Herbal Remedies Traditionally Used Against Malaria in Ghana: Bioassay-Guided Fractionation of Microglossa pyrifolia (Asteraceae)§

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Different extracts from 11 West African plants traditionally used against malaria in Ghana were tested against both the chloroquine-sensitive strain PoW and the chloroquine-resistant clone Dd2 of Plasmodium falciparum. Due to the promising in vitro activity of the lipophilic extract [IC₅₀: 10.5 µg/ml (PoW); 13.1 µg/ml (Dd2)], Microglossa pyrifolia (Lam.) Kuntze (Asteraceae) was chosen for further phytochemical investigation. From active fractions 13 compounds were isolated; their structures were established on the basis of spectroscopic methods. 1-Acetyl-6E-geranylgeraniol-19-oic acid and sinapyl diangelate represent new natural compounds. The two diterpenes 6E-phytol [IC₅₀: 8.5 µm (PoW); 11.5 µm (Dd2)], and 6E-geranylgeraniol-19-oic acid [IC₅₀: 12.9 µm (PoW); 15.6 µm (Dd2)] proved to be the most active constituents in our test system.

Introduction

In continuation of our research on medicinal plants with antiplasmodial activity we investigated 11 plants from Ghana, where malaria causes many deaths per year, particularly in young children (Browne et al., 2000). Gomphrena celosioides (Amaranthaceae), Picralima nitida (Apocynaceae), Pergularia daemia (Asclepiadaceae), Emilia sonchifolia (Asteraceae), Microglossa pyrifolia (Asteraceae), Adansonia digitata (Bombacaceae), Euphorbia hirta, Phyllanthus nirurioides (Euphorbiaceae), Tetrapleura tetraptera (Mimosaceae), Mitrugyna inermis, and M. stipulosa (Rubiaceae) are widespread in the Greater Accra Region of Ghana and are used against malaria or fever by herbalists (Bruce, 1998 and 2000). Based on our screening results (Table I) we chose Microglossa pyrifolia (Lam.) Kuntze (syn.: Conyza pyrifolia Lam., Asteraceae) for further investigation. M. pyrifolia is a shrub widespread in tropical Asia and Africa (Do-kosi, 1998). In Ghana an aqueous leaf decoction is used against fever and malaria. In other West African countries the leaf extract is taken against abdominal pains, rheumatism, diarrhoea and many other diseases (Neuwinger, 1994). Subsequent fractionation of the lipophilic extract coupled with an antiplasmodial bioassay (Desjardins et al., 1979) was performed to detect the active principles responsible for the antiprotozoal activity of M. pyrifolia.

Experimental

General experimental procedures

For fractionation, silica gel 60 (63–200 µm) and reversed phase material (LiChroprep® RP-18, 40–63 µm) were used. Preparative high performance liquid chromatography (HPLC) was performed on a Knauer Eurochrom 2000 with Knauer pumping system and a Knauer WellChrom DAD K-2700 UV-detector equipped with an Eurosphere 100 C-18 (10 µm, 22 × 250 mm) column. For preparative thin layer chromatography (TLC) aluminium sheets (20 × 20 cm) coated with silica gel 60
F₂₅₄ were used. Mass spectra were determined with a Finnigan MAT CH7A (220 °C, 70 eV) and FAB-MS were recorded on a VARIAN MAT CH5DF (Xenone, 7 kV). ¹H-NMR spectra, ¹H-¹H-COSY, and HMBC experiments were obtained using acetone-d₆ and CDCl₃ as solvents with a Bruker AVANCE DPX 400 (400 MHz, TMS as internal standard). Cultures of *P. falciparum* were harvested with an Inotech cell harvester and IC₅₀ values determined by a liquid scintillation counting.

**Plant material**

The plant species were collected in the Greater Accra Region from February to March 2000 and April 2001 by scientists of the Department of Botany, Legon-University Accra in Ghana. Voucher specimens of the following plants have been deposited in the herbarium of the Department of Botany, Legon-University Accra: *Gomphrena celosoides* (GC 39071), *Picrostigma nitida* (GC 39064), *Pergularia daemia* (GC 39065), *Emilia sonchifolia* (39072), *Microglossa pyrifolia* (GC 47681), *Euflorbia hirta* (GC 39068), *Phyllanthus nirurioides* (GC 39069), *Tetrapleura tetraptera* (39065), *Mitragyna inermis* (GC 39066), *Mitragyna stipulosa* (GC 39067), *Adansonia digitata* fruits were collected in April 2001 in Navrongo (Upper East Region).

**Extraction and isolation**

For the screening program, the air dried plant material (20 g) was crushed and extracted three times for 2 h with 150 ml petrol ether-EtOAc (1:1, v/v) at room temperature to gain the lipophilic extracts. Afterwards the plant material was air dried and treated three times with 150 ml MeOH to afford the hydrophilic extracts. The preparation of the aqueous extracts was carried out according to (Bruce, 2000): Stem barks and roots were boiled for one hour in an aqueous solution of NaHCO₃ (1 teaspoon/300 ml). Vegetative aerial parts or fruits were extracted by an infusion with such a solution.

For further investigations of *M. pyrifolia*, vegetative aerial parts (500 g) were ground and extracted for 24 h at room temperature with petrol ether-EtOAc 1:1 (21). This procedure was repeated three times. Solvents were evaporated at 40 °C under reduced pressure; the residue was subjected to the antiplasmodial test. The oily residue (30 g) was purified by column chromatography on RP-18 material and sequentially eluted with MeOH-H₂O mixtures (4:6; 5:5; 6:4; 7:3; 8:2; 9:1, v/v), MeOH, and CHCl₃ (800 ml, each). Fractions 6–10 eluting with MeOH-H₂O (8:2; 9:1, v/v, 300 ml each) and MeOH (500 ml), respectively, proved to be the most active ones in the antiplasmodial assay. Fraction 6 (residue 120 mg) was chromatographed on silica gel (7 g) with CH₂Cl₂, CH₂Cl₂-MeOH (9:1, v/v) (200 ml each) to yield compound 13 (3 mg). Further purification of the remaining fraction 6A by HPLC with MeOH-H₂O (30:70, v/v) to MeOH within 40 min lead to 11 (60 mg, Rₜ = 29.64 min). Fraction 7 (250 mg) was separated on silica gel (12 g) with petrol ether-EtOAc gradient (3:1 and 1:1, v/v respectively; 170 ml each) and CH₂Cl₂-MeOH (10:1, v/v, 200 ml) and finally by preparative TLC (CH₂Cl₂-MeOH 10:1, v/v) to yield acacetin 1 (3 mg, Rₜ = 0.42), compound 4 (5 mg, Rₜ = 0.52) and 5 (10 mg, Rₜ = 0.48). Fraction 8 (1 g) was purified on silica gel (40 g) with petrol ether-EtOAc (3:1 and 1:1, v/v respectively; 170 ml each) and CH₂Cl₂-MeOH (10:1, v/v, 200 ml) and finally by preparative TLC (CH₂Cl₂-MeOH 10:1, v/v) to yield acacetin 1 (3 mg, Rₜ = 0.42), compound 4 (5 mg, Rₜ = 0.52) and 5 (10 mg, Rₜ = 0.48). Fraction 8 (1 g) was purified on silica gel (40 g) with petrol ether-EtOAc gradient (10:1; 3:1; 1:1, v/v, respectively; 200 ml each) and yielded the active fractions 8A and 8B. Compound 6 (12 mg, Rₜ = 0.49) was isolated from 8A by preparative TLC (petrol ether-EtOAc 2:1, v/v). Purification of 8B by preparative TLC (CH₂Cl₂-MeOH 10:1, v/v) lead to 7 (30 mg, Rₜ = 0.57). Fraction 9 (6 g) was purified on silica (60 g) with petrol ether-EtOAc and CH₂Cl₂-MeOH gradients, respectively (10:1, 5:1 and 40:1, 20:1, v/v, respectively; 250 ml each) and lead to the active fractions 9A and 9B. Compounds 8 (3 mg, Rₜ = 0.60), 9, and 10 (6 mg, Rₜ = 0.62) were isolated from 9A by preparative TLC (CH₂Cl₂-MeOH 10:1, v/v). From fraction 9B, using preparative TLC (CH₂Cl₂-MeOH 10:1 and 20:1, v/v, respectively), we isolated compound 12 (12 mg, Rₜ = 0.62). Fractionation of 10 (1.5 g) on silica (20 g) lead to linoleic acid 2 (15 mg); fraction 10A was purified by preparative TLC (CHCl₃-MeOH 9:1, v/v) and yielded 3 (2 mg, Rₜ = 0.47). Fractionation of the inactive aqueous extract by column chromatography on RP-18 material with MeOH-H₂O mixtures of decreasing polarity (up to 90% MeOH) lead to active fractions, which contained compounds 7 and 11.

**1-Acetyl-6E-geranylgeraniol-19-oic acid** (12):

³¹HNMR (400 MHz), acetone-D₆: δ 6.77 (1H, t, J = 7 Hz, H-6); 5.39 (1H, tq, J = 7 Hz, 1 Hz, H-2); 5.19 (1H, tq, J = 7 Hz, 1 Hz, H-10); 5.10 (1H, tq, J = 7 Hz, 1 Hz, H-14); 4.59 (2H, d, J = 7 Hz, H-1);
2.41–2.33 (4H, m, H-8, H-9); 2.25–2.12 (4H, m, H-4, H-5); 2.05 (3H, s, OAc); 1.98 (4H, obscured by solvent, H-12, H-13); 1.72 (3H, s, H-20); 1.66 (3H, s, H-16 or H-17); 1.59 (3H, s, H-17 or H-16) ppm; (-)-FAB-MS: 361 [M-H]⁻.

**Sinapyl diangelate (13):** HNMR (400 MHz), acetone-D₆: δ 6.90 (2 H, s, H-2, H-6), 6.73 (1 H, brd, J = 16 Hz, H-7), 6.47 (1H, td, J = 6 Hz, 16 Hz, H-8), 6.23 (1 H, m, H-3) ppm; EI-MS: [M]⁺ 374 (5), 292 (12), 83 (100), 55 (46); HR-MS: m/z = 374.17273 (calculated 374.17294 for C₂₁H₂₆O₆), 292.13099 (calculated 292.13108 for C₁₆H₂₀O₅), 83.04966 (calculated 83.04969 for C₅H₇O).

### Table I. Activity of lipophilic plant extracts against *Plasmodium falciparum*.

<table>
<thead>
<tr>
<th>Plant family</th>
<th>Species</th>
<th>Plant part</th>
<th>Traditional uses</th>
<th>Mean IC₅₀ values [µg/ml]²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthaceae</td>
<td><em>Gomphrena celosioides</em> Mart.</td>
<td>whole plant</td>
<td>antimalarial (Bruce, 1998)</td>
<td>36.9</td>
</tr>
<tr>
<td>Apocynaceae</td>
<td><em>Picralima nitida</em> (Stapf) Th. &amp; H. Durand</td>
<td>stem bark</td>
<td>substitute for quinine (Neuwing, 1994), antimalarial (Bruce, 1998), (Francois et al., 1996) remedy for cold (Dokosi, 1998), and fever (Bruce, 2000) fever remedy (Abbiw, 1990), antimalarial (Azuine, 1998) fever remedy (Abbiw, 1990), antimalarial (Bruce, 1998)</td>
<td>&gt; 50 &gt; 50</td>
</tr>
<tr>
<td>Asclepiadaceae</td>
<td><em>Pergularia daemia</em> (Forssk) Chiov.</td>
<td>stem bark</td>
<td>remedy for cold (Dokosi, 1998), and fever (Bruce, 2000) fever remedy (Abbiw, 1990), antimalarial (Azuine, 1998) fever remedy (Abbiw, 1990), antimalarial (Bruce, 1998)</td>
<td>36.9 43.6</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Emilia sonchifolia</em> (L.) DC.</td>
<td>aerial parts</td>
<td>antimalarial (Bruce, 1998), antimalarial (Dokosi, 1998), and fever (Bruce, 1998)</td>
<td>10.5 13.1</td>
</tr>
<tr>
<td>Bombacaceae</td>
<td><em>Adansonia digitata</em> L.</td>
<td>fruit pulp⁵b</td>
<td>antimalarial (Bruce, 1998), antimalarial (Azuine, 1998) antimalarial (Dokosi, 1998)</td>
<td>&gt; 50 &gt; 50</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td><em>Euphorbia hirta</em> L.</td>
<td>whole plant</td>
<td>antimalarial (Bruce, 1998), antimalarial (Dokosi, 1998), and fever (Bruce, 1998)</td>
<td>&gt; 50 &gt; 50</td>
</tr>
<tr>
<td>Mimosaceae</td>
<td><em>Tetraptera tetraptera</em> (Schuhmach. &amp; Thonn.) Taub.</td>
<td>fruits</td>
<td>antimalarial (Neuwing, 1994)</td>
<td>&gt; 50 &gt; 50</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td><em>Mitragyna inermis</em> (Willd.) Kuntze</td>
<td>leaves, stem bark, root</td>
<td>analgesic properties (Abbiw, 1990), antimalarial (Bruce, 1998), (Mustafa et al., 2000) antimalarial (Bruce, 1998), antidote to poison, e.g. (Abbiw, 1990)</td>
<td>&gt; 50 34.6 39.6 32.6 36.1 48.7</td>
</tr>
<tr>
<td></td>
<td><em>Mitragyna stipulosa</em> (DC.) Kuntze</td>
<td>leaves, stem bark, root</td>
<td>analgesic properties (Abbiw, 1990), antimalarial (Bruce, 1998), (Mustafa et al., 2000) antimalarial (Bruce, 1998), antidote to poison, e.g. (Abbiw, 1990)</td>
<td>&gt; 50 34.6 39.6 32.6 36.1 48.7</td>
</tr>
</tbody>
</table>

### Antiplasmodial activity

The antiplasmodial assay was performed by means of the microculture radioisotope technique as described previously (Jenett-Siems et al., 2000). The concentration at which growth was inhibited by 50% (IC₅₀) was estimated by interpolation. IC₅₀ values > 50 µg/ml for extracts and IC₅₀ values > 25 µg/ml for fractions, respectively, were considered inactive (O’Neill et al. 1985).

### Results and Discussion

Lipophilic extracts of all tested plant species exhibited a moderate antiplasmodial activity with *M. pyrifolia* aerial parts as the most active ones [IC₅₀: 13.1 µg/ml (Dd2), 10.5 µg/ml (PoW)]. Bioassay-guided fractionation of the aerial parts of *M. pyrifolia* revealed the fractions eluting from a RP-18...
column with 80% to 100% MeOH to be most active with IC$_{50}$ values ranging from 2.5 to 18.7 µg/ml against a chloroquine-sensitive strain (PoW) and a chloroquine-resistant clone (Dd2) of Plasmodium falciparum. Further separation by several chromatographic methods yielded 13 compounds. We isolated acacetin (1), linoleic acid (2), E-phytol (3), benzyl 2,6-dimethoxybenzoate (4) (Lu et al., 1993), 13-hydroxy-octadeca-9Z,11E, 15Z-trien-oic acid (5) (Reddy et al., 1994), and 1-hydroxy-calamene as a mixture of two isomers (6) (El-Seedi et al., 1994). Furthermore, we obtained the following furanoditerpenes: strictic acid (7) (Tandon and Rastogi, 1979), hardwickii acid (8) (Heymann et al., 1994), 10α-nidoresedic acid (9) and 10β-nidoresedic acid (10). The spectral data of 9 and 10 were quite similar to those of the already known methyl esters (Singh et al., 1988, Bohlmann and Fritz, 1978). Several active fractions yielded 6E-geranylgeraniol-19-oic acid (11) (Zdero et al., 1990). The $^1$H NMR of 12 was very similar to that of 11. An additional singlet at 2.06 ppm (3H, s, OAc) pointed to an acetate group. Due to the presence of the acetate group, the signal for the two protons at C-1 displayed a chemical shift of 4.59 ppm (2H, d, $J = 1$ Hz) versus the signal for H-1 of 11 at 4.09 ppm (2H, d, $J = 7$ Hz, H-1). The (−) FAB MS gave a parent ion [M-H]$^-$ at m/z 361 thus confirming the structure and lead to the identification of 12 as 1-acetyl-6E-geranylgeraniol-19-oic acid. The isolation of the 6E-isomer has not yet been reported, in contrast to its 6Z-isomer (Herz and Kulanthaivel, 1985). The EIMS spectrum of 13 showed a molecular ion peak at m/z 374. The ion fragment peak at m/z 292 indicated the loss of an angelate group. In the $^1$H NMR compound 13 displayed a singlet at 6.90 ppm (2H, s, H-2, H-6) and two singlets for two methoxyl groups at 3.84 ppm (6H, s, OMe-3 and OMe-5), belonging to a methoxylated aromatic system. Additionally a doublet at 6.73 ppm (1H, d, $J = 16$ Hz, H-7) and a triplet doublet at 6.47 ppm (1H, td, $J = 6$ Hz, 16 Hz, H-8) pointed to a trans-configurated double bond in conjugation with the aromatic system. The chemical shift of the allylic methylene group at 4.81 ppm (2H, dd, $J = 6$ Hz, $J = 1$ Hz) indicated an esterification at this position. Additionally typical signals for two angelate moieties could be observed: 6.23 ppm (1H, m, H-3), 6.12 ppm (1H, qq, $J = 1$ Hz, 6 Hz, H-3′ or H-3″), 2.20 ppm (3H, m, H-4″ or H-5″), 1.97 ppm (3H, dq, $J = 7$ Hz, 1.5 Hz, H-4′), 1.90 ppm (3H, quint, $J = 1.5$ Hz, H-5′). Comparison with literature data of known sinapyl alcohol derivatives (Bohlmann et al., 1969) lead to the identification of 13 as sinapyl diangelate, which represents a new natural compound. This is the first report on the occurrence of 1-10, 12, and 13 from M. pyrifolia.

In our antiplasmodial test system the compounds linoleic acid (2), E-phytol (3), 13-hydroxy-octadeca-9Z,11E,15Z-trien-oic acid (5), and 6E-geranylgeraniol-19-oic acid (11) exhibited an activity against P. falciparum with IC$_{50}$ values between 2.5 µg/ml and 13.7 µg/ml (Table II). From several active fractions we isolated the diterpene 6E-geranylgeraniol-19-oic acid (11). Thus, we assume that the aliphatic unsaturated compounds of M. pyrifolia are representing the antiprotozoal principle of this species. In our test system the methanolic and aqueous extracts of all tested species did not show any activity. However, fractionation of the aqueous extract of M. pyrifolia lead to the detection ($^1$H NMR and EIMS spectra) of 7 and 11 in active fractions. Thus, 11 is detectable in the aqueous extract despite of its rather lipo-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean IC$_{50}$ values* PoW</th>
<th>Dd2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid (octadeca-9,12-dienoic acid)* (2)</td>
<td>6.1</td>
<td>21.8</td>
</tr>
<tr>
<td>E-Phytol (3)</td>
<td>2.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Benzyl 2,6-dimethoxybenzoate (4)</td>
<td>9.0</td>
<td>33.1</td>
</tr>
<tr>
<td>13-Hydroxy-octadeca-9Z,11E,15Z-trien-oic acid (5)</td>
<td>6.7</td>
<td>22.8</td>
</tr>
<tr>
<td>6E-Geranylgeraniol-19-oic-acid (11)</td>
<td>4.3</td>
<td>12.9</td>
</tr>
<tr>
<td>Chloroquine × 2 H$_3$PO$_4$</td>
<td>0.008</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Table II. Antiplasmodial activity of compounds isolated from Microglossa pyrifolia against Plasmodium falciparum in vitro.

* Performed in triplicate.
* Previously tested (Köhler et al., 2002).
philic character. Therefore, we conclude that 6E-geranylgeraniol-19-oic acid (11) is mainly responsible for a moderate antiplasmodial activity of a traditional plant preparation from *M. pyrifolia*.

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