Preliminary Phytochemical and Antimicrobial Screening of the Leave Extracts of *Uvaria Chamae*

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ABSTRACT

Phytochemical screening and antimicrobial activities of aqueous and ethanol leave extract of *U. chamae*, were studied using paper disc diffusion method against *Streptococcus pyogen, Escherichia coli* and *Salmonella thypi.* The results of the antimicrobial studies indicated that the extracts inhibited the growth of one or more tested pathogens. The ethanolic extract showed a broad spectrum of antimicrobial activity. Phytochemical investigation revealed the presence of tannins, alkaloids, glycosides, flavonoids, carbohydrates and terpenes. Anthraquinone and glycoside were not present. Inhibition zone by the extracts ranges from 6.0 mm to 29 mm. The Minimum Inhibitory concentration (MIC) ranges from 100 mg/mL to 6.25 mg/mL. *Uvaria chamae* leave may be able to produce antimicrobial agents in drug delivery.

Keywords: Medicinal Plant; Antimicrobial Activity; Phytochemcials; Uvaria, Chamae

INTRODUCTION

Despite the great advances witnessed in modern medicine in recent decade, plants still make an important contribution to health care (Poojary *et al.,* 2016). Of the 300,000 plant species acclaimed worldwide only about 5% have been investigated scientifically for their medicinal purposes (Adeyanju *et al*., 2011a). Researcher has reported that developing countries rely mainly on plants for the treatment of their prevailing ailments especially in rural areas where hospital are not accessible (Adeyanju *et al*., 2011a).

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Adeyanju *et al*., 2011b). The active principle of many drugs found in plants are secondary metabolites, therefore basic phytochemical investigation of plant extracts for major phytoconstituents is also vital. *Uvaria chamae* belongs to the family *Annonaceae* and it is a scandant shrubs or small tree of about 4.5 m high. The fruit carpel's are in finger-like clusters, the shape giving rise to many vernacular names translated as "bush banana" or the like implying wildness. The Sierra Leone Krio name lingers' and 'finger-root' for the roots are also from the fruit shape. The fruits are yellow when ripe and have a sweet pulp (Oluremi *et al*., 2010).

The plant has the common name bush banana. Local ethnic groups in Nigeria such as the Igla named it as Ayilokok, then in Yoruba it is named Okooja, and in Hausa it is Kaskaifi (Oluremi *et al.,* 2010). In Ghana severe abdominal pain is treated by a root infusion with native pepper in gin and the root with Guinea grains is used in application to the fontanelle for cerebral diseases. Among the Fulani people of Senegal, the root has a reputation as the "medicine of Riches" and is taken is taken conditions of lassitude and senescence. It is also considered to be a woman's medicine used for amenorrhea and to prevent miscarriage and in Togo a root-decoction is given for pains of childbirth. It is used for the treatment of jaundice in Ivory-coast. In Sierra Leone, the root is reputed for having purgative and febrifugal properties (Oluremi, *et al.,* 2010). In Nigeria however, the root – back is used for the treatment of bronchitis, and gonorrhea in addition to its being used internally for catarrhal inflammation of mucous membranes (Oluremi, *et al*., 2010). In the present study, the water and ethanolic

extracts of *U.chamae* leaves were screened for phytochemical constituents and antimicrobial activities against gram-positive and gram-negative microorganism.

MATERIALS AND METHODS

Plant used for this study was collected from Jos, Plateau State, Nigeria. The plant materials were identified by the Biological Science Department, University of Jos and a Voucher specimen No. 46BA was deposited in the research laboratory of chemistry Department, University of Jos, Nigeria.

Preparation of Plants Extracts

The plant material was dried at room temperature and then powdered using a grinder. The powdered sample (100 g) was subjected to soxhlet extraction using 300 mL of each of the solvents (water and ethanol). The resulting extracts were concentrated on a hot water bath and kept for further investigation.

Phytochemical Screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods. The extracts were screened for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, anthraquinones and terpenes.

Test Organisms

Standard strain of *S.pyogen*, *E. Coli* and *Salmonella* typhi were obtained from the department of medical microbiology, university of Jos teaching hospital, Jos, Nigeria.

Antimicrobial Screening Test

The paper disc diffusion method was used to determine the antimicrobial activity of the extract from *Uvaria chamae* using standard procedures (Adeyanju *et al*., 2011b; Bauer *et al.,* 1996). Solutions of the extract of varying concentrations, ranging from 200 to 500 mg/mL were prepared. Nutrient agar was prepared, sterilized and used as the growth medium for the microorganisms. 20 mL of sterilized medium was poured into each sterilized petri-dish covered and allowed to solidify. The Mueller-Hinton sensitivity agar plate was then seeded with the test microorganisms by the spread plate technique, and was left for about 30 minutes to dry. The sterilized paper discs were soaked in the prepared solution of the extracts with varying concentration and were dried at 50 °C. The dried paper discs were then planted on the nutrient Agar seeded with the test microorganisms. The plates were incubated at 37 °C for 24 h and then inspected for zones of inhibition of growth. The zones of inhibition were measured and recorded in millimeters. A control experiment was also set up using pure DMSO for each tested organisms.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the ethanolic and aqueous extract of *U. Chamae* which showed the highest antibacterial activity in the disc diffusion assay were determined based on broth dilution technique with a standard method (Krivoshan *et al.,* 1989). The inocula of microorganisms were prepared from 12 h broth cultures. Stock solutions of extracts (200 mg/mL) were diluted with nutrient broth cultures. Stock solutions of extracts (200 mg/mL) were diluted with nutrient broth in serial tenfold dilutions using nutrient broth to make dilution ranging from 200 mg/mL to 0.2 mg/mL and inoculated with 0.2 mL of the test microorganisms. The inoculated tubes were then incubated at 37 ^{0}C for 24 hours and were inspected for non-turbidity. The least concentration of the extract which prevented visible growth was noted and recorded as minimum inhibitory concentration (MIC).

RESULTS

The results of the phytochemical screening, antimicrobial tests and minimum inhibitory concentrations for the water and ethanolic extracts are presented in Tables 1 to 5.

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Table 1: Phytochemical screening of *U. chamae* water and ethanol leaf extracts.

Phytochemicals	**Water extract**	**Ethanol extract**
Tannins	+	++
Carbohydrate	++	++
Alkaloids	++	+++
Glycoside	-	-
Flavonoid	+	+++
Terpenes	+	++
Saponins	+	-
Anthraquinones	-	-

+++= High concentration; ++ = moderate concentration, + = low concentration; - = absent.

Table 2: Inhibition zone of *U. chamae* water extract against the tested microorganisms.

Zone of Inhibition (mm)			
Extract/drug (mg/mL)	*Streptococcus pyogen*	*E. coli*	*Salmonella typhi*
200	-	10±0.02	-
300	4.0±0.01	10.6±0.01	13±0.00
400	7.0±0.00`	11±0.00	13.4±0.01
500	9.0±0.02	13±0.01	15.3±0.03
250 GTC	20±0.02	22±0.00	21±0.00

GTC = Gentamicin, Results are means of triplicate determination ± standard deviation. - = No inhibition

Table 3: Inhibition zone of *U.chamae* ethanol extract against the tested microorganisms.

	Zone of Inhibition (mm)		
Extract/drug (mg/mL)	*Streptococcus pyogen*	*E. coli*	*Salmonella typhi*
200	4±0.00	15±0.01	16±0.01
300	4±0.00	16±0.00	18±0.02
400	7±0.01	19±0.02	20±0.02
500	10±0.01	20±0.00	21±0.02
250 GTC	22±0.00	24±0.01	25±0.01

GTC = Gentamicin, Results are means of triplicate determination ± standard deviation.

Table 4: Minimum inhibitory concentration (MIC) of *U. chamae* (water extract) against the tested microorganisms .

Extract/drug (mg/mL)	***Concentration mg/mL***						
	100	50	25	12.5	6.25	3.12	1.56
Streptococcus pyogen	+	+	0+	-	-	-	-
Escherichia coli	+	+	0+	-	-	-	-
Salmonella typhi	+	0+	-	-	-	-	-

+ = inhibition, 0+ = minimum inhibition, - = no inhibition/turbidity

Table 5: Minimum inhibitory concentration (MIC) of *U. Chamae* (ethanol extract) against the tested microorganisms

Extract/drug (nig/mL)	***Concentration mg/mL***						
	100	50	25	12.5	6.25	3.12	1.56
Streptococcus pyogen	+	+	+	+	0+	-	-
Escherichia coli	+	+	+	+	0+	-	-
Salmonella typhi	+	+	+	0+	-	-	-

+ = inhibition, 0+ = minimum inhibition, - = no inhibition/turbidity

DISCUSSION

The phytochemical screening (Table 1) revealed presence of tannins, alkaloids, glycoside, flavonoid terpenes. Glycoside and anthranquenone were not present. The chemical constituents present in the extract have many therapeutic values. Tannins are metabolites well known for their antimicrobial properties (Adeyanju *et al*., 2011b). Flavonoids have both antifungal and antibacterial activities. They possess anti-inflamatory activity (Ogundaini, 2005) Flavonoids, terpenes and steroids are known to have antimicrobial and bactericidal properties against several pathogens (Usman *et al*., 2007; Hassan *et al*., 2004). In the antimicrobial studies, the majority of the organisms were more sensitive to the ethanol leaf extract of *U. Chamae*t. According to Adeyanju *et al.(*2011a), the anti-bacterial activity and inhibitory effect of plant extracts may be due to the presence of secondary metabolites.The results of the sensitivity test, (zone of inhibition) table 2 and table 3 demonstrated that ethanol extract has higher inhibitory effect on all the microorganisms tested than the water extract. For example the zone of inhibition at the least concentration 200 mg/mL of ethanol extract against E. Coli was 15.0±00 mm while that of water extract against E. Coli at the same concentration of 200mg/ml was 10.00±0.02 mm. The lager zones of inhibition exhibited by the ethanol extract of *U.chamae* may be due to the presence of variety of active compounds in the plant such as Flavonoid, Tannins, Alkaloid, Saponins etc as described by Abo *et al.* (2000).The ethanol extract of *U.chamae* was active against the entire micro*organisms. S. Pyogen, E. coli and S. typ*hi. It has MIC value of 6.25 mg/mL and *S. Pyogen* and 12.5 mg/mL *S. Typhii*. The ethanol extract was more potent and active even at low concentrations against the tested microorganisms than the water extract.(Table 4 and 5).This may be due to the higher concentration of the phytochemicals in the ethanol extract. These findings are consistent with Etuk *et. al. (2009; who* reported that the bark extract of Plant had antidiarrhae activity in vivo. Previous reports have demonstrated the antidiarrheae activity of tannins (Murkherjee et. al., 1995), flavonoids (*Galvez et al*., 1993; and saponins (Otshudi *et al*., 2000).

CONCLUSION

The result of the experiment showed that the *U. Chamae* leaves may have some valuable antimicrobial activities against gram positive and gram negative microorganisms. This property tends to support the traditional medicinal stage in the treatment of bacterial infections. The result of the study justified the use of the plant in the treatment of diseases of microbial herbal medicine.

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