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The Antiprotozoan Potential of Flavonoids

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ABSTRACT

Flavonoids are a large group of plant secondary metabolites known for their wide range of biological and pharmacological activities including antioxidant, cytotoxic, anticancer, cardioprotective, hepatoprotective, neuroprotective, antibacterial and antimicrobial properties. However, although not always recognized, they also displayed strong antiprotozoan potentials. The purpose of this paper is to provide comprehensive review of the antiprotozoan activities of flavonoids with an emphasis on their antimalarial, antileishmanial, antitrypanosomal and antiameial properties.

KEY WORDS: Flavonoids, antimalarial, antileishmanial, antitrypanosomal and antiameial activities.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds having a C6-C3-C6 arrangement as basic skeleton, with C6 being a benzene ring. It is a large family of compounds comprising about 12 classes, different from each other by the degree of oxidation of the C ring (viz. flavones, flavonols, flavans, flavanones, flavanols, chalcones and dihydrochalcones, isoflavones, aurones, anthocyanins and anthocyanidins, catechins etc... see Scheme 1). Previously known mainly as responsible for flowers pigmentation, flavonoids are more and more reported from different other parts of the plant (fruits, nuts, seeds, stem-barks, roots etc...). Till date, more than 6500 flavonoids and flavonoid glycosides have been reported from natural sources (1-2). They are known to display a wide range of biological and pharmacological activities including antioxidant, cytotoxic, anticancer, cardioprotective, hepatoprotective, neuroprotective, antibacterial and antimicrobial properties (3-7). These biological and pharmacological properties are usually attributed to their free radical scavenging efficacies, metal complexation capabilities, and their ability to bind to proteins with a high degree of specificity. In this review, focus is given to their antimalarial, antileishmanial, antitrypanosomal and antiameial activities. Their putative drug target and possible mechanism of action are also explored.

Scheme 1: Different classes of flavonoids and their possible interchangeability
MALARIA

Malaria is one of the major growing protozoan diseases in many tropical and subtropical regions of the world, with over 500 million clinical episodes, and claiming more 1.5 million deaths annually, mostly young children and pregnant women (8). The incidence of malaria is now increasing because of the ever widening level of *Plasmodium falciparum* resistance to nearly all the available antimalarial drugs. The Roll Back Malaria initiative recently established by WHO aims to combat the disease through effective global partnership and cooperation (9), and the major thrust in this initiative is the identification of new targets that are critical to the disease process or essential for the survival of the parasite. The identification and/or the design of novel chemical entities specifically affecting these targets could lead to the availability of better drugs for the treatment of malaria.

Natural products have proven to be a great source of new biologically active compounds. Thus, in an effort to discover new lead antimalarial compounds, several research group screen plant extracts to detect secondary metabolites with relevant biological activities that could served as templates for the development of new drugs. Flavonoids have been isolated and characterized from many medicinal plants used in malaria endemic areas (10). However, controversial data have been obtained regarding their antiplasmodial activity, probably because of their structural diversity (11-13). More recently, several flavonoids have been isolated from *Artemisia afra* (14) and *Artemisia indica* (15), two plants related to *Artemisia annua*, the famous traditional Chinese medicinal plant from which artemisinin is isolated.

**Antimalarial chalcones**

Chalcones, or 1,3-dihydro-2-propen-1-ones are one of the major classes of natural products of the flavonoid family. Structurally, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system (see Scheme 1). They are known to possess many biological and pharmacological properties (4, 16-17). Licochalcone A, isolated from *Glycyrrhiza inflata*, was initially proposed as new antimalarial agent in 1994 (18), and since then, many chalcones, naturally occurring or synthesized, have been identified as potential antimalarial agents, using both molecular modelling and *in vitro* testing against active parasites.

Crotatoxin from *Crotalaria orixensis*, medicagenin from *Crotalaria medicagenia*, crotaramosmin, crotaramin and cotrin from *Crotalaria ramossissima*, five prenylated chalcones, were evaluated *in vitro*, at different concentrations (2, 10 and 50 µg/mL), as inhibitors of schizont maturation against NF-54 strain of *P. falciparum* (19). Crotatoxin exhibited 100% inhibition of maturation of parasites from ring to schizont stage, at 10 µg/mL concentration. Medicagenin was the most potent with complete inhibition at 2 µg/mL while the chromenodihydrochalcones (crotaramosmin, crotaramin and cotrin) displayed lower order of activity (19). Bartericins A and B, stipulin, 4-hydroxychalconarpin, isobavachalcone and kanzonol B, six chalcones from *Dorstenia barteri var. subtriangularis*, were evaluated in culture against W2 strain of *P. falciparum*. Only bartericin A, stipulin and 4-hydroxychalconarpin showed some antimalarial activity with relatively low IC₅₀ values (2.5, 5.1 and 3.4 µM, respectively) (20). A series of unusual chalcones (hostmannins A, B and C together with Z,6-dihydroxy-4'-methoxydihydrochalcone and (E)-methylindoleralin isolated from the leaves of *Piper hostmannianum var. berbicense* were evaluated in *vitro* against both chloroquine-sensitive and -resistant strains of *P. falciparum* (F32, FcB1), and *in vivo* against *P. vinckei petteri* in mice. Z,6-Dihydroxy-4'-methoxydihydrochalcone exhibited the most potent antiplasmodial activity against both chloroquine-sensitive and -resistant strains of *P. falciparum* (F32, FcB1). But, *in vivo* at a dose of 20 mg/kg/day, none of the compounds were active (21).

As result of the interesting antimalarial activity of naturally occurring licochalcone A, and in order to build some structure activity relationship, several chalcone derivatives have been prepared and evaluated (*in vitro* and *in vivo*) for their antimalarial activity. Li *et al* (22), using molecular modeling, have designed and prepared a series of chalcone derivatives, many of which were identified as potential antimalarials using *in vitro* testing against active parasites. These compounds were screened *in vitro* against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*, and shown to be active at the nanomolar range concentrations. The most active compound was (E)-3-(2-chloro-6,7-dimethoxyquinolin-3-yl)-1-(2,4-dichlorophenyl)prop-2-en-1-one, with an IC₅₀ value of 200 nM against both chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains (22). The resistance indexes for all compounds were substantially lower than that of chloroquine, suggesting that this series of compounds will be active against chloroquine-resistant malaria (22). 2,4-Dimethoxy-4'-butoxychalcone, an analog of licochalcone A prepared by Chen *et al* (23), also appeared to inhibit the *in vitro* growth of both a chloroquine-susceptible (3D7) and a chloroquine-resistant (Dd2) strains of *P. falciparum* in a [*H]*hypoxanthine uptake assay. When administrated either orally, intraperitoneally or subcutaneously to rats infected with *P. berghei* for 5 days, this compound appeared to protect the mice from otherwise lethal infection of this parasite, through a significant reduction of the parasitemia (23). However, in a study performed by Gutteridge *et al* (24), on sixteen 3-naphthalenyl-1-phenyl-2-prop-1-enones and ten 1-phenyl-3-pyridinyl-2-prop-1-enones, a series of chalcone analogs in which the 3-phenyl group is replaced by either a pyridine or naphthalene, no inhibitors with submicromolar efficacy against *P. falciparum* were identified (24). Some retinoid-like chalcones and diverse derivatives related to licochalcone A were also synthesized and evaluated for their antimalarial potency (25). The 4-hydroxy-chalcone-like compound was the most active, with IC₅₀ values of 4.9 and 8.5 µM against K1 and Thai strains, respectively (25). The chalcones drug targets in malaria parasites are not yet clearly identified. Parasites have exploited unique energy metabolic pathways as adaptations to the natural host habitat. In fact, the respiratory systems of parasites typically show greater diversity in electron transfer pathways than do those of host animals. These unique aspects of parasite
mitochondria and related enzymes represent promising targets for chemotherapy. In contrast to the potent inhibitory effect of chalcones on the mitochondrial functions of Leishmania, their action on Plasmodium is questionable since the mitochondria of the blood-stage malaria parasites was historically considered to be functionally quiescent (25). However recent bioenergetic experiments (26) and data from genome project (27) suggest that the mitochondria of Plasmodium blood stage is able to perform oxidative phosphorylation, and could harbor full tricarboxylic acid cycle enzymes (25). Thus, the inhibitory effect of licochalcone A on the bc, complex (ubiquinol-cytochrome c reductase) as well as complex II (succinate ubiquinone reductase, SQR) of P. falciparum mitochondria was investigated (28). Licochalcone A appeared to inhibit bc, complex activity at very low concentrations (28).

Through computational approach, a series of chalcones were also identified as potential inhibitors of P. falciparum cysteine protease (29). Sixteen antimalarial alkoxylated and hydroxylated chalcones were then tested for their ability to inhibit recombinant plasmepsin II in vitro. The best inhibitory compounds appeared to carry either a chloro or dimethylamino group at the 4-position of Ph ring A of the chalcone template (30). However, subsequent investigations showed that the antiplasmodial activity of chalcones does not necessarily correlates with their inhibition of Plasmodium's cysteine protease properties (31).

A series of alkoxylated and hydroxylated chalcones previously reported to have antiplasmodial activities in vitro were also investigated for their effects on the new permeation pathways induced by the malaria parasite in the host erythrocyte membrane (32). Of 21 compounds with good antiplasmodial activities, 8 members were found to inhibit sorbitol-induced lysis of parasitized erythrocytes to a significant extent at a concentration close to their antiplasmodial IC50 (32). Structure-activity analysis suggested that activity was governed to a greater extent by a substitution on ring B than on ring A of the chalcone template. Most of the active compounds were those carrying methoxy or dimethoxy groups on ring B. However, more than half of the chalcones with good antiplasmodial activities did not inhibit the parasite-induced channels to a significant degree (32). This observation reinforces the point that structurally different chalcones may exert their antiplasmodial activities by different routes or additional pathways in the parasitized cell. Lipophilicity did not appear to be an important determinant for activity. Although they are not exceptionally potent as inhibitors (lowest IC50, 1.9 μM), chalcones compare favorably with other more potent inhibitors in terms of their selective toxicities against Plasmodia, and their neutral character is also an important asset (32). It is important to notice that these series of compounds had negligible effects on the degradation of methemoglobin by crude Plasmodium extracts and did not inhibit malaria cysteine protease falcipain 2 or interfere with heme assembly process (32).

The malaria parasite has a limited capacity for de novo amino acids synthesis, and its survival is dependent on hemoglobin proteolysis. The amino acids derived from the hydrolysis of globins are incorporated into the parasite’s proteins and appear to be available also for energy metabolism (33). This digestion of hemoglobin releases heme, which is oxidized to hematin, also known as ferricoprotoporphyrin IX (33). Free hematin can damage cellular metabolism by inhibition of enzymes, peroxidation of membranes, and production of oxidative free radicals in the acidic environment of the digestive vacuole (34). Lacking the heme oxygenase that vertebrates use for heme catabolism, plasmodial species sequester this toxic by-product into a chemically inert crystal (34). The product formed, hemozoin, is an aggregate of dimeric units of hematin linked by carboxylate-iron(III) and carboxylate-carboxylate coordinated bonds (35-37). The aggregation process is a mechanism of detoxification that can be used as a target for antimalarial therapy (38-40). Thus, several antimalarials are proposed to work by complexing, and thus metabolically stabilizing a derivative form of hematin, avoiding the association of these units into hemozoin (42-43). The death of the parasite arises as a consequence of the toxicity of the free hematin (44-48). A series of sulphonamide chalcone derivatives (49) and phenylurenyl chalcone derivatives (50) were prepared and their ability to inhibit B-hematin formation investigated. Although some inhibition was observed, the mechanism of action of these compounds did not appear to be related to haemoglobin hydrolysis or heme detoxification (50).

An alternative heme detoxification mechanism in which non-polymerized hematin exits the food vacuole into the parasite’s cytosol where it is degraded by glutathione (GSH), is also described (48). Xanthohumol, a well known antimalarial chalcone, and seven derivatives were evaluated for their antiplasmodial activity as well as for their influence on the (GSH)-dependent hematin degradation. Although many of these compounds appeared to display relatively good antimalarial activity, only three of them were additionally active in the (GSH)-dependent hematin degradation assay (51). Again, their antiplasmodial activity did not appear to be related to their effect on (GSH)-dependent hematin degradation pathway (51).

Although significant efforts have been deployed in order to elucidate the antimalarial mechanism of action of chalcones as well as their putative drug target(s), the subject remains controversial and extremely confusing. It may be that the real drug target(s) and the mechanism of action of chalcones are still to be found, or that the structural diversity of chalcones implies different drugs targets and different impact on different pathways, or may be that all chalcones act simultaneously on different drug targets through different mechanisms of action. Whatever the case may be, the undisputable truth is that this subject requires further investigations.
Other antimalarial flavonoids

Chalcones are not the only flavonoids that have displayed useable antimalarial activity. Several flavones, flavanones, isoflavones and flavonoids glycosides are also reported. Artocarpones A and B, artonin A, cyclohetophyllin, artindonesianins E, R and A-2, heterophyllin and heteroflavanone C, 9 flavonoids isolated from of Artocarpus champeden were found to possess strong antimalarial activity against 3D7 clone strain of *P. falciparum*, with IC₅₀ values comprise between 0.001 and 1.3 µM (52). A series of flavone derivatives containing a piperazinyl side chain were synthesized and tested for their antiplasmodial activity against chloroquine-sensitive (Thai) and -resistant (FCB1, K1) strains of *P. falciparum*. These compounds displayed different levels of activity, with the most potent being those having a 2,3,4-trimethoxybenzylpiperazinyl side chain attached to the flavone at the 7-phenol group (53). Dehydrodiosylbin and 8-(1;1)-dimethylallyl-kaempferide, while evaluated in *vitro* for their antiplasmodial activity by real time PCR against five *P. falciparum* strains (3D7, HB3, Dd2, W2 and 7G8), revealed significant antimalarial activity against all the different strains (2). Four lavandulyl flavanones, (2S)-2'-methoxykurarinone, sophoraflavanone G and leachianone A, isolated from Sophora flavescens, were found to display moderate antimalarial activities, with EC₅₀ values of 2.4, 2.6, and 2.1 µM, respectively (54). Exiguaflavones A and B, two geranylated flavanones isolated from Artemisia indica also exhibited moderate *in vitro* antimalarial activity, with IC₅₀ values of 4.60 and 7.1 µg/mL, respectively (15). From six isoflavones (biochanin A, calycosin, formononetin, genistein, pratensein, and prunetin) isolated from Andira inermis, only calycosin and genistein appeared to display moderate *in vitro* activity against the chloroquine-sensitive strain POW (IC₅₀ 4.2 and 2.0 µg/mL, respectively) and the chloroquine-resistant clone Dd2 (IC₅₀ 9.8 and 4.1 µg/mL, respectively) of *P.
falciparum (55). 8-Prenylmucronulatol, glyasperin H and smiracinin, 3 isoflavans from Smirnowia iranica were also found to possess moderate antimalarial activity against 3D7 strain of P. falciparum (56), while 4,6,4'-triacetyl-3',5'-dimethoxy-2-aurone was found to display strong antimalarial activity, with IC\textsubscript{50} values of 0.007 and 0.18 µM against K1 and NF54 strains of P. falciparum, respectively (57). Interestingly, the multiple drug-resistant P. falciparum strain K1 appeared to be more sensitive to the tested aurones than the drug-susceptible strain NF54 (57).

A series of glycosylflavones isolated from Clidemia sericea were evaluated for their antimalarial activity against a chloroquine-resistant P. falciparum strain. Among them, 2",6"-O-digalloylvitexin and 2"-O-galloylvitexin showed a marginal antimalarial activity (24 and 38 µM, respectively) (58). Kaempoferol 3-O-α-L-rhamnopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-galactopyranoside, kaempferol 3-O-β-rutinoside, kaempferol 3-O-β-D-xylopyranosyl-(1→2)-β-D-galactopyranoside, rutin, isorhamnetin 3-B-O-rutinoside, multiside A, multiside A acetate, quercitrin and isoquercitrin also showed characteristic proliferation inhibition of P. falciparum at significantly low concentration without showing any cytotoxicity (59).

Some biflavonoids have also been reported for their antimalarial activity. Sikokianin B and C (IC\textsubscript{50} 0.54 and 0.56 µg/mL, respectively) from Wikstroemia indica (60), lanaroflavone (IC\textsubscript{50} 0.20 µg/mL and selectivity index = 159), bilobetin (IC\textsubscript{50} 6.7 µg/mL), ginkgetin (IC\textsubscript{50} 2.0 µg/mL), isoginkgetin (IC\textsubscript{50} 3.5 µg/mL) and sciadopitysin (IC\textsubscript{50} 1.4 µg/mL), a series of biflavonoids isolated from Campnosperma panamensis (61-62), liquiritigenin (IC\textsubscript{50} 0.08 µg/mL) and isoliquiritigenin (IC\textsubscript{50} 5.2 µg/mL) isolated from Ochna integrerrima (63), showed interesting antimalarial activity against several and different chloroquine-resistant strains of P. falciparum.

<diagram of chemical structures>
LEISHMANIASIS
Protozoan parasites of the genus Leishmania cause a broad spectrum of diseases ranging from self-healing cutaneous lesions in *L. major* infection to life threatening visceral disease in *L. donovani* infection. These parasites differentiate from proliferative promastigotes in the sandfly vector gut to infective metacyclic promastigotes in the insect foregut. Then, while taking a blood-meal, they are inoculated as parasites suffer tremendous oxidative stress due to a respiratory burst of macrophages producing reactive oxygen and reactive nitrogen species (64-65). The subset of the parasites that survives this substantial alteration of their respiratory burst of macrophages producing reactive oxygen and reactive nitrogen species (64-65). The subset of the parasites that survives this substantial alteration of their environment subsequently differentiate into intracellular amastigotes, and multiply as such, and finally leads to the disease manifestation. Despite the enormous progress made in understanding the biology of *Leishmania* and clinical possibilities presented by some experimental chemotherapeutic agents, no new drugs have been developed for the treatment of leishmaniasis since the introduction of the pentavalent antimonials more than 80 years ago. It is estimated that 12 million peoples suffer from the disease, with 400 000 new cases and 100 000 deaths each year (66).

Antileishmanial chalcones
Recently, a number of synthetic and naturally occurring chalcones with potential antileishmanial activity have been reported. Licochalcone A, one of the many flavonoids isolated from the roots of Chinese licorice, and some related chalcones were shown to inhibit the in vitro growth of both *L. major* and *L. donovani* promastigotes together with a remarkably ability to kill intracellular amastigote form of both parasites (67). The growth of *L. major* promastigotes was totally inhibited after a 20 hours incubation period with licochalcone A at 5 µg/mL. At a concentration of 0.5 µg/mL, licochalcone A markedly reduced the infection rate of human peripheral blood monocyte-derived macrophages and U937 cells infected with *L. major* promastigotes, and exhibited a strong intracellular killing of the parasite, with no toxicity to the host cells (67). Intraperitoneal administration of licochalcone A at doses of 2.5 and 5 mg/kg of body weight per day completely prevented lesion development in BALB/c mice infected with *L. major*, while in hamsters infected with *L. donovani*, intraperitoneal administration of licochalcone A at a dose of 20 mg/kg of body weight per day for 6 consecutive days resulted in more than 96% reduction of parasite load in the liver and the spleen, compared with values from untreated control animals (68). When orally administered at concentrations of 5 to 150 mg/kg of body weight per day for 6 consecutive days, licochalcone A achieved more than 65 and 85% reductions of *L. donovani* parasite loads in the liver and the spleen, respectively, compared to the untreated control hamsters (68).

Licochalcone A is not the only chalcones known for its antileishmanial activity. A variety of chalcones was shown to exhibit different levels of activity against *Leishmania* parasites. A series of 20 chalcones isolated from different plants were screened for their in vitro leishmanicidal activity against extracellular promastigotes of *L. donovani*, *L. infantum*, *L. enrietti* and *L. major*, and against intracellular amastigote *L. donovani* in murine macrophages (69). Most compounds were active against the extracellular *Leishmania* parasites, with EC\textsubscript{50} values between 0.07 and 2.01 µg/mL, and...
some of these chalcones, 2',4'-dihydroxy-4'-methoxychalcone, 2'-hydroxy-3,4-dimethoxychalcone, and 2-hydroxy-4',4'-dimethoxychalcone also inhibited the intracellular survival of *L. donovani* parasites, with EC50 values between 0.39 and 0.41 µg/mL (69). When tested against murine bone marrow-derived macrophages as a mammalian host cell control, all compounds with antileishmanial activities also proved to be cytotoxic to varying extents (EC50 0.19-2.06 µg/mL) (69). 2',6'-Dihydroxy-4'-methoxychalcone isolated from *Piper aduncum* appeared to inhibit intracellular *L. amazonensis* growth *in vitro* and *in vivo* in infected BALB/c mice (70), while (E)-1-[2,4-hydroxy-3-(methyl-2-butenyl)phenyl]-3-[4-hydroxy-3-(e-methyl-2-butenyl)phenyl]-2-propen-1-one, a chalcone isolated from Chinese licorice roots, also displayed potent antileishmanial activity (71). 2',6'-dihydroxy-4'-methoxy- and 4,2',6'-trihydroxy-4'-methoxydihydrochalcone, 2 dihydrochalcones isolated from *Piper elongatum* Vahl together with 20 synthetic related compounds, were also evaluated for their *in vitro* antileishmanial activity against extracellular promastigotes of *L. braziliensis* (72). Many of these compounds proved to be more active than the reference drug ketoconazol (IC50 34 µg/mL), used as positive control, with 4',2',6'-triacetoxy-4'-methoxy-, 4',2',6'-triacetoxy-4'-hydroxy- and 4',2',4',6'-tetraacetoxydihydrochalcone being the most active compounds, with IC50 values of 0.66, 0.44 and 0.70 µg/mL, respectively (72). A number of synthetic chromenochalcones were also screened against *in vitro* extracellular promastigotes and intracellular amastigotes of *L. donovani*. Among them, (E)-1-[5-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2H-chromen-6-yl]-3-(pyridin-3-yl)prop-2-en-1-one was the most potent with 99% inhibition of promastigotes at 10 µg/mL, 82% at 0.25 µg/mL and 96% at 10 µg/mL concentration against the amastigote form of the parasite (73).

Electron microscopic studies showed that licochalcone A and some oxygenated chalcones altered the ultrastructure of *L. major* promastigote and amastigote mitochondria in a concentration-dependent manner without damaging the organelles of macrophages or the phagocytic function of these cells (74-75). Studies on the function of the parasite mitochondria showed that these compounds inhibited the respiration of the parasite, as shown by inhibition of O2 consumption and CO2 production by the parasites. Moreover, these compounds inhibited the activity of the parasite mitochondrial dehydrogenase, and the inhibition of the activity of the parasite mitochondrial enzyme correlated well with the changes in the ultrastructure of the mitochondria shown by electron microscopy (74-75). Licochalcone A also inhibited the activity of fumarate reductase (FRD) in the permeabilized *L. major* promastigote and in the parasite mitochondria, and it also inhibited solubilized FRD and a purified FRD from *L. donovani* (76). Two other chalcones (2,4'-dimethoxy-4'-allyloxychalcone and 2,4'-dimethoxy-4'-butoxychalcone), also exhibited inhibitory effects on the activity of solubilized FRD in *L. major* promastigotes.

Although licochalcone A inhibited the activities of succinate dehydrogenase (SDH), NADH dehydrogenase (NDH), and succinate- and NADH-cytochrome c reductases in the parasite mitochondria, IC50 of licochalcone A for these enzymes were at least 20-fold higher than that for FRD (76). The IC50 of licochalcone A for SDH and NDH in human peripheral blood mononuclear cells were more than 70-fold higher than that for FRD. These findings indicate that FRD, one of the enzymes of the parasite respiratory chain, might be the specific target for chalcones, and since FRD exists in the Leishmania parasite and does not exist in mammalian cells, it could be an excellent target for antiprotozoal drugs development (76).

**Other antileishmanial flavonoids**

Other classes of flavonoids and flavonoid glycosides are also reported for their antileishmanial activity. When evaluated a series of flavonoids for their antileishmanial activity, the majority of the metabolites tested showed remarkable leishmanicidal potential (77). Fisetin, 3-hydroxyflavone, luteolin, and quercetin were the most potent, with IC50 values of 0.6, 0.7, 0.8, and 1.0 µg/mL, respectively. 7,8-Dihydroxyflavone and quercetin appeared to ameliorate parasitic infections in mouse models, and globally, these compounds lacked cytotoxicity *in vitro* and *in vivo* (77). 7,3'-Dihydroxy-4'-methoxy-isoflavone (LD50 = 140 nM) isolated from *Centrolobium sclerophyllum* (78), 2'-methoxy-4',5'-methylenedioxy-7,8-[2-(1-methylethenyl) furyl] isoflavone, (-)maackiain, 6,7-dimethoxy-3',4'-methylenedioxy-isoflavone and 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone isolated from *Millettia pugensis* (79), displayed interesting antileishmanial activity.
Numbers of flavonoid glycosides have also displayed strong antileishmanial activity against different strains of *Leishmania* parasites. Quercitrin (quercetin 3-O-α-L-rhamnopyranoside) (80), kaempferol 3-O-α-L-arabinopyranosyl (1→2) α-L-rhamnopyranoside (a), quercetin 3-O-α-L-arabinopyranosyl (1→2) α-L-rhamnopyranoside (b) and 4',5-dihydroxy-3',8-dimethoxyflavone 7-O-β-L-glucopyranoside (c) (81), isolated from *Kalanchoe pinnata*, were tested against *L. amazonensis* amastigotes in comparison with quercetin and afzelin. Quercitrin exhibited significant antileishmanial activity at 0.01 µg/mL, with an IC₅₀ value of 1 µg/mL and without significant toxicity (80), while the other flavonoid glycosides displayed only a marginal activity (81). Kaempferol 3-O-(2',3'-di-O-galloyl)-β-D-glucopyranoside (d), kaempferol 3-O-β-D-glucopyranoside (e), quercetin 3-O-B-D-glucopyranoside (f), quercetin 3-O-B-D-galactopyranoside (g), kaempferol 3-O-(2' O-galloyl)-β-D-glucopyranoside (h), quercetin 3-O-(2'-O-galloyl)-β-D-glucopyranoside (i) and quercetin 3-O-(2',3'-di-O-galloyl)-β-D-glucopyranoside (j), 7 flavonol glycosides from *Geranium pyrenaicum* Burm, when evaluated for antileishmanial activity against a panel of *Leishmania* species (*L. major*, *L. donovani* and *L. amazonensis*), exhibited very strong in vitro activities (IC₅₀ 4-27 nM) when compared with Pentostam (IC₅₀ 10 nM) (82).

Tumor necrosis factor-α (TNF), produced principally by activated macrophages and monocytes, plays a crucial role in the host defense against various pathogens (83). While testing the above mentioned flavonol glycosides (kaempferol 3-O-
D-glucopyranoside and quercetin 3-O-(2''-O-galloyl)-B-D-glucopyranoside, quercetin 3-O-B-D-glucopyranoside, quercetin 3-O-D-glactopyranoside, kaempferol 3-O-(2''-O-galloyl)-B-D-glucopyranoside, quercetin 3-O-(2''-O-galloyl)-B-D-glucopyranoside and quercetin 3-O-(2'',3''-di-O-galloyl)-B-D-glucopyranoside) in non-parasitized RAW 264.7 cells, the amounts of TNF induced by the tested compounds were below the detection limit (82). Importantly, all these compounds, when evaluated for their antileishmanial activity and macrophage activation for the release of nitric oxide (NO), tumour necrosis factor (TNF) and interferon (IFN)-like properties (87). Several functional bioassays were employed including an in vitro model for leishmaniasis in which murine bone marrow-derived macrophages were infected with the obligate intracellular parasite L. donovani, an extracellular Leishmania proliferation assay, a fibroblast-lysis assay (TNF-activity), and a biochemical assay for NO. Except for catechin (EC_{50} 25 - 68 nM), all polyphenols tested significantly inhibited the intracellular survival of L. donovani amastigotes (EC_{50} 0.4-14 nM) when compared with the clinically used agent, sodium stibogluconate (EC_{50} 10 nM) (87). Although NO is an important effector molecule in macrophage microbicidal activity, the inducing potential of the tested compounds for its release was found to be very moderate, ranging from 7-54 µM (IFN-γ + LPS 119 µM). On the other hand, inhibition of NO production had no apparent effect on intracellular leishmanicidal activity of these compounds (87).

**Trypanosomiasis**

Human African trypanosomiasis, also known as sleeping sickness, is caused by Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense, and is one of the major causes of mortality and morbidity in sub-Saharan Africa. About 60 millions people living in 36 African countries are believed to be at risk of contacting this disease (88). Trypanosoma cruzi is the causative agent of American trypanosomiasis (also known as Chagas’ disease), which is endemic in Latin America. An estimated 18-20 million people in South America are infected with T. cruzi, with 50,000-100,000 in the United States (89).

The Trypanosoma parasite is exclusively extra-cellular. It lives and multiplies in blood and tissue fluids of their mammalian host. The major problem in the treatment of sleeping sickness and Chagas’ disease is the second stage (invasion of the central nervous system) for sleeping sickness, and cell distribution of intracellular T. cruzi amastigotes during both acute and chronic phases for Chagas’ disease. Since the available trypanocidal drugs are unsatisfactory as they are often associated with severe side effects, several synthetic and naturally occurring flavonoids have been evaluated for their antitypansomal activities.

In the survey of a large set of flavonoid aglycons and glycosides for their in vitro activities against T. brucei rhodesiense and T. cruzi, the best in vitro trypanocidal activity for T. brucei rhodesiense was exerted by 7,8-
methoxyflavone, 5,4'-dihydroxy-3,6,7-trimethoxyflavone, 5.0 µg/mL. Generally, the tested compounds lacked cytotoxicity in vitro and in vivo (77). 5,4'-Dihydroxy-7-methoxyflavanone, 5,4'-dihydroxy-3,6,7-trimethoxyflavone, 3,5,4'-trihydroxy-7-methoxyflavanone and 5,4'-dihydroxy-3,6,7,8-tetramethoxyflavone, 4 flavones from Trixis vauthieri were evaluated against Trypanosoma cruzi, the protozoan responsible of Chagas’ disease. Only 5,4'-dihydroxy-7-methoxyflavanone and 5,4'-dihydroxy-3,6,7-trimethoxyflavone displayed 100 and 86 % growth inhibition of the parasite, respectively, at 500 µg/mL, with the 2 remaining compounds being inactive (90). Sophoraflavone G (3.7 µM), (-)-kaempurone (14 µM), kushenol L (7.1 µM), 2'-methoxycoumarin (6.9 µM), 7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (71 µM), leachianone A (5.5 µM), 8-prenyl-naringenin (18 µM), noranhydroicaritin (3.6 µM) and alopecurone G (4.4 µM), a series of flavonoids from Sophora flavescens, displayed different levels of activity against epimastigotes form of T. cruzi (91), while hispidulin, from Ambrosia tenuifolia, and santin, from Eupatorium pilion, were moderately active against both epimastigotes (IC50 47 µM) and trypomastigotes (IC50 62 and 42 µM, respectively) forms of T. cruzi (92). Tectochrysin, pinostrobin, pinobanksin, pinobanksin 3-acetate, pinocembrin, chrysin, galangin 3-methylether, quercetin 3-methylether, chrysoeriol and vicenin-2 from Lycnophora staavioidea Mart, also showed interesting trypanocidal activity against T. cruzi. The most active compound was quercetin 3-methylether, which also showed no blood lysis activity, thus represents a promising compound for use against T. cruzi in blood banks (93). Pinobanksin, pinobanksin-3-acetate, pinocembrin, galangin, galangin-3-methylether, pilon, tectochrysin, luteolin, luteolin-7-methylether and quercetin-3-methylether, a series of flavonoids from Lycnophora pohlii, when evaluated against the trypomastigotes form of T. cruzi, displayed only moderate activities (94). Quercetin-7,3',4'-trimethylether was shown to possess some trypanocidal activity in mice infected with T. cruzi, with no antinociceptive activity on mice (95). Vicenin-2, isorhamnetin-3-O-glucoside and isorhamnetin-3-O-(6'-p-coumaroyl)-glucoside from Lycnophora pohlii (94), calycoperterin, xanthomieric, isokaempferide, luteolin, apigenin, luteolin 7-O-B-D-glucopyranoside, luteolin 3'-O-B-D-glucuronide, apigenin 4'-O-B-D-glucopyranoside, and acacetin 7-O-B-D-glucopyranoside from Draccephalum kotschyi (96) showed only marginal lethality against epimastigotes form of T. cruzi.
Some isoflavones and chalcones are also reported for their trypanocidal activity. 7,2'-Dihydroxyisoflavone was shown to be active against the bloodstream trypanostigote form of T. cruzi (97), while 7-methoxybenosin, griffonianone E, calopogonium isoflavone B and 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone isolated from the seeds of Millettia griffoniana exhibited only moderate trypanocidal activity against T. b. brucei (98). 2-Geralnyl-3-hydroxy-8,9-methylenedioxypterocarpan, 3-geranyl-2,3',4,4'-tetrahydroxychalcone and 4'-dehydroxy-carbenegrin A-I were assayed against epimastigote forms of T. cruzi, using the tetrazolium salt (MTT) as an alternative method (99). All the compounds displayed moderate trypanocidal activity, with IC_{50} values of 12.2, 10.6 and 13 µg/mL, respectively, with no or little effect on human peripheral blood cells (99).

The trypanocidal effect of green tea catechins against two different developmental stages of T. cruzi is also reported. Catechin, epicatechin, gallicatechin, epigallocatechin, catechingallate, epicatechingallate, galloclatechingallate, and epigallocatechingallate were tested on the nonproliferative bloodstream trypanostigote and the intracellular replicative amastigote parasite forms (100). All the tested compounds displayed not only an outstanding in vitro activity (MIC_{50} between 0.12 to 85 pM), but were also capable to lyse more than 50% of the parasites present in the blood of infected BALB/c mice at the same concentrations (100). The most active compounds were galloclatechingallate and epigallocatechingallate, with minimal inhibition concentrations that inhibited 50% (MIC_{50}) of isolates tested of 0.12 and 0.53 pM, respectively (100). The same compounds were assayed for their capacity to inhibit trypanostigote infection in Vero cells, and amastigote intracellular replication. These compounds displayed very good activity at nanomolar range concentrations (100). In order to understand the biochemical interaction that could explain the trypanocidal activity of these compounds, the effects of these catechins on the recombinant T. cruzi arginine kinase, a key enzyme in the energy metabolism of the parasite, were assayed. The activity of this enzyme was inhibited by about 50% by nanomolar concentrations of catechinalate or gallocatechingallate, whereas the other members of the group were less effective (100). Upon intraperitoneal administration of daily doses of 0.8 mg/kg/day of epigallocatechingallate for 45 days, mice survival rates increased from 11% to 60%, while parasitemia diminished to 50%, with no obvious side effects on treated animals (101). 50% Inhibition of epimastigotes growth was achieved with 311 µM of epigallocatechingallate, 120 hours after drug addition. However, no lysis, total culture growth inhibition or morphological changes were observed upon addition of 1-3 mM epigallocatechingallate at 24 hours (101). This treatment also produced oligosomal fragmentation of epimastigotes DNA, suggesting a programmed cell death (PCD)-like process as possible mechanism of action of this compound (101).

AMEBIASIS

Amebiasis, also called dysentery, is a parasitic infection of the gastrointestinal tract caused by Entamoeba histolytica, a microscopic one-celled protozoan parasite. Symptoms include fever, vomiting, abdominal pain and diarrhea. In contrary to malaria, leishmaniasis and trypanosomiasis that are vector...
borne diseases, amebiasis infection occurs typically by ingestion of *E. histolytica* cysts through contaminated food or water. These cysts often live in the large intestine without causing any symptoms, but sometimes they can differentiate into trophozoites, multiply by simple division and invade the lining of the large intestine, causing intestinal amebiasis. This disease is most prevalent in tropical and subtropical countries where standards of public hygiene and sanitation may be low. In these countries, approximately 50 million cases of invasive *E. histolytica* disease occur each year, with up to 100,000 deaths (102).

As a result of their significant antiprotozoal activity, a handful of flavonoids have also been tested for their antiamebic activity. Apigenin, xanthomircil, galangin, kaempferol, quercetin, myricetin, tilisorside, hyperfine, reynoutrin, rutin, fisetin, 3,6-dimethoxykaempferol, 4',5,7-trihydroxyflavanone, pinocembrin, pinostrobin, catechin, epicatechin and epigallocatechin were evaluated for their activity against *E. histolytica* (103). These compounds displayed different levels of activity, with IC50 values ranging from 1.9 μg/mL for epicatechin to 274 μg/mL for xanthomicrol (103). Epicatechin (IC50 1.9 μg/mL), epigallocatechin (IC50 6.9 μg/mL) and kaempferol (IC50 7.9 μg/mL) were the most potent compounds (103). The structure-activity relationship revealed that the 2,3-double bond and the 4-keto group of ring C of the flavonoid template were not essential for the activity. However, 2,3-cis (a) stereochemistry might be an important asset for the activity since trans-configured compounds were less active than the cis-configurated ones (103). 5,7-Dihydroxylated A ring and the presence of a 3-hydroxy group were the common structural features of most active compounds. However, when the degree of oxygenation increased in the B-ring, the antiamebic activity significantly decreased (103).

**CONCLUSION**

Flavonoids are a large group of polyphenolic compounds that have been known for a long time to exert diverse biological effects. In this review are presented numerous evidences of their strong antiprotozoal activity, and the limited number of examples mentioned above should not be considered as an exhaustive list of antiprotozoan flavonoids, but rather as an illustrative summary of their potential and possible applications. In that respect, flavonoids represent a great source of new antiprotozoan drug candidates. However, their low bioavailability indicates the need for further investigations, including (1) the preparation and the evaluation of analogs for a better understanding of other aspects of their structure-activity relationship, (2) detailed mechanistic studies and (3) the development of parasite-specific formulations and delivery systems, using the above listed compounds as templates.

**REFERENCES**


