Natural Products Published in 2009 from Plants Traditionally Used to Treat Malaria

Introduction

There were an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly of children under 5 years (WHO, 2008). This disease, transmitted by an Anopheles mosquito, is caused by Plasmodium species. The parasite is now resistant to a number of antimalarials but plants can offer new metabolites with an original mode of action such as artemisinin from Artemisia annua which can be active on resistant strains. In this review, all antiplasmodial metabolites new or already known and isolated from plants to treat malaria and published in 2009 are described and organised according to their phytochemical classes. All the activities were determined in vitro on Plasmodium falciparum strains unless specified and bio-guided fractionation was also based on this antimalarial test. Activities were assessed on different strains among which some are chloroquine sensitive (NF54, 3D7, D6, F32, D10, Ghana, TM4), chloroquine resistant (FcB1, W2, FCM29, Dd2, FCR-3) and/or multidrug resistant (K1) strains, to find effective compounds on resistant malaria. We considered that those having an IC50 ≤ 11 µM may have some interest for further development, while those with a lower activity were less interesting. That is why we only give structures for these promising compounds, the others are cited in tables. Compounds tested in vivo are also cited. We also analysed the phytochemical classes of these metabolites published in 2009 and the families of plant from which they were isolated and compared these data with those of compounds published from 2005 to 2008. Other reviews already exist for compounds published before 2005 [1–7], or before 2009 [8–10].

Phenolic Derivatives

**Flavonoid derivatives (Fig. 1)**
The hexane extract of ground fruits of Neuraputia magnifica var. magnifica (Engl.) Emmerich (Rutaceae) was fractionated to obtain 2′-hydroxy-3,4,4′,5,6′-pentamethoxychalcone (1) which exhibited an antiplasmodial activity with an IC50 of 6.9 µM on 3D7 [11].

An new β-hydroxydihydrochalcone named (S)-elatadihydrochalcone (2) was isolated from the seedpods of Tephrosia elata Deflers. (Leguminosae) and showed antiplasmodial activity with IC50 values of 7.9 and 15.5 µM, respectively, on D6 and W2 [12].

A new isoprenylated flavone, artopeden A (3) was isolated from the bark of Artocarpus champeden Spreng. (Moraceae) and showed antiplasmodial activity with an IC50 of 0.11 µM against 3D7 [13].
**Baccharis dracunculifolia** D.C. (Asteraceae) contains ermanin (4) having an IC$_{50}$ of 8.3 µM on D6 and of 7.0 µM on W2 [14].

**Xanthones** (Fig. 2)

Two new xanthones, butyraxanthones A and B (5 and 6), were isolated from the stem bark of *Pentadesma butyracea* Sabine (Clusiaceae), together with four known xanthones: xanthone I (7), rubraxanthone (8), garcinone E (9) and 3-isomangostin (10). They exhibited antiplasmodial activity against FcB1 with IC$_{50}$ values of, respectively, 6.3, 5.8, 4.7, 8.3, 6.0 and 7.6 µM but were cytotoxic against a human breast cancer cell line (MCF-7) with IC$_{50}$ values of, respectively, 7.3, 7.1, 9.6, 6.3, 3.2 and 2.9 µM [15].

Three xanthones: gerontoxanthone I (11), macluraxanthone (12) and formoxanthone C (13) were isolated from the stem bark of *Cratoxylum muntingyi* Dyer (Clusiaceae) and another one [fusca-xanthone E (14)] from the fruits of *Cratoxylum cochinichinense* Blume (Clusiaceae). They displayed antimalarial activity against K1 with IC$_{50}$ values of 4.2, 3.4, 3.0 and 7.9 µM, respectively [16].

A new xanthone, 1,5-dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone (15) was obtained from *Garcinia griffithii* T. Anderson (Clusiaceae). It showed antiplasmodial activity with an IC$_{50}$ of 7.3 µM on a Ghana strain [17].

**Coumarins** (Fig. 3)

The methanolic extract of the rhizomes of *Angelica purpuraeefolia* T.H. Chung (Apiaceae) was investigated and two natural khellactones, (+)-4′-decanoyl-cis-khellactone (16) and (+)-3′-decanoyl-cis-khellactone (17) were isolated. These two compounds were evaluated for antimalarial activities and showed growth inhibitory activity against D10 with IC$_{50}$ values of 1.5 and 2.4 µM, respectively [18].
Other phenolic derivatives (Fig. 4)

Compound 18 was isolated from the dichloromethane extracts of the leaves of *Piper heterophyllum* Ruiz & Pav. and *P. aduncum* L. (Piperaceae) and exhibited activity with an IC$_{50}$ of 7.0 µM on F32 [19].

The petroleum ether extract of *Viola websteri* Hemsl. (Violaceae) was investigated and the main antiplasmodial compound was 6-(8′Z-pentadecenyl)-salicylic acid (19) with an IC$_{50}$ of 10.1 µM (D10). Given intraperitoneally, 19 showed in vivo a 63% suppression of parasitemia in *P. berghei* infected mice treated at 10 mg/kg/day. When used prophylactically a suppression of 70.1% at the same dose was recorded [20, 21].

The bioassay-guided purification of the CH$_2$Cl$_2$ extract of the bark of *Tapirira guianensis* Aubl. (Anacardiaceae) led to the isolation of two cyclic alkyl polyol derivatives: 4,6,2′-trihydroxy-6-[10′(Z)-heptadecenyl]-1-cyclohex-2-one (20) and 1,4,6-trihydroxy-1,2′-epoxy-6-[10′(Z)-heptadecenyl]-2-cyclohexene (21). The antiplasmodial activity of a mixture of these two compounds showed an IC$_{50}$ of 4.7 µM on F32 and 5.4 µM on FcB1 [22].

A new chromone, 10,11-dihydroanhydrobarakol (22), which showed antiplasmodial activity against 3D7 (IC$_{50}$ = 2.3 µM), was isolated from flowers of *Cassia siamea* Lam. (Caesalpinaceae) [23].

Studies on *Baccharis dracunculifolia* D.C. (Asteraceae) allowed the isolation of viscidone (23) which showed an IC$_{50}$ of 8.1 µM on D6 and of 9.8 µM on W2 [14].

Three phenolic compounds, vismione B (24), F (25) and E (26) were isolated from the fruits of *Cratoxylum cochinchinense* Blume (Clusiaceae) and displayed antimalarial activity against K1 with IC$_{50}$ values of 1.86, 4.76 and 10.97 µM, respectively [16].

Acyl phloroglucinols [isogarcinol (27), cycloxanthochymol (28), 7-epi-isogarcinol (29), coccinone A (30), B (31), C (32), D (33) and E (34), and 7-epi-garcinol (35)] from *Moronobea coccinea* Aubl. (Clusiaceae) exhibited an activity with IC$_{50}$ values of 3.5, 2.1, 5.1, 4.3, 5.5, 9.0, 7.0, 4.9 and 10.1 µM, respectively, on FcB1 [24].

Isoxanthochymol (36) was obtained from *Garcinia griffithii* T. Anderson (Clusiaceae) and showed antiplasmodial activity with an IC$_{50}$ of 4.5 µM on a Ghana strain but it was also cytotoxic against MRC-5 cells (IC$_{50}$ = 7.5 µM) [17].

A new phenanthenone, 9-O-demethyltrigonostemone (37), and a new phenanthropolone (38) were isolated from the roots of *Strophioblachia fimbriata* Boerl. (Euphorbiaceae) and displayed antiplasmodial activity (IC$_{50}$ values of 8.7 and 9.9 µM, respectively) against K1 [25].

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Bioassay-guided fractionation of an ethanol extract of the bark of *Scutia myrtina* Kurz (Rhamnaceae) led to the isolation of three new anthrone–anthraquinones dimers, scutianthraquinones A (39), B (40) and C (41), one new bisanthrone–anthraquinone trimer, scutianthraquinone D (42) and the known anthraquinone, aloesaponarin I (43). These compounds exhibited antiplasmodial activities with IC50 values of 1.23, 1.14, 3.14, 3.68 and 5.58 µM, respectively, on Dd2 and 1.2, 5.4, 15.4, 5.6 and > 50 µM, respectively, on FCM29 [26].

A phytochemical study of the stem bark of *Vismia laurentii* De Wild. (Clusiaceae) resulted in the isolation of a known compound, vismiaquinone A (44) which showed antimalarial activity of 1.42 µM against W2 [27].

A new compound named globiferin (45) was isolated from root extracts of *Cordia globifera* W.W. Sm. (Boraginaceae) with coradiachrome B (46), coradiachrome C (47) and cordiaquinol C (48). Antimalarial activities (IC50) were 8.7, 6.2, 0.8 and 1.2 µM, respectively, on K1 [28].

Bioassay-guided fractionation from extracts of roots of *Scleria striatonux* de Wild. (Cyperaceae) and possessed IC50 values of 1.8, 1.8, 5.6, 4.9 µM, respectively, on W2, D6, K1, NF54 [29].

An antiplasmodial bioguided investigation of the EtOAc extract of the aerial parts of *Teucrium ramosissimum* Desf. (Lamiaceae) led to the isolation of homalomenol C (50). Its IC50 was 4.7 µM on FcB1 [30].

The ethyl acetate extract of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae) rhizomes was fractionated to isolate a novel furanoterpenoid (51). This compound showed antimalarial activity with IC50 values of 13.9 and 7.2 µM, respectively, on D10 and K1 [31].

Bioassay-guided fractionation led to the isolation of two new sesquiterpene lactones (52 and 53) from an extract of *Distephanus angulifolius* (DC.) H. Rob. & B. Kahn (Asteraceae). The isolated compounds showed IC50 values of 1.9 and 1.55 µM on D10 and 3.24 and 2.10 µM on W2, respectively [32].

Bioactivity-guided fractionation of the dichloromethane extract of *Xanthium brasilicum* Vell. (Asteraceae) resulted in the isolation of three bioactive sesquiterpene lactones: 8-epixanthatin 1β,5β-epoxide (54), and the dimers pungiolide A (55) and B (56). They showed IC50 values of 6.5, 5.0 and 6.5 µM against K1 [33].
Fractionation of the ethyl acetate extract of *Carpesium cernuum* L. (Asteraceae) yielded four characterised sesquiterpenoid lactones among which 11(13)-dehydroivaxillin (57) and 11-epi-ivaxillin (58) exhibited antiplasmodial activity against D10 with IC₅₀ values of 2.0 and 9.3 µM [34].

*In vivo* antimalarial activity of 57 showed a suppression of parasitemia of 58.6% with a dose of 2 mg/kg/day in the four-day test [35].

Diterpenes (Fig. 7)
A new diterpene, 12-0-deacetyl-6-O-acetyl-19-acetyloxycoleon Q (59), and a known one (60) were isolated from the aerial parts of *Anisochilus harmandii* Doan (Lamiaceae) and exhibited antimalarial activity with IC₅₀ values of 6.5 and 9.1 µM on K1 [36]. A known compound, caniojane (61), was isolated from the roots of *Jatropha integerrima* Jacq. (Euphorbiaceae) and was evaluated for its antimalarial activity: IC₅₀ of 9.6 µM against K1 [37].

*Baccharis dracunculifolia* D.C. (Asteraceae) was shown to contain hautriwaic acid (62) and hautriwaic acid lactone (63) which had IC₅₀ values of 9.0 and 2.5 µM, respectively, on D6 and 7.8 and 7.0 µM, respectively, on W2 [14].

Triterpenes (Fig. 8)
A new quassinoid named simalikalactone E (64) was extracted from *Quassia amara* L. (Simaroubaceae) leaves and inhibited *Plasmodium falciparum* with IC₅₀ values of 24 nM on W2, 45 nM on FcB1 and 68 nM on F32 [38].

Two new quassinoiids, delaumonones A (65) and B (66) were isolated from the bark of *Launoniera bruceadelpha* Noot. (Simaroubaceae) and showed an antimalarial activity on 3D7 (IC₅₀ = 0.6 and 1.1 µM) [39]. Four other quassinoids were isolated from the dichloromethane extract of *Quassia amara* L. (Simaroubaceae): picrasin B (67), H–J (68–70). These compounds have antimalarial activities with IC₅₀ values of, respectively, 0.8, 3.4, 2.6 and 4.2 µM on W2 [40]. Isobrucein B (71) isolated from *Picrolemma sprucei* Hook. f. (Simaroubaceae) was tested for its antimalarial activity against the K1 strain (IC₅₀ = 2.1 nM) [41].

*Garcinium bronis* D (72) was obtained from *Garcinia celebica* L. (Clusiaceae) and showed an activity with an IC₅₀ of 7.7 µM on a Ghana strain [17].

Three new limonoids, ceramicines B–D (73–75), were isolated from the bark of *Chisochiton ceramicus* Miq. (Meliaceae). Ceramicines exhibited an antimalarial activity with IC₅₀ values of 0.56, 4.8 and 5.1 µM, respectively, on 3D7 [42]. A phytochemical study of the stem bark of *Vismia laurentii* De Wild. (Clusiaceae) resulted in the isolation of a tetracyclic triterpene, tirucalla-7,24-dien-3-one (76) which showed antimalarial activity of 1.18 µM against W2 [27].

*Garicium bronis* D.C. (Asteraceae) was shown to contain 2α-hydroxyursolic acid (77) which presented an IC₅₀ of 6.8 µM on D6 and 6.4 µM on W2 [14].
Alkaloids

Ornithine and lysine derivatives (Fig. 9)
Researches on *Albizia schimperiana* Oliv. (Leguminosae) allowed the isolation of the new bioactive macrocyclic spermine alkaloid, namely 5,14-dimethylbudmunchiamine L1 (78). This compound demonstrated antimalarial activity against D6 and W2 with IC50 values of 0.27 and 0.34 µM [43].

Four new indolizidines: prosopilosidine (79), prosopilosine (80), isoprosopilosine (81) and isoprosopilosidine (82) and a known one, juliprosopine (83) were isolated from *Prosopis glandulosa* Torrey var. *glandulosa* (Leguminosae). These compounds exhibited potent activity with IC50 values of 62, 191, 132, 67 and 350 nM, respectively, on D6 and 152, 366, 238, 192 and 604 nM, respectively, on W2. Prosopilosine also showed in vivo antimalarial activity, exhibiting 48% suppression of parasitemia at 2 mg/kg/day/i.p. against *Plasmodium berghei* after 3 days of treatment [44].

Phenylalanine and tyrosine derivatives (Fig. 10)
A known alkaloid, cheilanthifoline (84), from *Corydalis calliantha* D.G. Long. (Papaveraceae) showed antiplasmodial activity against TM4 and K1 strains with IC50 values of 2.8 and 3.8 µM, respectively [45].

The dichloromethane extract of *Doryphora sassafras* Endl. (Monimiaceae) was fractionated to obtain a quaternary benzylisoquinoline alkaloid, 1-(4-hydroxybenzyl)-6,7-methylenedioxy-2-methylisoquinolinium trifluoroacetate (85) which presented an antiplasmodial activity of 4.4 µM on Dd2 and 3.0 µM on 3D7 [46]. Two new dimeric alkaloids, cassiarins D (86) and E (87), which showed antiplasmodial activity against 3D7 (IC50 = 3.6 and 7.3 µM), were isolated from flowers of *Cassia siamea* Lam. (Caesalpinaceae) [23].

Tryptophane derivatives (Fig. 11)
Flinderole A (88) was isolated from *Flindersia acuminata* C.T. White (Rutaceae), flinderoles B–C (89–90) from *F. amboinensis* Poir., and isoborreverine (91) and dimethylisoborreverine (92) from *F. fournieri* Pancher & Sebert. They have selective antimalarial activities with IC50 values of 1.42, 0.15, 0.34, 0.32 and 0.08 µM, respectively, on Dd2 while on cancer cells (HEK-293) their IC50 values were 19.97, 2.13, 9.75, 8.99 and 4.09 µM, respectively [47]. Three new alkaloids, alstiphyllanes B–D (93–95), were isolated from *Alstonia macrophylla* Wall. (Apocynaceae) and showed antiplasmodial activity against 3D7 with IC50 values of 0.6, 10.0 and 4.5 µM, respectively [48].

Other N-containing compounds (Fig. 12)
Studies on the CH2Cl2/MeOH extract from the roots of the Australian tree *Mitrephora diversifolia* Miq. (Annonaceae) resulted in the purification of the known 5-hydroxy-6-methoxyonychine (96) which displayed IC50 values of 9.9 and 11.4 µM, respectively, on 3D7 and Dd2 [49].

Other Metabolites (Fig. 13)
The flower extracts of *Goniothalamus laoticus* (Fin. & Gagnep.) Bân (Annonaceae) were fractionated to obtain a styryllactone, (+)-3-acetylaltholactone (97). This compound was evaluated for its antiplasmodial activity against K1 (IC50 = 9.5 µM) but it showed cytotoxicity against human cancer cell lines with IC50 values of 10.6, 3.3 and 6.6 µM, respectively, on KB, BC1 and NCI-H187 [50]. A linear polyacetylenic diol (98) was isolated from *Bidens pilosa* L. (Asteraceae), and exhibited antiplasmodial properties in vitro with IC50 of 1.8 µM on FCR-3 as well as antimalarial activity by in-
travenous injection in vivo, which was carried out in mice infected with the *Plasmodium berghei* NK-65 strain. The average parasitemia of 32.8% in the control red blood cells was decreased significantly to 12.1% by the administration of 0.8 mg/kg/day of the compound for four days [51].

### Discussion and Conclusions

In traditional medicine, traditional healers use plants for the treatment of malaria or several symptoms of the disease. This review focusing on publications of 2009 shows that some promising new antimalarial compounds can be isolated by the ethnomedical and bioguided fractionation approaches. Various extracts of plants were fractionated to obtain 146 compounds which were evaluated for antimalarial activity in vitro. Among them, 41 possessed low (11 < IC_{50} < 50 µM), 65 moderate (2 < IC_{50} < 11 µM) and 31 promising (IC_{50} < 2 µM) activity in vitro against various strains of *Plasmodium falciparum* which is responsible for the most severe form of malaria. The activity of some of these compounds was tested against various cell lines, normal or cancer cells, but only a few of them for their in vivo antimalarial activity. Nevertheless, in these cases, the promising in vitro activities could be confirmed by in vivo tests. Among them, a phenolic compound: 6-(8′Z-pentadecenyl)-salicylic acid, a sesquiterpene lactone: 11(13)-dehydroivaxillin, a tryptophane derivative: prospilosine, and a linear polyacetylenic diol seem promising.

Among the compounds we reviewed, only a few of them exhibited a good activity and should be considered as lead compounds for further investigations (Table 1). In 2009, most of the highly active compounds were found in the alkaloid and terpene chemical classes, which was also the case in 2005–2008 (Fig. 14). The same trend was observed when considering families from which active compounds were isolated (Fig. 15). Most active alkaloids published in 2009 were isolated from the Leguminosae and Rutaceae families [10]. In our previous review, Leguminosae was also identified as a family allowing isolation of a significant number of active alkaloids, while Rutaceae was not found to be particularly interesting although it is a family whose activity is often due to the presence of alkaloids [6].

The more active triterpenes were obtained from Simaroubaceae which seem to have been well studied in 2009 and were not identified as an interesting family in 2005–2008. However, other reviews confirmed that the active antimalarial molecules of the Simaroubaceae are mainly quassinoids [6,9]. Quassinoids often displayed anticancer activity but the antimalarial activity does not seem to be correlated with the cytotoxicity [54].

When considering highly active compounds and families from which they were isolated and comparing with the results of 2005–2008, we observed that in 2009 no highly active diterpene was isolated, although this class was pointed out to be very interesting in 2005–2008. The same is observed with the Caesalpinaceae family from which they were isolated. This may be explained by the fact that one team focused in 2005–2008 on diterpenes from Caesalpinaceae leading to Kalauni et al. [55] and Linn et al. [56].

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**Fig. 11** Tryptophane derivatives with moderate or promising activity in vitro against various strains of *Plasmodium falciparum*.

**Fig. 12** Other N-containing compounds with moderate activity in vitro against various strains of *Plasmodium falciparum*.

**Fig. 13** Other metabolites with moderate or promising activity in vitro against various strains of *Plasmodium falciparum*.
Three of the most active sesquiterpenes are lactones and were obtained from a plant of the Asteraceae family as it was the case for the “famous” artemisinin. Among families from which most highly active compounds were isolated in 2005–2008, Menispermaceae and Asphodelaceae are not represented in 2009 while from Moraceae, only one interesting flavonoid (artopoden A) was isolated. These observations and comparisons show that it is often difficult to assess general rules concerning interesting classes of compounds or interesting families as it may depend highly on the activity of specific research groups. Nevertheless, we can indicate that during 2009, alkaloids from the Leguminosae and Rutaceae families, quassinoids from Simaroubaceae and well-known sesquiterpene lactones from Asteraceae were described as interesting antimalarial compounds with original structures which could be considered as lead compounds for new drugs against resistant malaria.

Supporting information
Tested phenolic derivatives, terpenic compounds, alkaloids and other metabolites presenting low or no activity in vitro against various strains of Plasmodium falciparum (Tables 1S–4S) are available as Supporting Information.

### Table 1: Tested compounds presenting promising activity with IC50 < 2 µM.

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Family</th>
<th>Plant</th>
<th>Compound</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Rutaceae</td>
<td>F. acuminata C. T. White</td>
<td>Fliderole A</td>
<td>1.42 (Dd2)</td>
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<tr>
<td>Alkaloids</td>
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<td>F. amboinensis Poir.</td>
<td>Fliderole B</td>
<td>0.15 (Dd2)</td>
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<td>Rutaceae</td>
<td>F. amboinensis Poir.</td>
<td>Fliderole C</td>
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<td>Rutaceae</td>
<td>F. fumieri Pancher &amp; Sebert</td>
<td>Isoborrevnine</td>
<td>0.32 (Dd2)</td>
</tr>
<tr>
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<td>Rutaceae</td>
<td>F. fumieri Pancher &amp; Sebert</td>
<td>Dimethylisoborrevnine</td>
<td>0.08 (Dd2)</td>
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<td>A. macrophylla Wall.</td>
<td>Astiphyllanine B</td>
<td>0.60 (3D7)</td>
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<td>A. schimperiana Oliv.</td>
<td>5,14-Dimethylbudmunchiamine-L1</td>
<td>0.27 (D6)</td>
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<td></td>
<td></td>
<td></td>
<td>0.34 (W2)</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Leguminosae</td>
<td>P. glandulosa Torrey</td>
<td>Prospilosidine</td>
<td>0.06 (D6)</td>
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<td></td>
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<td></td>
<td>0.37 (W2)</td>
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<td></td>
<td></td>
<td></td>
<td>0.24 (W2)</td>
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<tr>
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<td>0.07 (D6)</td>
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<td>0.19 (W2)</td>
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<td>Jujiliprosine</td>
<td>0.35 (D6)</td>
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<td>0.60 (W2)</td>
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<td>Coumarins</td>
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<td>(+)-4-Decanoyl-cis-khellactone</td>
<td>1.5 (D10)</td>
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<td>Artopeden</td>
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<td>Other metabolites</td>
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<td>Polyacetylenic diol</td>
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<td>Other phenolic derivatives</td>
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<td>Vismione B</td>
<td>1.86 (K1)</td>
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<td>Boraginaceae</td>
<td>C. globifera W. W. Sm</td>
<td>Cordiachrome C</td>
<td>0.8 (K1)</td>
</tr>
<tr>
<td>Quinones</td>
<td>Boraginaceae</td>
<td>C. globifera W. W. Sm</td>
<td>Cordiaquinol C</td>
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<td>Quinones</td>
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<td>1.2 (FCM29)</td>
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<td>5.0 (NF54)</td>
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<td>3.24 (W2)</td>
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<td>Simalkalactone E</td>
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References


Fig. 14 Number of compounds with good activity (IC50 < 2 µM) in vitro against various strains of Plasmodium falciparum, classified according to their chemical classes.

Fig. 15 Number of compounds with good activity (IC50 < 2 µM) in vitro against various strains of Plasmodium falciparum, classified according to the plant family from which they were isolated.


28 None from stem bark of Rosenthal PJ, Lobstein A, Tsamo E.

27 None from Pedersen MM, Chukwujekwu JC, Lategan CA, Van Staden J, Smith PJ.


23 Chung IM, Moon HI. Antiproliferative and antimalarial anthraquinones of nux bicyclic cyclofarnesylsesquiterpene endoperoxide from Fallopia japonica. Phytochemistry 2009; 70: 305–311


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