The Antimalarial Potential of Three Ghanaian Medicinal Plants

Abstract

Objective: Malaria is a major public health problem in Ghana and many indigenes, especially those in rural areas, resort to the use of medicinal plants to treat the disease. The plants: *Persea americana* Mill. (Lauraceae), *Theobroma cacao* L. (Malvaceae) and *Tridax procumbens* (L.) L. (Compositae) are used solely or in combination with other medicinal plants to manage malaria and its associated conditions. The leaves of the plants which are normally the main parts employed, were studied for their phytochemistry and antiplasmodial activity to establish their chemical profile and verify the antimalarial claim.

Methods: Plant materials were subjected to basic phytochemical screening to identify the major secondary metabolites. The aqueous extracts were evaluated against chloroquine-sensitive 3D7 *P. falciparum* and chloroquine-resistant W2 *P. falciparum* strains, using the fluorescence-based SYBR® green I method to determine their antiplasmodial activity.

Results: Basic phytochemical screening of the leaves revealed the presence of tannins, flavonoids and alkaloids in all three plant materials. *T. cacao* and *P. americana*, in addition, contained purine base alkaloids, triterpenoids including saponins. The aqueous extracts of the leaves showed antiplasmodial activity against the chloroquine-sensitive 3D7 *P. falciparum* (9.50 ± 1.38 ≤ IC₅₀ ≤ 10.15 ± 0.45 µg/mL) and against chloroquine-resistant W2 *P. falciparum* strains (6.40 ± 1.94 ≤ IC₅₀ ≤ 44.94 ± 1.12 µg/mL). The aqueous extract of *T. cacao* was the most active and was more active against W2 than 3D7 *P. falciparum*. Only *T. procumbens* displayed cytotoxicity (CC₅₀<25 µg/mL).

Conclusion: *T. cacao*, *T. procumbens* and *P. americana* possess antiplasmodial activity. The activity illustrates their antimalarial potential, and provides rationale for their use in traditional malaria therapy in Ghana. It thus paves the way for further study of these plants for antiplasmodial lead compound(s).

Keywords: *Persea americana*; *Theobroma cacao*; *Tridax procumbens*; Antiplasmodial; Phytochemistry; Malaria; Traditional; Medicinal plants

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Introduction

The use of medicinal plants in the treatment of diseases has a long history worldwide. In developing countries, about 80% of the population relies on traditional medicine for the treatment of various ailments including life-threatening ones such as malaria. In Sub-Saharan Africa, medicinal plants, which constitute the mainstay of traditional medicine, have significant medicinal usages, though with little or no supportive scientific data. Plants used in malaria and fever account for 6% of the medicinal plants of the Ghanaian domestic market [1] and some of these plants have been formulated into commercial phytotherapeutics [2]. According to Komlaga et al. [2], about 6%, 8% and 7% of herbalists in the Bosomtwe and Sekyere East Districts of Ghana, respectively, employ Persea americana Mill. (Lauraceae), Theobroma cacao L. (Malvaceae) and Tridax procumbens (L.) L. (Compositae) in the traditional treatment of malaria. We therefore evaluated the antiplasmodial activity of the aqueous extracts of the three plants in order to provide scientific validation for their antiplasmodial use in traditional medicine. Persea americana is cultivated for its nutritious fruit which contains vitamins and minerals [3]. The leaf is used for the treatment of malaria [3-5], dysentery, coughs, liver obstructions and high blood pressure [6]. It is used to enhance menstrual flow and reduce high levels of uric acid in the body [6]. The skin of the fruit is used to treat intestinal parasitic worms infestation, cancer of the labia and other tumours [3]. The fruit pulp has emollient and carminative properties, and lowers blood cholesterol levels. The mashed fruit pulp has aphrodisiac properties, and lowers worms infestation, cancer of the labia and other tumours [3]. The leaf is used for the treatment of malaria [3-5], dysentery, coughs, liver obstructions and high blood pressure [6]. It is used to enhance menstrual flow and reduce high levels of uric acid in the body [6]. The skin of the fruit is used to treat intestinal parasitic worms infestation, cancer of the labia and other tumours [3].

Materials and Methods

Reagents and chemicals

Chemicals including RPMI (Roswell Park Memorial Institute) 1640, hypoxanthine, N-2-hydroxyethylpiperazine-N’-2-ethane-sulphonic acid (HEPES), glucose, albumax II, SYBR® green I-lysis buffer, DMEM-F-12 (Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12), streptomycin, fetal bovine serum (FBS), Trypsin, Ethylenediaminetetraacetic acid (EDTA), were obtained from Gibco/Invitrogen Life Technologies, France. Non-infected O+ blood was kindly donated by Bichat Hospital (Paris, France).

Selection, collection and preparation of plant materials

The leaves of Persea americana Mill. (Lauraceae), Theobroma cacao L. (Malvaceae) and Tridax procumbens (L.) L. (Compositae) (Table 1), selected from an earlier survey, were harvested in July 2013 in Kumasi, Ghana. They were selected because high percentage of herbalists employ them in the traditional treatment of malaria in the Bosomtwe and Sekyere East Districts of Ghana [2]. The plant materials were authenticated by Dr G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Sciences and Technology (KNUST), Kumasi, Ghana, and herbarium specimen with Voucher numbers (Table 1) deposited in the same Department. The harvested plant materials were air dried under shade at ambient temperature (28-35°C) for 7 days. They were then coarsely powdered using an electric mill and stored in air-tight containers under room temperature until required for use.

Extraction of plant materials

For each plant material, 100 g of powder was boiled in 2 L of water in accordance with traditional preparations for 30 minutes. The decoction was then strained through double-layered white cotton material and subsequently through filter paper. The filtrate was lyophilized and the dry material kept at 4°C until needed for analysis.
Preliminary phytochemical analysis of materials

Plant materials were subjected to qualitative phytochemical screening to determine the presence of the major phytochemical constituents. The screening was performed according to standard procedures [21,22].

Plasmodium culture maintenance

Parasite cultures consisting of chloroquine-sensitive 3D7 *P. falciparum* and chloroquine-resistant W2 *P. falciparum* were maintained according to the method described by Trager & Jensen [24,25]. The parasites were separately cultivated at 37°C in a candle jar on complete culture medium of RPMI 1640, containing 2.5% hematocrit, hypoxanthine, HEPES, glucose, albumax II, and buffered with NaHCO₃. Fresh culture was maintained for at least 96 hours (2 complete life cycles) before being used for assays. Cultures were synchronized with 5% D-sorbitol (Sigma) and at 96 hours (2 complete life cycles) before being used for assays. The frozen plates were then taken through a 3 freeze-thaw cycles in a candle jar at 37°C, the plates were frozen at -80°C overnight. Obtaining 11 concentrations each in triplicate. After 42 h incubation, cells were incubated in a medium containing aqueous plant extracts in a dose-titration range of 0.098 - 100 µg/mL. After 24 h incubation, 20 µL MTT (5 mg/mL) was added to each well and cells were incubated for another 3 h. Finally, the culture medium containing MTT solution was removed and the formazan crystals were dissolved in 100 µL of dimethylsulfoxide (DMSO). Absorbance was read with an Eppendorf plate reader at 546 nm. Drug concentration that reduced the number of viable cells by 50%, CC₅₀, was calculated using GraphPad Prism Software (Version 5.0, San Diego, CA, USA).

Cytotoxicity on HUVEC using MTT assay

Human umbilical vein endothelial cells (HUVECs) were cultured in Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12) medium in the presence of fetal bovine serum (FBS; 10%) plus streptomycin (1%) and incubated in 5% CO₂ at 37°C [28,29]. The cytotoxicity of aqueous plant extracts was evaluated using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) colorimetric method [30]. HUVEC were seeded in a 96 well plate at 15000 cells/well and incubated for 24 h when cells reached >80% confluence. After discarding the old medium, the cells were incubated in a medium containing aqueous extracts of the plant materials in a dose-titration range of 0.78-100 µg/mL. After 24 h incubation, 20 µL MTT (5 mg/mL) was added to each well and cells were incubated for another 3 h. Finally, the culture medium containing MTT solution was removed and the formazan crystals were dissolved in 100 µL of dimethylsulfoxide (DMSO). Absorbance was read with an Eppendorf plate reader at 546 nm. Drug concentration that reduced the number of viable cells by 50%, CC₅₀, was calculated using GraphPad Prism Software (Version 5.0, San Diego, CA, USA).

Results

Phytochemical screening

All three plant materials tested positive for flavonoids, tannins, and alkaloids. However, *Theobroma cacao* and *Persea americana* also showed the presence of purine base alkaloids and terpenoids (Table 2).

Table 1 Plant for antimalarial assessment.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Family</th>
<th>Local name (Ewe/Twi)</th>
<th>Voucher specimen number</th>
<th>Part investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Persea americana</em> Mill.</td>
<td>Lauraceae</td>
<td>Peya</td>
<td>HM/032/13</td>
<td>Leaf</td>
</tr>
<tr>
<td><em>Theobroma cacao</em> L.</td>
<td>Malvaceae</td>
<td>Koko</td>
<td>HM/033/13</td>
<td>Leaf</td>
</tr>
<tr>
<td><em>Tridax procumbens</em> (L.) L.</td>
<td>Compositae</td>
<td>Nantwi bini</td>
<td>HM/034/13</td>
<td>Whole plant</td>
</tr>
</tbody>
</table>

Table 2: Phytochemical screening of the leaves of the plants.

<table>
<thead>
<tr>
<th>Constituents</th>
<th><em>P. americana</em></th>
<th><em>T. cacao</em></th>
<th><em>T. procumbens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Purine base alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Keys: +: present; -: absent, in the conditions used.

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In vitro antiplasmodial activity of aqueous extracts

The extracts were assessed at concentrations up to 100 µg/mL against 3D7 and W2 strains of *Plasmodium falciparum*. All three extracts showed antiplasmodial activity. Activity against 3D7 *P. falciparum* strain was generally around 10 µg/mL. The extract of *T. cacao* was particularly more active against the chloroquine-resistant W2 *P. falciparum* strain (IC<sub>50</sub>: 6.40 ± 1.94 µg/mL). However, *P. americana* and *T. procumbens* extracts were respectively 3 and 4 folds less active when compared to their activity against 3D7 *P. falciparum*. Resistant index (RI) was generally low and exceptionally low (RI<1) for *T. cacao*. Only *T. procumbens* demonstrated cytotoxicity (CC<sub>50</sub>: 24.89 ± 3.68). The extracts of *P. americana* and *T. cacao* generally showed high selectivity index (SI>2.9) whereas *T. procumbens* displayed largely low SI (Table 3).

Discussion

The use of medicinal plants in malaria therapy and also, in the therapy of many other diseases is commonplace at the primary healthcare level in many developing countries. In Ghana, medicinal plants have been used traditionally to manage malaria; some without scientific evidence of efficacy or safety. *Theobroma cacao*, *Persea americana* and *Tridax procumbens*, examples of such plants, were evaluated for their phytochemical profile, antiplasmodial activity and cytotoxicity.

The leaves contained various secondary metabolites including, tannins, alkaloids, flavonoids, and terpenoids (Table 2). Many compounds belonging to these phytochemical groups had shown antiplasmodial activity [31] and may indicate the same for constituents of the plants studied.

The aqueous extracts of the three plants demonstrated varying degrees of activity against chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of *P. falciparum* (Table 3). According to Philippe et al. [32], the antiplasmodial activity of plant extracts can be classified as highly active (IC<sub>50</sub> ≤ 5 µg/mL); moderately active (5<IC<sub>50</sub> ≤ 15 µg/mL); weakly active (15<IC<sub>50</sub> ≤ 50 µg/mL) and inactive (IC<sub>50</sub> >50 µg/mL). Against this backdrop, all the extracts were considered to be moderately active against 3D7 *P. falciparum*. On the other hand, only the leaf extract of *T. cacao* showed moderate activity against W2 *P. falciparum*; that of *P. americana* and *T. procumbens* were only weakly active against the same strain.

The aqueous extract of *P. americana* and *T. cacao* leaf extracts exhibited no cytotoxicity against HUVECs up to the highest concentration (100 µg/mL) tested, and displayed good selectivity for the parasite (SI>2.9). Conversely, *T. procumbens* showed significant cytotoxicity and very low selectivity especially for the chloroquine-resistant parasite (Table 2). *T. procumbens* can therefore be described as generally cytotoxic since it demonstrated cytotoxicity to both parasite and human cells. This extract needs further investigation regarding potential toxicity and evaluation of benefit/risk ratio in man. Until this is done, *T. procumbens* should be used in traditional treatment of malaria with caution since it could be harmful to the host especially in high doses. Extracts with high selectivity index offer the potential of safer therapy [27,28]. Therefore with appropriate dosage regimen, *T. cacao* in particular could serve as an important malaria therapy. However, preclinical studies and clinical studies would be required to ascertain potential usefulness.

The resistance index (RI) was determined to assess the levels of resistance demonstrated by the resistant strain of the parasite against the respective extracts. The chloroquine-resistant W2 *P. falciparum* displayed no resistance to *T. cacao* while it showed low level of resistance to *P. americana* and *T. procumbens* extracts. This may suggest *T. cacao* a more appropriate remedy in the traditional treatment of malaria particularly at a time that *P. falciparum* resistance is a challenge in the treatment of the disease [33,34].

Appiah-Opong et al. [18] reported an inferior antiplasmodial activity (IC<sub>50</sub> >500 µg/mL) for the aqueous extract of *T. procumbens* against chloroquine-resistant Dd2 *P. falciparum* strain while in this study, a more stronger activity (IC<sub>50</sub>: 44.94 ± 1.12 µg/mL) was established against the chloroquine-resistant W2 *P. falciparum* strains. Many factors could account for this difference among which could be the strain of *P. falciparum* used and plant-associated factors such as the geographical source of plant, the season of collection, the age when collected, preparation and storage condition (where applicable) of extract.

The aqueous extract of *P. americana* leaf is reported for the first time for antiplasmodial activity; likewise, the leaf aqueous extract of *T. cacao*. However, the bean (cocoa) powder has been shown to have antiplasmodial activity [35].

Aside the direct effects of drugs on the malaria parasite in

<table>
<thead>
<tr>
<th>Extract</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;: 3D7</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;: W2</th>
<th>Cytotoxicity</th>
<th>SI, 3D7</th>
<th>SI, W2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>9.93 ± 0.86</td>
<td>34.20 ± 5.80</td>
<td>3.4</td>
<td>&gt;100</td>
<td>&gt;10.1</td>
</tr>
<tr>
<td>TC</td>
<td>9.50 ± 1.38</td>
<td>6.40 ± 1.94</td>
<td>0.7</td>
<td>&gt;100</td>
<td>&gt;10.5</td>
</tr>
<tr>
<td>TP</td>
<td>10.15 ± 0.45</td>
<td>44.94 ± 1.12</td>
<td>4.4</td>
<td>24.89 ± 3.68</td>
<td>2.5</td>
</tr>
<tr>
<td>CQ</td>
<td>0.02 ± 0.00</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Keys: PA: aqueous extract of *Persea americana* leaf, TC: aqueous extract of *Theobroma cacao* leaf, TP: aqueous extract of aerial part of *Tridax procumbens*; 3D7: chloroquine-sensitive *P. falciparum* strain; W2: chloroquine-resistant *P. falciparum* strain; ND: not determined; CQ: chloroquine; Selectivity Index (SI) is defined as the ratio of CC<sub>50</sub> to IC<sub>50</sub>; Resistance Index (RI) is defined as the ratio of the IC<sub>50</sub> of the chloroquine-resistant *P. falciparum* line (W2 *P. falciparum*) to that of the parent (chloroquine-sensitive) strain (3D7 *P. falciparum*); the concentration of CQ is expressed in µM; IC<sub>50</sub> and CC<sub>50</sub> are expressed in µg/mL ± SD.
chemotherapy of the disease, alleviation of the feverish symptoms, such as joint pains, body weakness and headache among other associated symptoms are important. Adeyemi et al. [36] reported the analgesic and anti-inflammatory activities of the leaf aqueous extract of P. americana. Also T. procumbens has shown anti-inflammatory [20] and anti-arthritic activities [21]. These symptomatic non-parasite specific effects of the extracts together with their antiplasmodial activity could account for the relief from the disease during therapy. This study thus justifies the traditional use of these plants in malaria treatment in Ghana.

Conclusion

The aqueous extracts of Theobroma cacao (leaf), Tridax procumbens (whole plant) and Persea americana (leaf) possess antimalarial effect. They were active against both chloroquine-sensitive 3D7 and chloroquine-resistant W2 P. falciparum. Only T. procumbens displayed cytotoxicity. The study supports the traditional use of the three plants as a therapy for malaria in Ghana, and provides scientific justification for further study of the plants [36].

P. falciparum has displayed resistance to existing antimalarial drugs including artemisinin and its derivatives [37], emphasizing the need for new effective but affordable antimalarials. The three plants could be considered for formulations into standardized antimalarial phytomedicines using the ‘reversed pharmacology’ approach [38] to afford cheaper alternatives to malaria therapy. Also, different organic solvent extracts of the plant materials could be evaluated to identify solvent specific extracts with promising antiplasmodial activity. Such extracts can become a subject of activity-directed fractionation to isolate active compounds. Compound showing significant activity could be modified chemically to afford molecule with high efficacy and safety. Isolated compounds can also be assessed in combination with themselves and classical antimalarial drugs for synergistic effect against P. falciparum.
References


