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ORIGINAL ARTICLE

OVICIDAL AND LARVICIDAL ACTIVITIES OF *PROSOPIS AFRICANA* LEAF AND FRACTIONS ON *HAEMONCHUS CONTORTUS*

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study was carried out to investigate the anthelmintic efficacy of the crude extract and fractions of *Prosopis africana* leaves against the gastrointestinal parasitic ova and larvae of *Haemonchus contortus*. The methanol crude extract of *P. africana* was partitioned into four fractions: N-butanol, petroleum ether, ethyl acetate, and aqueous. The concentrations of the extracts and fractions used in the treatment groups were 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml. At the concentration of 12.5 mg/ml the result showed that the egg hatch and larval development inhibition were 99.5% and 96%, respectively. This shows a significant difference ($p < 0.05$) when compared with the 100% inhibitory effect of albendazole. In the assay for ovicidal activity, all the fractions showed no significant difference ($P > 0.05$) when compared with albendazole. However, a significant difference ($P < 0.05$) was observed with all the fractions against larvae of *H. contortus*. This indicates a dose-dependent increase in the ovicidal and larvicidal activity of the crude extract and the fractions of *P. africana* leaves. However, this study confirms that the crude extract and fractions of *P. africana* possess anthelmintic compounds against *H. contortus*.

Keywords: crude; fractions; *Haemonchus contortus*; larvicidal; ovicidal; *Prosopis africana*

INTRODUCTION

Ruminant (sheep and goat) production is one of the main economic and farming activities for rural people in both the tropics and subtropic regions. Their challenges in ruminant production are due to gastrointestinal (GI) nematodes infections, caused by *Haemonchus contortus*, which are some of the numerous GI parasites causing great economic losses and threats to the livestock sector [1, 2, 3, 4]. In Nigeria, helminthosis, indiscriminate or irregular use of synthetic anthelmintics, and high cost of anthelmintic production is one of the major problems in ruminant produc-

tion, leading to the development of resistance by GI parasites to anthelmintic control. This has led to investigations and developments of new anthelmintics from medicinal plants using *Prosopis africana* as an alternative source to combat these problems [5, 6, 7, 8].

Prosopis africana (*P. africana*) (Guill and Perr) Taub (syn: *P. oblonga* Benth) grows wildly in the Middle Belt and Northern parts of Nigeria and some other African countries (Aremu et al., [9]; Ken, [10]). *P. africana* leaves, stem barks, and roots are used in the treatment of headache, toothache, rheumatism, naso-pharyngeal infections, cutaneous and subcutaneous parasitic infections (parasitic Loranthaceae) [10, 11].

MATERIALS AND METHODS

Plant collection, identification and extraction

The plant part used for this study was collected in the field from Kwali Area Council of Abuja, Federal Capital Territory, in months of March and April, 2024. Plants were identified by Mr. Alfred Ozioko, a botanist with the Bio-resources Development and Conservation Program (BDCP), Nsukka, and voucher specimens of each plant species were deposited at the Department of Pharmacology, Faculty of Veterinary Medicine, University of Abuja. The plant materials were air-dried, weighed, and pulverized. The extraction process was carried out according to the method described by Olayemi et al. [12] and Simon et al. [13].

Extraction of plant material

The pulverized plant material was weighed and extracted according to the method described by Olayemi et al. [12] and Simon et al. [13]. Three hundred grams of each plant material were weighed and wrapped in a tumble sack, placed in the Soxhlet apparatus and extracted with one litre of 80% methanol. The plant crude extract was collected in a flask attached to the Soxhlet apparatus, and poured into an evapourating dish and concentrated to dryness over water baths at 50–64 ˚C.

Solvent partitioning of the crude extract

The crude extract was then subjected to column fractionation and partitioning with the solvents petroleum ether, ethyl acetate, and N-butanol in order of increasing polarity, using 150 ml of each solvent. The whole process was repeated three times for each solvent, and the methanol-water portion was concentrated to dryness as described by Olayemi et al. [14] and Simon et al. [13].

Fig. 1. *Prosopis africana* leaves

In vitro assay

The Egg Hatch Assay (EHA) was performed using the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines [14]. Adult *Haemonchus contortus* were obtained from the abomasum of naturally infected sheep slaughtered at the Dogarawa abattoir, Zaria. Female *H. contortus* were identified, individually picked, separated, and suspended in distilled water and later crushed with mortar and pestle to liberate the parasite eggs (Simon et al. [15] and Taylor et al. [16]

Approximately 200 (*H. contortus*) eggs contained in 0.08 ml were pipetted into a 96-flat-bottomed microtitre plate. While 0.5 ml at different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml of the crude extract and the fractions were added. Similarly, the same process was repeated for the positive control albendazole, 0.5 ml at different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml, whereas 0.5 ml of the distilled water was used for the negative control. The concentrations were then incubated for 48 hours at 27°C. Each concentration was done in triplicate as described by Cala et al. [17].

$$\text{Eggs (\%)} = \frac{\text{Eggs + Larvae 1 – Larvae 1 x 100}}{\text{(Eggs + Larvae 1)}}$$ (Cala et al. [17]).

Evaluation of larvicidal activity of the extracts

The evaluation of the larvicidal activities of the different portions of the extracts and fractions was done according to methods described by Ameen et al. [18]. One hundred (100) larvae of *H. contortus* contained in 0.1 ml of suspension were added into each of a labeled 96-well flat-bottom microtitre plate. Thereafter, 0.5 ml of the different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml of the extracts and fractions were added. For albendazole 0.5 ml at different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml were added. 0.5 ml of the distilled water was used for the negative control. Each concentration was carried out in triplicate. Thereafter, the content of each well was stirred and pipetted onto a clean glass slide and then examined under the microscope to determine for mortality.

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae X 100}}{\text{Number of larvae in culture}}$$

(Ameen et al. [18])

Table 1. Mean (±SD) percentage of ovicidal inhibition of crude extracts at different concentrations (mg/ml)

Treatment groups	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
Prosopis africana	94 ± 1.00^{a}	96 ± 1.00^{a}	97.33 ± 0.76^{a}	98.33 ± 0.76^{a}	99.5 ± 0.50^{a}
Albendazole	96.8 ± 1.04^{b}	98 ± 0.50^{b}	99 ± 0.00^{b}	99.8 ± 0.29^{b}	100 ± 0.00^{b}
DW	6.2 ± 2.33				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Table 2. Mean (±SD) percentage of larvicidal inhibition of crude extract at different concentrations (mg/ml)

Treatment groups	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
Prosopis africana	62 ± 3.00^{a}	72 ± 2.00^{a}	77.6 ± 2.52^{a}	84 ± 2.00^{a}	96 ± 1.00^{a}
Albendazole	73.47 ± 2.25^{b}	77 ± 1.73^{b}	91.3 ± 3.2^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}
DW	13 ± 1.15				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Table 3. Mean (±SD) percentage of ovicidal inhibition by fractions at different concentrations (mg/ml)

Fractions	Treatment group	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
N-Butanol	*P. africana*	86.17 ± 1.76^{a}	88.50 ± 1.50^{a}	90.17 ± 3.75^{a}	94.5 ± 1.00^{a}	97.17 ± 0.76^{b}
Ethyl acetate	*P. africana*	88.3 ± 5.11^{c}	90.6 ± 4.93^{c}	93.3 ± 4.37^{c}	94.6 ± 4.30^{c}	96.6 ± 3.80^{b}
Petroleum ether	*P. africana*	90.33 ± 1.53^{d}	92.83 ± 1.44^{d}	93 ± 2.18^{d}	95 ± 2.18^{d}	97.6 ± 2.05^{b}
Aqueous	*P. africana*	88 ± 1.00^{e}	90.5 ± 0.87^{e}	92.5 ± 0.87^{e}	94.5 ± 1.32^{e}	97.6 ± 1.76^{b}
	Albendazole	96.8 ± 1.04^{b}	98 ± 0.5^{b}	99 ± 0.00^{b}	99.8 ± 0.28^{b}	100 ± 0.00^{b}
	DW	6.2 ± 2.33				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Table 4. Mean (±SD) percentage of larvicidal inhibition of fractions at different concentrations (mg/ml)

Fractions	Treatment group	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
N-Butanol	*P. african*	57.0 ± 2.00^{a}	55 ± 2.00^{a}	65 ± 4.58^{a}	74 ± 2.60^{a}	82.0 ± 2.00^{a}
Ethyl acetate	*P. africana*	58.6 ± 6.59^{c}	59.3 ± 2.03^{c}	63.6 ± 10.7^{c}	80.2 ± 2.01^{c}	94.2 ± 2.00^{c}
Petroleum ether	*P. africana*	59.2 ± 3.00^{d}	69.9 ± 1.00^{d}	76 ± 2.00^{d}	80 ± 2.00^{d}	96.6 ± 3.0^{d}
Aqueous residue	*P. africana*	59.3 ± 2.00^{e}	61.2 ± 2.00^{e}	60.5 ± 0.00^{e}	84.5 ± 2.00^{e}	95 ± 3.00^{e}
	Albendazole	73.47 ± 2.25^{b}	77 ± 1.73^{b}	91.3 ± 3.20^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}
	DW	13 ± 1.15				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Data management and statistical analysis

Comparison of mean percentages of egg hatch and larval development inhibition assays, at different concentrations with the controls, was performed by one-way ANOVA. The Post hoc statistical significance test employed was least square difference (LSD), the difference between the means is considered significant at $p < 0.05$.

RESULTS

The results observed for qualitative phytochemical analysis showed that saponins, alkaloids, carbohydrates, steroids, triterpines, cardiac glycosides, condensed tannins, flavonoids and phenols were positive metabolites contained in *P. africana* leaves except for anthraquinones which were negative. The quantitative phytochemical analysis showed the contents of the flavonoids, alkaloids, tannins, phenols and saponins to be 10.9%, 4.50%, 10.7%, 19.7%, and 1.3%, respectively.

DISCUSSION

The selection of *P. africana* plant was based on a literature survey and the use of these plants in traditional medicine in some parts of Nigeria. This study showed that phenols had the highest percentage (19.7%) of the metabolites, while saponins had the lowest (1.3%) The results observed in this study revealed that the crude extracts and fractions of *P. africana* significantly ($p < 0.05$) had ovicidal and larvicidal inhibitory effects on the parasite in a concentration-dependent manner.

The crude extract and fractions of *P. africana* leaves showed a more significant ($p < 0.05$) inhibitory effect on the hatching of *H. contortus* eggs than on the larvicidal development. Even though there were differences in the activity between the extracts of the plant, that was shown to be statistically non-significant ($p > 0.05$) with the effect observed with albendazole. In this regard, this study agrees with the previous study investigated by Enejoh [20]. *P. africana* shows no significant difference ($p > 0.05$) in ovicidal activity on the egg hatching of *H. contortus,* while the fractions of *P. africana* showed significant difference ($p < 0.05$) in larvicidal activity when compared to the larvicidal activity of albendazole against the *H. contortus* parasite.

This study observed that the ovicidal inhibitory effects of crude extract and fractions of *P. africana* against *H. contortus* were more effective than larvicidal inhibitory activity. The higher ovicidal inhibitory effect produced by these crude extracts and fractions could be attributed to the penetration of the phytochemicals, such as tannins, flavonoids, and saponins, into the parasite's eggshell which completely affects the biology of the egg's composition, as previously revealed by Enejoh [19]; Soetan et al. [20]; Dotto and Abihudi, [21]; and Kollins et al. [22]. The larvicidal inhibitory effect produced by *P. africana* extracts was likely due to the penetration of the active chemical constituents of the extracts across the cuticle of the larvae into their circulatory system when the larvae had contact with the extracts, as reported earlier by Payne et al., [23].

The present study on *P. africana* is in agreement with previous studies carried out by Aliyu et al. [24] and Kipyegon [25], who observed that as the treatment concentration increases, the anthelmintic activity of *P. africana* crude extract and fractions increases in a dose-dependent manner. Suteky and Dwatmadji [26] also studied the *in vitro* anthelmintic activity of *Melastoma malabatricum* extract against *H. contortus*, which showed significantly increased anthelmintic activity as the concentration dose increased. Therefore, this study showed that there was a significant increase in the mean percentage inhibition of egg hatch and larval development against the *H. contortu*s at different concentrations.

CONCLUSION

The results from this study suggested that the crude and fraction extracts of *P. africana* had significant anthelmintic activities on ovicidal and larvicidal inhibitory effects against *H. contortus*. The anthelmintic activities of the plants selected for this study showed promising novel drug potential. Therefore, further *in vivo* studies are required to profile the toxicity level and safety of this plant for use as an anthelmintic drug model against the *H. contortu*s parasite.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Conflict of Interest

The authors hereby wish to declare to the editor of this journal, that there is no conflict of interest between the authors of this manuscript.

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