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PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTHELMINTIC POTENTIALS OF XYLOPIA AETHIOPICA (DUNAL) A. RICH (ANNONACEA) FROM NIGERIA

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ABSTRACT

The main aim of the research work was to investigate the phytochemical constituents of the aqueous, ethanol and methanol extracts and the anthelmintic activity of the extracts of the fruits against *Eudrilus eugeniae*. The four concentrations (10, 20, 50 and 100 mg/ml) of extracts were studied *in vitro* in the bioassay for their anthelmintic activity in experimental worm, *Eudrilus eugeniae* which involved the determination of time of paralysis and time of death of the experimental worms. Albendazole (15 mg/ml) was used as a standard reference drug in the assay. At the concentration of 100mg/ml, the aqueous, ethanol and methanol extracts showed very significant activities as compared to the standard drug Albendazole (15 mg/ml), the time of paralysis and death being 1.63±0.36 and 6.77±0.11 in the case of the aqueous extract, 2.91±0.10 and 8.86±0.66 in the case of ethanol extract, 3.19±0.56 and 6.44±0.83 in the case of the methanol extract and 32.00±0.87 and 38.87±0.65 as in the case of the standard drug Albendazole respectively. The extract of *X. aethiopica* produced a significant anthelmintic activity.

Keywords: *Xylopia aethiopica*, phytochemical constituents, anthelmintic activity, *Eudrilus eugeniae*

INTRODUCTION

Spices are a group of exoteric food adjunct that have been in use for thousands of years to enhance the sensory qualities of foods. The quality and variety consumed in tropical countries is particularly extensive. These spice ingredients imparts characteristics flavour, aroma or piquancy and colour to foods. Some spices can also modify the texture of foods. Spices are used in wines, beverages, foods, cosmetics, tooth pastes, and in medicines as adjuvant. Some have antimicrobial and soothing properties **[1]**. A spice being a vegetable substance of indigenous or exotic origin, being aromatic, is used to enhance the flavour of food. They are derived from rhizomes, bark of fruits, seeds, leaves, fruits, and other parts of plants **[2]**. The inhibitory effect of spices oils could be attributed to the presence of aromatic nucleus containing a polar functional group. *Xylopia aethiopica* has been reported in literature to possess medicinal and nutritional values **[3]**. Chemical constituents include essential oils, resins, annonacin, reberoside, avicien, rebersole, alkaloids, tannins, oxalate, and flavonoids. The fruits are used as spices and aqueous decoction is used especially after child birth probably for its antiseptic properties and to arrest bleeding. This plant has a wide spectrum of biological activities and has played a crucial role in traditional medicines because of their valuable physiological and pharmaceutical properties **[4]**.

The fruits have been found to contain volatile aromatic oil, fixed oil and rutin **[5]**. It is used in the treatment of digestive system motility (Diarrhoea), bronchitis, stomach aches, febrile pains, and rheumatism. This fruit of *X. aethiopica* has been reported to act as antioxidant, hypolipidaemia and hypoglycaemic agents, hence, confirming its use as an antidiabetic agent **[6]**.

Therefore, the present study was carried out to determine the phytochemicals composition of the aqueous, ethanol and methanol extracts of *X. aethiopica* and to establish its anthelmintic potential as a medicinal food.

MATERIAL AND METHODS

Plant Materials

The dry fruits of *X. aethiopica* were purchased from a local market in Owerri, Imo State, Nigeria in the month of November, 2011. They were identified and authenticated by Mr A. Ozougwu, a Botanist at the Department of Botany and a voucher specimen was preserved at the Department of Botany herbarium,

University of Nigeria Nsukka for further reference.

Extract from the Plant Material

The *X. aethiopica* fruits were sorted cleaned and milled using a laboratory mill (Retsch, 5657, GmbH, Germany). A quantity, about 300 g of the dried and ground *X. aethiopica* fruits was defatted by shaking it with a volume, 2 L of n-hexane for 1 hour, 3 times to extract the oil. The defatted *X. aethiopica* fruit flour was then dried in a desiccator under vacuum until all traces of the n-hexane is removed. The aqueous, ethanol, and methanol extract were obtained by stirring each of 100 g of the dry defatted *X. aethiopica* flour with 300 ml of distilled hot water, ethanol and methanol for each of the extracts respectively at room temperature (27 ± 1^0C) for 24 hours. The mixtures were then filtered using a clean muslin cloth and then whatman No.1. The mixtures were evaporated to dryness. The extracts were then stored at 4^0C for further use.

Standard Drug Used

For the present study, Albendazole was used as the standard drug **[7]**. The concentration of the standard drug was prepared in normal saline to give 15 mg/ml concentration.

Worm Collection and Authentication

Adult African worms of the genius and species *Eudrilius eugeniae* (Family: Eudrildae) were used to study the anthelmintic activity. The earthworms were obtained from nearby areas of faculty of biological sciences, university of Nigeria, Nsukka in the month of November, 2011 and authenticated at the department of Zoology. They were washed with normal saline to remove all the traces of faecal matter and waste surrounding their body. The African earthworm (*Eudrilius eugeniae*) 3 – 7 cm in length and 0.1 – 0.3 cm in width weighing 0.6 – 5.01 g were used for all experiment protocols. The earthworms resembled the intestinal roundworm both anatomically and physiologically and hence used to study the anthelmintic activity **[8]**. Ethical approval was obtained from the animal ethics committee of the department of Veterinary medicine, University of Nigeria, Nsukka (Ref No. VM-096). Procedures were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental animals **[9]**.

Phytochemical Analysis of the Extracts

The phytochemical study for the presence or absence of phytochemicals in the extracts was carried out according to the method described by Harbone **[10]**.

Alkaloids

A quantity, 0.2g of each of the extracts was added to 5 ml of 2% hydrochloric acid and heated on boiling water for 10 minutes. They were then allowed to cool and then filtered. To 1 ml of the filtrate in a test tube was tested with alkaloids reagent, Wagner's and Mayer's reagent and results compared to blank. Turbidity or precipitation indicated the presence of alkaloids.

Tannins

A quantity, 0.2 g of each of the extracts was boiled with 5 ml of 45% ethanol for 5 minutes. The mixture was filtered hot using a filter paper and filtrate collected in a beaker. 2 ml of the filtrate was mixed with 10 mL of distilled water and then a drop of Iron Chloride solution was added. A blue-black or blue-green precipitate indicates the presence of tannins.

Saponin

A quantity, 0.1 g of each of the extracts was measured into a beaker and 20 mL of distilled water was added, the beaker was heated in a water bath for over 5 min. The mixtures were filtered using a filter paper into another beaker to obtain a filtrate. 2 ml of each of the filtrate was measured to another test tube and 10 mL of distilled water was added, it was shaken vigorously for over a minute. Frothing which persist on warming indicated the presence of Saponin.

Resins

A weighed quantity, 0.2 g of each of the extracts was poured into 20 ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.

Steroids

A quantity, 0.1 g of each of the extracts were added a mixture of 10 ml of lead acetate solution (90% w/v) and 20 ml of 50% aqueous ethanol in a 200 ml conical flask. The mixtures were placed on boiling water for 2 minutes, cooled and filtered. The filtrate was extracted twice with 15 ml chloroform. Then 5 ml of the chloroform extract was evaporated to dryness on a water bath. To the residue, 2 ml of 3, 5 - dinitrobenzoic acid solution (2% in ethanol) and 1 ml of 1N sodium hydroxide solution were added. A reddish brown interphase shows the presence of steroids.

Glycosides

A quantity, 0.2 g of each of the extracts was mixed with 30 ml of water and heated on a water bath for 5 minutes and filtered. 5 ml of a mixture of equal parts of Fehling's solutions A and B were added to 5 ml of the filtrate until it turned alkaline (with litmus) and then boiled on a water bath for 5

minutes. A brick red precipitate indicated the presence of Glycosides.

Cyanogenic Glycosides

A quantity, 0.1 g of each of the extracts in a conical flask was added 10 ml of water and 1.0 ml dilute HCl. Picrate papers were suspended above the mixtures and contents of the flask were warmed at 45^0C for 1 hour. A control without the extracts was set up. A colour change from yellow to reddish purple of the picrate paper was indicative of a positive test.

Flavonoids

A quantity, 0.1 g of each of the extracts was added a mixture of 10 ml of lead acetate solution (90% w/v) and 20 ml of 50% aqueous ethanol in a 200 ml conical flask. The mixtures were placed on boiling water for 2 minutes, cooled and filtered. A volume, 5 ml of dilute ammonia was added to a portion of the aqueous filtrate followed by the addition of concentrated sulphuric acid (1 mL) to 2 mL of potassium hydroxide solution and allowed to mix. Then into acid base mixture a small quantity of aqueous filtrate of the sample was added and observed for colour change.

Anthelmintic Activity Study

Fourteen groups of approximately equal sized earthworms consisting of six earthworms in each group were released in to 50 ml of desired formulation. The extract was suspended in 1% Dimethyl sulphoxide (DMSO) in normal saline at 10, 20, 50, and 100 mg/ml concentrations. Each group was treated with one of the following; control (1% DMSO in normal saline), Albendazole (15 mg/ml) or extracts (10, 20, 50, and 100 mg/ml). Observations were made for the time taken to paralyze and / or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour.

Statistical Analysis

Numerical data were presented as mean±standard deviation and analysed using simple students' T-test. Values of $P < 0.05$ were considered as significant. All analysis was done using SPSS (Statistical Package for Social Science) software, version 17.0 by IBM (International Business Machine), USA.

RESULTS

The percentage yield of the aqueous and ethanol, methanol extracts of *X. aethiopica* seed flour after solvent evaporation were approximately 5.0%, 17.0**%** and 16.0**%** respectively, **Table 1**.

Preliminary phytochemical screening of the aqueous extract showed that it contained alkaloids, tannins, saponins, resins, cyanogenic glycosides, glycosides and flavonoids. The ethanol extract revealed the presence of alkaloids, tannins, saponins, resins, cyanogenic glycosides, glycosides and flavonoids while the methanol extract also contained alkaloids, tannins, saponins, resins, cyanogenic glycosides, glycosides and flavonoids, **Table 2**.

The anthelmintic activity of the aqueous extract of *X. aethiopica* was more potent than the ethanol extract of *X. aethiopica*, which was more potent than the methanol extract. At the concentration of 100mg/ml, the aqueous, ethanol and methanol extracts showed very significant activities as compared to the standard drug Albendazole (15 mg/ml), the time of paralysis and death being 1.63±0.36 and 6.77±0.11 in the case of the aqueous extract, 2.91±0.10 and 8.86±0.66 in the case of ethanol extract, 3.19±0.56 and 6.44±0.83 in the case of the methanol extract and 32.00±0.87 and 38.87±0.65 as in the case of the standard drug Albendazole respectively. The seed extracts of *X. aethiopica* produced a significant anthelmintic activity in dose dependent manner as shown in **Table 3**.

Table 1: The Colour, Consistency and Yield of Different Extracts of the Seeds of *X. aethiopica*

S/No.	SOLVENT EXTRACTS	COLOUR	CONSISTENCY	YIELD (%)
1	Aqueous Extracts	Brown	Dry	5.0%
2	Ethanol Extracts	Brown	Sticky	17.0%
3	Methanol Extracts	Brown	Sticky	16.0%

Table 2: Qualitative Phytochemical Analysis of Different Extracts

Phytochemicals	Aqueous extracts	Ethanol extracts	Methanol extracts
Alkaloids	+++	++	++
Tannins	+++	+++	+++
Saponins	+++	+++	+++
Resins	+++	++	++
Steroids	-	-	-
Glycosides	+	+	+
Cyanogenic glycosides	+	+	+
Flavonoids	++	++	++

Key: +++ = present in high amount, ++ =present in moderately high amount,

+ =present in trace amount, − = Absent

Table 3: The Anthelmintic Potentials of *X. aethiopica* Fruit Extracts

TREATMENT	GROUPS	CONCENTRATION	TIME OF PARALYSIS (MIN) (MEAN±SD)	TIME OF DEATH (MIN) (MEAN±SD)
Control	1	-	-	-
Albendazole	2	15 mg/ml	32.00±0.87	38.87±0.65
Aqueous extract	3	10 mg/ml	5.44±0.76*	11.04±0.22*
Aqueous extract	4	20 mg/ml	4.63±0.01*	10.61±0.67*
Aqueous extract	5	50 mg/ml	2.44±0.89*	8.76±0.44*
Aqueous extract	6	100 mg/ml	1.63±0.36*	6.77±0.11*
Ethanol extract	7	10 mg/ml	10.84±0.36*	16.81±0.13*
Ethanol extract	8	20 mg/ml	7.22±0.44*	15.88±0.90*
Ethanol extract	9	50 mg/ml	4.03±0.56*	12.61±0.17*
Ethanol extract	10	100 mg/ml	2.91±0.10*	8.86±0.66*
Methanol extract	11	10 mg/ml	10.44±0.70*	14.46±0.12*
Methanol extract	12	20 mg/ml	8.67±0.83*	13.06±0.30*
Methanol extract	13	50 mg/ml	6.11±0.50*	12.01±0.14*
Methanol extract	14	100 mg/ml	3.19±0.56*	6.44±0.83*

Values are Mean ± SD for each group of five rats. *Means significantly different at P<0.05 compared with the Albendazole treated group

DISCUSSION

Parasitic helminths affect animals and man, causing considerable hardship, malnutrition and stunted growth. Preliminary phytochemical tests of the crude extracts of *Xylopia aethiopica* revealed the presence of tannins, flavonoids and alkaloids, among other constituents contained within it.

Tannins have been shown to produce anthelmintic effects. The anthelmintic effect of plants containing tannins actually depends on the type and content of tannins in the plant **[11]**. It has been reported that Sheep fed *ad libitum* with forages high in condensed tannins had increased food intake and had weight gain as compared to sheep fed on forages low in condensed tannins **[12]**. It is therefore reasonable to assume that food taken freely due to presence of tannins might be another way with which to control the detrimental effects of gastrointestinal parasitism.

Chemically, tannins are polyphenolic compounds **[13]**. Some synthetic phenolic anthelmintics example niclosamide, oxyclozanide and bithiol are known to interfere with energy generation in helminth parasites by uncoupling parasite specific fumarate reductase mediated oxidative phosphorylation reaction. It could be possible that tannins contained in our extracts produced similar effects. From the result of our analysis, there is moderately high amount of tannin in our aqueous,

ethanol and methanol extracts. Another feasible effect of tannins is that they can bind to free proteins in the gastro intestinal tract of the host animal as glycoprotein on the cuticle of the parasite **[14]** and cause death to it.

Alkaloids may have acted on the central nervous system of the earth worms causing paralysis **[15]**. This study suggests that the effect could also be due to presence of the steroidal alkaloids oligosaccharides which have been reported to suppress the transfer of sucrose from the stomach to the small intestine. This could diminish the availability of glucose to helminths together with its antioxidant effect which is capable of reducing the nitrate generation. The extracts may have also induced possible inflammatory effect in the gastric and intestinal mucosal which could have interfered in local homeostasis, essential in the development of helminths.

The main biologic activity ascribed to saponins based on recent research is their membrane permeability property. The main possible actions of saponins are changes in membrane permeability and pore formation, similar with two conventional anthelmintic drugs such as praziquantel and toltrazuril. The anthelmintic drug affects the permeability of the cell membrane of earth worm, causing vacuolisation and disintegration of the teguments **[7]**.

CONCLUSION

The present study has confirmed that the extract of *X. aethiopica* possess anthelmintic activity against the worm used in this study. Further studies to isolate and reveal the active compounds contained in the organic extracts of *X. aethiopica* and to establish the mechanism of action are required to be done in future.

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