of flavonoids may involve procedures of various complexities. Flavonoids are present in significant amounts in plants. Thus, extracts of *Camellia sinensis,* gold-green tea, are a popular dietary supplement. Approximately 1 kg of *C. sinensis* leaves yield 100 g of solids, about 10 % of which are polyphenols corresponding to 50-65 % epigallocatechin-3-O-gallate, 13-20 % epicatechin-3-O-gallate, 2-4 % epicatechin and 1.5-3 % epigallocatechin.

However, the flavonoids in plant extracts are unstable due to the presence of free phenolic groups, which oxidize readily after contact with oxygen or light and lose all activity. The material can be stabilized by forming esters. This has the advantage of ensuring stability and rendering the material liposoluble without affecting its antioxidant and anti-enzyme properties. *In vivo*, esterases should cleave the ester group, releasing the original flavonoid and the organic acid used to form the ester.

Many flavonoids initially extracted and identified in plants are now chemically synthesized. Chemical modifications (addition of halogens, sulfur, nitrogenous or aromatic groups) have made it possible to increase the activities of many molecules against various parasites [91, 92]. Nevertheless, the use of synthesized chemicals identical to those found in plants but stabilized by esterification brings us back to the debate between chemists and consumers about the natural or artificial nature of compounds.

Another way of increasing the efficacy of flavonoids is to use a special formulation. Attempts have been made to increase the efficacy of quercetin for treating experimental leishmaniasis *in vivo* in hamsters and to reduce its toxicity by means of nanocapsulation [93]. The nanocapsulated formulation is more potent, and its hepatotoxicity and renal toxicity are lower than those of the uncapsulated drug.

The large number of flavonoids available also makes the screening of their activity time-consuming and repetitive, even if some data are available for the establishment of structure-activity relationships. There are many cellular targets, differing for different genera and species of parasites. Flavonoid activity has often been compared with that of reference products, which differs considerably between protozoa, helminths and ectoparasites. This makes comparative analyses of efficacy against various families of parasites difficult.

CONCLUSION

Parasitic diseases affect millions of people and animals throughout the world. They cause particularly severe problems

in Africa, Asia, and South America, where available drugs are often too expensive for widespread use. Moreover, hopes of eradicating these diseases were dashed in the last few decades by the emergence of multidrug resistance to most of the available chemical treatments in these parasites. No effective vaccine against these parasites has yet been produced. The production of new natural substances with low toxicity in mammals, such as flavonoids, provides a new hope for improvements in treatment efficacy at low cost.

ABBREVIATIONS

DNA	=	DesoxyriboNucleic Acid
HPLC	=	High Performance Liquid Chromatography
IC_{50}	=	Inhibitory Concentration for 50 %
MDR	=	MultiDrug Resistance
NADH	=	Nicotinamide Adenine dinucleotide
NADPH	=	Nicotinamide Adenine Dinucleotide Phosphate
NO	=	Nitric Oxyde
PKs	=	Protein Kinases

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