African Journal of Microbiology Research Vol. 4 (13), pp. 1391-1394, 4 July, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Comparative assessment of antibacterial activity of uvaria chamae parts

B. B. Oluremi, M. O. Osungunna and O. O. Omafuma

Department of Pharmaceutical Microbiology, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

Accepted 15 October, 2009

Methanolic extracts of the root, stem and leaf of *Uvaria chamae* were evaluated for their antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA), Staphylococcus aureus, *Escherichia coli*, *Klebsiella* spp, *Proteus* spp, and typed strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC27853 using standard agar diffusion method at 50, 100, 150, 200 and 250 mg/ml. Preliminary phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides and cyanogenic glycosides. In general, the stem bark extract inhibited the growth of all the tested organisms with leaf extract showing the least antibacterial activity. The MIC of methanolic stem extract on *E. coli,* MRSA, *Klebsiella* spp, *Proteus* spp, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 were 5.62, 12.59, 200, 35.48, 28.18, 5.62 and 19.95 mg/ml respectively while that of root extract with the exclusion of *Klebsiella* spp were 31.62, 10.0, 3.55 2.82, 12.59 and 39.81 mg/ml respectively. Methanolic leaf extract was found to have MIC 200, 19.95, 250, 250, 250 and 200 mg/ml respectively with no MIC value against *P. aeruginosa*.

Key words: Methicillin-resistant, phytochemical screening, alkaloids, cyanogenic, flavonoids.

INTRODUCTION

The search for more potent and efficacious antimicrobial agents will continue except that the menace of development of antibiotic resistance by microorganisms can be eliminated. But since many microorganisms have the intrinsic ability to resist the action of antibiotics, it therefore follows that resistance can only be curtailed.

One of the sources by which 'lead' compounds can be obtained is through screening plants for their bioactive molecules. In fact, plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines (Nair et al., 2005).

Although the use of plants as source of medicine in treating disease is an ancient practice but in recent times, attention has been reverted back to plants as sources of therapeutic agents due to obvious reasons as reduced cost, relative lower incidence of adverse reactions compared to modern conventional pharmaceuticals (Karachi, 2006) and ready availability among others. A large number of plants in different location around the world have been extracted and semi-purified to investigate individually their antimicrobial activity (Draughon, 2004). Nevertheless, the increased incidence of diseases for which there is yet no known cure calls for more screening programmes. One of such screened plants is *Uvaria chamae,* screened for its antimicrobial activity.

U. chamae belongs to the family Annonaceae and it is a scandant shrub 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 'fingers' and 'finger-root' for the roots are also from the fruit shape. The fruits are yellow when ripe and have a sweet pulp.

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 Fulai people of Senegal, the root has a reputation as the "medicine of Riches" and is taken for 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

*Corresponding author. E-mail: yomosun2002@yahoo.co.uk.

Table 1. Extraction yield of *Uvaria chamae*.

Plant part	Solvent used	Weight of crude extract (g)	Percentage yield
Root	Methanol	2.5	12.61
Stem bark	Methanol	2.12	10.6
Leaf	Methanol	1.58	7.91

the treatment of jaundice in Ivory- coast. In Sierra Leone, the root is reputed for having purgative and febrifugal properties.

In Nigeria however, the root-bark is used for the treatment of bronchitis, and gonorrhea in addition to its being used internally for catarrhal inflammation of mucous membranes.

MATERIALS AND METHOD

Plants collection and identification

Fresh root, stem bark and leaves of *U. chamae* were collected from Eruwa, Oyo State, Nigeria. The plant parts were identified and authenticated by Forestry Research Institute of Nigeria (FRIN), Ibadan with number 107901.

Sample preparation and extraction

The fresh root, stem bark and leaves were sun-dried for about 3 weeks and separately grounded into fine powder using a mechanical grinder.

The method of Dupont et al. (2005) was adopted for extraction with little modification.

Briefly, 20 g portions of the powdered plant parts were separately weighed and soaked in 100 ml methanol at ambient room temperature for 72 h under regular shaking condition. The extract was then filtered using Whatman filter paper No 1. The filtrates were evaporated to dryness at 35°C using evaporating dish and percentage yield calculated.

Phytochemical screening

The following phytochemical tests were carried out on the extracts.

Test for alkaloids was performed using Wagner's and Dragendoff's reagents (Sofowora, 2008). 0.5 g of the extract was added to 5 ml of 1% aqueous hydrochloric acid on a steam bath. This was filtered and 1 ml portion treated with a few drops of Draggendoff's reagent and another 1 ml portion similarly treated with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

The blood haemolysis test was used to test for Saponnins (Sofowora, 2008).

The test for anthraquinones was done by shaking 0.5 g of the extract with 5 ml chloroform for 5 min. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink, violet or red color in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones. Test for tannins was done using ferric chloride test. A deep green coloration showed the presence of tannins (Evans, 2009).

The Keller-Kiliani test was used to test for the presence of cardenolides. 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underplayed with 1 ml of concentrated sulfuric acid, H_2SO_4. A brown ring obtained at the interphase indicated the presence of a deoxysugar typical of cardenolides.

About 1 g of the extract was dissolved in 5 ml of 2% potassium hydroxide and then filtered. Formation of precipitate on addition of 10% hydrochloric acid to the filtrate confirms the presence of flavonoids.

Antimicrobial screening

The pure cultures of the organisms used were collected from Medical Pathology and Microbiology Department of Olabisi Onabanjo University Teaching Hospital, Sagamu. These include: *Escherichia coli,* Methicillin-resistant *Staphylococcus aureus, Proteus* spp, *Klebsiella* spp, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. All were collected in slants in McCartney bottles containing Mueller - Hinton agar and appropriately stored until needed.

A known weight of each extract was reconstituted in sterile distilled water to give the desired concentration of extract in milligram (mg). The bacterial suspensions were cultured in peptone water for 24 h. 0.2 ml of 10^6 cfu/ml was used as inoculum size for all organisms. Each inoculum was mixed with 20 ml of Mueller-Hinton agar in Petri dishes. Wells (6 mm in diameter) were punched in the agar medium using sterile glass cork borer before being filled with 0.1 ml of plant extracts. On each plate, gentamycin was used as positive control while methanol was used as negative control. The plates were incubated for 24 h at 37°C and the diameter of inhibition zones measured. Each experiment was done in duplicates.

Determination of minimum inhibitory concentration (MIC)

This was determined by agar dilution method. Varying concentrations of the extract were prepared by serial dilution and allowed to set in plates. Each plate containing different concentration was streaked with test organism and incubated at 37°C for 24 h. The minimum concentration inhibiting the growth of test organism was recorded as the MIC.

RESULTS AND DISCUSSION

Table 1 shows the yields of the plant's parts used with root giving the highest yield of 12.61% and leaf the least value, 7.91%w/w.

Preliminary phytochemical screening reveals the presence of tannins, alkaloids, cardiac glycosides, cyanogenic glycosides as well as flavonoids. Moreover, the crude extracts of all the plant parts screened displayed activity, to varying degree, against both the Gram-positive

Table 2. Effect of *Uvaria chamae* methanolic extracts on test organisms at different concentrations.

Plant part	Concentration of crude extract (%w/v) (mg/ml)	Inhibition zone diameter (mm) against						
		E. coli	*S. aureus ATCC* 25923	*P. aeruginosa* ATCC 27853	*MRSA*	*Proteus* spp	*Klebsiella* spp	*E. coli ATCC* 25922
Root	50	7.5	10.0	6.5	16.5	12.0	-	11.5
	100	11.5	13.0	7.0	18.0	12.5	-	12.0
	150	12.5	13.0	7.5	18.0	13.0	-	13.0
	200	13.0	14.5	7.5	19.0	14.5	-	13.0
	250	15.0	15.5	9.0	21.0	15.5	-	14.5
Stem bark	50	11.5	12.0	7.0	12.5	7.0	-	10.0
	100	13.0	13.5	7.0	15.0	10.0	-	11.0
	150	14.5	15.0	7.0	17.0	11.0	-	13.0
	200	14.5	15.5	7.5	19.0	12.0	11.5	16.0
	250	15.5	16.0	8.5	20.0	13.0	14.5	18.0
Leaf	50	-	-	-	9.0	-	-	-
	100	-	-	-	13.5	-	-	-
	150	-	-	-	14.0	-	-	-
	200	10.0	9.0	-	15.0	-	-	-
	250	11.0	11.0	-	15.5	10.5	8.0	10.5
Gentamycin	0.25	12.0	11.5	8.0	-	-	8.0	-
	0.5	13.0	13.5	9.0	8.0	-	10.0	9.0
	1.0	13.5	16.5	14.5	11.5	9.0	11.5	11.5
	2.0	15.0	19.5	17.5	13.5	12.0	13.0	13.0
	4.0	16.5	22.5	20.5	15.0	13.5	14.5	14.5

and Gram-negative bacteria used for the study as seen in Table 2.

In general, the stem bark extract displayed highest degree of antibacterial activity, being active against all the organisms used although *Klebsiella* spp displayed lack of sensitivity at lower concentration of between 50 and 150 mg/ml.

Root extract had no activity against *Klebsiella* spp at the concentrations used for the study while other organisms were susceptible to the activity of the root extract.

The leaf extract displayed the least activity as it was only active on Methicillin-Resistant *S. Aureus* (MRSA) at all the concentrations used for the study while other organisms were not sensitive to the activity of the leaf extract as shown in the Table. *P. aeruginosa* ATCC 27853 was not susceptible to the activity of the leaf extract at all the concentrations studied.

The MIC of the methanolic root extract on *E. coli,* MRSA, *Proteus* spp, *E. coli* ATCC 25922*, S. aureus ATCC 25923 and P. aeruginosa ATCC 27853* were31.62, 10.0, 3.55, 2.82, 12.59 and 39.81 mg/ml respectively. MIC of the stem bark extract on *E. coli,* MRSA, *Klebsiella* spp*, Proteus* spp, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 were 5.62,

12.59, 200, 35.48, 28.18, 5.62 and 19.95 mg/ml respectively. The values of the MIC for the leaf extract against *E. coli,* MRSA, *Klebsiella* spp, *Proteus* spp *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were 200, 19.95, 250, 250, 250 m and 200 mg/ml respectively.

However, the fact that all the extracts from leaf, stem bark and root showed activity against MRSA is a major breakthrough especially in the management of Methicillin-Resistant *S. aureus*, a major cause of both community-acquired and nosocomial associated infections.

Nonetheless, that some organisms were not susceptible to the activity of the extracts corroborated the fact that resistance to antimicrobial agents cannot be eliminated but curtailed since some organisms are intrinsically resistant.

Conclusion

The antimicrobial activity of *U. chamae* cannot be ignored most especially in its use in the treatment of diarrhea, dysentery and its application on wounds and sores to promote healing.

Its activity on *MRSA* has made the plant a promising source of more efficient and efficacious chemotherapeutic agent and cheaper alternative to orthodox antibiotics to which organisms frequently and continually develop resistance. (Singleton, 1999).

REFERENCES

Draughon FA (2004): Use of Botanicals as biopreservative in foods. Food Technol., 58: 20–28.

Evans WC (2009). Trease and Evans pharmacognosy. 15th Edition WB Saunders Ltd. London pp 137–159.

Karachi (2006). Importance of Medicinal plants highlighted (2006) retrieved June 18th fro http//www.dawn.com/2006/01/07/Local/html

Nair R, Kalariya T, Chanda S (2005). Antibacterial activity of some selected Indian Medicinal Flora, Turk J. Biol 29:41–47.

Singleton P (2004). Bacteria in Biology, Biotechnology and Medicine 6th Edition John Wiley and Sons Ltd, New York.

Sofowora EA (2008). Medicinal plants and Traditional Medicine in Africa. 3rd Edition Spectrum Books Ltd, Nigeria. pp. 181–207.