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In vitro and *in vivo* antimalarial studies of *Striga hermonthica* and *Tapinanthus sessilifolius* extracts.

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Summary

The antimalarial activities of the methanol extracts of *Striga hermonthica* (whole plant) and *Tapinanthus sessilifolius* (leaves), commonly used in Northern Nigeria for the treatment of malaria, were evaluated. In the *in vitro* antiplasmodial analysis, the extracts of *T. sessilifolius* and *S. hermonthica* utilized in the study, displayed mild to weak activities with IC_{50} values of 200.5 and 274.8 µg/ml respectively. This was investigated, using the multidrug resistant *Plasmodium falciparum*, K1 strain, in the parasite lactate dehydrogenase assay. The murine model *in vivo* antimalarial activity of the tested extracts, using chloroquine-sensitive *Plasmodium berghei* (ANKA P1), in the 4-day suppressive test, showed that both plants had intrinsic antimalarial properties, that were dose-dependent. At a dose of 400mg/kg weight of mice, extract of *S. hermonthica* exhibited a higher intrinsic antimalarial activity (68.5 % suppression) than that of *T. sessilifolius* (51.3 %). Chloroquine, the standard reference drug, had an average suppression of 78.0 % at a dose of 10 mg/kg weight of mice while normal saline was used as control. Preliminary phytochemical screening of the extracts indicated the presence of saponins, tannins, flavonoids, volatile oils and cardiac glycosides.

Keywords: *Striga hermonthica*; *tapinanthus sessilifolius*; *Plasmodium falciparum*; *plasmodium berghei*; antimalarial activities.

Résumé

Les activités antipuladiénnes des extraits du méthanol du *striga hermonthica* (plante entiere) et *Tapinanthus sessilifolicus* (feuille), plus utilisés au Nord du Nigéria pour le traitement du paludisme étaient evalués. L'analyse in vitro des activités antipaludiénnes de ces extraits montrait des valeurs d'IC50 moderés ou faible au *P.falciparum* de 200.5 et 274.8ug/ml respectivement. Ceci était investigué utilisant la souche résistante K du *P.falciparum* dans le test du lactase dehydrogenase. L'in vivo du modéle de murine de l'activité antipludiénne des extraits testés utilisant la souche sensitive du P.*Berghei* (Anka P1) dans le test de suppréssion de 4 jours montrait que les 2 plantes ont des propriétés antipaludiénnes intrinsique et a dose dependente. A la dose de 400mg/kg poids des souris, l'extrait de *S herronthica* démontrait une plus grande activité antipaludiénne intrinsique (63.5%suppression) que la meme dose du *T. sessilifolus* (51.3%). La chloroquine, médicament de reférence avait une moyenne de suppréssion de 78.0% a une dose de 10mg/kg poids corporel des souris et la solution normale était utilisée comme controle. Le dépistage phytochimique préliminaire de ces extraits indiquent la presence de la saponine, tanine, flavines, l'huile végetale et glycosides cardiaques.

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Introduction

Malaria is one of the highest causes of morbidity and mortality in the developing world, especially in the tropical countries. In Africa, it is estimated that malaria is the cause of the death of between 1.5 and 2.7 million people annually, mainly due to an increase in parasite resistance [1,2]. The therapeutic potential of plants used traditionally as antimalarial remedies cannot be over-emphasized [3,4,5] and the effective utilization of existing tools and development of new strategies are critical in the attainment of significant reduction in global malaria mortality by the end of the 1st decade of the 21st century [6]. More than ever before, it is imperative to develop new chemotherapeutic agents for the treatment of malaria or novel chemical structures with different mechanism of action from existing antimalarial drugs, more so to deal with the spread of drug-resistant *Plasmodium falciparum* [7,8, 9].

Striga hermonthica (Del) Benth (Scrophulariaceae) is a plant used commonly in northern Nigeria for the treatment of malaria. It is also used to soften beans while cooking and as an adjuvant to indigo to deepen co lour [10]. The whole plant is used for skin infections in Sudan [11]. *Tapinanthus sessilifolius* (P. Beav) van Tiegh (Loranthaceae) is a semi-parasitic plant widely distributed throughout the northern part of Nigeria. An aqueous extract prepared from the fresh leaves is used as remedy for hypertension and Diabetes mellitus [12]. The macerated leaves of related specie is taken with lemon juice as beverage in West Africa [13].

Materials and methods

Plant collection, authentication and preparation

The fresh plant sample of *Tapinanthus sessilifolius* (leaf) was collected in Jos, in February 2001, while that of *Striga hermonthica* (whole plant) was collected in Abuja in September 2001 in the middle belt zone of Nigeria. Mr T. K. Odewo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, authenticated the plants. The voucher specimens were deposited at the herbarium of FRIN under FHI 106102 (*S. hermonthica*) and FHI 106103 (*T. sessilifolius*). After air-drying and powdering, 400g samples were extracted by maceration in re-distilled methanol (BDH) for 72 hours, respectively. After removal of solvent, extracts were weighed and stored in the refrigerator till needed for analysis.

Phytochemical screening
The extracts were screened for the presence of carried out for various plant secondary metabolites. The extracts were screened for presence of alkaloids, flavonoids, saponin glycosides, tannins and volatile oils using standard procedures [14].

Antimalarial activity determination
In vitro antiplasmodial assay:
In the *in vitro* test, *Plasmodium falciparum* (multi-resistant strain K1) was used in the parasite lactate dehydrogenase assay. The parasites were maintained in human A$^+$ erythrocytes suspended in RPMI 1640 medium supplemented with A$^+$ serum and D-glucose according to previously published methods [15,16]. Cultures containing predominantly early ring stages were used for testing. The extracts were dissolved in DMSO and further diluted with RPMI 1640 medium (1:1 ratio). Serial dilutions were made in 96-well microtitre plates in duplicate, and infected erythrocytes were added to give a final volume of 100µl with hematocrit 2.5% and 1% parasitaemia. Chloroquine diphosphate was used as a reference drug, and uninfected and infected erythrocytes without extracts were included in each test. Plates were placed into a modular incubator gassed with 93% nitrogen, 3% oxygen and 4% carbon dioxide and incubated at 37°C for 48hrs. The lactate dehydrogenase activity was used for assessment of parasite growth [17]. The reagent used contained the following in each milliliter: 0.74mg of acetyl pyridine adenine dinucleotide (APAD), 19.2mg of lithum lactate, 0.1mg of diaphorase, 2µl of triton X-100, 1mg of nitro blue tetrazolium and 0.5mg of phenazine ethosulfate. Fifty micro liters of this reagent was added to each well and mixed and the plates were incubated for 15 min for 37°C. Optical densities were read at 550nm using a Dynatech laboratories MRX micro plate reader, and percent inhibition of growth was calculated by comparison with control values. IC_{50} values were determined using linear regression analysis (Microsoft Excel). Readings were taken in quadruplicates.

In vivo animal antimalarial assay:
This was carried out using Peter's 4-day suppressive test against chloroquine-sensitive strain of *P. berghei* (ANKA P1 clone) infection in mice [18]. Using this procedure, treatment with the extract commenced immediately after the mice had been inoculated (early infection, D0). Thirty Swiss albino mice (male) weighing between 18-22 g were chosen and divided into six groups of five mice each. Each mouse received approximately 1 x 10^7 infected erythrocytes intra-peritoneally. Four doses of extracts (100, 200, 300, and 400 mg/kg) were administered once daily for 4 consecutive days starting from the first day (D0) and continuing on D1, D2 and D3 by oral route to four groups of mice, the fifth group was treated with chloroquine diphosphate, while the last group of mice, served as the infected control and received only normal saline. On day 5 of the test, thin blood smears were taken and the blood films were fixed in methanol. The films were stained with 4% geimsa at pH 7.2 for 30min and examined microscopically. The percent suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Chloroquine diphosphate, the standard reference drug (10 mg/kg/wt mouse), was used as a positive control, while normal saline was used as a negative control.

Results
The preliminary phytochemical screening of both plants revealed the presence of saponins, tannins, flavonoids, volatile oils and cardiac glycosides. Alkaloids and authraquinones were absent in both plant extracts.

Table 1: Antiplasmodial activities of *Striga hermonthica* and *Tapinanthus sessilifolius* methanol extracts using multi resistant *Plasmodium falciparum* (K1) strain

Plant/Drug/Part[a]	Family	Yield (%)	IC_{50} (µg/ml) ± SEM[b]
Striga hermonthica(h)	Scrophulariaceae	7.4	274.8 ± 8.74
sessilifolius(l)	Loranthaceae	9.1	200.5 ± 7.31
Chloroquine	-	-	0.21 ± 0.002

a. h = whole herb; . l = leaves
b. SEM; n=10

Crude methanol extracts of *Striga hermonthica* and *Tapinanthus sessilifolius* were tested *in vitro* against *P. falciparum* in culture (Table 1). The extracts of *S. hermonthica* (whole plant) caused a slight *in vitro* inhibition of *P. falciparum* growth with an IC_{50} of 200.0 µg/ml whereas *T. sessilifolius* showed little or no inhibition at 274.8 µg/ml compared with chloroquine disphosphate (IC_{50} = 0.21µg/ml).

Table 2: The 4-day suppressive test of methanol extracts of *S. hermonthica* and *T. sessilifolius* using *Plasmodium berghei* (ANKA P1 clone) in mice

Extract/ Drug dose[a] (mg/kg/day)	Activity against *P. berghei* in Mice (%)[b]			
	S. hermonthica		*T. sessilifolius*	
	Parasitaemia	Suppression	Parasitaemia	Suppression
100	13.57 ± 4.51	34.82	18.77 ± 2.85	2.85
200	12.06 ± 4.05	42.07	18.20 ± 4.76	5.80
300	7.05 ± 1.90	66.14	10.78 ± 14.20	44.20
400	6.55 ± 2.14	68.54	9.14 ± 2.55	51.29
CQ	4.35 ± 1.08	79.10	1.35 ± 1.08	77.48
Con	20.82 ± 3.29	-	19.32 ± 3.95	-

a. CQ = chloroquine diphosphate (10 mg/kg/day); Con = control; normal saline
b. Values are mean ± SEM, n = 5

The suppressive activity of *S. hermonthica* and *T sessilifolius* extracts against *P. berghei* in mice is displayed in Table 2. The extract of *S. hermonthica* at 100mg/kg and 400mg/kg gave 34.8% and 68.5% suppression of parasitaemia, respectively. On the other hand, extract of *T. sessilifolius* gave 51.29% suppression at 400mg/kg. In the analysis, chloroquine

disphophate produced suppressions of 77.5% and 79.1% respectively, in the 4-day suppressive test. Please see Table 2 for details.

Discussion

The *in vitro* and *in vivo* antimalarial activities of the methanol extracts of *S. hermonthica* and *T. sessilifolius* were investigated in this study. The *in vitro* study showed that the extract of *S. hermonthica* was slightly more active than *T. sessilifolius* as shown in Table 1. The *in vivo* study in mice, the extract of *S. hermonthica* exhibited a better antimalarial activity using chloroquine-sensitive *P. berghei* than the extract of *T. sessilifolius*. The activity might be attributed to the presence of flavonoids that has been shown to be the major constituent identified in *S. hermonthica* [19, 20].

From the result of the study, it was observed that in the suppressive test, the two extracts displayed a dose-dependent suppression of parasitaemia, suggesting that the two extracts possess schizonticidal activity.

Conclusion

Preliminary results from this study seem to justify the use of the plant extracts as remedies in the treatment of malaria infection. Further investigation and subsequent isolation of these antimalarial components are imperative in other to make a meaningful contribution to drug discovery from the studied plants.

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