In vitro Antimicrobial Assay and Phytochemical Analysis of Ethanolic Extracts of *Voacanga africana* Seeds

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Abstract: Dried and pulverized seeds of *Voacanga 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, cardiac glycosides, phenols, phlobatanins, starch and tannins. The crude extracts exhibited antimicrobial activity against *Escherichia coli* (34.61 and 25%), *Serretia marcescens* (45.08 and 29.16%) and *Staphylococcus aureus* (42.10 and 34.21%). Others are *Alternaria solani* (33.33 and 25%), *Aspergilus flavus* (33.33 and 22%), *A. niger* (25 and 00%) *Candida albicans* (29.62 and 25.92 %) and *Rhizopus stolonifer* (22.58 and 19.35 %); relative to the standard antibiotics, Gentamicin and Clotrimazole; in the Agar Well. Diffusion sensitivity test. The efficacy of the hot extract was greater than the cold extracts in the test organisms, except in *Pseudomonas aeruginosa* where they appeared equipotent. [Journal of American Science 2010; 6(6):119-122]. (ISSN: 1545-1003).

Key words: *Voacanga africana*, phytochemical, bioactive, equipotent.

1. Introduction

Knowledge and application of ethno-medicinal properties of plants dates back to about 300 years BC. (Makhubu, 1998; Ogbonna *et al.* 2007). Plants therapeutic essence is secondary metabolites, known as phytochemicals. These organic chemical substances are stored in matured cells of the various organs, such as roots, stems, leaves, flowers, fruits and seeds.(Sofowora, 1982).Some of the phytochemicals implicated in this exercise; alkaloid, flavonoids, glycosides, phenols, phlobatanins, saponins, tannins, etc.., had been found in crude extracts of some plant species, called medicinal plants (Okwu, 2001; Ano and Ubochi, 2007). Among these plants is a tropical shrub called *Voacanga africana*

V. africana is a deciduous, mesophytic, sap-woody, perennial, aborescent shrub of the primary and secondary forest, within the Tropical Rain Forest and the Guinea Savannah woodland belt. A mature *V. africana* crop is not more than 10m tall, lowly branched, stem, with smooth, grayish white bark. Slash exudes milky latex. Leaves are simple, petiolate and decussately arranged. Inflorescence, terminal, lax, pedunculate, cyme. Flower, pedicellate and mildly scented; corolla lobe, with overlapping aestivation. Stamen, pentamerous and epipetalous. Ovary, superior and bicarpellary. Fruit, globose berry with brownish – white blotches. Seed, dark, bean –shape with denticulate ornamentation. (Duru, 2009).

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; Duru, 2009). This stimulated interest to further investigate this plant, with a view to determining the antimicrobial activity of the seed extracts in *in vitro* culture as well as the phytochemical composition of the crude extracts.

2. Materials And Methods

Collection of Plant Materials:

Matured fruits of the plant were harvested from the wild and identified as *Voacanga africana* Stapf by a plant Taxonomist, at the Department of Biology, Federal University of Technology, Owerri, Imo State, Nigeria. The fruits were slit open and seeds extricated. The seeds were oven- dried at 40^{o}C for seven (7) days, pulverized and stored in air-tight sterile bottle.

Test Organism:

Clinical isolates of the Bacteria- *Escherichia coli, Pseudomonas aeruginosa, Serretia marcescen, Staphylococcus aureus* and the test Fungi- *Candida albicans*, were collected from the Department of Microbiology, Federal Medical Centre, Owerri, Imo State, Nigeria; while the other test fungi- *Aspergilus flavus, A.niger, Alternaria solani* and *Rhizopus 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 re-subcultured on Nutrient Agar and Sabouraud Dextrose Agar media, respectively and stored at 4O^{o}C for further studies.

Extraction of Active Principles:

Cold and hot absolute ethanol was used in the extractions. The cold process followed the method of Boakye-Yiadom (1979). While the hot process, followed the methods of Harborne (1973) and Ogbonna *et al*. (2007). In the cold percolation, 20g of the dried, blended seeds were weighed out, transferred into a beaker, and 100ml of absolute alcohol added. The mixture were agitated and allowed to extract at laboratory temperature for 48hrs.The mixture was then filtered in a flask, using Whatman's No 1 filter paper. The filtrate was evaporated at 40 ^{o}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), 20g of the dried powdered seeds were Fed into the Soxhlet extractor and extracted for 24hrs at 80 ^{o}C in 200ml of absolute ethanol. The extracts were allowed to cool, and stored at 4 ^{o}C in a sterile bottle.

Phytochemical Screening:

The screening procedure adopted, followed the methods described by Trease and Evans (1983), Banso and Adeyemo (2007).

Microbial Susceptibility Test:

The agar well diffusion technique was used in the investigation, following the procedure described by Russell and Fur (1977), Boakye- Yiadom (1979), Banso and Adeyemo (2007), and Radhika, *et a*l. (2008). Five (5) wells, 8mm 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, Pseudomonas aeruginosa, Serratia marcescen* and *Staphylococcus aureus* were seeded on the surface of the nutrient agar medium while *Candida albicans, Aspergillus flavus, A. niger, Alternaria solani* and *Rhizopus stolonifer* were seeded on the Sabouraud Dextrose Agar (SDA) medium, using swab stick. The cut agar discs were removed with the aid of sterile forceps. Concentrations of 25g/ml, 50g/ml, 100g/ml, 150g/ml, 200g/ml, 250g/ml, and 500g/ml of the extracts were separately introduced into separate cavities. Three (3) control holes were set up, one, empty, one filled with gentamicin and the other filled with clotrimazole, to serve as positive control for the bacteria and fungi, respectively.

The plates were incubated at 37 ^{o}C for 24hrs and 15days respectively for the bacterial 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), Brown (1994), and Radhika, *et al*. (2008). Extracts concentrations of 10g/ml, 15g/ml, 25g/ml, 50g/ml, 100g/ml, 125g/ml, 150g/ml, 200g/ml, 250g/ml and 500g/ml were used in the exercise. The lowest concentration of each of the extracts in each treatment, showing zero growth after 24hrs for the bacteria and 15 days for the fungi, were recorded as the MIC values.

Minimum Cidal/Static Concentration:

The determination of the minimum bactericidal (MBC) and fungicidal (MFC) concentrations of the extracts were done according to the procedure described by Rotimi, *et al*. (1988), Alade and Irobi (1993), and Banso and Adeyemo (2007). The inoculums from the pure 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^{o}C for 24hrs and 15days, respectively for the bacteria and fungi. The lowest concentration of the extracts with out any growth was noted as the minimum cidal concentration (MBC / MFC).

3. Results

The results of the phytochemical screening are shown in Table 1

Table 1: Phytochemical Analysis of the Seeds Extracts of *Voacanga africana*

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

Key: +ve = present ; -ve = absent

The phytochemical screening test, showed the presence of some active principles; Alkaloids, Anthranoids, Anthraquinone, Cardiac glycosides, phenol, phlobatanins, Starch and Tannins.

At the end of the incubation periods of 24 hours and 15 days respectively, for the bacteria and fungi sets. The zones of inhibition of *E. coli, P. aeruginosa, Serretia marcescen* and *Staphylococcus aureus; Candida albicans, Aspergilus flavus, A. niger, Alternaria solani* and *Rhizopus stolonifer* were determined, and the result was shown in Table 2.

Table 2: Sensitivity Test for The Bacterial and Fungal species on the seeds extracts of *Voacanga africana*

Test Organisms	Zones Of Inhibition (Mm)						
	HEE		CEE		EH	GH	CH
	100 mg/ ml	200 mg/ ml	100 mg/ ml	200 mg/ ml			
Escherichia. coli	9	12	6.5	8	00	26	00
Pseudomonas. aeruginosa,	6.5	8	6.5	7.5	00	24	00
Serretia marcescen	11	12	7	8	00	24	00
Staphylococcus aureus	8	8	6.5	6.5	00	19	00
Candida albicans,	8	10	7	8	00	00	27
Aspergillus flavus	9	9	6	7	00	00	27
Aspergillus. niger	7	7	-	-	00	00	28
Alternaria solani	10	11	75	8.5	00	00	30
Rhizopus stolonifer	7	9	6	6.5	00	00	31

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Key:
HEE ------Hot Ethanol Extract
CEE ------Cold Ethanol Extract
EH ------ Empty Hole
GH ------ Gentamicin Hole
CH -------Clotrimazole Hole

The antimicrobial sensitivity test, using Agar Well Diffusion technique, showed that there was no inhibition on the growth of *Aspergilus niger* by the cold ethanolic extracts. However, all the test microbes were susceptible to the extracts. With mean inhibition diameter ranging from 6.5mm – 12mm in the hot ethanolic extract and 6.mm – 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 25g/ml – 100g/ml in hot ethanol extract and 50g/ml – 200g/ml in the cold extract. (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of The Seeds Extracts of *Voacanga africana*

Test Organism	Extracts Concentration (g/ml)	
	HEE	CEE
Escherichia coli	25	50
Pseudomonas aeruginosa	50	100
Serretia marcescen	25	50
Staphylococcus aureus	25	100
Aspergillus flavus	50	100
Aspergillus niger	50	-
Alternaria solani	100	100
Candida albicans,	100	100
Rhizopus stolonifer	100	200

4. Discussion

Absolute ethanol was used as the extraction agent because it wais readily available and cheap to procure. Some seeds contain oil and fatty acid that may not be soluble in water. The extracts had antibacterial activity against, *Escherichia coli, Pseudomonas aeruginosa, Serretia marcescen* and *Staphylococcus aureus.* It also demonstrated antifungal activity against *Alternaria solani, Aspergilus flavus, A. niger , Candida albican* and *Rhizopus stolonifer* as such suggesting that the seeds extracts of *V. africana* has a broad spectrum antimicrobial potency. The antibacterial and the antimycotic potency may be due to the presence of some active principles, like Alkaloids, Anthranoids, Anthraquinone, Cardiac glycosides, Phenols, Phlobatanins, Starch and Tannins. This result agrees with the report of Ebena, *et al.* (1991), Trease and Evans (2005), and Banso and Adeyemo (2007).

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. At 100g/ml concentration, we had 34.61% (HEE) and 25% (CEE), against *E. coli*. 27.08% (HEE) and 27.08% (CEE) against *Pseudomonas aeruginosa.* 45.08% (HEE) and 29.16% (CEE), against *Serretia marcescen.* 42.10% (HEE) and 34.21% (CEE), against *Staphylococcus aureus.* 33.33% (HEE) and 25% (CEE), against *Alternaria solani*. 33.33% (HEE) and 22.22% (CEE) against *Aspergilus flavus,* 25% (HEE) and 00% CEE against *A. niger* . 29.62% (HEE) and 25.92% against. *Candida albican*, 22.58% (HEE) and 19.35% (CEE), against *Rhizopus stolonifer*. However they were equipotent against *Pseudomonas aeruginosa* 27.08% for both HEE and CEE treatments. Generally, the reduced efficacy of the extracts, relative to the standard antibiotics, used in the study may be due to the fact that, they are still crude and require further purification.

The cold extracts did not elicit antimicrobial activity, against *A. niger*. These effects could be due to the fact that the concentration quotient was too minimal to elicit cidal activity on the test fungi.

Seeds of *Voacanga africana*, which hitherto, waste in our forest contain medicinally, useful phytochemicals, such as Alkaloids, anthranoids, anthraquinones, cardiac glycosides, phenols, phlobatanins, starch and tannins. These substances are antimicrobial and could be extracted for bacterial and fungal diseases management, pharmaceutical exploits, research in Microbiology, Biotechnology and general Medicine.

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References

1. Ano AO. Ubochi CI. Phytochemical composition of vegetable cowpea genotype. Advances in Science and Technology, 2007; 1(1): 1-7.
2. Banso A, Adeyemo SO. Phytochemical and antimicrobial evaluation of ethanolic extracts *Draclena mannii* bark. Nig J Biotech. 2007; 18: 27-32.
3. Boakye-Yiadom IK, Antimicrobial activity of two flavonone Isolates from the Cameroonian plant *Enythiana sigmoides.* Planta Medicia. 1979; 54 (2): 126-212.
4. Brown B. Developments in antimicrobial susceptibility testing. 1994; 5: 65-75.
5. Burkill HM. The useful plant of west Tropical Africa 2nd Ed. The white Frairs press ltd, London. 1995; 190 –191.
6. Duru CM. Studies on certain genera of Apocynaceae in relation to Taxonomy. *PhD. Thesis, University of Port Harcourt.* Nigeria. 2009.
7. Ebena RUB. Madunagu BE, Ekpe ED, Otung IN. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola, Boreria ocymoides, Kola nitida* and *Citrus aurantifolia.* J Appl Bacterio 1991; 71:398-401.
8. Egorov I. The antimicrobial activity of the leaf extracts of *Calotropis procera.* Letters in Applied Microbiology, 1985; 55: 205-210.
9. Harborne JB. Phytochemical Methods. A guide to modern technique of plant analysis. Cambridge University press, Cambridge . UK. 1973.
10. Makhubu L. Bioprospecting in an African context. Science, 1998;282: 41 – 43.
11. Ogbonna AI. Makut MD, Gyar SD, Adamu EU. Antimicrobial activity of the ethanolic extracts of the seed of *Ricinus cummunis L.* Nig J Biotech 2001;18: 40-43.
12. Okwu DE. Evaluation of the chemical composition of indigenous species flavouring agent. Global Journal of Pure and Applied Sciences, 2001;39:69-72.
13. Radhika P, Sastry BS, Harica B. Madhu. Antimicrobial screening of *Andrographis paniculata* (Acanthaceae) root extracts. Res J Biotech. 2008; 3(3):62-63
14. Sofowara A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books ltd, Ibadan, Nigeria. 1993.
15. Trease GE, Evans IC. *Pharmacognosy 12th Ed*. Boinlliene Tinnal, London. 1983.

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