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ANTIMALARIAL ACTIVITY IN CRUDE EXTRACTS OF SOME CAMEROONIAN MEDICINAL PLANTS.

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Abstract

Fifteen crude extracts from the stem bark and seeds of four medicinal plants, viz: *Entandrophragma angolense*, *Picralima nitida*, *Schumanniophyton magnificum* and *Thomandersia hensii* were tested *in vitro* for their antimalarial activity against the chloroquine-resistant *Plasmodium falciparum* W2 strain. The results showed that the extracts of these plants possessed some antimalarial activity, the methanol extract of *Picralima nitida* demonstrating the highest activity *in vitro*. Further isolation and identification of some active compounds from these plants will justify their common use in traditional medicine for the treatment of malaria or fever in Cameroon.

Key words: *Entandrophragma angolense*; *Picralima nitida*; *Schumanniophyton magnificum*; *Thomandersia hensii*; *Plasmodium falciparum*; Antimalarial activity.

Introduction

The spread of chloroquine-resistant *Plasmodium falciparum* and the alarming emergence of multi-drug resistant strains have raised an urgent need to search for new, safe and effective antimalarial drugs (O 'Neill et al., 1986; Weenen et al., 1990). So, screening of herbal potions used in traditional medicine was indicated. Medicinal plant extracts as well as their occurring products have been used in the treatment of fever or other symptoms associated with malaria throughout the tropics and subtropics, with a folklore encompassing centuries of practical experience (Weenen et al., 1990). The continuing clinical use of quinine isolated from *Cinchona* species (Rubiaceae) and the recent discovery of a highly effective anti-malarial substance, artemisinin, from a Chinese herb, *Artemisia annua* L. (Compositae) have stimulated the interest in medicinal plants as sources of new potential antimalarial drugs.

Since 1980, large-scale *in vitro* and *in vivo* screenings of plants all over the world have been intensified and some of these have shown high antimalarial activities (Fandeur et al., 1985; Noster and Kraus, 1990; Koumaglo et al., 1991; Nkunya, 1992; Gessler et al., 1993).

In Cameroon, many plants are traditionally used as antimalarials or antipyretics (Tantchou et al., 1986; Kouamouo, 1988). A recent study using a crude extract and purified compounds of *Khaya grandifoliola* (Meliaceae) (Bickii et al., 2000) has yielded interesting results and stimulated us to investigate more plants from other families, traditionally used for the treatment of malaria or fever. *Entandrophragma angolense* (Welw) CDC (Meliaceae), *Picralima nitida* Stapf, 1910 (Apocynaceae), *Thomandersia hensii* De Wild and Th. Dur (Acanthaceae), and *Schumanniophyton* (Rubiaceae) (Letouzey, 1985) are some of these plants usually used in Cameroonian traditional medicine as antimalarials or antipyretics. Although, their therapeutic properties are claimed or evidenced in traditional medicine, few studies have been realised in our country to assess the activities of these medicinal plants.

In the present study, the *in vitro* antimalarial activity of these traditional medicinal plants, used in Cameroon, was assessed against *P. falciparum* W2 (Indochina I/CDC) chloroquine-resistant strain.

Materials and methods

Plant materials

Plants were selected on the basis of their ethnomedical studies and endemicity. Stem bark of *Entandrophragma angolense* Welwitsch C. D. C. (Meliaceae) was harvested in April 1999 in Yokadouma in the eastern Cameroon. Stem bark of *Thomandersia hensii* De Wild and Th. Dur (Acanthaceae) was harvested in march 2003 in Ngomedzap, southern Cameroon. Stem bark of *Schumanniophyton magnificum* Harms (Rubiaceae) and the seeds of *Picralima nitida* Stapf (Apocynaceae) were collected in April 2003 in Eseka, southern Cameroon. Plants were authenticated in the Cameroon National Herbarium, Yaoundé for *E. angolense* (N° 29932) and in the Herbarium of the Research Centre for Medicinal Plants and Traditional Medicine, Laboratory of Botany, Yaoundé, for *P. nitida* (LB Pn 0301), *T. hensii* (LB Th 0301) and *S. magnificum* (LB Sm 0301), where different Voucher specimens were conserved.

Extraction procedures

Plant materials were dried and pulverised. The following extracts were made: hexane, 50% dichloromethane-methanol, methanol and water-ethanol (1: 1 v/v) of the stem bark of *E. angolense*; hexane, 50% dichloromethane-methanol and methanol extracts of the seeds powder of *P. nitida*; hexane, ethyl acetate, 50% dichloromethane-methanol and methanol extracts of the stem bark powder of *T. hensii* and hexane, 50% dichloromethane-methanol, methanol and water-ethanol (1: 1) extracts of the stem bark powder of *S. magnificum*.

Dry and powdered stem bark of *E. angolense*, *S. magnificum*, *T. hensii* and the seeds of *P. nitida*, were extracted separately with a mixture of water-ethanol (1: 1) (*E. angolense* and *S. magnificum*) and hexane. The defatted material obtained after hexane extraction was then extracted successively with ethyl acetate, dichloromethane – methanol (1:1), and methanol.

Hence, dried and ground plant material (3.34 kg *E. angolense*, 2.5 kg *S. magnificum*) were macerated with ethanol-water (1:1) mixture for 48h and 96h. The extracts were filtered and the filtrate were freeze-dried to yield (2.62 % $^{w}/_{w}$ *E. angolense*, 3.28 $^{w}/_{w}$ *S. magnificum*) respectively.

For the hexane, ethyl acetate, dichloromethane-methanol (1:1) and methanol extracts, the ground materials (8 kg of each plant) were dried and successively extracted with hexane, ethyl acetate, dichloromethane-methanol (1:1) and methanol at room temperature for 48h and 96h. The extracts were filtered and the filtrates were submitted to concentration by a rotavapor. The solvents were evaporated *in vacuo* to yield hexane extracts (2.85 % $^{w}/_{w}$ *E. angolense*, 1.14 % $^{w}/_{w}$ *P. nitida*, 0.30 % $^{w}/_{w}$ *S. magnificum*, 0.67 % $^{w}/_{w}$ *T. hensii*), ethyl acetate extract (6.7 % $^{w}/_{w}$ *T. hensii*), dichloromethane – methanol extracts (4.28 % $^{w}/_{w}$ *E. angolense*, 5 % $^{w}/_{w}$ *P. nitida*, 1.97 % $^{w}/_{w}$ *S. magnificum*, 6.2 % $^{w}/_{w}$ *T. hensii*) and methanol extracts (3.57 % $^{w}/_{w}$ *E. angolense*, 3.57 % $^{w}/_{w}$ *P. nitida*, 1 % $^{w}/_{w}$ *S. magnificum*, 5.83 % $^{w}/_{w}$ *T. hensii*).

In vitro evaluation assay

Parasites cultivation

The *P. falciparum* chloroquine-resistant strain W2 was maintained in continuous culture in sealed flasks according to the technique described by Trager and Jensen (1976). The clone W2 was cultivated in the culture medium (RMPI 1640, 25 nM Hepes and 25 nM $NaHCO_3$, supplemented with heat inactivated 10 % human serum) and the parasites were incubated at 37 °C in a 3 % O_2, 5 % CO_2 and 91 % N_2 atmosphere. These were synchronised in the ring stage by serial treatment with 5 % sorbitol (Sigma) (Lambros and Vanderberg, 1979) and studied at 1 % parasitemia.

Preparation of tested extracts

Extracts were prepared as 20 mg/ml stock solutions in DMSO (dimethyl sulfoxide, Sigma Chemicals Co, S^{t} Louis, MO, USA) filtered, diluted as needed for individual experiments and tested in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2 % DMSO in the final reaction medium. Seven concentrations were tested: 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml.

Flow cytometric analysis

An equal volume of 1 % parasitemia, 4 % hematocrit culture was thereafter added and gently mixed thouroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1 µM

chloroquine diphosphate (Sigma chemicals Co, S[t] Louis, MO, USA). Culture were incubated at 37 °C for 48 hrs (1 parasite erythrocytic life circle). Parasite at ring stage were thereafter fixed by replacing the serum medium by an equal volume of 1 % formaldehyde in PBS (Sigma chemicals Co, S[t] Louis, MO, USA). Aliquots (50 µl) of each culture were added to 5 ml round- bottomed polystyrene tubes containing 0.5 ml 0.1 % Triton X- 100 and 1 nM YOYO nuclear dye (Molecular Probs) in PBS.

Parasitemias of treated and negative cultures were compared using Beckton Dickinson FACSort Flow cytometer to count parasitized erythrocytes. Data acquisition was performed using Cell Quest software. These data were normalized to percent control activity and 50% inhibitory concentrations (IC_{50}s), calculated using Prism 3.0 software (Graph Pad) with data fitted by non linear regression to the variable slope sigmoidal dose-response formula $Y = 100/ [1 + 10^{(logIC50-x)H}]$, where H is the Hill coefficient or slope factor (Singh and Rosenthal, 2001). This activity was analysed in accordance with the norms of plants antimalarial activity of Rasoanaivo et al. (1998). According to these norms, an extract is very active if IC_{50} < 5 µg/ml, active 5µg/ml < IC_{50} <50 µg/ml, weakly active 50 µg/ml < IC_{50} < 100 µg/ml and inactive IC_{50} > 100 µg/ml.

Results

The IC_{50} value of chloroquine for the clone W2 was 133 nM. The IC_{50} values of the plants extracts are shown in Table 1. These extracts demonstrated that they possess antimalarial activity. No significant differences were observed between the activity of methanol and water-ethanol extracts of *E. angolense* and *S. magnificum*. The methanol extract of *P. nitida* was more active than those of the other plants. However, nearly all the extracts showed antimalarial activity *in vitro*.

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

Plants	**Extract**	Extract Yield (% $^{w}/_{w}$)	**IC_{50}** (µg/ml)* (Mean ± SD)
Entandrophragma angolense	Hexanic extract	2.85	33.4 ± 0.5
	50% dichlomethane/MeOH	4.28	18.4 ±1.8
	Methanolic extract	3.57	26.2 ± 3.2
	50 % Water-EtOH extract	2.62	26.4 ± 2.8
Picralima nitida	Hexanic extract	1.14	129.6 ± 1.7
	50%dichloromethane/MeOH	5.00	20.1 ± 2.4
	Methanolic extract	3.57	10.9 ± 1.1
Thomandersia hensii	Hexanic extract	0.67	53.9 ± 3.2
	Ethyl acetate extract	6.70	24.7 ± 0.8
	50% dichloromethane/MeOH	6.20	77.2 ± 1.5
	Methanolic extract	5.83	68.2 ± 1.4
Schumanniophyton magnificum	Hexanic extract	0.30	78.1 ± 1.5
	50% dichloromethane/MeOH	1.97	47.8 ± 1.3
	Methanolic extract	1.00	28.7 ± 1.9
	50 % Water-EtOH extract	3.28	25.5 ± 2.0

(*) The results are expressed as geometric means ± SD from triplicate experiments.

Discussion

Entandrophragma angolense is locally used in the treatment of malaria and some other diseases (Irvine, 1961; Obih et al., 1986; Bray et al., 1990). The stem bark, in aqueous decoctions, is used for the treatment of fever or malaria in Cameroon and Ivory Coast. The IC_{50} of the 50% dichloromethylene-methanol extract of *E. angolense* was more active than the methanol and the water-ethanol extracts. This higher activity could be explained by the fact that some extracting compounds show relative affinity with some solvents. Although there was no significant difference between the IC_{50} values of the methanol and the water-ethanol extract of *E. angolense*, our result justifies the use of palm wine, instead of water in the preparation of traditional remedies.

The stem bark, the roots, the seeds and the fruits of *Picralima nitida* are always used in our traditional medicine to cure malaria or fever. The stem bark, in aqueous decoctions is also used for the treatment of

diarrhoea, intestinal worms, and gonorrhoea in many Central and West African countries, and for the antimalarial and their anti-inflammatory activities (Ezeamuzie et al., 1994; Fakeye et al., 2000). The methanol extract of the seeds of *P. nitida* showed the highest antimalarial activity (IC_{50} = 10 µg/ml), although this IC_{50} value is very low compared to those reported in other studies (François et al., 1996).

Thomandersia hensii is extensively used in traditional medicine (Burkill, 1985). A leaf decoction is used in Cameroon and Zaïre as a remedy of diarrhoea and colitis. The irritant sap is the treatment of choice for furuncles, abscesses and syphilis ulcers. A decoction of roots and leafy twigs is used for uro-genital disorders and intestinal parasites and as a tonic in case of debility and tiredness. Pulped roots are used for the treatment of oedema, rheumatism and sometimes ear and eye inflammations (Ngadjui et al., 1994). Although the water-ethanol extract was not tested, the antimalarial testing of the ethyl acetate extract showed some activity (IC_{50} = 24.7 µg/ml) which is similar to the results obtained from the methanol and the water-ethanol extracts of *E. angolense* in our study.

Shumanniophyton magnificum is a plant widely used in Africa ethno-medicine for the treatment of various diseases, particularly malaria and fever. A decoction of the bark is in great repute among some tribes of Cameroon as a remedy for dysentery and used as an enema (Tane et al., 1990), while other tribes use it as a lotion after circumcision (Irvine, 1961). The juice is used as a snake bite remedy in Nigeria and the protective effects of the extract against snake venom have been demonstrated (Akunyili and Akubue, 1986; Houghton et al., 1992). This plant is also well-known as a repellent for snakes (Okogun et al., 1983). The methanol and the water-ethanol extracts were active against *P. falciparum* W2 strain. Their activities were similar to those of the same extracts of *E. angolense*.

Conclusion

The results of our study show that the extracts of *E. angolense*, *P. nitida*, *T. hensii* and *S. magnificum* possess some antimalarial activity. The methanol and the water-ethanol extracts showed the highest activities, thus justifying the use of water and ethanol (palm-wine, spirits) as common solvents in traditional medicine. Further pharmacological screening with chemical fractions of methanol and water-ethanol extracts of *E. angolense*, *P. nitida* and *S. magnificum* and the ethyl acetate extract of *T. hensii* would permit the isolation and the identification of active compounds of these plants.

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