Full Length Research Paper

In vitro anthelmintic potentials of *Xylopia aethiopica* and *Monodora myristica* from Nigeria

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Monodora myristica and *Xylopia aethiopica* are two spices that have been traditionally used as a vermifuge in Ayurveda. The main aim of the research work was to investigate the phytochemical constituents of the aqueous, ethanol and methanol extracts of the two spices and the anthelmintic activity of the extracts of their seeds against *Eudrilus eugeniae*. The four concentrations (10, 20, 50 and 100 mg/ml) of each of the extracts from the two spices were studied in the bioassay which involved the determination of time of paralysis and time of death of the worm. Albendazole (15 mg/ml) was used as a standard reference drug in the assay. At the concentration of 100 mg/ml, the aqueous, ethanol and methanol extracts of the two spices showed very significant activities as compared to the standard drug Albendazole (15 mg/ml). The seed extracts of *M. myristica* and *X. aethiopica* produced a significant anthelmintic activity.

Key words: *Monodora myristica*, *Xylopia aethiopica*, phytochemicals, anthelmintics, *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 (Srinivasan, 2005). 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 (Kochlar, 1986). The inhibitory effect of spices oils could be attributed to the presence of aromatic nucleus containing a polar functional group.

Monodora myristica commonly called African nutmeg is

a perennial edible plant that grows wild in evergreen West Africa forests (Burubai et al., 2009). Its seeds usually embedded in a white sweet smelling pulp, was reported to possess valuable economic and medicinal value (Okafor, 1987; Okigbo, 1977). In Nigeria and other African countries, the kernel obtained from the seeds is a popular spicing agent as well as an aromatic stimulating addition to medicine and snuff (Ekeanyanwu et al., 2010). Also, the seeds when ground into powder and taken acts as stimulant, relieving constipation and can as well be sprinkled on sore especially those caused by guinea worm (Burkill, 1985).

Xylopia aethiopica has been reported in literature to possess medicinal and nutritional values (Nwachukwu, 2000). 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 (Ogbonnia

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et al., 2008). The fruits have been found to contain volatile aromatic oil, fixed oil and rutin (Burkhill, 1985). It is used in the treatment of digestive system motility, 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 (Ameyaw and Owusu-Ansah, 1998).

The helminth which infect the intestine are Cestodes example tape worms (*Taenia solium*), nematodes example hookworm (*Ancyclostoma duodenate*), round worm (*Ascaris lumbricoides*) and trematodes or flukes (*Shistosoma mansoni* and *Schistosoma hematobolium*). The disease originated from parasite infection causing severe morbidity includes lymphatic filariasis, ochocerciasis, and schistosomiasis (Mali and Mahta, 2008). Traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in tropical countries including Nigeria. It is in this context that the people consume plants or plant derived preparations to cure helminth infections (Satyavati, 1990). Ideally an anthelmintic agent should have broad spectrum of action, high percentage cure with a single therapeutic dose, free from toxicity to the host and should be cost effective. None of the synthetic drugs available meets the requirement (Mali and Mahta, 2008). Resistance of these parasites to existing drugs (Walter and Prichard, 1985) and their high cost warrants search for newer anthelmintic agents.

Therefore, the present study was carried out to determine the phytochemicals composition of the aqueous, ethanol and methanol extracts of *M. myristica* and *X. aethiopica* and to estimate their anthelmintic activity.

MATERIALS AND METHODS

Plant materials

The dry fruits of *M. myristica* and *X. aethiopica* were collected between the month of June and November, 2011. They were identified and authenticated at the department of Botany, University of Nigeria Nsukka through comparison with a voucher specimen present in the herbarium.

Extract from the plant materials

The *M. myristica* and *X. aethiopica* fruits were sorted cleaned and milled using a laboratory mill (Retsch, 5657, GmbH, Germany). A quantity, about 300 g each of the dried and ground *M. myristica* and *X. aethiopica* fruits were defatted by shaking them with a volume, 2 L of n-hexane for 1 h, 3 times to extract the oil respectively. The defatted fruit flours of *M. myristica* and *X. aethiopica* were then dried separately in desiccators under vacuum until all traces of the n-hexane is removed. The aqueous, ethanol, and methanol extracts of *M. myristica* and *X. aethiopica* were obtained by stirring each of 100 g of the dry defatted flour of both spices with 300 ml of distilled hot water, ethanol and methanol for each of the extracts respectively at room temperature (27±1°C) for 24 h. The mixtures

were then filtered using a clean muslin cloth and then whatman No.1 and evaporated to dryness. The extracts were then stored at 4°C for further use.

Standard drug used

For the present study, Albendazole was used as the standard drug (Wang, 2010). 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 (*E. eugeniae*) 3 to 7 cm in length and 0.1 to 0.3 cm in width weighing 0.6 to 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 (Lakshananam et al., 2011). Ethical approval was obtained from the animal ethics committee of the Department of Veterinary Medicine, University of Nigeria Nsukka. Procedures were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental animals (Anonymous, 2004).

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 Harborne (1984).

Alkaloids

A quantity, 0.2 g of each of the extracts was added to 5 ml of 2% hydrochloric acid and heated on boiling water for 10 min. 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 min. 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 min, 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 1 N 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 min 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 min. 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°C for 1 h. 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 min, 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 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

Twenty six groups of approximately equal sized earthworms consisting of six earthworms in each group were released into 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), *M. myristica* extracts (10, 20, 50, and 100 mg/ml) and *X. aethiopica* 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 Statistical Package for Social Science (SPSS) software, version 17.0 by International Business Machine (IBM), USA.

RESULTS

The percentage yield of the aqueous, ethanol and methanol extracts of *M. myristica* seed flour after solvent evaporation were approximately 6.16, 1.17 and 3.01% respectively, while the percentage yield of the aqueous, ethanol and 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 extract of *M. myristica* showed that the aqueous extracts contained alkaloids, tannins, saponins, resins, steroids, glycosides, flavonoids, cyanogenic glycosides, oxalates, and phytates. The alcohol extract revealed the presence of alkaloids, tannins, saponins, resins, glycosides, flavonoids, cyanogenic glycosides, oxalates and phytates, while the methanol extract also contained phytochemicals such as alkaloids, tannins, saponins, resins, glycosides, flavonoids, cyanogenic glycosides, oxalates and phytates (Table 2). The preliminary phytochemical screening of the *X. aethiopica extracts* showed that the aqueous extract 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).

Anthelmintic drugs like Albendazole are reported to cause paralysis of the worms so that they are expelled in the faeces of man and animals (Tiwari, 2011). The extracts not only demonstrated this property, but caused the death of the worms. The anthelmintic activities of the aqueous extract of *M. myristica* and *X. aethiopica* were more potent than the ethanol extracts of *M. myristica* and *X. aethiopica*, which were more potent than the methanol extracts. The various extracts were more potent than the standard drug Albendazole at 15 mg/ml (Table 3). At the concentration of 100 mg/ml, the ethanol, methanol and aqueous extracts of *M. myristica* showed very significant activities as compared to the standard drug Albendazole (15 mg/ml), the time of paralysis and death being 1.98±0.67 and 7.23±0.19 as in the case of aqueous extract, 2.30±0.28 and 8.30±0.34 in the case of ethanol extract and 4.06±0.60 and 6.30±0.88 as in the case of

Table 1. The colour, consistency and yield of the different extracts of the seeds of *M. myristica* and *X. aethiopica*.

Solvent extracts	Colour	Consistency	Yield (%)
Xylopia aethiopica extracts			
Aqueous extracts	Brown	Dry	5.0
Ethanol extracts	Brown	Sticky	17.0
Methanol extracts	Brown	Sticky	16.0
Monodora myristica extracts			
Aqueous extract	Reddish brown	Dry	6.16
Ethanol extract	Reddish brown	Dry	1.17
Methanol extract	Reddish brown	Sticky	3.01

Table 2. Qualitative phytochemical analysis of the different extracts of *M. myristica* and *X. aethiopica*.

Phytochemical composition	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract
	Monodora myristica extracts			*Xylopia aethiopica* extracts		
Alkaloids	+++	++	+	+++	++	++
Tannins	++	++	++	+++	+++	+++
Saponins	+++	+++	+++	+++	+++	+++
Resins	++	+	+	+++	++	++
Steroids	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+
Flavonoids	+++	+++	+++	+	+	+
Cyanogenic glycosides	+	+	+	++	++	++
Oxalates	+	+	+	+++	++	++
Phytates	+	+	+	+++	+++	+++

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

methanol extract respectively. The same trend was observed in *X. aethiopica* extracts where the anthelmintic activity of the aqueous extract of *X. aethiopica* was found to be more potent than the ethanol extract of *X. aethiopica*, which was more potent than the methanol extract. At the concentration of 100 mg/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 *M. myristica* and *X. aethiopica* produced a significant anthelmintic activity in dose dependent manner.

DISCUSSION

Parasitic helminths affect animals and man, causing considerable hardship, malnutrition and stunted growth.

For a drug to be considered a good anthelmintic drug, it must be able to penetrate the cuticle of the worm or gain access to the alimentary tract. Anthelmintic drugs are known to act by causing paralysis of the worm, or damaging cuticle, leading to partial digestion or to rejection by immune mechanism. Anthelmintic drugs also interfere with the metabolism of worm, and since the metabolic requirement of these parasites vary greatly from one species to another (Aisawarya et al., 2010).
Albendazole has been known to affect worms by destroying the cytoskeletal structure of the worm thereby causing paralysis (Nikesh et al., 2011). The cytoskeletal structure of the helminth includes microfilaments, microtubules and β-tubulins. Under normal conditions, microtubule assembly is dependent on β-tubulin function where the β-tubulin dimmers are continually being polymerised from one end and then depolymerised at the other end of the microtubule (Tiwari, 2011). Albendazole is known to bind to the β-tubulin, preventing their assembly. This results to depletion of parasites glycogen stores, reducing the formation of ATP, disrupting the metabolic pathways and ultimately leading to the parasites death.

Table 3. The Anthelmintic activities of *M. myristica* and *X. aethiopica* extracts.

Treatment	Groups	Concentration (mg/ml)	Time of paralysis (min) (Mean±SD)	Time of death (min) (Mean±SD)
Control	1	-	-	-
Albendazole	2	15	32±0.87	38.87±0.65
Monodora myristica extracts				
	3	10	6.39±0.66*	12.86±0.11*
	4	20	5.01±0.91*	11.77±0.83*
Aqueous extract	5	50	3.66±0.23*	10.68±0.14*
	6	100	1.98±0.67*	7.23±0.19*
	7	10	8.48±0.18*	13.78±0.26*
	8	20	6.39±0.30*	12.39±0.02*
Ethanol extract	9	50	4.59±0.02*	12.15±0.33*
	10	100	2.30±0.28*	8.30±0.34*
	11	10	10.47±0.36*	13.41±0.47*
	12	20	8.46±0.86*	11.26±0.31*
Methanol extract	13	50	6.07±0.77*	11.26±0.31*
	14	100	4.06±0.60*	6.30±0.88*
Xylopia aethiopica extracts				
	15	10	5.44±0.76*	11.04±0.22*
	16	20	4.63±0.01*	10.61±0.67*
Aqueous extract	17	50	2.44±0.89*	8.76±0.44*
	18	100	1.63±0.36*	6.77±0.11*
	19	10	10.84±0.36*	16.81±0.13*
	20	20	7.22±0.44*	15.88±0.90*
Ethanol extract	21	50	4.03±0.56*	12.61±0.17*
	22	100	2.91±0.10*	8.86±0.66*
	23	10	10.44±0.70*	14.46±0.12*
	24	20	8.67±0.83*	13.06±0.30*
Methanol extract	25	50	6.11±0.50*	12.01±0.14*
	26	100	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.

Preliminary phytochemical tests of the crude extracts of *M. myristica* and *X. aethiopica* revealed the presence of tannins, flavonoids and alkaloids, among other constituents contained within them. Phytochemicals such as tannins, alkaloids, flavonoids and saponins have been demonstrated to possess anthelmintic activities. Chemically, Tannins are polyphenolic compounds (Bate-smith, 1962). Some synthetic phenolic anthelmintics example niclosamide, oxyclozanide and bithionol 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 anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of the host animal (Athanasiadou et al., 2001) as glycoprotein on the cuticle of the parasite (Thompson and Geary, 1995) and cause death to it.

Alkaloids may have acted on the central nervous system of the earth worms causing paralysis (Roy, 2010). This study suggests that the effect could 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 which 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 (Wang, 2010).

Conclusions

The traditional claim that the seeds of *M. myristica* and *X. aethiopica* possess vermicidal property has been confirmed as our various extracts from the two spices displayed activity against the worm used in the study. Further studies to isolate and reveal the active compounds contained in the organic extracts of *M. myristica* and *X. aethiopica* seeds and to establish the mechanism of action are required to be done in future.

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