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The antiplasmodial and radical scavenging activities of flavonoids of *Erythrina burttii*

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**ABSTRACT**

The acetone extract of the root bark of *Erythrina burttii* showed *in vitro* antiplasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* with *IC* 50 values of 0.97 ± 0.2 and 1.73 ± 0.5 μg/ml respectively. The extract also had radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with an *EC* 50 value of 12.0 μg/ml. The isoflav-3-enes burtinol-A and burtinol-C, and the 2-arylbenzofuran derivative burtinol-D were identified as the most active antiplasmodial (*IC* 50 < 10 μM) and free radical scavenging (*EC* 50 ca. 10 μM) principles. The acetone extract of *E. burttii* at 800 mg/kg/day, in a 4-day *Plasmodium berghei* ANKA suppressive test, showed *in vivo* antimalarial activity with 52% chemosuppression. In the same *in vivo* test, marginal activities were also observed for the extracts of the root and stem bark of *Erythrina abyssinica* and the root bark of *Erythrina saxleuixii*.

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**1. Introduction**

Half a century after elaborate eradication efforts were launched (Narasimhan and Attaran, 2003; Talisuna et al., 2004), malaria continues to elude control and treatment efforts and sustains devastating morbidity and mortality rates (Hay et al., 2010). Drug resistance has significantly increased in the second half of the 20th century, prompting the change of malaria treatment from chloroquine to sulfadoxine/pyrimethamine and then to artemisinin based combination therapies (ACTs), which are currently the preferred malaria treatment methods (Grimberg and Mehlota, 2011). Despite the optimism and confidence brought by ACTs, the cost and emergence of resistant parasite strains to this treatment (Grimberg and Mehlota, 2011), heralds the need for new drugs with different structural features and mode of action.

The success of the natural product-based antimalarials such as quinine and artesimisin has showed the importance of plants as potential source of antimalarial agents. Consequently, numerous efforts to identify antiplasmodial activities from plants traditionally used as remedies for fever and malaria (Chen et al., 1997; Havsteen, 2002; Kaur et al., 2009, Lacroix et al., 2011), coupled with biosassay guided isolations of active principles have resulted in the identification of limonoids, coumarines, alkaloids, flavonoids (including chalcones), quassinoids, xanthenes, terpenes, quinones and peptides as antimalarial principles (Kaur et al., 2009).

Flavonoids display a wide range of biological and pharmacological activities (Havsteen, 2002), some of which are attributable to their free radical scavenging efficacies, metal complexion and protein binding abilities (Fotie, 2008). Flavonoids have been isolated and characterized from many medicinal plants used to treat...
infections around the world (Nundkumar and Ojewole, 2002), among which are *Erythrina* species (Mitscher et al., 1987). Since the identification of the chalcone licochalcone A, as a lead structure in antimalarial drug development (Ziegler et al., 2004), there is a growing interest in antimalarial activity of chalcones and other flavonoids. Ferreira et al. (2010) have pointed out that artemisinin (in its original form) is accompanied by flavonoids which act in supporting the activity of artemisinin. Unfortunately, these flavonoids are lost in the process of the preparation of semisynthetic artemisinin derivatives.

The antioxidant activities of flavonoids are well documented (Han et al., 2004; Kapche et al., 2009). This property of flavonoids appears to be important in malaria treatment since oxidative stress normally follows malaria infection (Griffiths et al., 2001). This is due to elevated production of reactive oxygen species (Griffiths et al., 2001; Muller, 2004). Therefore, it may be beneficial to protect cells from oxidative burden through the use of effective antioxidants in malarial patients; however, more studies are needed to determine the true clinical value of flavonoids in malaria treatment.

*Erythrina* species contain prenylated flavonoids with anti-infective activities (Mitscher et al., 1987). We have reported several flavonoids with antiplasmodial activity from the roots and stem bark of *Erythrina abyssinica* (Yenesew et al., 2003, 2004) and the stem bark of *Erythrina sacleuxii* (Andayi et al., 2006). The radical scavenging activities of some flavonoids and isoflavonoids isolated from *Erythrina* species have also been reported (Yenesew et al., 2009). Here we report the antiplasmodial and radical scavenging activities of flavonoid derivatives isolated from the root bark and stem bark of *Erythrina burttii* (Yenesew et al., 2002, 1998). We also report the in vivo antimalarial activity of the root extract from this plant along with the extracts from *E. abyssinica* and *E. sacleuxii*.

## 2. Materials and methods

### 2.1. Plant material

The authentication of *E. burttii* (Yenesew et al., 1998), *E. abyssinica* (Yenesew et al., 2004) and *E. sacleuxii* (Andayi et al., 2006) has been described previously. The plants were identified at the University Herbarium, Botany Department, University of Nairobi, where voucher specimen (AY-SGM-2008-01 for *E. burttii*, AY-SGM-2008-02 for *E. abyssinica*, AY-SGM-2008-03 for *E. sacleuxii*) are deposited.

### 2.2. Extraction and isolation

The crude extracts from the root bark and stem bark of *E. burttii*, *E. abyssinica* and *E. sacleuxii* were prepared by soaking 200 g of each of the plant material in acetone for 24 h by cold percolation. These extracts were filtered and the solvent removed under reduced pressure. The extracts were kept at 4 °C before testing for antimalarial activities. The isolation and characterization of pure compounds from the root bark and stem bark of *E. burttii* have been previously described (Yenesew et al., 1998, 2002).

### 2.3. In vitro antimalarial activity assay

The crude extract and pure compounds were assayed using a non-radioactive assay technique (Smilkstein et al., 2004) with modifications (Johnson et al., 2007) to determine 50% growth inhibition of cultured parasites. Briefly, two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *Plasmodium falciparum* were grown as described (Johnson et al., 2007). Drugs and compounds were dissolved in 99.5% dimethylsulfoxide (DMSO) (Sigma–Aldrich) and diluted in complete Roswell Park Memorial Institute 1640 series of Cell Culture Medium (RPMI 1640) prepared as described by Akala et al. (2011). Briefly, the basic culture medium was prepared from RPMI 1640 powder (10.4 g; Invitrogen, Inc. augmented with 2 g glucose (Sigma Inc.) and 5.95 g of HEPES (Sigma Inc.), dissolved to homogeneity in 1 liter of de-ionized water and sterilized with 0.2 µm filter. Complete RPMI 1640 media, used for all parasite culture and drug dilutions, consisted of basic RPMI 1640 media with 10% (v/v) human ABO pooled plasma. 3.2% (v/v) sodium bicarbonate (Thermo Fisher Scientific Inc.) and 4 µg/ml hypoxanthine (Sigma Inc.). Complete RPMI 1640 media was stored at 4 °C and used within 2 weeks.

Concurrently, two-fold serial dilutions of chloroquine (1.953–1000 ng/ml), mefloquine (0.488–250 ng/ml) and test sample (97.7–50,000 ng/ml) were prepared on a 96-well plate, such that the amount of DMSO was equal or less than 0.0875%. The culture-adapted *P. falciparum* at 2% hematocrit and 1% parasitemia, were added on to the plate containing dose range of drugs and incubated in gas mixture (5% CO2, 5% O2, and 90% N2) at 37 °C. The assay was terminated 72 h later by freezing at −80 °C and parasite growth inhibition quantified as described (Johnson et al., 2007) and presented as mean ± standard deviation (Mean IC50 ± SD).

### 2.4. In vivo antimalarial test

#### 2.4.1. Animals

Permission to carry out this study was approved by Kenya Medical Research Institute (KEMRI) animal care and use committee, reference number KEMRI/RES/7/3/1. Male Swiss Albino mice were obtained from Kenya Medical Research Institute (KEMRI) animal house. The animals were housed in experimental room in standard Macrohon type II cages clearly labeled with experimental details at 22°C and 60–70% relative humidity. They were fed on commercial rodent feed and water ad libitum.

#### 2.4.2. Drug and extracts preparation

The reference drug (chloroquine) and plant extracts were freshly prepared on the day of treatment by suspending in a 70% TWEEN-80 (d = 1.08 g/ml) and 30% ethanol (d = 0.81 g/ml) solution, then diluted 10-fold in water.

#### 2.4.3. 4-Day *P. berghei* ANKA suppressive test

The four-day suppressive test described by Peters et al. (1975) was adopted. Briefly, the animals were randomly infected intraperitoneally (ip) with blood containing 2 × 107 parasitized red blood cells containing the *P. berghei* ANKA strain in 0.2 ml inoculum on day zero (D0). Mice were divided into 3 groups of 5 mice each (test, untreated, and positive control). At 4 h post infection, the test animals were treated orally with a single dose of plant extract (800 mg/kg/day) or pure compound (100 mg/kg/day) at a volume of 0.01 ml/gram mouse. The positive control received 10 mg/kg/day of chloroquine. Drug administration was repeated 24, 48, and 72 h post infection. On day 4 (96 h post infection), blood smears were taken by making a thin film from a tail snap of each mouse, fixed in methanol and stained with 10% Giemsa to estimate the level of parasitaemia. Parasitaemia was determined by microscopic examination and counting the parasites in 4 fields of ~100 erythrocytes per view of thin blood film. The difference between the mean number of parasites per view in the control group (100%) and those of the experimental groups was calculated and expressed as percent parasitaemia suppression (PS) according to the formula

\[
\text{% Chemosupression} = \left( \frac{A - B}{A} \right) \times 100
\]

where A = the mean % parasitaemia in the untreated control group; B = the mean % parasitaemia in the test group (Tona et al., 2001).
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antiplasmodial activity</th>
<th>RSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 (µM)</td>
<td>EC50 (µM)</td>
</tr>
<tr>
<td></td>
<td>D6a</td>
<td>W2a</td>
</tr>
<tr>
<td>Crude extracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. burttii (root bark)</td>
<td>0.97 ± 0.2</td>
<td>1.73 ± 0.5</td>
</tr>
<tr>
<td>E. burttii (stem bark)</td>
<td>2.6 ± 0.8</td>
<td>2.9 ± 1.4</td>
</tr>
<tr>
<td>Isoflav-3-enes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burttinol-A (1)</td>
<td>7.6 ± 0.3</td>
<td>8.5 ± 0.6</td>
</tr>
<tr>
<td>Burttinol-B (2)</td>
<td>19.1 ± 0.6</td>
<td>21.1 ± 0.8</td>
</tr>
<tr>
<td>Burttinol-C (3)</td>
<td>9.3 ± 0.9</td>
<td>9.1 ± 1.2</td>
</tr>
<tr>
<td>Eryvarin H (4)</td>
<td>13.3 ± 2.5</td>
<td>20.3 ± 6.1</td>
</tr>
<tr>
<td>2-Arylbenzofuran</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burttinol-D (5)</td>
<td>4.9 ± 0.3</td>
<td>6.1 ± 1.5</td>
</tr>
<tr>
<td>Flavanones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'-O-Methylsigidomoid B (6)</td>
<td>12.4 ± 1.7</td>
<td>12.7 ± 2.3</td>
</tr>
<tr>
<td>Abyssinone V (7)</td>
<td>5.7 ± 0.5</td>
<td>6.6 ± 1.3</td>
</tr>
<tr>
<td>Abyssinone V methyl ether (8)</td>
<td>10.7 ± 2.4</td>
<td>11.5 ± 2.1</td>
</tr>
<tr>
<td>Pericarpian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caloparcarpin (9)</td>
<td>19.4 ± 1.8</td>
<td>17.0 ± 1.5</td>
</tr>
<tr>
<td>Reference drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.009 ± 0.001</td>
<td>0.08 ± 0.001</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.04 ± 0.02</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Quercetin</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT – not tested. The acetate derivatives, burttinol-A diacetate (1a), burttinol-B acetate (2a), burttinol-C diacetate (3a), ervarin H diacetate (4a) and burttinol-D diacetate (5a) were inactive in both assays with IC50 and EC50 values being above 100 µM.

a D6 and W2 are chloroquine-sensitive and -resistant clones of Plasmodium falciparum respectively.
b DPPH: 2,2-diphenyl-1-picrylhydrazyl.

The IC50 and EC50 values of the crude extracts are given in µg/ml.

2.5. Radical scavenging activity test

Pure compounds and crude extracts were tested for radical scavenging activity using a the method described in Ohnishi et al. (1994). Briefly, to a methanolic solution of DPPH (3 ml of DPPH at 100 µM), 0.5 ml of the test compound was added and the mixture was shaken and left to stand for 30 min. The radical scavenging activities were estimated as the percent decrease of the absorbance of DPPH (100 µM) at 517 nm (Ohnishi et al., 1994). Compounds were tested at seven different concentrations: 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µM (for the crude extracts the concentrations were in µg/ml). In all cases the mean values were used from triplicate experiments. Effective concentration (EC50) values were calculated using Finney’s probit analysis for quantal data (McLaughlin et al., 1991).

3. Results and discussion

The crude acetone extracts of the root bark and stem bark of E. burttii demonstrated significant in vitro antimalarial activities against the D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of P. falciparum (Table 1). The major compounds (Fig. 1) previously isolated from the root bark (Yenesew et al., 2002) and stem bark (Yenesew et al., 1998) were also tested. These include four isoflav-3-enes [burttinol-A (1), burttinol-B (2), burttinol-C (3) and ervarin H (4)], a 2-arylbenzofuran [burttinol-D (5)], three flavanones [4’-O-methylsigidomoid B (6), abyssinone V (7) and abyssinone V methyl ether (8)] and a pericarpian [caloparcrain (9)]. The isoflav-3-enes burttinol-A (1) and burttinol-C (3), the 2-arylbenzofuran burttinol-D (5) as well as the flavanone abyssinone V (7) were the most active with IC50 values less than 10 µM against both strains (Table 1). Some of the in vitro activities listed in Table 1 are comparable to the activity reported for licochalcone A (IC50 = 5.6 ± 0.6 µM), the most promising antimalarial flavonoid/chalcone derivative (Ziegler et al., 2004). It is worth to note that among the most active compounds, burttinol-A (1) and burttinol-D (5) contain the relatively uncommon 1,1-dimethylylallyl substituent as licochalcone A. The antiplasmodial activities of flavanones (Yenesew et al., 2003, 2004) and some 2-arylbenezofuran derivatives (Kraft et al., 2001) have been reported. However, this appears to be the first report on the antiplasmodial activity of isoflav-3-enes.

The crude extract remains more active than any of the pure compounds, which suggests that additional antimalarial compounds may exist within the roots of E. burttii; high potency of the crude extract may also be due to synergistic effect of multiple compounds. Future studies should aim to elicit any synergistic effects that combinations of compounds within the crude extract have on the growth of the malaria parasite.

In a Radical Scavenging Activity (RSA) assay against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, using spectrophotometric method, the crude acetone extract of the root bark of E. burttii had an EC50 value of 12 µg/mL. The flavonoids isolated from this plant were tested and showed EC50 values between 9 and 76 µM (Table 1). Compounds 1, 3 and 5 (EC50 value of ca. 10 µM) are again the most active compounds in this assay, with the reference sample quercetin showing EC50 value of 5.0 µM. The high radical scavenging activity of compounds 1, 3 and 5 appear to be related to the common structural features in these compounds – the presence of a double bond in ring C which extends the conjugation in the three rings (rings-A, -B and -C, Fig. 1). Upon loss of two hydrogen radicals from the hydroxyl groups at C-4’ and C-7 to DPPH, the molecule will be resonance stabilized due to extended conjugation across the three rings. We have earlier proposed the same reasoning for the high radical scavenging activities (EC50 ca. 10 µM) of the structurally related pericarpianes isolated from the roots of E. abyssinica (Yenesew et al., 2009).

Burttinol-C (3) appear to be the biogenetic precursor of burttinol-B (2), where intramolecular cyclization involving the hydroxyl group at C-7 and the prenyl group at C-8 of compound 3 forms compound 2. This cyclization causes a four-fold loss of radical scavenging activity, accompanied by similar loss of antimalosomal activity, suggesting the importance of the free phenolic groups at C-4’ and C-7 in compound 3 for the relatively higher antimalosomal and radical scavenging activities. When the phenolic groups are completely blocked through acetylation, the acetate derivatives 1a, 2a, 3a, 4a and 5a (Fig. 1) were inactive (IC50 > 100 µM) in both in vitro antimalosomal and radical scavenging activity tests (data not shown in Table 1). In order to see if this observation applies to structurally related compounds, the acetate derivatives ervarin L diacetate and erycristagallin diacetate were prepared from ervarin L and erycristagallin isolated from E. abyssinica (Yenesew et al., 2003, 2009). These acetate derivatives (eryvarin L diacetate and erycristagallin diacetate) were again inactive (IC50 > 100 µM, data not shown in Table 1) while the parent compounds were reported to be active (Yenesew et al., 2003, 2009). This clearly showed the importance of free phenolic groups for antimalosomal and radical scavenging activities for these isoflavonoid derivatives.

Overall, the isoflavonoids 1, 3 and 5 (Fig. 1) having two free phenolic groups, a C5 substituent adjacent to a hydroxyl group and a double bond at C-3, showed significant antimalosomal and radical scavenging activities, bringing into focus the dual role these isoflavonoids can play in malaria control. It is worth to mention that quercetin which is used as a positive control here (EC50 = 5.0 µM) in radical scavenging activity, also have antimalosomal activity with IC50 value of 10 µM (Gupta et al., 2010).
The crude extract of the roots of *E. burttii*, the roots and stem bark of *E. abyssinica* and the root bark of *E. sacleuxii* were tested in an *in vivo* 4-days *P. berghei* ANKA suppressive test (Peters et al., 1975), at 800 mg/kg/day. All the extracts showed weak to moderate parasite suppression activities (Table 2). In our hands the only compound with enough quantity for *in vivo* testing was the flavanone abyssinone V methyl ether (8). When tested, this compound unfortunately did not show significant activity at 100 mg/kg/day. It is worthwhile to test the *in vivo* activities of the isoflav-3-enes burttinol-A (1) and burttinol-C (3), the 2-arylbenzofuran burttinol-D (5) and the flavanone abyssinone V (7) isolated from this extract, since these compounds have shown significant *in vitro* activities comparable to that of licochalcone A, a chalcone/flavonoid which is known for its *in vivo* activity (Ziegler et al., 2004). The flavanone abyssinone V (7) is also one of the active principles in the roots and stem bark of *E. abyssinica* (Yenesew et al., 2003, 2004).

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**References**


