Nig J. Biotech. Vol. 20 (2009) 61 – 65
ISSN: 0189 17131
Available online at http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Phytochemical Analysis and Antimicrobial Activity of the Bark Extracts of *Voacanga africana Stapf*

Christopher, M. D., Uchechukwu, E. E.* and Ernest, A. A.

Department of Biotechnology Federal University of Technology, P.M.B. 1526, Owerri, Nigeria

Abstract

Phytochemical analysis and antimicrobial activity hot water, hot and cold ethanolic extracts of the bark of *Vocanga Africana* stapt. on *Escherichia coli, Serratia marcescen, Staphyloccus aureus, Alternaria solani , Aspergellius niger* and *Penicillium notatum* were investigated using paper disc diffusion technique. Results showed that the bacteria *Escherichia coli,* Serratia *marcescens, Staphylococcus aureus* were susceptible to hot ethanol extract, *E.coli and Serretia marcescen were susceptible to hot water extract, S. aureus was not. Pencillium notatum* was susceptible to hot water, hot and cold ethanol extracts, *A.niger was* susceptible *to* only the ethanol extract; while *Alternaria solani* was resistant to the bioactivity of the extract. The presence of active principles, Alkaloids, Anthranoid, cardiac glycosides, Polyphenol, saponin and starch, detected in the phytochemical analysis might be responsible for the antimicrobial activity of the extract.

Key Words: Phytochemical, antimicrobial, susceptibility, Vocanga

**Correspondence: ucheezeji@yahoo.com*

Introduction

The medicinal properties of some plants have been implicated and explored in both folk (traditional) and orthodox medicine. The potency of these plants has been traced to some active principles; alkaloids, anthranoids, etc. referred to as secondary metabolites. These active ingredients are contained in leaves, stem bark, roots, seed, of higher plants. Phytochemical screening of these organs helped to advance the course of science and medicine (Urquiag and Leighton, 2000).

The plant species of *Voacanga africana* stepf. belong to the family of *Apocynaceae.* They constitute the luxuriant flora of the tropical woodland and savannah ecosystem. A matured V. *africana* is an aborescent shrub of between 3-11 metres tall and 4-40cm in girth. It has a tap root system. The bole is soft and woody but relatively shorter than that of a tree. It branches several and, regularly in a bifurcate arrangement. The fork appears very close to the base. Each prong of the fork in turn begets another fork and continued until it terminates at the twig. Thus, forming a low widely spreading crown supported by a network of didymous branches. The bark is whitish - brown and the slash yellowish white. The leaves are simple, petiolate, with decussate phyllotaxy, usually concentrated towards the twig. The flowers are born on a long pair of pedunculate, cymose inflorescence at the last fork of the branches. The fruit is a spherical berry, dark green in colour with light greenish white blotches and a single line of dehiscene.

The pericarp is well developed with a thick fleshy epicarp enclosing a mass of soft fibrous yellow mesocarp within which are embedded numerous seeds. The seeds are ellipsoidal or slightly spherical, cotyledonous and measure between 5-10mm long and 3-5mm broad (Green, 1994). The twig and root decoction of this plant have been implicated in the treatment of malaria, blannorrhoea and diarrhea (Dalziel, 1937; Oliver, 1960). But in spite of all these advances, work on phytochemical analysis and antimicrobial

activities of *V. africana has* not been fully explored. This study, therefore aimed at screening crude extracts of the bark of *V. africana* of some phytochemicals; Alkaloids, Anthroquinone, Tannins, Saponins, starch, Polyphenol, Phlobatannins and cardiac glycosides as well as their antimicrobial activities.

Materials and method

Plant Material: The plant materials were collected from a plant at the premises of Federal University of Technology Owerri, and Identified as *Voacanga africana* at the Department of Biology and Biotechnology of the Federal University of Technology, Owerri.

Test Organism: The stock culture of the bacteria: *Escherichia coli Staphylococcus aureus, Serratia marcescen* used in the study were obtained from Federal Medical Centre, Owerri, Imo State, Nigeria while the fungi: *Aspergellus niger, Alternaria solani* and *Penicillium notatum* were obtained from Federal University of Agriculture, Umudike, Abia State, Nigeria.

Extraction of active ingredients: The bark specimen was oven dried at 40°C for seven days. The dried specimen was pulverized with an electric grinder (Lexus Millenium Blender; SBG 320). The powder was transferred into a bottle and preserved for future use.

Hot Ethanol Extraction: 20g of the blended materials were weighed out and transferred into the thimble and inserted into the soxhlet apparatus and extracted for 24 hours with 100mls of Absolute ethanol. The extracts obtained was allowed to cool and stored in a sterile bottle and labeled.

Cold Ethanol Extraction: 20g of the leaf powder was weighed out and transferred into a beaker and 100ml of Absolute ethanol added. The mixture was agitated and allowed to extract at laboratory temperature for 48 hours. The mixture was filtered into a Petri dish using Whatman No 1 filter paper. Alcohol was evaporated from the filtrate at 40°C on a hot plate. The supernatant extract was allowed to cool and stored into a sterile bottle, for further use.

Hot Water Extraction: 32g of the bark powder were boiled in 100ml of distilled water for 25 minutes, allowed to cool and filtered into a sterile bottle, labeled and stored.

Phytochemical analysis

Test for Athranoid: 0.2g of the bark powder were boiled with 0.5ml of 0.5m potassium hydroxide [KOH] for 2 minutes in a test-tube and allowed to cool and filtered through cotton wool. Six drops of acetic acid and 5ml of toluene were added to the filtrate and the mixture shaken to mix properly. Two layers were formed and the upper layer was carefully pipetted into another test tube and 2mls of 0.5 KOH was added. A red colour indicates the presence of Anthranoid.

Test for Athraquinone: A mixture of 5g of the bark powder and 10mls of benzene was shaken vigorously and filtered through a Whatman No 1 filter paper. 5ml of 10% ammonia solution was added to the filtrate and shaken vigorously. The presence of a pinkish or reddish colour indicates the presence of Anthraquinone.

Test for Cardiac Glycoside: A mixture of 5g of the bark powder and 2ml of chloroform was shaken vigorously in a text tube and 2mls of concentrated sulphuric acid added and carefully shaken, the presence of reddish-brown colouration indicate the presence cardiac glycoside.

Test for Phlobatannins: 2g of the bark powder was boiled with 5mls of 1% hydrochloric acid (HCl). The appearance of reddish precipitate showed the presence of phlobatannins.

Test for Polyphenol: A mixture of 2g of the bark powder and 10ml of distilled water was heated for 30minutes, allowed to cool and filtered through cotton wool. 1ml of 1% ferric chloride plus 1ml of potassium ferrocyanide were added to 1ml of the filtrate. A bluish-green colouration indicates the presence of polyphenol.

Test for Saponin: A mixture of 5g of the bark powder and 5 mls of distilled water was shaken vigorously in a test tube to produce frotting, then boiled for 30 minutes at 60^0C, persistent frothing, indicates the presence of saponin.

Test for Tannins: A mixture of 2g of the bark powder and 10ml of distilled water in a test tube was stirred and filtered using Whatman

No.1 filter paper. 2ml of ferric chloride added to 2ml of the filtrate. A blue - black or grayish blue precipitate indicates the presence of Tannins.

Test for Alkaloids: Concentrated ammonium solution was added to 10g of the leaves powder to moisten it. After 10 minutes, ethanol; chloroform (1:1) mixture was added to soak the powder. The mixture was stirred for 2 minutes with a glass rod then filtered using cotton wool. The filtrate was evaporated to dryness on a water bath. The impure crystalline substance was allowed to cool. The impure crystalline substances were dissolved in 5ml of pure chloroform; 3ml of sulphuric acid was added and carefully shaken. A layer appears and was allowed to separate. The lower chloroform layer was removed, and the upper layer retained. This step was repeated until the upper layer become colourless. Concentrated ammonia was added to make it alkaline. 3ml of chloroform was added to the extract and evaporated to dryness; the pure crystals are the alkaloids.

Determination of the Antimicrobial Activities of the Bark Extracts: The Disc Diffusion technique was used in the investigation following methods described by Pelezar and Chan (1977), Cheesbrough (1984) and Miller *et al.* (1984). Circular disc of 7mm diameter were punched out from whatman No.1 filter paper, the discs were wrapped in an aluminum foil and kept in a glass plate, the plate and the forceps were autoclaved for 15min at 121°C. The sterile paper discs were placed in Petri dishes and impregnated with 0.lml of the leaf extract using sterile pipette. They were then dried in hot air oven at 40'C for 20 minutes. A sterile swab stick was used to seed the agar plate with the test organisms. The plates were incubated by inverting them in an incubator set at 37^0C for 24 hours for bacteria and 72 hours for fungi. The diameter of the observed inhibition zones were measured with transparent metric ruler.

An unimpregnated sterile disc was used as control experiment.

Results and discussion

Phytochemical screening of the bark extract of *Voacanga africana* revealed that the extract contains alkaloid, anthroquinone, cardiac glycoside, saponin and starch (Table 1). A lot of research has been carried out on the screening of different medicinal plants for active principals or phytochemicals (Umar *et al*., 2000; Gill, 1992; Okorondu *et al*., 2006). These phytochemicals are thought to be responsible for the medicinal effects of these plants (Omenka *et al*., 2004). Not much work has however be done to determine the presence of phytochemicals in *Voacanga africana* even though it is used for the local cure of some diseases.

Result of the susceptibility test of some selected microorganisms (*Escherichia coli, Serretia marcescen, Staphylococcus aureus, Alternoria solaria, Aspergellus niger, Penicillium notatum)* to hot ethanol, cold ethanol and hot water extract of the test plant is shown is table 2. The greatest number of the organisms was susceptible to the hot ethanol extract, followed by hot water extract. The cold ethanol extract was found to affect only on one organism (*Penicillium notatum*). There was no zone of inhibition on the growth of the plant pathogenic fungi *Alternaria solani.* For the three extracts however, all the extracts were found to be active against *Penicillium notatum* with almost equal efficacy as shown on the zones of inhibition 7.5mm and 7.5mm and 7.0mm for the hot water, hot and cold ethanol extracts respectively.

Only the hot ethanol extract was effective against the fungi *Aspergellius niger, Escherichia coli, Serratia marcescen* and *Staphylococcus aureus* with zones of inhibition of 8.0mm,15.0mm, 14.0mm and I0.0mm respectively.

Table 1: Result of phytochemical analysis

TEST	REMARK
Alkaloid	+
Anthranoids	-
Anthraquinone	+
Cardiac glycoside	+
Phlobatanins	-
Polyphenol	+
Saponin	+
Tannins	-
Starch	+

Key: + = Present; - = Absent

Table 2: Sensitivity test

ORGANISM	*ZONE OF INHIBITION (MM)*		
	HEE	CEE	HWE
Escherichia coli	15.0	-	10.0
Serretia marcescen	14.0	-	10.0
Staphylococcus aureus	10.0	-	-
Alternoria solaric	-	-	-
Aspergellus niger	8.0	-	-
Penicillium notatum	7.5	7.5	7.0

Key:
HEE = Hot Ethanol Extract; CEE = Cold Ethanol Extract; HWE = Hot Water Extract

References

Cheesbrough, M. (1984). *Medical Laboratory Manual for Tropical Countries, Vol. 11. Butterworth*. Heinemann Ltd., Oxford. Pp. 449.

Dalziel, J. M. (1955). Useful plants of West Tropical Africa. Crown agents London.

Gill, L.S. (1992). Ethno-medical uses of plants in Nigeria. Uniben Press, P. 276.

Green, B. 0. (1994). Taxonomic and ethnobotanical studies on the family Apocynaiese in Nigeria Ph.D thesis, University of Port-Harcourt, Nigeria.

Miller, J.M., Thornsberry, C. and Baker, C.N. (1984). *Disc Diffusion Susceptibility Test. Trouble Shooting Guide Laboratory Manual*, 15: 183.

Oliver, B. (1960). Medicinal Plants in Nigeria, paper presented to the Pharmacy Department, Nigeria College of Arts, Science and Technology, Ibadan.

Okorondu, S.I., Braide, W., Ogbulie, T.E and Akujobi, C.O. (2006). Antimicrobial and phytochemical properties of some traditional spices. Nig. Journ. Microb., 20 (3): 1301-1308.

Omenka, C.A., Ukagwu, N., Ali, B.A. and Onyemenonue, C. (2004). Antimicrobial Activity and phytochemical analysis of Phyllantus niruri. *Inter-World Journal of Science and Technology*, 1(1): 160-168.

Pelezar, M.J. Jnr. And Chan, E.C.S. (1977). *Laboratory Exercises in Microbiology*. Black Dot Inc., USA.

Umar, A.I., Madia, B.I., Buratai, B. and Karumi,

Y. (2000). The effect of a combination of Garlic (Allium sativa) powder and wild honey on lipid metabolism in insulin. *Nig. Journ. Exp. Appl. Biol*. 1: 37-42.

Urquiag, I. and Leighton, F. (2000) Plant Polyphenol Antioxidants and Oxidative Stress. *Biological Research 33*: 159 – 165.