Document heading   doi:

In vitro antiplasmodial activity of crude extracts from Togolese medicinal plants

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ARTICLE INFO

ABSTRACT

Objective: To investigate the antimalarial effect of a few plants in Togo folk medicine. **Methods:** After ethnobotanical survey, *Opilia celtidifolia*, *Pavetta corymbosa (P. corymbosa)* and *Tamarindus indica (T. indica)* were selected for screening. *In vitro* antimalarial tests were performed on crude extracts against fresh clinical isolates of *Plasmodium falciparum* using the semi microtest. **Results:** Different IC_{50} values of the extracts ranged from 2.042 to 100.000 μ g/mL. According to the results, the methanol extract of aerial part of *P. corymbosa* followed by aqueous extract of fruit of *T. indica* were the most active (IC_{50} of 2.042 and 4.786 μ g/mL, respectively). Qualitative test revealed the presence of alkaloids in the leaves of *P. corymbosa* that may be responsible for the activity of the plant. **Conclusions:** Our study provides scientific evidence for usage of plant in the folk medicine, and further studies are needed for identification and purification of the active principles.

1. Introduction

Application of plants for medicinal purposes dated back to prehistory and this tradition has been handed down from generation to generation[1]. Until today, plants continue to play a key role in many health care systems particularly in developing countries where modern drugs are not often affordable for the majority of the populations[2]. In Togo, many plants are used in the treatment of several diseases including malaria, infectious diseases and AIDS opportunistic infections. In the country, plant materials are directly sold in market places or transformed in several concoctions by traditional healers in their houses before sale, but there are very few ethnobotanical and pharmacological studies of these plants[3].

Malaria is a disease causing major public health problem in Togo. Indeed the disease is well known and treated by traditional healers. Approximately 500 millions cases and 1.5 to 2 millions deaths are annually recorded[1].

It is estimated that about 12 billions euros are paid for the annual cost due to malaria disease in developing countries[4]. Despite intensive efforts made by politics and scientists, the incidence of the disease does not decrease because of increasing resistance of the parasite to available antimalarial drugs and the mosquitoes to insecticides[5–7]. The situation has brought to the continuous need for new powerful antimalarial agents. Many investigations show that plants are often the main sources of new agents[2,8].

In this study, three medicinal plants of traditional medicine in Togo were screened for *in vitro* antiplasmodial activity. The plants were selected after ethnobotanical survey conducted in 2005 and 2006[9].

2. Materials and methods

2.1. Chemicals

RPMI 1640, bovine foetal serum, HEPES and chloroquine phosphate were obtained from Sigma Chemical Company (St. Louis). L–Glutamine and streptomycin/penicillin were obtained from Gibco BRL. All the other chemicals were of analytical grade.

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2.2. Plant materials

A previous ethnobotanical survey has identified 61 plants assumed to cure malaria in traditional medicine of Togo[9]. In this study three most cited and not well studied plants were selected. The following plant materials were used:
− Aerial part of *Opilia celtidifolia (O. celtidifolia) (G. and Perr.)* Endl. (Opiliaceae), voucher specimen N° CB/TOGO05524, harvested in the gallery forest of Tsévie, 32 km in north of Lomé;
− Aerial part of *Pavetta corymbosa (P. corymbosa)* (cd.) F.N. Will. (Rubiaceae), voucher specimen N° CB/TOGO00232, bought in the medicinal plants market of Tsévié;
− Leaves and fruits of *Tamarindus indica (T. indica)* Flax. (Caesalpiniaceae), voucher specimen N° CB/TOGO07643, the leaves were harvested in the Marian sanctuary of Togoville and the fruits were bought from stalls of medicinal plants market of Hédzranawoé, Lomé.

The samples were botanically authenticated at the Department of Botany and Ecology of University of Lomé where the voucher specimens were deposited.

2.3. Extractions

Plant samples were collected from December 2007 to January 2008. These collected samples were dried in laboratory at room temperature and grinded into powder for several extractions. Aqueous extraction was made by boiling 25 g powder in 250 mL distilled water for 30 minutes. After cooling at room temperature, the extract was filtered with wattman N°1 and lyophilised. Methanolic extraction was performed by maceration of the 25 g powder in 250 mL of 70% (v/v) methanol for 24 hours. Afterwards methanol was evaporated under reduced pressure with a rotary evaporator and the extract was lyophilised. Qualitative tests were carried out to detect major chemical components as described by Ciulei[10]. Thus, the following methods were used:

Bouchardat, Mayer and Dragendorff reagents for alkaloids; Reaction of Liebermann for Steroids/triterpene; Reaction with ferric chloride and sodium hydroxide for tannins and polyphenols; Cyanidine test for flavonoïds; Mechanic agitation of the solution for saponins.

2.4. Parasites

Fresh clinical isolates of *Plasmodium falciparum* were obtained from Laboratoire de Biologie Médicale Saint Camille in Ouagadougou. Giemsa−stained thin smears were examined for *Plasmodium* species identification. The parasite density was determined by counting the number of infected erythrocytes among 20 000 erythrocytes. From each donor, 4 mL of venous blood was collected in a tube coated with EDTA (Greiner Labortechnik). Samples with monoinfection due to *Plasmodium falciparum* and a parasite density between 1% and 2% were used for the *in vitro* antimalarial tests.

2.5. In vitro antimalarial tests

Plasmodium falciparum was grown in 96−well plates as described by Trager and Jensen[11]. Blood cells were washed three times with RPMI 1640 before culture. Erythrocytes were then suspended in RPMI supplemented with HEPES (25 mM), bovine foetal serum (10% (v/v)), streptomycin (100 μ g/mL) and penicillin (100 IU/mL). The haematocrit was 5%. The *in vitro* antimalarial tests were performed under light microscopy using Giemsa−stained smears as described by Le Bras and Deloron[12]. Lyophilised powders were dissolved in dimethyl sulfoxide (DMSO). Plant extracts were then diluted with culture medium to a final concentration of 0.5% (v/v) DMSO in the first wells. Chloroquine phosphate was dissolved in distilled water. The aliquots of drug solutions were added in duplicate. A control experiment was performed separately using 0.5% DMSO to check the effect of these solvents on parasite maturation. Drug concentrations in the wells ranged from 200 to 1.6 μ g/mL for the extracts and from 0.2 to 0.003 μ g/mL for chloroquine phosphate. The final volume in the wells was 200 μ L. The plates were incubated at 37 ℃ with 5% CO_2 in a CO_2 incubator.

2.6. Evaluation of the activity

Parasite maturation was determined by counting mature schizonts among all asexual parasites in 20 000 erythrocytes. The percentages of parasite maturation were plotted against the logarithm of drug concentrations. The concentrations causing 50% inhibition of the maturation (IC_{50} values) were determined by regression equations.

3. Results

A total of 8 different extracts were obtained by aqueous extraction and methanolic extraction from collected plant material. Both water soluble and alcohol soluble compounds were more abundant in *T. indica* fruits than these in other samples (30.04% and 37.16% for aqueous and methanol extracts, respectively). Aerial part of *O. celtifdifolia* and leaves of *T. indica* were found to be richer in water soluble compounds (20.66% versus 10.66% and 22.66% versus 13.33% for the two plants, respectively), however, there were more alcohol soluble compounds than water soluble compounds in the aerial part of *P. corymbosa* (11.33% versus 9.33%).

Qualitative tests were performed for the detection of several chemical components including alkaloids, flavonoids, tannins, saponins triterpens and sterols. Table 1 displayed the major chemical components detected in the extract. Globally triterpen and sterols were not detected in the extracts. However, alkaloids, flavonoids, tannins and saponins were detected in several extracts.

The height extracts were tested for the *in vitro* antiplasmodial activity against fresh clinical isolates of

Table 1
Main chemical components detected in three Togolese medicinal plant extracts.

Extracts		Saponins	Alkloids	Flavonoids	Steroids/triterpenes	Tannins
O. celtidifolia	MetOH	+++	++	+	−	+
	Aqueous	+++	++	++	−	++
P. corymbosa	MetOH	+	+++	++	−	+++
	Aqueous	++	+++	+	−	++
*T. indica (*leaves)	MetOH	++	−	−	−	−
	Aqueous	++	−	−	−	−
T. indica (fruits)	MetOH	−	−	−	−	++
	Aqueous	−	−	−	−	++

Relative abundance; −:absent, +: trace, ++: abundant, +++: very abundant.

P. falciparum with chloroquine phosphate as standard antimalarial drug. According to the results, aerial part of *P. corymbosa* methanol extract followed by the fruits of *T. indica* aqueous extract were the most active (IC$_{50}$ values of 2.041 μ g/mL and 4.786 μ g/mL, respectively). According to international standards[13], methanol extracts of the aerial part of *P. corymbosa* and the aqueous extract of fruits of *T. indica* were very active on the strains of *P. falciparum*; the aqueous extracts of the aerial part of *P. corymbosa* was active (IC$_{50}$ = 6.025 μ g/mL); the aqueous extract of *O. celtidifolia* (IC$_{50}$ = 83.176 μ g/mL) and the methanol extract of *T. indica* (IC$_{50}$ = 55.544 μ g/mL) were moderately active; the other extracts (IC$_{50}$ > 100 μ g/mL) were devoid of intrinsic antiplasmodial activity.

4. Discussion

O. celtidifolia is not a well−documented plant for its pharmacological properties. The present study revealed a weak antiplasmodial activity of both aqueous and methanol extracts of the plant. Qualitative tests revealed the presence of saponins as major components of the extracts. Saponins are well known for their tensioactive properties but they are not potent antimalarial agents. The present results are in accordance with these observations and they corroborated previous reports on the antimalarial activity of the plant. Indeed, Kamanzi−Atindehou et al[14] showed that the ethanolic extract of the plant was inactive on the chloroquine−resistant strain of *P. falciparum* K1, however, Okpekon et al[15] found that dichloromethane extract of leaves was more active (IC$_{50}$ =17.4 μ g/mL) than the methanol extract (IC$_{50}$ >20 μ g/mL) on *P. falciparum*.

There are very few studies on the antiplasmodial activity of *T. indica*. In the present study, the leave extract failed in the inhibition of parasite growth. However, the aqueous extract of fruit showed significant activity on the parasites. Qualitative analysis showed that tannins were found to be the major components of the extract. Tannins are not potent antimalarial agents; however, some tannins−rich plants of the same *Caesalpiniaceae* family showed significant antiplasmodial activity. Main examples are *Cassia occidentalis*[16−18] *Cassia siamea*[19], *Cassia alata*[18] and *Cassia hirsuta*[20]. However, *Caesalpinia bonduc*[17],

Canthium selosum[21], *Entanda macrophylla*[18] and *Guilbourtia demensii*[20] of the same family did not show significant inhibition of parasite growth.

P. corymbosa is a *Rubiaceae* that showed the best activity in the present screening. This antiplasmodial activity may be due to alkaloids detected in the plant extract as major components. Weniger et al[21] reported good antiplasmodial activity of leave extract of the plant. In fact the synthesis of alkaloids is common in *Rubiaceae* family, of course these plants are considered as potent antimalarial plants. *P. crassipes* from the same genus has been studied by several authors who found moderate antiplasmodial activity[19,22]. Several species of the same family are well known for their uses in African traditional medicine as antimalarial plants. *Nauclea latifolia*[18,23,24], *Morinda lucida*[16,17,20,23], and *Mitragyna imernis*[23−26] showed significant activities (IC$_{50}$ between 2 and 10 μ g/mL). Alkaloids are often found to be responsible for the activity of the plants in the majority of these studies. An alkaloid is a plant−derived compound that is toxic or physiologically active. It contains nitrogen in a heterocyclic ring, and has a complex structure. Besides, it is of limited distribution in the plant kingdom. Many alkaloids have been found to possess antimalarial properties. The main example is quinine isolated from *Cinchona* species. Their antiplasmodial activity may be through different mechanisms. Some such as chloroquine, a chemical derivative of quinine, act by inhibiting the detoxification of heame in red blood cells[27]. This is supported by a fluorescent microscopy study. It suggested that cryptolepine accumulates into parasite structures that may correspond to the parasite nucleus[28]. The others such as cryptolepine, which is the main indoloquinoline alkaloid and occurs in *Sida acuta*[29], is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition[30,31].

Our study showed that *P. corymbosa* is very active in treating *Plasmodium falciparum*. This activity may be due to alkaloids that are found to be common in *Rubiaceae* family and responsible for the activity of several members of the family. Our study provides scientific evidence for the use of this plant in the folk medicine, and further studies are needed for identification and purification of the active principles.

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