Afr. J. Trad. CAM (2006) **3 (1):** 75 - 81

Research Paper
ISSN 0189-6016©2006

Afr. J. Traditional,
Complementary and Alternative Medicines
www.africanethnomedicines.net

ANTIMALARIAL ACTIVITY OF *SWARTZIA MADAGASCARIENSIS* DESV. (LEGUMINOSAE), *COMBRETUM GLUTINOSUM* GUILL. & PERR. (COMBRETACEAE) AND *TINOSPORA BAKIS* MIERS. (MENISPERMACEAE), BURKINA FASO MEDICINAL PLANTS.

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Abstract

Swartzia madagascariensis, *Combretum glutinosum* and *Tinospora bakis* are three plants of the folk medicine used by healers in Burkina Faso for the treatment of malaria. A scientific validation of this utilization was not previously made. Aqueous, methanol, hydromethanol extracts from the roots bark of *S. madagascariensis*, methanol and hydromethanol extracts from the leaves of *C. glutinosum* and aqueous and alkaloidal extracts from the roots of *T. bakis* were also made and their antimalarial activity was screened against *Plasmodium falciparum* chloroquine-resistant strain W2 in vitro. The screening showed that the methanol and hydromethanol extracts of *Swartzia madagascariensis*, hydromethanol extracts of *Combretum glutinosum* and alkaloidal extracts of *Tinospora bakis* were active (5µg/ml < IC50 < 50 µg/ml).

Key words: Antimalarial activity; *Swartzia madagascariensis; Combretum glutinosum; Tinospora bakis.*

Introduction

Malaria is the worldwide most important parasitic disease with an incidence of almost 300 millions clinical cases and over one million deaths per year. Almost 90% of these deaths occur in sub-saharan Africa where young children are the most affected. Malaria is directly responsible for one in five childhood deaths in Africa and indirectly contributes to illness and

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deaths from respiratory infections, diarrhoeal disease and malnutrition (WHO, 1999). While we wait for malaria vaccine, effective chemotherapy remains the mainstay of malaria control (Winstanley, 2000). *Plasmodium falciparum*, the potentially lethal malaria parasite has shown itself capable of developing resistance to nearly all used antimalarial drugs (WHO, 1999) and resistant strains have rapid extension (Plowe et al. 1995).

The lost of effectiveness of chemotherapy constitute the greatest threat to the control of malaria. Therefore, to overcome malaria, new knowledge, products and tools are urgently needed; especially new drugs are required (Omulokoli et al. 1997; Rasoanaivo et al.1999). Traditional methods of malaria treatment could be a promising source of new antimalarial compounds. In Africa, more than 80% of people use traditional medicines and most families have recourse to this medicine based on plants extracts for the curative treatment of malaria (Wright and Phillipson, 1990). In fact, the traditional medicine of this continent constitutes an important source for ethnopharmacological investigation. In the present work, the antimalarial activity of three traditional medicinal plants used in Burkina Faso was evaluated against the Vietnamian *Plasmodium falciparum* chloroquine-resistant strain W2.

Material and methods

Plant materials

Plants were selected on the basis of their ethnomedical histories. The roots bark of *Swartzia madagascariensis* Desv. (Leguminosae) and the young leaves of *Combretum glutinosum* Guill. & Perr. (Combretaceae) were harvested in January 2004 in the district of Peni (Province du Houet). The roots of *Tinospora bakis* Miers. (Menispermaceae) were collected in February 2004 in the district of Oursi (Province de l'Oudalan). Plants were authenticated in the Department of Botany, Université de Ouagadougou, where voucher specimens were conserved: *S. madagascariensis* (LPa Sm01), *C. glutinosum* (LPa Cg01) and *T. bakis* (LPa Tb01). The roots bark of *S. madagascariensis*, leaves of *C. glutinosum* and root of *T. bakis* were washed thoroughly in tap water, shade-dried and powdered.

Plants crude extracts

The following extracts were made: Water, methanol and water-methanol (50:50 $^{v}/_{v}$) extracts from the powder of root and bark of *S. madagascariensis*, Methanol and water-methanol (50:50 $^{v}/_{v}$) extracts from the powder of leaves of *C. glutinosum*, Water and alkaloidal extracts from the root powder of *T. bakis*.

Aqueous extractions were performed three different times. Dried and ground plant materials (20 g) were extracted with distilled water (200 ml) for 1h, 24h or 48h with constant stirring. The extracts were filtered and the filtrates were freeze-dried to yield 15.24% $^{w}/_{w}$, (Sm1h), 19.22% $^{w}/_{w}$ (Sm24h) and 21.36% $^{w}/_{w}$ (Sm48h) of *S. madagascariensis* respectively. For the powder of root of *T. bakis*, only an extract of 24h duration was made. This extraction yield 9.93%, w/w referred as Tb24h of crude extracts of *T bakis*.

Before extraction with methanol and water-methanol (50:50 $^{v}/_{v}$), the powders of plants materials (20 g) were first macerated overnight at room temperature with dichloromethane in column and then washed with the same solvent in order to remove chlorophylls and lipids. The ground materials were dried under a chemical hood and successively extracted with methanol and water-methanol (50:50 $^{v}/_{v}$). Methanol extracts were obtained by maceration of dried grounds with the solvent (200 ml) at room temperature for 18h. After percolation, these extracts were mixed with H_2O (100 ml) and methanol was evaporated *in vacuo*. The aqueous

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fractions were lyophilized to yield crude methanol extracts: 17.04%, $^{w}/_{w}$ (SmMeOH) for *S. madagascariensis*; and 12.91%, $^{w}/_{w}$: (CgMeOH) for *C. glutinosum*. To obtain water-methanol extracts, grounds were extracted with water-methanol (50:50 $^{v}/_{v}$) overnight by maceration and methanol was evaporated *in vacuo*. Aqueous fractions were freeze-dried to yield crude water-methanol extracts (9.30% $^{w}/_{w}$: (SmMeOH-H_2O) for *S. madagascariensis,* and 5.17% $^{w}/_{w}$: (CgMeOH-H_2O) for *C. glutinosum*.

The alkaloid extracts of the dried and ground roots of *T. bakis* was made with 500 g of root powder according to the classical method of alkaloid extraction to yield 0.19% $^{w}/_{w}$ of crude alkaloid extracts as TbAlt. (Bruneton, 1993).

In vitro antimalarial assay
Parasites cultivation

The antimalarial activity of plant extracts was assessed against the Vietnamian *Plasmodium falciparum* strain W2 which is resistant to chloroquine, pyrimethamine and proguanil. This strain obtained from the Institut de Medecine Tropicale du Service de Santé des Armées (Marseille, France) was maintained in continuous culture in flask according to the methodology described by Tragger and Jensen (1976) and Tragger (1987). *P. falciparum* strain W2 were cultivated in human red blood cells (O^+), diluted to 2% hematocrit in RPS10 (RPMI 1640 medium supplemented with 25 mM HEPES and 25 mM $NaHCO_3$ and complemented with 10% human A^+ serum and 5% Neomycin). Incubations were done at 37°C 6%CO_2, 84%N_2, and 90% H_20. Hematocrits were adjusted at 2% and parasite cultures were used when they exhibit a parasitaemia of 2%.RPMI 1640, HEPES and $NaHCO_3$ were obtained from Gibco – BRL (Paisley, Scotland).

Preparation of tested extracts

The aqueous extracts were dissolved in ultrapure H_2O. Methanol, hydromethanol and alkaloidal extracts were dissolved in DMSO (dimethyl sulphoxide, Sigma Chemical Co, St Louis, MO, USA). These solutions were filtered through Millipore sterile filters (mesh 0.22 µm, Millipore SA, 67120, Molsheim, France) and diluted 1/20 in RPS10 to obtain the tests stock solutions. Five concentrations were tested: 50, 25, 12.5, 6.25 and 3.125 µg/ml.

Flow Cytometric analysis

Extracts were incorporated in triplicate cultures. Negative control (fresh red blood cells without *Plasmodium* diluted to 2% hematocrit) and positive control (parasitized blood cells culture not treated with plants extract) were added to each set of experiments. The antimalarial assays were performed three times in flat bottomed 96-well Nunc tissue culture plates (Brand products, Fisher, Paris, France) containing 200 µl of culture medium according to the method of Azas et al (2001). Parasitaemia was evaluated after 48 h without medium replacement, by a flow cytometer technique derived from the methods of Van der Heyde et al. (1995) using the vital dye hydroethidine (HE, Interchim, Montluçon, France) (Wyatt et al., 1991). Hydroethidine was diluted in DMSO to make a stock of HE (10 mg/ml) and stored at -20°C. The culture medium was removed from each well of parasite culture by flicking the plates and 160 µl of HE stock solution diluted 1/200 in phosphate-buffered saline (PBS, Sigma Chemical Co, St Louis, MO, USA) was added to each well. Plates were incubated for 20 min at 37°C in the dark. The cells were then washed three times with PBS by

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centrifugation at 1200 rpm (Sigma, laborzentrifugen 4K15, Bioblock) during 5 min and re-suspended in a final volume of 1 ml of PBS in the tubes for fluorescence-activated cell sorter (FACS) analysis. Flow cytometric data acquisition and analysis were made on a FACS sort (Becton-Dickinson, San Jose, CA) fit out with an argon laser of 15 mW, wavelength of 488 nm. The apparatus was run according to this program: forward scatter (FSC-H: voltage E-1, gain 1, Log mode), side scatter (SSC-H: voltage 250, gain 1, Log mode) and red fluorescence (FL2: voltage 459, gain 1, Log mode). Ten thousand cells were used for data acquisition and both infected and uninfected erythrocytes were gated in the analysis. The percentage of parasitaemia (number of infected erythrocytes/total erythrocytes x100) was determined using the LYSIS II program.

Antimalarial activity calculation and analysis

The antimalarial activity of plant extracts was expressed by the inhibitory concentrations 50 (IC_{50}), representing the concentration of drug that induced a 50% parasitaemia decrease compared to the positive control culture referred as 100% parasitaemia. The IC_{50} values were determined graphically on dose-response curves (concentration versus percent inhibition curves) with non-linear analysis by TableCurve 2D v5.0 software (Jandel Scientific, Paris, France). This activity was analysed in accordance with the norm of plants antimalarial activity of Rasaonaivo et al. (1992). According to this norm, an extract is very active if IC_{50} < 5µg/ml, active 5µg/ml < IC_{50} < 50µg/ml, weakly active 50µg/ml < IC_{50} <100 µg and inactive IC_{50} > 100 µg/ml.

Results

The IC_{50} of Chloroquine was 775.10nM. The IC_{50} values of plants extracts shown in table 1 demonstrated that they possess an antimalarial activity. The following extracts (Sm1h, Sm24h, Sm48h, CgMeOH and TbH_2O) were moderately active and there was not a big difference between the activities of the aqueous extracts of *S. madagascariensis*: Sm1h (IC_{50} = 56.5µg/ml), Sm24h (IC50 = 50.6µg/ml) and Sm48h (IC_{50} = 60.5µg/ml). SmMeOH, Sm MeOH-H_2O, Cg MeOH-H_2O and TbAlt were active. Alkaloid extracts from *T. bakis* were far more active than the aqueous extract of the same plant.

Discussion

Plasmodium W2 strain were successfully cultured and was chloroquine-resistant (IC_{50} > 500nM). Methanol extracts of *S. madagascariensis* showed the highest antimalarial activity. Previous pharmacological investigations dealt only with the molluscicidal activity of the seeds pods of this plant (Suster et al. 1986, Lwambo and Moyo, 1991, Hostettmann and Wolfender, 1997). The root bark is used in aqueous maceration for the curative treatment of fever with vomits and jaundice in Burkina Faso. Even if there was not a great difference among the activities of different time duration of the macerate of the root barks of *S. madagascariensis,* Sm24h seem to be the more active. It could mean that the optimal time for aqueous extraction is around 24 hours. Elsewhere, this duration corresponds to the duration of preparation advised by traditional healer whom gave this treatment. The weakness of the activity of Sm48h with regard to Sm24h could be explained by the denaturising of the active compounds because of the phenomena of fermentation which often occurred when this plants is macerated during 48h. However, further biological assays and chemical analysis with methanol extracts

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Table 1: IC_{50} value of plants extracts against *Plasmodium falciparum* chloroquine-resistant strain W2.

Plants	**Extract**		Extract Yield (% w/w)	**IC_{50}** (µg/ml) (moyenne ± SEM)
S. madagascariensis (ref. LPa Sm01)	Aqueous extracts	Sm 1h	15.24	56.29 ± 0.27
		Sm 24h	19.22	50.6 ± 1.10
		Sm 48h	21.36	60.5 ± 1.42
	Methanolic extract	Sm MeOH	17.04	15.5 ± 1.08
	Hydromethanolic extract	SmMeOH-H_2O	9.30	28.8 ± 3.49
C. glutinosum (ref. LPa Cg01)	Methanolic extract	Cg MeOH	12.91	53 ± 0.01
	Hydromethanolic extract	Cg MeOH-H_2O	5.17	43.6 ± 3.33
T. bakis (ref. LPa Tb01)	Aqueous extract	Tb H2O	9.93	59.8 ± 3.14
	Alkaloïds extract	Tb Alt	0.19	28.6 ± 1.04

could allow faster isolation and identification of the active components because these alcoholic extracts from *S. madagascariensis* were more active than the aqueous ones.

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The young leaves of *C. glutinosum* are used as diuretic and antihypertensive supplement. They possess a remarkable activity against fever, jaundice and lever illness (Kerharo and Adam, 1974). They are used against fever, malaria and jaundice in Burkina Faso (Thiombiano, 1996). The methanolic extracts from this plant showed antihepatitis B activity *in vitro* and contain gallic tannins (Pousset et al., 1993). This extract showed however a weak antiplasmodial activity (IC_{50} > 50 µg/ml). This result is in accordance with Asres et al. (2001) who found that tannins have intermediate activity against *P. falciparum*.

T bakis is in excellent traditional drug against fever with hematuric and gall vomits, jaundice and liver complaints. Alkaloid extracts (TbAlt, IC50=28.6 µg/ml) are more active than the aqueous extracts TbH_2O (IC50 = 59.8 µg/ml).

Conclusion

The experiment showed that the extracts of *S. madagascariensis*, *C. glutinosum* and *T. bakis* possess some measure of antimalarial activity. Methanol and alkaloid extracts showed higher activity than the aqueous extracts which are used in traditional medicine. Further pharmacological screenings with chemical fractions of methanol extracts of *S. madagascariensis*, hydromethanol extracts of *C. glutinosum* and alkaloid extracts of *T. bakis* could allow the isolation, and identification of active compounds of these plants.

Acknowledgements

We thank the AUF agency, for the post-doc proficiency research grant and the Republic of Japan through UNESCO-Japan grant which allowed us to carry out this research.

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