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Antimalarial activity of *Sida acuta* Burm. f. (Malvaceae) and *Pterocarpus erinaceus* Poir. (Fabaceae)

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Abstract

Among strategies to combat malaria, the search for new antimalarial drugs appears to be a priority. Sheering for new antimalarial activities, four plants of the traditional medicine of Burkina Faso: *Combretum micranthum*, *Khaya senegalensis*, *Pterocarpus erinaceus* and *Sida acuta*, were tested in vitro on fresh clinical isolates of *Plasmodium falciparum*. The screening showed that *Sida acuta* has a significant activity ($IC_{50} < 5$ μg/ml), and *Pterocarpus erinaceus* has a moderate activity (5 μg/ml $< IC_{50} < 50$ μg/ml). Further chemical screening showed that the activity of the most active plant, *Sida acuta*, was related to its alkaloid contents.

Keywords: Malaria; *Plasmodium falciparum*; *Sida acuta*; *Pterocarpus erinaceus*; Alkaloids

1. Introduction

Malaria is a parasitic disease caused by a protozoan of the genus *Plasmodium*. Most of the lethal cases are caused by *Plasmodium falciparum*, the most virulent of the four *Plasmodia* species that infect humans. Despite extensive control efforts, the incidence of the disease is not decreasing, principally in developing countries, where malaria remains a parasitic disease that causes major public health problems. Infection with *Plasmodium falciparum* is responsible for hundreds of millions of cases and more than 1 million deaths each year (Breman, 2001). Malaria also remains a major risk to travellers from industrialised to developing countries. The spread of multidrug-resistant parasites and insecticide-resistant mosquitoes has led to major difficulties in the treatment and in the control of the disease. Therefore, malaria control efforts may include the attempt to seek for effective vaccine, eradication of mosquito vectors and find out new antimalarial drugs (Oask et al., 1991; Olliaro et al., 1996). However, the development of an effective vaccine has proven very difficult and a highly effective vaccine may not be available soon (Hoffman and Miller, 1996). Although the use of insecticide-impregnated bed nets does appear to reduce malaria-related death rates, efforts to control *Anopheles* mosquitoes have had limited success (Alonso et al., 1997). In addition, methods to replace natural vector populations with mosquitoes unable to support the development of the parasites are under investigation (Collins and Besansky, 1994). To develop new antimalarial drugs, the ethnobotanical investigation in traditional medicine can be an important source of new leads. African traditional medicine uses numerous plants that can be source of new antimalarials. In the present work, we report the in vitro antimalarial activity of several plants used in the traditional medicine to treat malaria in Burkina Faso. Further investigation on the most active plant was performed to identify the active compounds.

2. Methodology

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. Parasites

Fresh clinical isolates of *Plasmodium falciparum* were obtained before treatment from paediatric patients of Laboratoire de Biologie Médicale Saint Camille de Ouagadougou. Enquiry was made on drug intakes of the patients to select those who did not take any antimalarial drug. 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 patient, 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% was used for the in vitro antimalarial tests.

2.3. Plant materials

Plant samples were chosen according to their traditional uses. A survey was made with traditional practitioners to select 50 widely used plants to treat fever. A previous study has been conducted on seven plants (Sanon et al., 2003a,b). The second study involved four plants. The following plant materials were used:

- leaves of *Combretum micranthum* G. Don (Combretaceae), harvested in July 2001;
- leaves and bark of *Khaya senegalensis* (Desr.) A. Juss. (Meliaceae), harvested in July 2001;
- leaves and bark of *Pterocarpus erinaceus* Poir (Fabaceae), harvested in July 2001;
- whole plant of *Sida acuta* Burm. F. (Malvaceae), harvested in August 2001.

All samples were harvested around Ouagadougou and were botanically authenticated at the Department of Plant Biology and Ecology of the University of Ouagadougou and voucher specimens were deposited at the Laboratoire de Pharmacologie et de Biochimie Clinique, CRSBAN, Université de Ouagadougou. Their code numbers are: BC-cm01, BC-ks01, BC-pe01 and BC-sa01 for *Combretum micranthum*, *Khaya senegalensis*, *Pterocarpus erinaceus* and *Sida acuta*, respectively.

2.4. Extraction of antimalarial compounds

Samples were washed with water and dried in the laboratory at room temperature (20–25 °C). Afterwards samples were ground to pass a sieve of 1 mm. They were then percolated in 70% (v/v) ethanol for 24 h. The solvent was evaporated with a rotary evaporator. Extracts were diluted with distilled water and lyophilised. The first antimalarial tests were performed with lyophilised samples. The two most active ethanolic extracts were diluted in water and brought under liquid–liquid separation with petroleum ether, chloroform and water. Ether and chloroform were evaporated from the corresponding fractions and the aqueous fraction was lyophilised. These isolated fractions were used for the second antimalarial tests.

Alkaloids from *Sida acuta* were extracted using the classical method of alkaloids extraction. The ground and sieved sample was made alkaline with ammonia and extracted with chloroform. The extract was made acidic with hydrochloride acid and extracted again with chloroform. Prior to biological tests, extracts were stored at −22 °C.

2.5. In vitro antimalarial tests

Plasmodium falciparum was grown in 96-well plates as described by Trager and Jensen (1976). Blood cells were washed three times with RPMI 1640 before use in culture. Erythrocytes were then suspended in RPMI supplemented with L-glutamine (4.2 mM), HEPES (25 mM), bovine foetal serum (10% (v/v)), streptomycin (100 μg/ml) and penicillin (100 IU/ml). The haematocrit was 5%.

The in vitro antimalarial tests were performed by light microscopy using Giemsa-stained smears as described by Le Bras and Deloron (1983).

Lyophilised powders were dissolved in dimethyl sulfoxide (DMSO) and alkaloids were dissolved in methanol. Plant extracts were then diluted with culture medium to a final concentration of 0.5% (v/v) DMSO and 0.1% (v/v) methanol 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 or 0.1% methanol to check the effect of these solvents on parasite maturation.

Drug concentrations in the wells ranged from 200 to 1.6 μg/ml for the ethanolic extracts, from 100 to 0.19 μg/ml for the separation fractions, from 1 to 0.03 μg/ml for the alkaloids of *Sida acuta* and from 0.2 to 0.003 μg/ml for chloroquine phosphate. The final volume in the wells was 200 μl. The plates were incubated at 37 °C in a candle jar for a total period of 36–40 h.

2.6. Evaluation of the activity

Parasite maturation was determined by counting mature schizonts among all asexual parasites for 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 with regression equations.

3. Results

A total of 38 clinical isolates were obtained from the patients for the antimalarial tests. Fifteen isolates were used for the activity of the ethanolic extracts, 13 for the isolated fractions and 10 for the alkaloids. All the isolates were used for the activity of chloroquine phosphate in these experiments.

Table 1
IC_{50} values of isolated fractions

Fractions	*Sida acuta* (μg/ml)	*Pterocarpus erinaceus* (μg/ml)
Ether fraction	57.04	7.38
Chloroformic fraction	0.87	1.93
Aqueous fraction	0.92	103.35
Alkaloids	0.05	Not found

The percentages of mature schizonts were always higher than 20% in the control wells. The presence of DMSO and methanol did neither decrease parasite maturations nor alter their morphology. At high concentration of extracts, parasites were completely obliterated and the few survivors were shrunken. However, erythrocytes showed no significant deformation. The response curves for the drugs over these ranges were characteristically sigmoidal after logarithmic transformation of drugs concentrations.

The IC_{50} of ethanolic fractions of *Sida acuta*, leaves of *Pterocarpus erinaceus*, *Combretum micranthum*, leaves of *Khaya senegalensis*, bark of *Khaya senegalensis* and bark of *Pterocarpus erinaceus* were 4.37, 14.63, 33.05, 58.48, 82.17 and 95.13 μg/ml, respectively. According to the norm that active extract has $IC_{50} < 5$ μg/ml and moderate active extract 5 μg/ml $< IC_{50} < 50$ μg/ml (Rosanaivo et al., 1992), the ethanolic extract of *Sida acuta* could be considered as active, and the extract of leaves of *Pterocarpus erinaceus* and *Combretum micranthum* moderately active. The ethanolic extract of *Khaya senegalensis* had no significant activity.

Sida acuta and leaves of *Pterocarpus erinaceus* have been used for the second part of the study. The results of these second antimalarial tests are shown in Table 1. The IC_{50} values ranged from 0.05 to 57.04 μg/ml for *Sida acuta* and from 1.93 to 103.35 μg/ml for *Pterocarpus erinaceus*. The IC_{50} values of chloroquine phosphate in each case were always less than 0.042 μg/ml, with an average of 0.0097 μg/ml.

4. Discussion

All the isolates of *Plasmodium falciparum* used in this study were chloroquine-sensitive strains according to IC_{50} values with chloroquine phosphate.

Ethanolic extracts displayed different activities on *Plasmodium falciparum* strains. The extract of *Sida acuta* appeared to be the most active. Empirically, this plant is used in decoction alone or in association with other plants to treat fever. This study confirms the antimalarial activity of this plant. The plant is also known to have a moderate activity on the venom of *Bothotrops atrox* (Otero et al., 2000).

The extract of leaves of *Pterocarpus erinaceus* showed a moderate antimalarial activity, however, the bark of the same plant is devoid of any antimalarial activity. This plant is also used in decoction alone or in association with other plants to treat fever. The bark of the plant is widely known to treat chronic diarrhoea (Kerharo and Adam, 1974), but cytotoxicity has been described for this plant (Abreu et al., 1999).

Combretum micranthum showed a moderate activity. Antiplasmodial activity was previously described for the plant by Benoit et al. (1996) and recently by Ancolio et al. (2002). Other investigation revealed that the plant has an antiviral activity (Ferrea et al., 1993).

Khaya senegalensis is widely used in West Africa to treat many diseases because the plant has many pharmacologic properties. Ethnobotanical investigations revealed that the plant has an anti-inflammatory activity (Thioune et al., 1999) and an activity against *Leishmania donovani* (Abreu et al., 1999). In the particular case of malaria, it was previously described an antimalarial activity for the plant against *Plasmodium falciparum* 3D7, a chloroquine-sensitive strain, and against *Plasmodium falciparum* Dd2, a chloroquine-resistant strain (El Tahir et al., 1999). In the present study, the plant showed no significant activity against fresh clinical isolates but the plant may treat malaria symptoms, like fever, since the plant has hypothermic activity (Lompo et al., 1995).

IC_{50} of chloroformic fraction was higher than the IC_{50} of the petroleum ether fraction of *Pterocarpus erinaceus*. This suggests that the active compounds were more soluble in chloroform than petroleum ether. However, there is no significant difference between the chloroformic and aqueous fraction in case of *Sida acuta*. Active compounds may have the same solubility in chloroform and in water or different compounds may be responsible for the antimalarial activity. Alkaloids were extracted because they are known to be soluble in organic solvents and in water according to pH and they are known to be potential antimalarial agents. The IC_{50} value obtained confirmed that the antimalarial activity of *Sida acuta* was due to alkaloids. Leaves of *Pterocarpus erinaceus* were devoid of alkaloids.

5. Conclusion

From selection of four plants, preliminary result showed that *Sida acuta* was the most active plant against *Plasmodium falciparum* followed by *Pterocarpus erinaceus*. The antimalarial activity of the most active plant, *Sida acuta*, may be due to alkaloids.

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