The Effects Of Ethanolic And Boiling Water Extracts Of Root Barks And Leaves Of *Uvaria Chamae* On Some Hospital Isolates

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ABSTRACT: Root barks and leaves of Uvaria chamae used traditionally in the treatment of diarrhea, cough and urinary tract infections (UTI), were extracted by maceration in ethanol and boiling water. The extracts were tested by agar diffusion method, for activity against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis* isolated from hospital patients. The agar diffusion method was used to determine the extracts' antibacterial potentials at different concentrations of 50 mg ml^{-1}, 100 mg ml^{-1}, 150 mg ml^{-1}, 200 mg ml^{-1} and 250 mg ml^{-1}. The results indicated that the extracts did not inhibit the growth of *B. subtilis* while the other isolates were inhibited to varying degrees. The largest zone of growth inhibition for the ethanol root bark extract was recorded with *S. aureus* with a zone diameter of 16.6 mm at 250 mg ml^{-1} while for the ethanol leave extract the largest zone of inhibition was 15.1 mm on *E. coli* at 250 mg ml^{-1} concentration. *Sa. typhi* was not inhibited by the 100 mg ml^{-1} and 50 mg ml^{-1} concentration of the ethanol extracts while *P. aeruginosa* was not inhibited by the 100 and 50 mg ml-1 concentrations of the leave extracts. The water extracts did not inhibit the growth of the test isolates at 150 – 50 mg ml^{-1} concentrations. However, the ethanol extracts were more inhibitory on the isolates than the water extracts. The minimum inhibitory concentration (MIC) of the ethanol root bark extracts on *E. coli*, *Staph aureus*, *P. aeruginosa* and *Sa. typhi* were 72.44 mg ml^{-1}, 50.19 mg ml^{-1}, 45.71 mg ml^{-1} and 131.83 mg ml^{-1} respectively while the MIC of the ethanol leaves extracts were 61.66 mg ml^{-1}, 79.43 mg ml^{-1}, 125.89 mg ml^{-1}, and 105.93 mg ml^{-1} respectively. The MIC of the water extracts ranged from 120.23 mg ml^{-1} observed on *S. aureus* to 131.83 mg ml^{-1} observed on *E. coli*. The observed antibacterial effects were believed to be due to the presence of alkaloids, tannins and flavonoids identified in the extracts. The results apparently justify their use in traditional medicine especially in the treatment of UTI and diarrhea, and are of significance in the health care delivery system. [The Journal of American Science. 2007;3(3):68-73]. (ISSN: 1545-1003).

Key words: *Uvaria chamea*; Root bark; Leaves; Agar diffusion; Inhibition; MIC; Ethanolic extracts

INTRODUCTION

Uvaria chamae belongs to the family Annonaceae (Irvine, 1961). It is a small tree that grows to about 4.5m high. It is commonly found in the savanna and rain forest regions of Nigeria and other African countries. It is called "M*mimi ohia*", "*Kas kaifi*" and "*Akisan*" amongst the Ibos, Hausas and Yorubas respectively (Adetunji, 1999). The fruits are yellow when ripe and have a sweet pulp which is widely eaten. The fruit carpels are in finger-like clusters. All parts of the plant are fragrant.

The root barks, stem barks and leaves have a wide spread medicinal use. In Nigeria a decoction of the stem is used on the treatment of diarrhea (Igoli *et al.*, 2005). Personal interaction with traditional medicinal practitioners indicated that they use the various parts of the plant in the treatment of cough, various stomach problems and urinary tract infections. They also apply the saps from the roots, stem and leaves to wounds and sores for quick and proper healing of wounds.

The use of this plant and its extracts in treatment of infections is very popular amongst the traditional medical practitioners of South Eastern Nigeria, even when the acclaimed antimicrobial effects have not been established. This work is therefore undertaken to authenticate the plant's antimicrobial potentials.

MATERIALS AND METHODS

Plant collection and identification

Fresh root barks and leaves of *Uvaria chamae* were collected from Nekede, Owerri West Local Government Area of Imo State, Nigeria. The plant was identified by Dr. I. I. Ibeawuchi of the Department of Crop Science Technology, Federal University of Technology, Owerri. Specimen vouchers were kept in the Department with number UC.CCO.003.

Sample preparation and extraction procedure:

The fresh root barks and leaves were air dried for about one week and separately ground into fine powder using a mechanical grinder. 20 g of the ground root barks and leaves were separately weighed into 250 ml of ethanol (95%) in conical flasks. These were covered, shaken every 30 mins. for 6 hr. and then allowed to stand for about 48 hr. for extraction. The solutions were subsequently shaken and filtered using Whatman filter paper. The filtrates were evaporated to dryness using a rotary evaporator (model type 349/2, Corning Ltd). Yields of 11.85% and 12.25% were obtained for the root barks and leaves in relation to the powdered materials. These were stored at 15^0C in amber coloured bottles.

Also for boiling water extraction method, 20 g of the ground root barks and leaves were separately weighed into 250 ml of distilled water. This was heated to 100^0C and maintained at this temperature for 15 min. The solutions were subsequently shaken and filtered using Whatman filter paper. The filtrates were evaporated to dryness. Yields of 5.6% and 5.35% were obtained for the leaves and root barks respectively. They were also stored at 15^0C in amber coloured bottles.

Preparation of crude extracts:

The methods of Akujobi *et al.* (2004) and Esimone *et al.* (1998) were adopted for the study. The crude extracts obtained were diluted with 20% dimethylsulphoxide (DMSO) and distilled water for the ethanol and water extracts respectively to obtain 250 mg ml^{-1}, 200 mg ml^{-1}, 150 mg ml^{-1}, 100 mg ml^{-1} and 50 mg ml^{-1} concentrations. These were stored at 15^0C in amber coloured bottles until required.

Test microorganisms and their sources:

The isolates *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Federal Medical Centre, Owerri while *Bacillus subtilis* was isolated from fermented African Oilbean seeds ("*Ugba*"). They were re-isolated, re-identified and the pure cultures subcultured on Nutrient agar slants. They were stored at 4^0C until required for the study.

Evaluation of antimicrobial activity:

The agar diffusion method as described by Esimone *et al.* (1998) and Osadebe and Ukwueze (2004) was adopted for the study. Nutrient broth cultures of the test isolates containing 1 x 10^7 cells ml^{-1} of organisms was prepared. 0.1 ml of the culture was introduced into a sterile petridish and 15 ml of molten Nutrient agar poured into the petridish. The content was thoroughly mixed and allowed to solidify. Three holes measuring 5.0 mm each in diameter were made in the plates using a sterile cork borer. Equal volumes of the plant extracts were transferred into the holes using a Pasteur pipette.

The plates were allowed to stand for one hour for prediffusion of the extracts to occur (Esimone et al., 1998). These were then incubated at 37^0C for 24 hr.

At the end of incubation the plates were collected and the zones of inhibition that developed were measured. The averages of the zones of inhibition were calculated. The minimum inhibitory concentrations (MIC) of the extracts on the isolates were calculated by plotting the logarithm of the concentrations against the square of zone of inhibition. The antilogarithm of the value at the intercept on the logarithm of concentration axis gave the MIC values (Osadebe and Ukwueze, 2004, Esimone *et al.*, 1998).

Two petridishes containing a particular microorganism were used for each concentration of the plant extracts.

Preliminary phytochemical screening of plant extracts:

Preliminary phytochemical analysis was carried out according to the methods described by Trease and Evans (1989). This was conducted for determination of the presence of saponins, tannins, alkaloids, flavonoids, cardiac glycosides and cyanogenic glycosides.

Statistical analysis:

The data obtained in the study were statistically analysed using Analysis of Variance (ANOVA). The means were separated using Fisher's Least Significant Difference (LSD).

RESULTS

Table 1 shows the results of the antimicrobial screening of the crude ethanolic extract of the root bark of *Uvaria chamae*. The results show that the extract did not inhibit the growth of *B. subtilis* at any of the concentrations administered. However, the growth of *E. coli*, *S. aureus* and *P. aeruginosa* were inhibited to varying degrees by the extract. The largest zone of inhibition was produced by the 250 mg ml^{-1} on *Staph aureus* with a zone diameter of 16.6 mm. The lowest zone of inhibition was produced by the 150 mg ml^{-1} concentration on *Sa. typhi* which gave a zone of growth inhibition measuring 5.2 mm.

Table 2 shows the results of the antibacterial effects of the ethanolic leave extracts on the test isolates. The largest zone of growth inhibition was produced by the 250 mg ml^{-1} concentration on *E. coli*. This gave a zone of growth inhibition of 15.1 mm. The growth of *B. subtilis* was not inhibited by any of the concentrations applied. In general the lower the concentration administered the lower the zone of growth inhibition. The 50 mg ml^{-1} concentration of the extract did not inhibit the growth of any of the isolates except *E. coli* which had a zone of growth inhibition measuring 6.2 mm.

Tables 3 and 4 show the results of the antibacterial effects of the water extracts of the root bark and leave extracts respectively. The largest zone of growth inhibition was observed on *E. coli* by the 250 mg ml^{-1} concentration of the root bark extract while the lowest zone of growth inhibition was observed on *S. typhi* by the 100 mg ml^{-1} concentration of the leave extract. None of the water extracts inhibited the growth of *B. subtilis*.

Table 5 shows the minimum inhibitory concentration (MIC) of the root bark and leave extracts of *U. chamae* on the test isolates. For the ethanolic root bark extracts *S. aureus* had the lowest MIC of 50.19 mg ml^{-1} while for the leave (ethanolic) extracts the lowest MIC was recorded with *E. coli* with a value of 61.66 mg ml^{-1}. The MIC of the boiling water extracts ranged from 120.23 mg ml^{-1} to 131.83 mg ml^{-1}.

Table 6 shows the results of the preliminary phytochemical analysis of the extracts of the root bark and leaves of *U. chamae*. No saponins were identified in the extracts while tannins, flavonoids, alkaloids, cardiac glycosides and cyanogenic glycosides were present.

Table 1: *Antimicrobial screening of the crude ethanolic extract of the root bark of Uvaria chamae

Concentration of extract (mg/ml)	Zone of inhibition (mm)				
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*Sa. typhi*
250	15.8^{a}	16.6^{a}	15.1^{a}	NI	10.0^{b}
200	13.7^{a}	15.7^{b}	13.8^{a}	NI	8.4^{c}
150	11.2^{a}	13.0^{b}	$12.9^{a,b}$	NI	5.2^{c}
100	8.7^{a}	10.2^{b}	$9.9^{a,b}$	NI	NI
50	5.4^{a}	7.1^{b}	5.3^{a}	NI	NI

* Values are means of triplicate readings

NI = No inhibition

a,b,c Values on the same row with different superscripts are significantly different (P = 0.05)

Table 2: *Antimicrobial screening of the crude ethanolic extract of the leaves of *Uvaria chamae*

Concentration of extract (mg/ml)	Zone of inhibition (mm)				
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*Sa. typhi*
250	15.1^{a}	13.3^{b}	9.4^{c}	NI	12.0^{b}
200	14.3^{a}	11.7^{b}	8.3^{c}	NI	10.4^{b}
150	11.7^{a}	9.9^{b}	5.4^{c}	NI	8.1^{b}
100	9.6^{a}	7.4^{b}	NI	NI	NI
50	6.2	NI	NI	NI	NI

* Values are means of triplicate readings
NI = No inhibition
a,b,c Values on the same row with different superscripts are significantly different (P = 0.05)

Table 3: *Antimicrobial screening of the crude boiling water extract of the root bark of *Uvaria chamae*

Concentration of extract (mg/ml)	Zone of inhibition (mm)				
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*Sa. typhi*
250	NI	8.7^{a}	NI	NI	8.1^{a}
200	NI	7.2^{a}	NI	NI	6.7^{a}
150	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
50	NI	NI	NI	NI	NI

* Values are means of triplicate readings
NI = No inhibition
a,b,c Values on the same row with different superscripts are significantly different (P = 0.05)

Table 4: *Antimicrobial screening of the crude boiling water extract of the leaves of *Uvaria chamae*

Concentration of extract (mg/ml)	Zone of inhibition (mm)				
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*Sa. typhi*
250	8.5^{a}	8.8^{a}	7.2^{b}	NI	NI
200	6.8^{a}	7.1^{a}	NI	NI	NI
150	NI	NI	NI	NI	NI
100	NI	NI	NI	NI	NI
50	NI	NI	NI	NI	NI

* Values are means of triplicate readings
NI = No inhibition
a,b,c Values on the same row with different superscripts are significantly different (P = 0.05)

Table 5: Minimum inhibitory concentrations of the ethanolic and hot water extracts of the root bark and leaves of *Uvaria chamae* on isolates

Plant extracts	Minimum inhibitory concentration (mg ml^{-1})				
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*Sa. typhi*
Root bark (ethanol)	$^{1}72.44^{a}$	$^{1}50.19^{b}$	$^{1}45.71^{b}$	NIL	$^{1}131.83^{c}$
Root bark (boiling water)	NIL	$^{2}120.23^{a}$	$^{2}123.03^{a}$	NIL	NIL
Leaves (ethanol)	$^{1}61.66^{a}$	$^{1}79.43^{a}$	$^{2}125.89^{b}$	NIL	$^{2}105.93^{c}$
Leaves (boiling water)	$^{2}131.83^{a}$	$^{2}127.35^{a}$	NIL	NIL	NIL

a,b,c Values with different superscripts on same row are significantly different ($P \leq 0.05$)
1,2,3 Values with different superscripts on same column are significantly different ($P \leq 0.05$)

Table 6: Preliminary phytochemical screening of the ethanolic extracts of the root bark and leaves of *Uvaria chamae*

Plant part	Saponins	Tannins	Flavonoids	Alkanoids	Cardiac glycosides	Cyanogenic glycosides
Root bark	-	+	+	+	+	+
Leaves	-	+	+	+	+	+

\+ Present
\- Absent

DISCUSSION

From the results obtained in this study it could be observed that the ethanolic and water extracts of the plant inhibited the growth of majority of the test isolates. This is an indication that the extracts posses substances that can inhibit the growth of some microorganisms. However, the observed inhibitory effects were more with the ethanolic extracts of the plant. Thus, ethanol is a better extraction solvent than water for the extraction of plant active principles. This is agreement with the statement of Obi and Onuoha (2000) who stated that ethanol was a better extraction solvent than water. The practitioners may have observed these in the past that they almost always recommend the use of ethanol (local gin) for the maceration and extraction of plants for native medicine. The lower inhibitory effects of the boiling water extracts could be that water did not extract much of the active principles or some of the active principles may have been lost during boiling. Some workers have shown that some plant active principles are volatile and are usually lost during boiling. *Ocimum gratissimum* leaves contain the substance thymol which has been found to possess antibacterial activities, but is volatile and usually lost during boiling of the leaves (Soforowa, 1982, Soforowa, 1970). Also the results indicated that in general the ethanolic root bark extracts had greater inhibitory effects on the isolates than the leave extracts. This is most evident on *S. aureus* and *P. aeruginosa*. it could then be concluded that the root barks contain more of the active principles than the leaves. This is of importance to the traditional medical practitioners. Thus, they could use more of the root bark extracts for treatment of infections.

Various other workers have also shown that extracts from some plants posses antimicrobial properties (Ajao *et al.*, 1985, Esimone *et al.*, 1998, Nweze *et al.*, 2004, Osadebe and Ukwueze, 2004). However, the inability of the extracts to inhibit the growth of *Bacillus subtilis* indicates that the organisms possesses a mechanism for detoxifying the active principles in the extracts or other mechanisms which include exclusion of the substance from the cell and modification of the target site of the substance. Some bacteria posses mechanisms for converting substances toxic to it into non-toxic substances. *Staph aureus* produces the enzyme penicillinase which converts penicillin to penicillinic acid which could not inhibit its growth (Singleton, 1999).

The observed antibacterial properties could be due to the presence of tannins, alkaloids and flavonoids which have been shown to posses antibacterial properties (Cowan, 1999, Draughon, 2004).

The low minimum inhibitory concentration (MIC) exhibited by the extracts of the plant's root bark on *Staph aureus* and *P. aeruginosa*, and the leave extracts on *E. coli* is of great significance especially in this region where the cost of obtaining medicare is high. Thus they can be used as alternatives to orthodox antibiotics as they are much cheaper, and also in cases of drug resistance by these microorganisms. These organisms frequently develop resistance to orthodox antibiotics (Signleton, 1999).

The observed antibacterial effects on the isolates corroborates their use in traditional medicine. Etahnolic and water preparations of the root bark, stem bark and leaves of the plant are used by traditional medical practitioners in the treatment of cough, stomach problems such as diarrhea, and urinary tract infections. The sap from the roots, stem and leaves are also applied to wounds and sores to promote rapid healing (Igoli *et al.*, 2005). *Staph aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds (Brande, 1982) while *E. coli* is responsible for a number of food related illness that manifest themselves in the form of diarrhea (Adams and Moss, 1999). Also *Staph aureus* is implicated in cases of urinary tract infections (Brande, 1982).

More work is however, recommended to ascertain their toxicity effects on humans and the dose levels that can be administered.

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