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In vitro Study on the Antimicrobial Activity and Phytochemical Analysis of Ethanolic Extracts of the Mesocarp of *Voacanga africana*

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Abstract: This study seek to establish the medicinal relevance of the fruit mesocarp of *Voacanga africana*, using standard methods of microbial sensitivity test and phytochemical analysis. Dried mesocarp samples of *V. africana* were extracted with hot and cold absolute ethanol. The extracts were screened for their phytochemical composition and antimicrobial activities. The results revealed the presence of some bioactive compounds; alkaloids, anthranoids, anthraquinone, glycosides, saponins, starch and tannins. The crude extracts exhibited antimicrobial activity against *Escherichia coli* (30.7%), *Serratia marcescens* (37.5%) and *Staphylococcus aureus* (39.47%). Others are *Alternaria solani* (30%), *Candida albicans* (26%) and *Rhizopus stolonifer* (20%); relative to the standard antibiotics, Gentamicin and Clotrimazole. The Agar Well Diffusion, sensitivity test, also showed zones of inhibition of 8 and 7.5 mm for hot and cold ethanol extracts, against *E. coli,* 9 and 00 mm; against *S. marcescens,* 7.5 and 6.5 mm; against *S. aureus,* 9 and 8 mm; against *A. solani*, 7 and 7 mm; against *C. albicans,* 6.5 and 00 mm, against *R. stolonifer*, at 100 g mL^{-1} concentration of the extracts. The hot and cold extracts were equipotent, against *C. albicans* but vary against other test organisms.

Key words: *V. africana*, mesocarp, phytochemical, bioactive molecule

INTRODUCTION

Over the years therapeutic manipulations in folk Medicine and Orthodox medical practices, has been dependent on pharmacopoeic formulations from plants. These plants, generally called Medicinal Plants, have been found to contain active secondary metabolites called Phytochemicals (Okwu and Josiah, 2006; Chukwuedo *et al*., 2007; Ogbonna *et al*., 2007; Ojo and Jide-Ojo, 2007; Nwadiaro and Nwachukwu, 2007; Deepthi *et al*., 2008; Oigiangbe *et al*., 2007; Duru *et al*., 2009).

Medicinal plants constitutes, the flora of wild and cultivated species of trees, shrubs, herbs and lianas. These groups of plants belong to different taxonomic families, among which is the Apocynaceae, which harbor the shrub-*V. africana* Stapf.

Voacanga africana is an abundant, deciduous, mesophytic, sap-woody, perennial, aborescent shrub of the primary and secondary forest, within the Tropical Rain Forest especially in Nigeria and the Guinea Savannah woodland belt. A mature *V. africana* crop is not more than 10 m tall, lowly branched, stem, with smooth, grayish white bark. Slash exudes milky latex.

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The leaves and roots decoction of this plant had been implicated in folk medicine for the treatment of malaria, diarrhea, infant convulsion, insane persons and heart arches (Burkill, 1995). This stimulated interest to further investigate this plant, with a view to determining the antimicrobial activity of the fruit mesocarp extracts in *in vitro* culture as well as the phytochemical composition of the extracts.

MATERIALS AND METHODS

Collection of Plant Materials

Ripe fruits of the plant were harvested from the wild and identified as *V. africana* Stapf. by a plant Taxonomist, at the Department of Biology, Federal University of Technology, Owerri, Imo State, Nigeria. The study was carried out from November, 2007 to March, 2009. The fruits were slit open and seeds extricated. The mesocarp were air-dried for 14 days, pulverized and stored in air-tight sterile bottle.

Test Organism

Clinical isolates of the Bacteria-*E. coli*, *P. aeruginosa*, *S. marcescens*, *S. aureus* and the test Fungi-*C. albicans*, were collected from the Department of Microbiology, Federal Medical Centre, Owerri, Nigeria while the other test fungi-*A. flavus*, *A. niger*, *A. solani* and *R. stolonifer*, were collected from the Plant Pathology Laboratory, National Root Crop Research Institute, Umudike, Abia State, Nigeria. They were separately sub-cultured and the pure culture resub-cultured on Nutrient Agar and Sabouraud Dextrose Agar media, respectively and stored at 4°C for further studies.

Extraction of Active Principles

Cold and hot absolute ethanol were used in the extractions according to the methods described by Biyiti *et al.* (1988), Harborne (1973) and Ogbonna *et al.* (2007), respectively.

In the cold percolation, 20 g of the dried, blended mesocarp were weighed out, transferred into a beaker and 100 mL of absolute alcohol added. The mixture were agitated and allowed to extract at laboratory temperature for 48 h. The mixture was then filtered in a flask, using Whatman's No. 1 filter paper (Ogbonna *et al.*, 2007). The filtrate was evaporated at 40°C on a hot plate till supernatant. The concentrated extracts were allowed to cool and stored in a sterile bottle. The hot ethanol extraction (Soxhlet), 20 g of the dried powdered mesocarp were fed into the Soxhlet extractor and extracted for 24 h at 80°C in 200 mL of absolute ethanol. The extracts were allowed to cool and stored at 4°C in a sterile bottle.

Phytochemical Screening

Standard screening procedures were followed as described by Trease and Evans (1983) and Ayoola *et al.* (2008).

Test for Reducing Sugars (Fehling's Test)

The aqueous ethanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for Anthraquinones

The 0.5 g of the extract was boiled with 10 mL of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipetted into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Saponins

To 0.5 g of extract was added 5 mL of distilled water in a test tube. The solution was shaken vigourously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

Tannins

Sample of the powdered mesocarp (3 g) was boiled in 5 mL of distilled water for 3 min on a hot plate. The mixture was filtered while hot and the resulting filtrate was used to carry out ferric chloride test. 1.0 g sample of the filtrate was weighed into a beaker and 10 mL of distilled water added. This was boiled for 5 min. Two drops of 5% ferric chloride ($FeCl_2$) was then added. Production of greenish precipitate indicated the presence of tannins (Trease and Evans, 1983).

Test for Alkaloids

The 0.5 g of extract was diluted to 10 mL with acid alcohol, boiled and filtered. To 5 mL of the filtrate was added 2 mL of dilute ammonia. Five milliliter of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for Cardiac Glycosides

To 0.5 g of extract diluted to 5 mL in water was added 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Microbial Susceptibility Test

Agar-well diffusion method was used in the investigation as described by Boakye-Yiadom (1979) and Radhika *et al.* (2008). Five wells, 8 mm each were made on solidified nutrient agar and sabouraud dextrose agar media plates, respectively with the aid of a sterile cork borer. 0.2ml of the log phase culture of the test microbes: *E. coli*, *P. aeruginosa*, *Serratia marcescen* and *S. aureus* were seeded on the surface of the nutrient agar medium while *C. albicans*, *A. flavus*, *A. niger*, *A. solani* and *R. stolonifer* were seeded on the sabouraud dextrose agar medium, using swab stick. The cut agar discs were removed with the aid of sterile forceps. Concentrations of 25, 50, 100, 150, 200, 250 and 500 mg mL^{-1} of the extracts were separately introduced into separate cavities. Three control holes were set up, one, empty, one filled with gentamycin and the other filled with clotrimazole, to serve as positive control for the bacteria and fungi, respectively.

The plates were incubated at 37°C for 24 h and 15 days, respectively for the bacteria and fungal cultures. The observed zones of inhibition were measured using transparent metric ruler.

Minimum Inhibitory Concentration of the Extracts

Determination of the Minimum Inhibitory Concentrations (MIC) followed the methods of Egorov (1985) and Radhika *et al.* (2008). Extracts concentrations of 10, 15, 25, 50, 100, 125, 150, 200, 250 and 500 mg mL^{-1} were used in the exercise. The lowest concentration of each of the extracts in each treatment, showing zero growth after 24 h for the bacteria and 15 days for the fungi, were recorded as the MIC values. Percentage (%) inhibition was calculated as extract concentration/concentration of standard (antibiotic e.g., Gentamycin) multiplied by 100.

Minimum Cidal/Static Concentration

The determination of the minimum bactericidal cum fungicidal concentrations of the extracts, was done according to the procedure described by Rotimi *et al.* (1988) and Alade and Irobi (1993). The innoculum from the culture tubes containing different concentrations of the extracts, showing no visible growth of the organisms from the MIC test, were subcultured in sterile nutrient agar and incubated at 37°C for 24 h and 15 days, respectively for the bacteria and fungi. The lowest concentration of the extracts without any growth was noted as the minimum cidal concentration (MBC/MFC).

RESULTS AND DISCUSSION

The phytochemical screening test, showed the presence of some active principles; Alkaloids, Anthranoids, Anthraquinone, Cardiac glycoside, Saponins, Starch and Tannins. Phenol and Phlobtanin were absent (Table 1). At the expiration of the incubation periods of 24 h and 15 days, respectively, for the bacteria and fungi sets. The zones of inhibition of *E. coli*, *P. aeruginosa*, *S. marcescen* and *S. aureus*, *C. albicans*, *A. flavus*, *A. niger*, *A. solani* and *R. stolonifer* were determined and the results are shown in Table 2. The antimicrobial sensitivity test, using Agar-Well Diffusion technique, showed that there was no inhibition on the growth of *A. flavus*, *A. niger* and *P. aeruginosa*, by the extracts. However, other test microbes were susceptible to the extracts. With mean inhibition diameter ranging from 6.5-10 mm in the hot ethanolic extract and 6.5-8.5 mm in the cold extract (Table 2). The minimum inhibitory concentration of the extracts against the test organisms susceptible to it range from 25-100 mg mL^{-1} in hot ethanol extract and 50-200 mg mL^{-1} in the cold extract (Table 3).

The choice of absolute ethanol as the extraction agent was due to the fact that it was readily available and cheap to procure. The vapourization temperature (78°C) is blow the thermolabile limit for most protein (Adebayo, 1990). Some fruits mesocarp contained oil and fatty acids that were not soluble in water (Ogbonna *et al.*, 2007). The low pH of between

Table 1: Phytochemical analysis of the mesocarp extracts of *V. africana*

Test	Remarks
Alkaloid	+
Anthranoid	+
Anthraquinone	+
Cardiac glycoside	+
Phenol	-
Phlobatanin	-
Saponin	+
Starch	+
Tannin	+

+ve = Present, -ve = Absent

Table 2: Sensitivity test for the bacteria and fungal species on the mesocarp extracts of *V. africana* (Percentage inhibition in bracket)

Test organisms	Zones of inhibition (mm)						
	HEE		CEE				
	100	200	100	200			
	(mg mL^{-1})				EH	GH	CH
E. coli	8 (30.76%)	10 (38.46%)	7.5 (28.84%)	8 (30.76%)	00	26	00
P. aeruginosa	-	-	-	-	00	24	00
S. marcescens	9 (37.50%)	9 (37.50%)	-(00%)	-	00	24	00
S. aureus	7.5 (39.47%)	8 (42.10%)	6.5 (34.21%)	6.5 (34.21%)	00	19	00
C. albicans	7 (25.92%)	8 (29.63%)	7 (25.92%)	8 (2.63%)	00	00	27
A. flavus	-	-	-	-	00	00	27
A. niger	-	-	-	-	00	00	28
A. solani	9 (30.00%)	9.5 (31.67%)	8 (26.67%)	8 (26.67%)	00	00	30
R. stolonifer	6.5 (20.97%)	7 (22.58%)	- (00%)	- (00%)	00	00	31

HEE: Hot ethanol extract, CEE: Cold ethanol extract, EH: Empty hole, GH: Gentamicin hole, CH: Clotrimazole hole

Table 3: Minimum inhibitory concentration of the mesocarp extracts of *V. africana*

Test organism	Extracts concentration (mg mL^{-1})	
	HEE	CEE
Escherichia coli	25	50
Serratia marcescens	25	50
Staphylococcus aureus	25	100
Alternaria solani	100	100
Candida albicans	100	100
Rhizopus stolonifer	100	200

4.7 and 4.9 showed that the extracts are weak acid medium, suggesting that the active principles in the extracts are potent in acid medium. This is in consonance with the report of Nwadiaro and Nwachukwu (2007), on the extracts of *Cymbopogon citratus*, *Ceiba pentandra* and *Loranthus bengwelensis*. The extracts had antibacterial activity against, *E. coli*, *S. marcescens* and *S. aureus*. It also demonstrated antifungal activity against *A. solani*, *C. albican* and *R. stolonifer*. Thus; suggesting that the mesocarp extracts of *V. africana* has a broad spectrum antimicrobial property. The antibacterial and the antimycotic potency may be due to the presence of some active principles: Alkaloids, Anthranoids, Anthraquinone, Glycosides, Saponins, Starch and Tannins. This is in consonance with the report of Ebena *et al*. (1991) on the extracts of *Garcinia kola*, *Boreria ocymoides*, *Kola nitida* and *Citrus aurantifolia*, Nwadiaro and Nwachukwu (2007) on the extracts of *C. citratus*, *C. pentandra* and *L. bengwelensis,* Duru *et al*. (2009), on bark extracts of *V. africana* and Deepthi *et al*. (2008), on the extracts of *Alstonia scholaris*.

The sensitivity test result, showed that the extracts were less potent than the standard antibiotics; Gentamicin and Clotrimazole, used in the study. The hot and the cold fractions were apparently not equipotent, The hot extraction method had an advantage over the cold method since it inhibited *S. marcescens* at same concentration. At 100 mg mL^{-1} extract concentration, the HEE inhibition values were 30.76, 37.50, 39.47, 30 and 20.97% against *E. coli*, *S. marcescens*, *S. aureus*, *A. solani* and *R. stolonifer*, respectively while that of CEE were 28.84, 00, 34.21, 26.67 and 0% *E. coli*, *S. Vmarcescens*, *S. aureus*, *A. solani* and *R. stolonifer*, respectively. However, they were equipotent against *C. albicans*, 25.92% for both HEE and CEE treatments.

Generally, the less potency, of the extracts, relative to the standard antibiotics may be due to the fact that it was crude and required further purification.

The results also showed that the extracts did not elicit antimicrobial activity against *P. aeruginosa*, *A. flavus* and *A. niger*. These effects could be either due to cultural composition of the media or the biochemical composition of the target structure of the organism Ogbonna *et al.* (2007). It is also possible, that the insensitivity of the non-susceptible test microbes, on the extracts could be due to short period of exposure, on the part of the test bacteria or the concentration quotient was minimal for cidal activity on the part of the test fungi.

The result obtained in this study had shown that the mesocarp of *V. africana*, which hitherto, waste in our forest contain medicinally useful phyto-chemicals, such as Alkaloids, anthranoids, anthraquinones, glycosides, saponins, starch and tannins. These substances are antimicrobial and could be extracted for disease therapy, pharmaceutical exploits, researches in Microbiology, Biotechnology and general medicine.

REFERENCES

Adebayo, L., 1990. Essential Microbiology for students and Practitioners of Pharmacy, Medicine and Microbiology. Amkra Books, Lagos, Nigeria, pp: 106.

Alade, P.I. and O.N. Irobi, 1993. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. J. Ethnopharmacol., 39: 171-174.

Ayoola, G.A., H.A.B. Coker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweya, E.C. Ezennia and T.O. Atangbayila, 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J. Pharm. Res., 7: 1019-1024.

Biyiti, L., D. Pesando and S. Puiseux-Dao, 1988. Antimicrobial activity of two flavonone Isolates from the Cameroonian plant *Erythrina sigmoidea*. Planta Med., 54: 126-128.

Boakye-Yiadom, K., 1979. Antimicrobial properties of some West African medicinal plants. II. Antimicrobial activity of aqueous extracts of *Cryptolepis sanguinolenta* (Lindl.) Schlechter. Q. J. Crude Drug Res., 17: 78-80.

Burkill, H.M., 1995. Useful Plants of West Tropical Africa. Vol. 5, 2nd Edn., Royal Botanic Gardens, Kew, England, ISBN-10:1900347407.

Chukwuedo, A.A., A. Usman and A.O. Olabode, 2007. Effects of *Nelsonia canecan* leaf extracts on the treatment of Foot-rot in ruminant. Nig. J. Biotech., 18: 16-18.

Deepthi, S.R., R. Remya and V. Thankamani, 2008. Antimicrobial activity studies and phytochemical screening on the methanol extracts of *Alstonia scholaris*. R. Br. Res. J. Biotech., 3: 40-43.

Duru, C.M., E.U. Ezeji and E.A. Anyalogbu, 2009. Phytochemical analysis and anti microbial activity of the bark extracts of *Voacanga africana* Stapf. Nig. J. Biotech., 20: 60-65.

Ebena, R.U.B., B.E. Madunagu, E.D. Ekpe and I.N. Otung, 1991. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Boreria ocymoides*, *Kola nitida* and *Citrus aurantifolia*. J. Applied Bacteriol., 71: 398-401.

Egorov, I., 1985. The antimicrobial activity of the leaf extracts of *Calotropis procera*. Lett. Applied Microbiol., 55: 205-210.

Harborne, J.B., 1973. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, Ltd., London.

Nwadiaro, P.O. and I. Nwachukwu, 2007. Inhibition of pathogenic fungi by Ethnobotanical extracts of *Cymbopogon citratus*, *Ceiba pentandra* and *Loranthus bengwelensis*. Nig. J. Biotechnol., 18: 61-65.

Ogbonna, A.I., M.D. Makut, S.D. Gyar and E.U. Adamu, 2007. Antimicrobial activity of the ethanolic extracts of the seed of *Ricinus cummunis* L. Nig. J. Biotech., 18: 40-43.

Oigiangbe, O.N., I.B. Igbinosa and M. Tamo, 2007. Bioactivity of extracts of *Alstonia boonei* (Apocynaceae) De Wild stem bark against *Maruca vitrata* (Lepidoptera: Pyralidae) *Fabriscus*. Adv. Sci. Technol., 1: 67-70.

Ojo, O.O. and C.C. Jide-Ojo, 2007. Efficacy of extracts of lemongrass (*Cymbopogon citratus*), on acetaminophen-treated rats. Nig. J. Biotechnol., 18: 56-60.

Okwu, D.E. and C. Josiah, 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotechnol., 5: 357-361.

Radhika, P., B.S. Sastry and B.M. Harika, 2008. Antimicrobial screening of *Andrographis paniculata* (Acanthaceae) root extracts. Res. J. Biotechnol., 3: 62-63.

Rotimi, V.O., B.E. Laughton, J.S. Bartlet and H.A. Mesadomi, 1988. Activities of Nigerian chewing stick extracts against bacterioid *Gingi valli* and Bacterioides *Melaniogenicus*. Antimicrob. Agents Chemother., 32: 598-600.

Trease, G.E. and I.C. Evans, 1983. Textbook of Pharmacognosy. 12th Edn., Bailliere Tindall, London, pp: 21-22.