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THE ANTHELMINTIC ACTIVITY OF *HUNTERIA UMBELLATA* K SCHUM (FAM. APOCYNACEAE) EXTRACTS

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Aqueous and methanolic extracts of the leaves, seeds and stembark of *Hunteria umbellata*. K. Schum, were tested for anthelmintic activity. The extracts of the leaves, seeds and stem bark have significant anthelmintic activity when compared with normal saline which acted as control. The methanolic extract of the stembark had the highest activity for all the extracts. However, the activity of the aqueous extract of the seeds was more effective than the aqueous extract of the leaves and stembark.

Key words: *Hunteria umbellata*, Helminthiasis, Aqueous extracts, Methanolic extracts.

Introduction

Hunteria umbellata. K. Schum, of the family Apocynaceae is a tree of about 12 meters height and 0.9 meter girth, with a dense evergreen crown. The tree branches are very low and crooked bole (Oliver 1960; Keay *et al* 1964). The root and stem bark are used as anthelmintic by tradomedical practitioners (Irvine 1961; Gill 1992). South-Western and Northern Nigeria are endemic with helminthiasis all year round especially those caused by guinea worm, filaria worm and schistosomiasis (Eboka and Njoku 1986). The government in collaboration with UNESCO has embarked on an eradication campaign of helminthiasis in the country. In the World Health Organisation magazine of 1998 helminthiasis (Onchocerciasis) is one of the target research areas. However, significant progress is yet to be made in this campaign; hence a plant-based remedy will not only be easily acceptable to the rural populace who are susceptible to this disease, but would also save some government currency spent on the importation of anthelmintic drugs. Moreso, as the plant would be a local one, it can be easily recognized and collected for use by the people without any formal education. In addition a plant based remedy will facilitate the eradication process of helminthiasis in Nigeria.

This project is to ascertain anthelmintic properties of this plant in South-West Nigeria and to provide a plant based anthelmintic remedy needed to facilitate helminthiasis eradication in Nigeria. The choice of earthworm in this study is justified by the work of Sollman (1957) who demonstrated that the susceptibility of different species of worms was quite different towards various anthelmintics. He therefore recommended the use of earthworms for anthelmintic screenings. Most drugs that are toxic to earthworms produce primary irritation or agitation resulting in the withdrawal of the worms from the neighbourhood of the poison (Munch 1931; Sollman 1957). It should be noted that *Ascaris* or any other worm on exposure to any anthelmintics do not always respond parallel to clinical efficacy even when identical worms are used (Sollman 1957). Finally the use of earthworms is cost-effective and saves time.

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Materials and Methods

Plant material. The leaves, seeds from the fruits and stembark were collected at Bolorunduro village in Ondo-East Local Government Area of Ondo-State of South-Western Nigeria. The plant was authenticated at the Herbarium in the Botany Department, University of Ibadan Nigeria. The plant parts were dried separately at a temperature of 50-60°C, and were reduced to fine powder with a hammer mill.

The powdered leaves (82.5g), seeds (52.3g) and stembark (98.3g) were macerated separately with the solvents: distilled water (to minic the traditional method of preparation) and methanol for 48 h. After extraction, the extracts were filtered and concentrated under reduced pressure with a percentage yield of 17.09, 16.82, 4.17 respectively for the aqueous extracts and 25.56, 15.40 and 17.71 for the methanolic extracts.

A stock solution of 250mg ml^{-1} was prepared for both the aqueous and methanolic extracts of the leaves, seeds and stembark

by dissolving 3.5 g of the extracts in 14ml of distilled water with continuous stirring at a temeprature of 40°C for 5 mins.

Bio-assay. Amodified method of Semper and Yagi (Munch 1931) for the screening of the anthelmintic activity of drugs was used in this study, which was based on the principle that all clinical anthelmintics are markedly toxic to earthworms. By this method the anthelmintic value of any drug can be determined and compared with the activities of different drugs.

In this study six groups of earthworms were used with each group having a total of ten worms. Four groups were treated with the aqueous extracts of the seeds at concentrations of 10-40 mg ml^{-1} and the other two groups which served as the control were treated with distilled water and normal saline. This experiment was replicated twice and the result analysed using a students T-test at a probability of 0.05.

The extracts of the stembark and leaves were subjected to similar procedure as the seed extract. The above experimental methodology for the aqueous extracts of the seeds, stembark and leaves were also executed using the methanolic extracts of the seeds, stembark and leaves.

Phytochemical sreening. Appropriate portions of the powdered leaves, seeds and stembark were subjected to phytochemical screening for the presence of alkaloid, tannins, saponins, anthraquinones using standard phytochemical procedures (Sofowora 1982; Harborne 1983; Evans 1987).

Test for alkaloids. The powdered plant 0.5g was boiled in 1M HCl in ethanol and filtered. The filtrate was allowed to cool and separated into four parts. These parts were separately tested for alkaloids using Mayer's, Dragendorff's Wagner's reagents and Hager's /picric acid solution. The presence of turbidity or precipitate in the solution mixture is an indication of the presence of alkaloids. (Sofowora 1982; Harborne 1983; Evans 1987).

Test for tannins. Powdered plant 5g was extracted with 20ml distilled water (5min). The mixture was filtered and to 5ml of this solution 6 drops of ferric chloride was added blue-black, green or blue-green precipitate is indicative of the presence of tannins. (Sofowora 1982; Evans 1987).

Test for anthraquinones. Powdered plant 5g was extracted with 10 ml benzene (shaking). The mixture was filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the appearance of a pink, red or violet colour in the ammonia phase is indicative of the presence of anthraquinones.

Powdered plant 5g was boiled with 10ml aqueous sulfuric acid and filtered hot. The filtrate was shaken with 5ml benzene and the benzene phase was separated. To this phase half it's volume of 10% ammonia solution was added. The presence of a pink, red or violet colour in the ammonia phase is indicative of the presence of anthraquinone derivatives. (Sofowora 1982; Evans 1987).

Test for saponins. Powdered plant 0.2g was boiled with 10ml of distilled water for 10min. The mixture was filtered hot and the filtrate was allowed to cool. About 5ml of the filtrate was allowed to cool. About 5ml of the filtrate was diluted with distilled water and shaken vigorously. The appearance of persistent froth, even on warming is a preliminary indication of presence of saponins. (Stahl 1973; Sofowora 1982).

To test solution of 4ml dilute H_2SO_4 2ml was added and boiled for 5min. The appearance of a light green solution indicates the presence of saponins. (Sofowora 1982; Harborne 1983).

Statistics. The administered doses were prepared using the formula:

$$D = \frac{a \times b}{c}$$

Where,

a = volume of the stock solution,
b = concentration of the stock solution,
c = volume of dose solution to be prepared and
D = concentration of the dose to be prepared.

A one tailed student t-test was used for the analysis at a probability of 0.05, using the formula.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S} \times \frac{\sqrt{n_1 n_2}}{\sqrt{n_1 + n_2}}$$

Where,

x_1 = mean of first sample of n_1 observations,
x_2 = mean of second sample of n_2 observation and
S = standard deviation

The standard deviation of the whole study was calculated from the variance using the formula,

$$S^2 = \frac{\Sigma X^2x - (\Sigma Xx)^{\frac{2}{n_1}} + \Sigma X^2a - (\Sigma Xa)^{\frac{2}{n_2}}}{n_1 + n_2 - 2}$$

where,

ΣX^2x = sum of squares of observations in the first sample,
ΣXx = sum of observations in the first sample,
ΣX^2a = sum of squares of observations in the second sample
ΣXa = sum of observations in the second sample and
S^2 = pooled variance of the two samples.

Results and Discussion

The aqueous extracts of the leaves, seeds and stembark showed significant anthelmintic action on earthworms when compared

with normal saline (control) as shown in Table 1, with its potency increasing proportionally with the dose. The dose oriented treatment of the seeds revealed that the 30 mg ml^{-1} dose is the most potent for earthworms with at-value of -4 for all the samples tested (Table 2). However, the aqueous extract of the seeds gave the highest activity of mortality time of 88.33 min as the potent dose of 30 mg ml^{-1} (Table 2). The activity of the aqueous extract proved to be dose dependent, except for the stembark extract having its highest potency at the 20mg ml^{-1} dose, (Table 3). The stembark was found to be relatively as active as the seeds at the dose of 40 mg ml^{-1} (Table 2-3). From a qualitative point of view, the seeds were more active, followed by the stembark and finally the leaves. The average mortality time range for the aqueous extracts of the stembark and those for the leaves were appreciably close between 140 - 390 min for the leaves (Table 1) and 137 - 380 for the stembark (Table 3). There was an increase in the mortality rate at higher concentrations for the aqueous extracts (Table 1).

The methanolic extracts of the plant parts showed significant anthelmintic activity (Tables 1) when compared with normal saline. The methanolic extract of the leaves gave a significant anthelmintic activity (t = -1.93) when comapared with normal saline (standard control) as shown in Table 1. The seeds gave the highest activity at the various dose ranges used especially at the 40 mg ml^{-1} dose with a t-value of - 3.47 (Table 2). The anthelmintic activity of the seeds increased proportionally with increase in dose (Table 2). The activity of the stembark proceed's that of the seeds, while the stembark gave the least activity (Table 1-3). The dose related investigation of the leaves gave inconsistent result, with the highest activity occuring both at the lowest and the highest doses used (i.e. 10 mg ml^{-1} and 40 mg ml^{-1}). However, a similar trend of effect was observed for the methanolic extract of the stembark. This deviation from the normal trend of action of the aqueous extract of the stembark and methanolic extract of the leaves could be due to biological variations in the individual worms used, which may have been caused by injury or physiological variations (Sollman 1957).

The aqueous and methanolic extracts of the leaves, seed and stembark possessed significant anthelmintic activity compared to normal saline and distilled water which were the control groups. When the activity of the seeds and the stembark of the methanolic extracts were compared, they were found to be significant. Gill (1992) reported the use of the root and stembark of this plant for anthelmintics. The result of this investigation confirms that the stembark has anthelmintic property coupled with the fact that it gave the highest activity for the methanolic extract, justifies its choice by the trado-medical practitioners. However, if the solvent for extraction is water, which is usually the case, in the trado-medical practice, then the seeds would be preferred since the seeds have a higher anthelmintic activity.

Though this investigation is a qualitative work, the seeds were found to be more potent in both mediums. However, Arecoline hydrobromide, an alkaloid from the nuts of *Areca catechu* Linne has been found to be responsible for its anthelmintic action (Sollman 1957; Tyler *et al* 1981; Reamongkol 1995). Water soluble alkaloids have been found to be present in all parts of the plant especially the seeds from the result of the preliminary phytochemical investigation conducted and this could be responsible for the observed activity. This also confirms the report of Schultes (1976) and David (1996) of the preseence of water soluble and insoluble alkaloids in the seed and stembark of *H.umbellata* as well as other species of this family.

Table 1

Anthelmintic activity of the leaf extracts of *H. umbellata*, at P = 0.05, DF = 4

Test drug	Dose (mg ml^{-1})	Average mortality time (min)	Standard deviation (S.D.)	t-value
Control				
Normal saline	50 ml	429.00	145.91	--
Distilled water	50 ml	1078.00	–	--
Aqueous	10	391.67	120.14	-0.34
extract	20	414.69	150.07	-0.14
	30	149.33	101.79	-2.72
	40	140.33	103.07	-2.79
Methanol	10	176.67	172.77	-1.93
extract	20	321.67	190.12	-0.78
	30	250.33	231.30	-1.13
	40	157.67	61.70	-2.97

Table 2
Anthelmintic activity of the seed extracts of *H. umbellata*, at P = 0.05, DF = 4

Test drug	Dose (mg ml^{-1})	Average mortality time (min)	Standard deviation (S.D)	t-value
Control				
Normal saline	50 ml	1079.20	145.91	--
Distilled water	50 ml	429.00	–	--
Aqueous	10	894.00	257.39	1.21
extract	20	308.67	26.58	-1.41
	30	88.33	22.50	-4.00
	40	189.00	35.51	-2.77
Methanol extract	10	217.00	122.31	-1.93
	20	181.33	29.67	-2.88
	30	181.00	100.00	-2.43
	40	126.33	39.11	-3.47

Table 3
Anthelmintic activity of the stembark extracts of *H. umbellata*, at P = 0.05, DF = 4

Test drug	Dose (mg ml^{-1})	Average mortality time (min)	Standard deviation (S.D)	t-value
Control				
Normal saline	50 ml	429.42	145.98	--
Distilled water	50 ml	1078.10	–	–
Aqueous	10	381.33	295.11	-0.25
extract	20	417.33	118.36	-0.11
	30	223.67	287.45	-1.10
	40	137.67	55.01	-3.24
Methanol	10	489.67	355.19	0.27
extract	20	126.67	29.67	-3.30
	30	92.67	100.00	-3.85
	40	110.67	47.49	-3.59

It should be noted that if a test drug fails to produce any irritation or cause the withdrawal of the earthworm from the medium, it simply indicates that it does not have any anthelmintic principle. Hence, there will be no need to embark on any form of clinical investigation.

The crude drugs (leaves and seeds) tested positive for saponin-glycosides using Molisch, Fehlings and the heamolysis test. Saponin was also found to be present in the stembark. The ferric chloride test for tannins indicated the presence of this class of compound in the seeds of *H. umbellata*. However, all parts of the plant were positive for alkaloidal test especially for tropane and indole type of alkaloids. The stembark was found to contain only alkaloids (both tropane and indole types).

Conclusion

The leaves, seeds and stembarks showed significant anthelmintic activity with the highest result obtained from the seeds at various dose ranges when compared with normal saline for both the aqueous and methanolic extracts.

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