Research Article

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In vitro evaluation of the anthelmintic and antibacterial activities of three Nigerian medicinal plants

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ABSTRACT

The methanol extracts of the leaves of *Asystasia gangetica*, roots of *Urtica dioica* and whole plant of *Spigelia anthelmia* were screened for anthelmintic and antibacterial activities using *in vitro* models. Anthelmintic test was done using the local earthworm *Nsukkadrilus mbae*, while antibacterial activity against laboratory strains of *Eschericia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were evaluated by agar diffusion technique. Extracts of the three plants caused a significant ($P<0.05$) concentration-related reduction in time of onset of paralysis and death of *Nsukkadrilus mbae* compared to piperazine. The magnitude of anthelmintic effect was in the order *S. anthelmia* > *A. gangetica* > *U. dioica*. In the antimicrobial activity test, only *S. anthelmia* inhibited microbial growth with magnitude of susceptibility in the order *E. coli* > *S. aureus* > *B. subtilis*, and minimum inhibitory concentrations (MIC) of 11.69, 12.59 and 14.42 mg/ml respectively. In conclusion, extracts of *A. gangetica*, *U. dioica* and S. *anthelmia* have significant anthelmintic activity, while *S. anthelmia* also possesses antibacterial activity against *E. coli*, *S. aureus* and *B. subtilis*.

KEY WORDS: Antibacterial, Anthelminthic, *Asystasia gangetica*, *Nsukkadrilus mbae*, *Spigelia anthelmia*, *Urtica dioica*

INTRODUCTION

Several plants are used traditionally in Nigeria for the control of helminthiasis in man and animals; however, few have been scientifically evaluated. Such plants include *Asystasia gangetica* (L) T. Anderson, sub-species *micrantha* (Nees) Ensermu (Acanthaceae), *Spigelia anthelmia* Linn (Loganiaceae) and *Urtica dioica L.* (Urticaceae). *Asystasia gangetica* is a flowery, fast-growing, spreading, herbaceous groundcover that grows 300 – 600 mm in height. It has green, oval-shaped leaves with rounded base occurring in opposite pairs. The flower is white – cream coloured with purple markings and the fruit is a club shaped capsule, splitting from tip to base. [1] It is widely distributed from tropical Asia to Africa. [2,3] In many parts of Africa, the leaves are popularly used in the management of asthma and helminthiasis. [4,5] Pharmacological studies have shown that the leaves of this plant possess bronchospasmolytic and anti-inflammatory properties. [5,6]

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The local inhabitants of South Western Nigeria often use *Spigelia anthelmia* for expelling worms. It has demonstrated anthelmintic activity against experimental *Nippostrongylus braziliensis* infection in rats [7] and gastrointestinal nematodes in sheep. [8] Also, extract of the plant has been shown to cause neuromuscular blockade, eliciting dose-related myotonia and muscular paralysis of rapid onset, [9] as well as inhibition of egg hatching and larval development of sheep and goat gastrointestinal nematodes. [10] These findings indicate its potential usefulness in controlling helminthiasis in livestock.[7,8,10]

Urtica dioica is a common herb that is used traditionally in various regions of the world as anthelmintic, [11] and to manage hypertension and diabetes, [12] rheumatic pain, urinary tract infections, bladder stone, gout, hair loss, and mild bleeding (particularly mild menorrhagia, for nose bleeding and as haemostatic). It has been shown to possess hypoglycemic, [13] anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, cardioprotective, hypocholesterolemic, [14] bradycardial, hypotensive, diuretic [15,16] and antiaggregant effects. [17] Extracts of the plant reduced platelet aggregation in healthy and type 2 diabetic donors attributed to inhibition of the production of endogenous reactive oxygen species, Ca^{2+} mobilization and protein tyrosine phosphorylation. [18]

This study was designed to evaluate the anthelmintic activity of these plants *in vitro*, as well as their antibacterial activity.

MATERIALS AND METHODS

Drugs and solvent

Albendazole (Zentel™, Smithkline Beecham, England), Piperazine (Antepar™, SKG-Pharma Limited, Nigeria), Gentamicin (Sigma Aldrich, Germany), Methanol (Sigma Aldrich, Germany).

Animals

Local species of earthworm, *Nsukkadrilus mbae*, were collected within the campus of the University of Nigeria, Nsukka and identified by Prof. C.C. Mba of the Department of Soil Science, University of Nigeria, Nsukka. The worms were kept in moist sand placed in glass jars and allowed to acclimatize to laboratory conditions for 48 h, prior to onset of the study.

Collection and authentication of plant material

Fresh leaves of *A. gangetica*, whole plant of *S. anthelmia* and roots of *U. dioica* were collected in August from Orba, Enugu State, Nigeria. The plants were identified and authenticated at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria, where voucher specimens were deposited. The voucher herbarium specimen numbers are; *A. gangetica* (INTERCEDD/200), *S. anthelmia* (INTERCEDD/972) and *U. dioica* (INTERCEDD/973).

Preparation of extracts and phytochemical analysis

The plant materials were dried under a shade and pulverized to coarse powder using an electric blender. *A. gangetica* leaf powder (584 g), *S. anthelmia* powder (34 g) and *U. dioica* (59.97 g) root powder were separately extracted by cold maceration in methanol at room temperature (28 ± 1 ºC) for about 48 h, and concentrated *in vacuo* to yield 60.96 g (10.44 % w/w), 8.14 g (23.94 % w/w) and 11.52 g (19.21 % w/w) of methanol extracts of *A. gangetica*, *S. anthelmia* and *U. dioica* respectively. The extracts were stored at – 4 ºC, and fresh solutions prepared as needed. The extracts were subjected to phytochemical analysis using standard procedures. [19,20]

In vitro anthelmintic study

This was done as described by Hukkeri et al., 1993 and Ghosh et al., 2007, with modifications. Six earthworms were placed in a Petri dish (diameter of 9 cm) containing 50 ml of a definite concentration (12.5, 25 or 50 mg/ml) of each extract dissolved in distilled water. Control group were placed in 50 ml of distilled water in a petri dish. However, due to the limited quantity, *S. anthelmia* extract was tested only at 50 mg/ml. The time taken for paralysis was noted as when the earthworms exhibited no sensitivity/response when touched with a sharp object (pin). Time of death was recorded as when the earthworms failed to move on transfer to warm water (50ºC). [21,22] Piperazine citrate (15 mg/ml) and albendazole (10 mg/ml) were used as standard reference anthelmintics, while distilled water was the negative control.

Bacterial sensitivity test

This was done using the agar diffusion technique reported by Lovian. [23] Briefly, sterile Muller Hinton agar plates were flooded with 1×10^6 cfu/ml suspension of laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*. Each extract (0.03 ml of 100 mg/ml) was placed in the wells made on the seeded agar plates and left to diffuse for 30 min. Control tests were carried out with 10% dimethylsulfoxide (DMSO) and gentamicin (1.4 mg/ml). The plates were incubated in an inverted position at 37ºC for 24 h and antimicrobial activity was determined by measuring the inhibition zone diameter (IZD). All tests were done in triplicate and average results recorded. Only *S. anthelmia* showed activity against *E. coli*, *S. aureus*, *B. subtilis* and was thus subjected to MIC determination.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of *S. anthelmia* was evaluated against laboratory strains of *S. aureus*, *E. coli*, and *B. subtilis* using the agar well diffusion method of Lovian. [22] Briefly, sterile Muller Hinton agar plates were flooded with 1×10^6 cfu/ml suspension of microorganism. Using a sterile cork borer (7 mm diameter), 6 wells were bored on the agar and three drops of different concentrations of *S. anthelmia* extract (100, 50, 25, 12.5, 6.25 mg/ml) dissolved in 10 % DMSO placed in the appropriate well. Gentamicin (1.4 mg/ml) was used as standard antibiotic, while DMSO (10 %) served as control. The plates were allowed 30 min for diffusion and incubated in an inverted position for 24 h at 37 °C. The test was done in triplicate. After incubation, the IZD for each well was measured horizontally and vertically and the mean obtained. The minimum inhibitory concentration (MIC) was determined as intercept on the concentration axis of concentration versus mean IZD^2 plot.

RESULTS

Phytochemical constituents of the extracts

Phytochemical tests showed that *S. anthelmia* tested positive for alkaloids, saponins, steroids and terpenes. *A. gangetica* gave positive reactions for alkaloids, saponins, flavonoids, resins and terpenes, while *U. dioica* tested positive for alkaloids, tannins, resins, steroids and terpenes (Table 1).

Anthelmintic activity assay

Extracts of the three plants produced concentration-related reduction in the time of paralysis and death of the treated worms compared to control and piperazine. The order of magnitude of anthelmintic effect was *S. anthelmia* > *A. gangetica* > *U. dioica*. The extracts showed significantly (P<0.05) higher activity than piperazine, but less than albendazole. All the earthworms in the control group were alive and active after 24 h (Table 2).

Table 1: Phytochemical constituents of the extracts

Phyto-constituent	Extract		
	S. anthelmia (23.97 %)	*A. gangetica* (10.44 %)	*U. dioica* (19.21 %)
Alkaloids	++	+++	++++
Saponins	+	++	-
Flavonoids	-	++	-
Tannins	-	-	++
Steroids	+	+	+++
Terpenes	+	+	+++
Resins	-	++	++

Values in parenthesis are extractive yields calculated relative to the weight of the plant material;
++++= abundantly present; +++ = conspicuously present; ++ = moderately present; + = present; - = absent.

Table 2: Anthelmintic activity of the extracts

Treatment	Concentration (mg/ml)	Time of paralysis (min)	Time of death (min)
A. gangetica	12.5	25.20±1.59	124.13±7.77*
	25.0	18.20±0.52	105.75±7.21*
	50.0	9.36±0.49	33.67±1.79*
U. dioica	12.5	34.20±1.77	88.33±14.57*
	25.0	22.50±1.18	49.03±1.01*
	50.0	16.33±0.32	36.18±1.59*
S. anthelmia	50.0	10.20±0.19	27.27±2.55*
Albendazole	10	7.18±0.21	19.20±0.62*
Piperazine	15	55.25±2.18	148.48±7.18
Control	-	NP	ND

*n=6; Results are expressed as Mean ± SEM; * P < 0.05 compared to piperazine; NP and ND = no paralysis and no death respectively 24 h after administration.*

Antibacterial activity assay

The antibacterial sensitivity test showed that only the extract of *S. anthelmia* inhibited the growth of *E. coli*, *S. aureus* and *B. subtilis* at the concentrations tested (Table 3). The extract exhibited a concentration-dependent inhibition of microbial growth against *E. coli*, *S. aureus* and *B. subtilis* with MIC of 11.69, 12.59 and 14.42 mg/ml respectively (Table 4).

Table 3: Antibacterial sensitivity of the extracts

	Inhibition zone diameter (mm)		
	A. gangetica (100 mg/ml)	*U. dioica* (100mg/ml)	*S. anthelmia* (100mg/ml)
E.coli	0	0	7.5±0.01
S.aureus	0	0	12.5±0.01
B.subtilis	0	0	12±0.00
P.aeruginosa	0	0	0
S. typhi	0	0	0

n=3; 0 = no inhibition;

Table 4: Minimum inhibitory concentration (MIC) of *S. anthelmia*

Treatment	MIC (mg/ml)		
	E.coli	*S. aureus*	*B. subtilis*
S. anthelmia	11.69	12.59	14.42
Gentamicin	0.36	0.04	0.02

DISCUSSION

The anthelmintic activity was evaluated on adult local earthworm, *N. mbae* due to its anatomical and physiological resemblance to the intestinal roundworm parasite of man (*Ascaris lumbricoides*). Due to easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro.* [24-29]

Extracts of leaves of *A. gangetica* and roots of *U. dioica* exhibited potent anthelmintic activity, while whole plant of *S. anthelmia* exhibited antibacterial and potent anthelmintic activity. The *in vitro* anthelmintic activity of *S. anthelmia* is consistent with reports from previous *in vivo* studies which used livestock parasites in an effort to validate its ethnomedicinal use in animal helminthiasis. [7-10] However, results of this study indicate potential for its use in helminth control in humans. The results point to the potential benefit of *A. gangetica*, *S. anthelmia* and *U. dioica* in the control of helminth infestation in man or animals.

Infection by helminthes may be limited solely to the intestinal lumen or may involve a complex process with migration of the adult or immature worm through the body before localization in a particular tissue. Furthermore, it is noteworthy that almost any kind of damage to the intestinal surface/mucosa is likely to be exploited by the normal intestinal microflora,[30] resulting in a variety of infections. For instance, some helminth infestations are known to result in septicaemia due to e.g *E. coli*, secondary to the bacteria entering the blood through breaches in the gastrointestinal mucosa made by the helminth as they invade the gastrointestinal mucosa en route the systemic circulation and extraintestinal tissues. The foregoing highlights the extra advantage offered by *S. anthelmia* in gastrointestinal infective diseases.

The anthelmintic and antibacterial activities of the respective plants may be due to one or more of the phytoconstituents. The alkaloids in *S. anthelmia* may account for its antimicrobial activity as alkaloids are amongst the important groups of compounds credited with a wide range of antimicrobial activity. [31,32] In addition, alkaloids have been implicated in anthelmintic activity. [33] Phenolic compounds including gallotannins, condensed tannins and flavonoids as well as saponins and alkaloids have been implicated in pharmacological activities such as anthelmintic, antimicrobial and anti-inflammatory activities (Makkar et al., 2007).[33] Isolation and characterization of the active principles of the plants are in progress.

In conclusion, results indicate potential benefits of *A. gangetica* and *U. dioica* in the treatment of helminthiasis, while *S. anthelmia* may be beneficial in the treatment of helminthiasis and various pathogenic diseases.

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