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A comparative study of the anti-protozoan activities of *Hyptis suaveolens* (L.) Poit and *Hyptis atrorubens* (L.) Poit.

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ABSTRACT

This study evaluated the anti-trypanosomal and chemo-suppressive anti-malarial activities of the leaf extracts of *Hyptis suaveolens* and *Hyptis atrorubens*. Methanol extracts of the leaves of both plants were obtained using cold extraction. The extracts were screened for anti-trypanosomal and chemo-suppressive anti-malarial activities using mice infected with *Trypanosoma evansi* and *Plasmodium berghei* (ANKA) respectively. The minimum lethal dose (LD_{50}) of both extracts was found to be greater than 5000 mg/kg body weight. A significant ($p < 0.05$) decrease in parasitaemia *(T. evansi)* level of infected mice occurred from 26.80 ± 1.11% to 5.00 ± 0.36% and 5.50 ± 0.57% for *H. suaveolens* and *H. atrorubens* respectively at 1000 mg/kg body weight. The standard drug (Tryponil® for trypanosomiasis at 3.5 mg/kg achieved 100 ± 0.00% parasitaemia clearance. The plasmodium-infected mice treated with 1000 mg/kg of *H. atrorubens* extract exhibited a higher percentage chemosuppression (86.10 ± 8.00) than *H. suaveolens* (67.95 ± 7.12) in the anti-malarial test model. Both were found to compare favourably with the standard drug (chloroquine) (54.57 ± 10.41). Extracts of *H. suaveolens* and *H. atrorubens* demonstrated moderate anti-trypanosomal and anti-malarial activities. The extracts showed no acute toxic effect on mice.

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***Keywords*:** trypanocidal, antiplasmodial, medicinal plants, *Hyptis suaveolens, Hyptis atrorubens*

1. INTRODUCTION

Infections caused by protozoan parasites include Chagas disease, African trypanosomiasis, leishmaniasis and malaria. They are responsible for considerable morbidity and mortality worldwide with devastating social and economic consequences[1]. African trypanosomiasis is a vector-borne parasitic disease caused by infection with protozoan parasites belonging to the genus *Trypanosome*. The African animal trypanosomiasis (AAT) also known as 'Nagana' or 'Surra' is a disease complex transmitted to humans or Livestock by tsetse-fly (*Glossina* genus) bites which have acquired their infection from human beings or from animals harbouring the pathogenic parasites[2]. AAT occurs most importantly in cattle but can cause serious losses in pigs, camels, goats, and sheep. Infection of cattle by one or more of the three types of AAT results in subacute, acute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhea, rapid loss of condition and often terminates in death[3].

Malaria on the other hand, is a life-threatening disease caused by *Plasmodium* parasites that are transmitted to man through the bites of infected female *Anopheles* mosquitoes[4]. There are five different types of parasites that infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi. P. berghei* infects rodents. Of these, *P. falciparum* and *P. vivax* are the most prevalent, and *P. falciparum* is the most dangerous, with the highest rates of complications and mortality. Each year, 219 million cases of malaria are estimated to occur around the world. According to WHO, there were roughly 212 million malaria cases and an estimated 429,000 malaria deaths in 2015[5]. In 2017, WHO[6] also reported that Nigeria accounted for 25% of malaria cases worldwide with children under 5years of age being the most vulnerable. The estimated number of malaria death in Nigeria stood at approximately 227,645 deaths in 1990 and 192,284 deaths recorded in 2015[7].

Many anti-malarial drugs used for the treatment of the disease include artemisinin, mefloquine, chloroquine among others. However, their use has led to resistance in many strains of *Plasmodium* mainly against *P. falciparum*. A more recent approach is the use of Artemisinin based Combination Therapies (ACTs). The use of the ACT as first-line treatment against uncomplicated malaria was recommended by the WHO in the mid-2000s and till date, ACT has been used extensively to manage the disease in most disease endemic countries[8]. Despite the successes recorded with the use of ACTs, herbal therapy is still widely used partly because they serve as cheaper alternatives. [9]reported that there are over 1200 plant species that are used for the treatment of malaria and fevers, and herbal preparations are potentially important sources of new anti-malarial treatments. There is need to uncover the phytochemicals responsible for such activity through scientific investigations.

The genus *Hyptis* belongs to the Lamiaceae family and is widespread in Australia (northern territory and Queensland), China, Indonesia, Papua New Guinea, Solomon Islands etc. It is widespread in West and Central Africa including Nigeria, where it is considered insidious species[10]. In some parts of France, the aerial parts of *H. atrorubens* are used for sore throat and flu, while the leaves are used topically against dermatitis and athlete's foot[11,12]. The volatile oil of the plant has been analysed by Maia and Andrade[13] to contain two essential oils chemotypes[14].

Isolated and identified four antibacterial compounds from the plant: rosmarinic acid, methyl rosmarinate, isoquercetin and hyperoside. *H. suaveolens* has been regarded as obnoxious weed distributed throughout the world[15]. Virtually every part of the plant is used to treat one form of ailment or the order. Many authors have reviewed the plant based on its pharmacological, phytochemical and nutritional profiles[16,17]. The aim of the study was to investigate two species of *Hyptis, H. suaveolens* and *H. atrorubens* for their *in vivo* trypanocidal and anti-plasmodium activities in mice.

2. MATERIALS AND METHODS:

Reagents and Chemicals

All the reagents used in this study were of analytical grades and include methanol (BDH Poole England), ethylacetate (BDH Poole England), n-Hexane (BDH Poole England), Tween-20, Tryponil® (interchemie, The Netherlands) chloroquine. Reference drugs were obtained from appropriate pharmacy shops. respectively.

Plant collection and identification

The fresh leaves of *Hyptis suaveolens* (L.) Poit and *H. atrorubens* (L.) Poit [28-32] were collected along Ibadan Express Way, Ile-Ife, Osun State and Saki, Oyo State respectively. The plants were identified by Mr I. Ogunlowo, Pharmacognosy Herbarium, Faculty of Pharmacy, OAU and Mr. Bernard Omomoh, Herbarium unit of Department of Botany, OAU. Voucher specimens (IFE HERBARIUM, 17427 and 17008 for *H. suaveolens* and *H. atrorubens* respectively) were deposited in the Herbarium unit, Department of Botany, OAU.

Preparation of plant extracts

H. suaveolens and *H. atrorubens* leaves were air dried at room temperature for about a week, milled into powder and 2 kg of each was extracted separately in 100 % methanol at room temperature for 72 hours with constant shaking using a laboratory electric shaker. The filtrates were concentrated *in vacuo* in a rotary evaporator and lyophilized to complete dryness. The crude extracts were then kept in a dark container to eliminate light reaction and stored at 4 °C.

Experimental animals

Mice weighing between 19-25 g were purchased from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife and acclimatized for 1 week in well ventilated cages in the laboratory. The animals were fed with pelletized growers mash (Grand Cereals Limited, Jos, Plateau State, Nigeria) and water given *ad libitum*.

Acute toxicity test

Lorkes[18] method was used to determine the acute toxicity of *H. suaveolens* and *H. atrorubens* via the oral route. Mice were divided randomly into 3 groups (n=3) and given oral doses of 10, 100 and 1000 mg/kg body weight of the extracts respectively. The control group received (10 % Tween-20). The animals were deprived of food for 12 hours prior to extract administration. The result obtained from this first phase led to a dose selection test (phase 2

test), using doses of 1600, 2900 and 5000 mg/kg. The animals were monitored continuously for the first 3 hours for the signs of toxicity such as reduction in locomotion, aggressiveness, social interactions and mortality. The surviving animals were monitored daily for 7 days for changes in food and water consumption. Toxicity was calculated using the formula:

$$LD_{50} = \sqrt{}\text{ (maximum dose for all survival} \times \text{minimum dose for all deaths).}$$

Antitrypanosomal assay:

Test organism

Trypanosoma evansi was obtained from Nigerian Institute for Trypanosomiasis and Onchocerciasis Research, Kaduna, Nigeria. The parasite was maintained in the laboratory in rats by continuous passage until when needed. Passage was considered necessary when parasitemia was in the range of 16-32 parasites per field.

Determination of parasitemia

Parasitemia was monitored in blood obtained from the tail. The number of parasites was determined microscopically at 40× magnification using the 'Rapid Matching' method of Herbert and Lumsden[19].

Test Procedure

For *in-vivo* screening of each plant, 25 mice were inoculated via the intraperitoneal route with 10^4 parasites in 0.2 ml of infected blood, they were divided into 5 groups of (n=5) animals each, treatment was commenced after 48 hours post inoculation at the appearance of parasitemia. Wet blood film was carried out every other day using blood obtained from the tail to estimate parasitemia changes. The negative control group received 10 % tween-20, groups 2, 3 and 4 were treated with doses of 250, 500, 1000 mg/kg of the extract while group 5 (positive control) received standard drug Tryponil® at 3.5 mg/kg (Each 2.36 granules with 1.05g of active ingredients: Diminazene aceturate, and 1.31g Phenazone). All treatment commenced when the parasitemia was averagely one parasite per field and treatment continued daily for 7 days. Parasitemia was monitored every other day and mortality was also recorded.

***In-vivo* Antimalaria tests**

Plasmodium berghei berghei

Chloroquine sensitive strain of *Plasmodium berghei* (ANKA) obtained from Institute of Malaria Research and Training (IMRAT), University College Hospital Ibadan, Nigeria was used. The parasites were maintained by serial passage of blood from infected mice to non-infected ones on weekly basis.

Suppressive test (4 – day test)

The model was carried out using the method of [20]. In this procedure, malaria parasite was obtained by collecting blood samples from donor mouse infected with *Plasmodium berghei (ANKA).* Mice divided into 5 groups (n=5) were infected with parasites by inoculating them intraperitoneally with 0.2 ml of the prepared blood solution. Treatment commenced 24 hours

later. Group 1 received 0.2ml normal saline (0.9 %), groups 2 to 4 were treated respectively with 250, 500 and 1000 mg/kg body weight of the extract while group 5 (positive control) was treated with 10 mg/kg body weight of chloroquine. The treatment continued daily for four consecutive days. On day 5, thin film was made from the caudal vein of each mouse, the number of the parasitized cells was determined microscopically, and the percentage suppression evaluated.

$$\text{Mean \% chemosuppression} = \frac{\text{Mean \% parasitaemia control} - \text{Mean \% parasitaemia in test group}}{\text{Mean \% parasitaemia in control}}$$

Statistical Analysis

The data obtained during the study were subjected to Analysis of Variance (ANOVA) and Probit Analysis, the significance was set at $p<0.05$. Values were expressed as Mean ± SEM (standard error of mean).

3. RESULTS AND DISCUSSION

The intractable problem of drug resistance due to antigenic variations in causative parasites in animal trypanosomiasis and malaria has led to the resurgence of interest in medicinal plants and plant derived drugs as newer sources of medicine to suppress or possibly eradicate these major tropical diseases amongst others. The results of this present study showed that the leaf extracts of *Hyptis atrorubens* and *H. suaveolens* demonstrated anti-trypanosomal and antiplasmodial activities by inhibiting multiplication of the parasite and suppressing parasite population in mice respectively.

The acute toxicity test results of oral administration of *H. suaveolens* and *H. atrorubens* showed that the LD_{50} of both plants were greater than 5000 mg/kg because no mortality observed at this dose, implying that the plant extracts could be considered relatively safe according to OECD 423 guidelines.

The antitrypanosomal profile of the extract of *H. suaveolens* and *H. atrorubens* are as shown in Figures 1 and 2. Both extracts demonstrated trypanocidal activities. Parasitaemia reductions were dependent on the doses used, especially at the higher doses of 500 and 1000 mg/kg. Parasitaemia reduction compared well with the positive control at the highest dose (1000 mg/kg) after seven (7) days of treatment.

Trypanocidal activities of *H. suaveolens* extract reduced parasitaemia level from 26.80± 1.11 to 5.00 ± 0.36 with survival time of 24.75 ± 1.78 days, while that *H. atrorubens* reduced parasitaemia from 26.80 ± 1.11 to 5.50 ± 0.57 with survival time of 21.00 ± 1.27 days. The extract of *H. suaveolens* was more effective at 1000 mg/kg, resulting in 80 % parasiteamia clearance in treated animals as against *H. atrorubens* extract at 1000 mg/kg which gave 67.95 % parasiteamia clearance.

There was no complete (100%) parasite clearance during the 7-day period. This suggests the need to increase the dosage or prolonge the treatment at 1000 mg/kg in order to achieve complete parasite clearance. Mozhiyarasi and Anuradha[21] revealed that the methanol extract of the leaf of *H. suaveolens* was rich in alkaloids, carbohydrates, glycosides, terpenoids, protein, steroids, flavonoids, phenols and tannins.

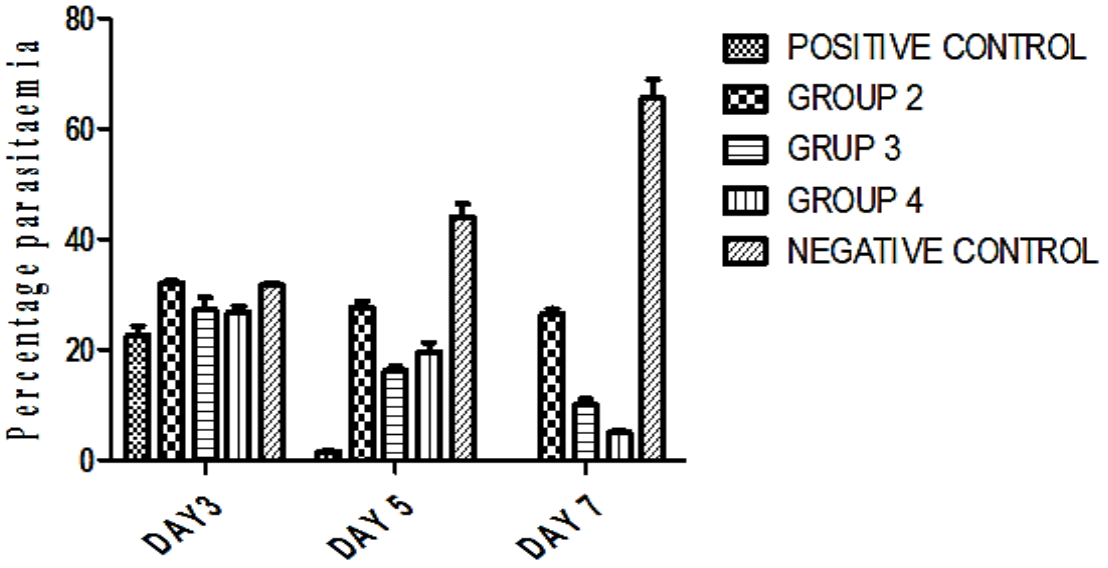

Figure 1. *In vivo* anti-trypanosomal activity of *Hyptis suaveolens* (L.) Poit.

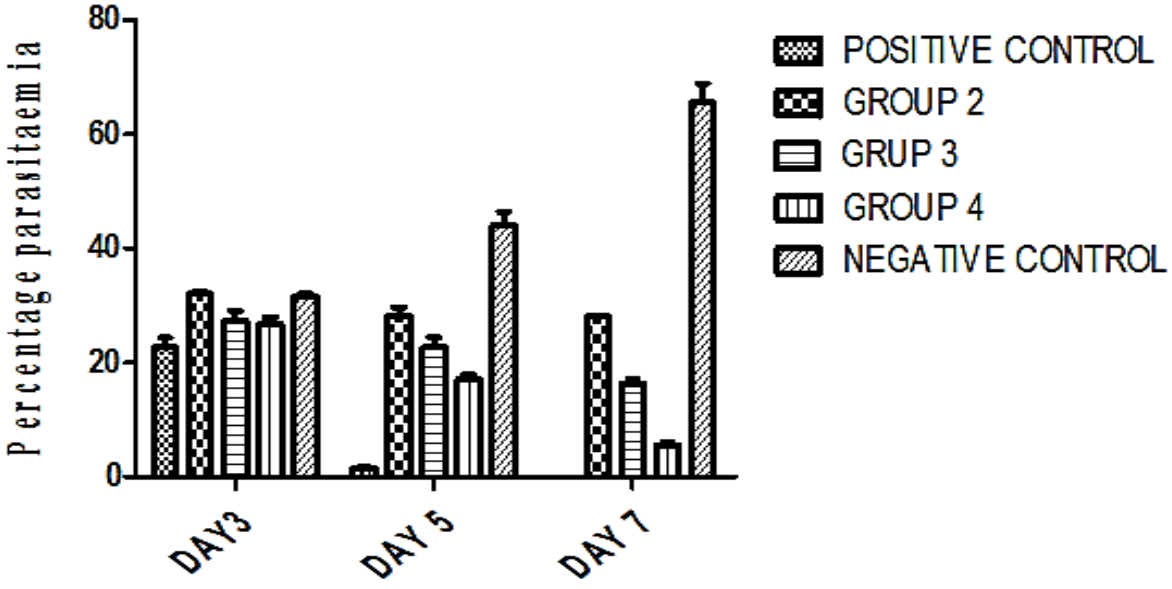

Figure 2. *In vivo* anti-trypanosomal activity of *Hyptis atrorubens* (L.) Poit.

These phytochemicals must have contributed to observed trypanocidal activity. A review of trypanocidal plants in Nigeria according to Ibrahim[22], showed that a total of 264 plant species belonging to 79 families were investigated for anti-trypanosomal activity. However, only 48 bioactive anti-trypanosomal compounds were successfully isolated in pure forms. Furthermore, some of the plants were investigated for possible ameliorative effects on the trypanosome-

induced pathological changes out of which 18 plants were reported to be effective while a few others were not.

Results of the antimalarial activities of *H. suaveolens* and *H. atrorubens* extracts are shown in Table 1. There was a significant difference between the treated mice and the untreated mice.

Table 1. The Antimalarial activity of *Hyptis suaveolens* (L) Poit and *H. atrorubens* (L) Poit.

Doses (mg/kg)	**Parasitaemia (%± SEM)**		**Chemo-suppression (%± SEM)**		**Survival time (%± SEM)**	
	HS	**HA**	**HS**	**HA**	**HS**	**HA**
NC	5.45±0.57		0.00±0.00[a]		2.00±0.63	
250	3.72±1.14	1.62±0.46	31.93±20.81[ab]	70.29±8.43[a]	2.80±18.30	9.00±1.79
500	1.54±0.28	2.09±0.15	71.70±5.05[a]	61.72±2.75[a]	12.60±3.98	8.00±3.35
1000	1.75±0.39	0.76±0.44	67.95±7.12[a]	86.10±8.00[ac]	12.60±8.71	15.60±6.86
CQ	2.48±0.57		54.57±10.41[a]		13.80±6.86	

KEY: Values with the same superscript letters are not significantly different from Chloroquine and from each other ($p>0.05$). Values with different superscript letters (ab and ac) are significantly different from each other.
NC: Negative control, **HS:** *Hyptis suaveolens,* **HA:** *Hyptis atrorubens,* **CQ:** Chloroquine

Both extracts also exhibited similar antiplasmodium activities. The parasitaemia level decreased with increasing doses. At 250 mg/kg, parasitaemia level dropped from 3.72±1.14 and 1.62±0.46 to 1.75±0.39 and 0.76±0.44 at 1000 mg/kg for *H. suaveolens* and *H. atrorubens* extracts respectively. Chemo-suppressive activities of both extracts were dose-dependent. Percentage chemosuppression of *H. suaveolens* and *H. atrorubens* extracts respectively increased from 31.93± 20.81 to 67.95±7.12 and from 70.29± 8.43 to 86.10± 8.00. The minimum survival times at 250 mg/kg were 2.80± 18.30 and 9.00± 1.79 while the maximum surviving days at 1000 mg/kg were 12.60± 8.71 and 15.60± 6.86 for *H. suaveolens* and *H. atrorubens* extracts respectively.

The chemosuppressive activities and survival time compared very well with the positive control (Chloroquine) at higher concentration. *H. suaveolens* has been widely reported to have been used in traditional medicine for the treatment of malaria[23,27]. The present findings validated its ethnobotanical use as an antimalaria plant. The report of [24,25] that hydroxyl derivative of dehydroabietic acid, dehydro-abietinol isolated from *H. suaveolens* was found to inhibit the growth of chloroquine-sensitive as well as chloroquine-resistant strains of *Plasmodium falciparum* in the erythrocytes *in vitro* (IC_{50} 26-27 μM). [26] also isolated abietane-type diterpenoid endoperoxide, 13*α-epi*-dioxiabiet-8(14)-en-18-ol from *H. suaveolens,* a molecule with high anti-plasmodial activity (IC_{50} = 0.1 μg mL^{-1}).

4. CONCLUSION

This study revealed that the methanolic extracts of *H. suaveolens* and *H. atrorubens* possessed both anti-trypanosomal and anti-malarial properties.

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