



***In vitro* Anthelmintic Activity of Crude Methanolic Leaf Extract of *Hyptis suaveolens* (L.) Poit (Bush mint) on *Ascaris suum*.**

Salami, O. S.^{*}, Kwanashie, H. O., Maje, I. M.

Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria, Nigeria.

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ABSTRACT

Ascariasis is a neglected tropical disease prevalent in areas with poor hygiene and low standard of living in tropical countries. Hyptis suaveolens is been used traditionally around the world for various ailments. The aim of the study was to evaluate the anthelmintic activity of the methanolic leaf extract of the plant on the three life cycle stages of Ascaris suum synonymous to Ascaris lumbricoides. The phytochemical analysis of the extract was evaluated. The in-vitro anthelmintic activity of the methanolic leaf extract of Hyptis suaveolens on the egg hatchability and larva inhibition of Ascaris suum were assessed using Bizimenyara inhibition method, where 100 eggs were counted and incubated in 5 different plate-wells containing positive control, negative control and 3 concentrations (50, 70 and 100 mg/ml) of extract. The plate wells were brought out after incubation and content examined under the microscope. The effect of the methanolic leaf extract and pyrantel pamoate on cuticle peeling, motility and mortality were evaluated using adult worms. The egg hatchability and larval development after incubation for 48 h and 21 days significantly ($p < 0.05$ for both) inhibited with 98% unhatched eggs and 95% undeveloped larva of A. suum respectively. There was significant ($p < 0.05$) visible peeling of cuticles, reduced motility and mortality of the adult worms after 30, 12 and 80 minutes respectively. It can be concluded that H. suaveolens has anthelmintic activity on A. suum which may be attributed to some of the phytochemical constituents.

Key words: Hyptis suaveolens, anthelmintic, Ascaris suum

INTRODUCTION

Helminthosis is a parasitic disease of humans and animals in which a part of the body is infected with parasitic worms which are sub-classified as Cestodes (tapeworms), Trematodes (flukes) and Nematodes (roundworms). Roundworms are the largest group of parasite that infects humans. Soil-transmitted helminthoses are the most common and are among the neglected tropical diseases [1]. Worm infestations especially with *Ascaris lumbricoides* in children are prevalent in the tropics and frequently occur with poor hygiene and sanitation [2]. A high infection rate of ascariasis has been observed in a large number of Asians (95%), Africans (73%), and South Americans (12%) [3], the prevalence has also been observed in several studies in different parts of Nigeria [4, 5]. Ascariasis is usually asymptomatic but symptoms may include loss of appetite, growth delay, retardation, wheezing, pneumonitis, intestinal obstruction, hepatobiliary and pancreatic injury [6]. Due to the negative effect of ascariasis on the immune system, it has been linked to worsen malaria and tuberculosis and other ailments that affect the immune system [7]. *Ascaris suum* is the large round worm parasitic nematode causing ascariasis in pigs, and it is considered to be the same as, or similar to *Ascaris lumbricoides* in humans by different researchers [8]. This may be due to their genetic and morphological similarities [9], some studies have established that cross infection between human and pig has been established [10]. *Hyptis suaveolens* (L.) Poit commonly called 'bush mint' is a plant or shrub found along roadsides, farmsteads and waste ground in different parts of the world mainly in the tropics and subtropics. Several studies have been carried out on this plant with either the whole plant or plant parts, which demonstrated anthelmintic activity against

earthworm (*Pheretima posthuma*) [11] and *Ascardia galli*. However, anthelmintic studies have not been carried out on *Asacris lumbricoides* or the similar and readily obtained *Ascaris suum* using the methanolic leaf extract of *Hyptis suaveolens*. This study used the crude methanolic leaf extract of *Hyptis suaveolens* to assess the *in vitro* anthelmintic effect using the three lifecycle (egg, larva and adult) stages of *Ascaris suum* obtained from pigs.

MATERIALS AND METHODS

Plant collection, Identification and Extraction

The plant was collected in November 2017 in Lugbe area of Abuja, Federal Capital Territory (FCT). The whole plant was harvested and taken to the Herbarium of the Department of Botany Faculty of Life Science, Ahmadu Bello University, Zaria. It was identified and authenticated as *Hyptis suaveolens* by a taxonomist (Namadi Sanusi) with a voucher no 11923. The leaves were air dried under shade at room temperature for a week [12], powdered and extracted with methanol using cold maceration [13] then evaporated to dryness on a steam bath at 45°C.

Parasite collection

The *Ascaris* worms from pigs were obtained from various abattoirs in Kaduna state in a beaker containing normal saline which is the physiological environment of the worm while in the pig's stomach.

Phytochemical screening

The qualitative screening of the leaf of *Hyptis suaveolens* extract was carried out to determine the presence of secondary metabolites as described by [13].

Egg hatch inhibition Assay

100 fresh eggs were placed in 0.2ml of water in each of the 15 wells of the flat bottom micro titre plate. A negative control- Dimethyl sulphoxide (DMSO), three different concentrations of extract were prepared by dissolving 30µg, 60µg and 120µg in 60µl of 4% DMSO [14] and made up to 300µl with distilled water, a positive control (pyrantel pamoate 50 µL of 1 µg/ml) was also included. All groups were assigned number 1, 2, 3, 4, 5 respectively. Each group was separately distributed into three replicate micro titre plate wells containing the eggs. The negative control was 60µg of 4% DMSO while the positive control was pyrantel pamoate 50 µl of 1µg/ml per well. The eggs were incubated in these mixtures for 48 h at 27 °C in an incubator. After 48 h, 10% Lugol's iodine solution was added to stop the eggs from further hatching. Pasteur pipette was used to mix the content in the wells and pipetted on a dry slide and placed on the microscope. All the unhatched eggs and first stage larvae (L_1) were counted. Data were expressed as percentage unhatched eggs using the formula described by [15].

$$E(\%) = \frac{(Eggs + L_1) - L_1}{(Eggs + L_1)} \times 100$$

Where E – unhatched eggs, L_1 – first stage larva

In-vitro larval development inhibition Assay

The larval development inhibition assay was conducted as described by [16]. 100 eggs were counted into each of the 15 wells with 10µl amphotericin B solution to prevent fungal growth and plates were incubated for 48 h at 27 °C. It was brought out and the graded concentrations of the extract, pyrantel pamoate and DMSO were applied and each replicated 3

times. These wells were then returned into the incubator and brought out after 21 days. The larvae were counted by separating the larvae into: L_3 , L_2 and L_1 . The percentage inhibition of larval development was calculated using the formula proposed by [16] below.

$$E(\%) = \frac{(L_1 + L_2 + L_3) - L_3}{(L_1 + L_2 + L_3)} \times 100$$

Where E – larva inhibition, L_1 – first stage larva, L_2 – second stage larva, L_3 – third stage larva

In-vitro anthelmintic Assay

Fresh *Ascaris suum* obtained from the abattoir was quickly brought to the laboratory in a beaker containing normal saline. The worms were transferred and separated into 5 different petri dishes containing 5 worms each. After that, three different concentrations of the extract, normal saline (negative control) and 50 µL of 1 µg/ml pyrantel pamoate (positive control) was added into each of the petri dish content. All the 5 petri dishes were left for an hour after which observation were made on three parameters namely; Reduced motility (a sign of weakness and paralytic action of the extract), cuticle peeling and mortality. These observations were made at hourly interval for 4 hours.

RESULTS

Qualitative analysis of the methanolic extract of *Hyptis suaveolens* leaves showed the presence of alkaloids, phenols, glycosides, saponins, tannins, flavonoids, carbohydrates, cardiac glycosides, steroids and triterpenes.

Table 1: Effect of *Hyptis suaveolens* leaf extract on *Ascaris suum* egg hatch inhibition. Mean percentage of hatched and unhatched eggs

Concentration	Hatched egg (%)	Unhatched eggs (%)
-ve control	98	02
50mg/ml HS	22	78*
70mg/ml	11	89*
100mg/ml	0	100*
Pyrantel pamoate	0	100*

Data presented as mean $\pm$ SEM, analysed using repeated measure ANOVA and Tukeys *post hoc*, $P < 0.05$ is significant for *

-ve control - 60ug of 4% DMSO, +ve control - 50ul of 1ug/ml Pyrantel pamoate, HS – *Hyptis suaveolens* methanol leaf extract

Table 2: Effect of *Hyptis suaveolens* leaf extract on *Ascaris suum* Larva development Mean percentage developed and undeveloped larva

Concentration	Developed larva (%)	Undeveloped larva(%)
-ve control	100	00
50mg/ml HS	07	93*
70mg/ml	04	96*
100mg/ml	0	100*
Pyrantel pamoate	01	99*

Data presented as mean $\pm$ SEM, analysed using repeated measure ANOVA and Tukeys *post hoc*, $P < 0.05$ is significant for *

-ve control - 60ug of 4% DMSO, +ve control - 50ul of 1ug/ml Pyrantel pamoate, HS – *Hyptis suaveolens* methanol leaf extract

Table 3: Effect of *Hyptis suaveolens* leaf extract on Adult *Ascaris suum* Mean Time in minutes

Concentration	Reduced motility	Cuticle peeling	Death
DMSO	0	0	0
50mg/ml HS	60*	120	170
70mg/ml HS	35*	70	140
100mg/ml HS	15	60*	120*
Pyrantel pamoate	30*	80*	130*

Data presented as mean $\pm$ SEM, analysed using repeated measure ANOVA and Tukeys *post hoc*, $P < 0.05$ is significant for *

-ve control - 60ug of 4% DMSO, +ve control - 50ul of 1ug/ml Pyrantel pamoate, HS – *Hyptis suaveolens* methanol leaf extract

DISCUSSION

The phytochemical screening of the methanolic leaf extract of *Hyptis suaveolens* revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins and triterpenes. *Hyptis suaveolens* methanol leaf extract was found to have inhibited the egg hatching, larva development and *Ascaris suum* worm significantly. The result of the anthelmintic activity of the methanol extract on the egg hatchability, larva development and on adult worm of *A. suum* showed some activity even at a lower dose of 50 mg/ml when compared to a standard anthelmintic, pyrantel pamoate which showed activity at 50 µL of 1 µg/ml. The egg hatchability and larva development were significantly inhibited after incubation at 50, 70 and 100 mg/ml. The adult worm was also inhibited by the extract with visible peeling of cuticles, reduced motility and eventual death occurring during the period of observation. Previous study done using aqueous, ethanol and methanol extract of *Hyptis suaveolens* on *Pheretima posthuma* and *Ascardia galli* showed significant anthelmintic effect especially with methanol extract of the plant [11].

The result of this study has established that the plant has some active principles against ascariasis. This activity may be attributed to the presence of phytochemicals. Further research into the isolation and characterization of these active principles could lead to development of herbal drugs in the prevention and treatment of ascariasis.

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