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Phytochemical screening and antimicrobial studies of *uapaca togoensis (pax)* stem bark extracts

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----------**ABSTRACT**----------

Air-dried stem bark of UapacatogoensisPax (Phyllanthaceae, a segregate of Euphorbiaceaesensulato) was powdered and successively extracted using the three selective solvents;n-hexane, ethyl acetate and methanol by Soxhlet extraction. The n-hexane extracted 0.44%, ethyl acetate extracted 0.85% and methanol extracted 17.63% of the plant material. Preliminary phytochemical screening of the extracts revealed that U.togoensis stem bark contains carbohydrates, glycosides, cardiac glycosides, saponins, tannins, flavonoids, alkaloids, steroids and triterpenes. Antimicrobial activities studies showed that all the extracts are active against the same set of bacteria and fungi. The extracts have activity against MRSA,S,aureus, C. ulcerans, E. coli, S. typhi, S. dyseateriae,P. mirabilis, N. gonorrhoae, C. albicans, C. krusei and C. tropicalis but have no activity against VRE, S. pyogenes, P. aeruginosa and C.stellatoidea. Thus, the U. togoensis stem bark extract is both antibacterial and antifungal. The MIC of the extracts are generally lower than the MBC/MFC. In most cases, the MIC of the extracts is 10mg/mL and the MBC/MFC is 20mg/mL

KEY WORDS: *Phytochemical screening, Uapacatogoensis, antimicrobial activity.*

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I. INTRODUCTION

The World Health Organisation (WHO) supports the use of herbal medicine provided they are safe and efficacious (Omale*et al*, 2010). However, the use of herbal medicine has continued to suffer antagonism from allopathic medicine practitioners who claim that it has no scientific basis. In comparison to allopathic medicine, herbal medicines cost less, are more often used to treat chronic diseases, and the occurrence of undesirable side effects seems to be less frequent (Bandaranayake, 2006). Herbal remedies often contain a variety of biochemicals (phytochemicals or active ingredients) found naturally in the plants which are responsible for the plants medicinal properties. In most cases, the active ingredients responsible for the pharmacological/biological activity of the herbal products are unknown to the traditional herbal medicine providers and thus lack standardization and dosage is usually estimated based on experience (Agyare*et al*, 2006; Bandaranayake, 2006).

The correct identification of the herbal material and the active ingredients is essential to quality control, safety and efficacy, acceptability and possible integration into the national healthcare system of herbal remedies (Bandaranayake, 2006). Furthemore, the knowledge of the phytochemicals of plants is important to many disciplines (Borokini and Ayodele, 2012). The knowledge is important to botany for studying systematics and evolution as an aid in botanical classification; to ecology for studies on structural variation of secondary metabolites in space which could lead to the discovery of adaptive mechanisms, coevolution of organisms in their ecosystems, defense, pollination, and dispersion strategies of plant; to pharmacology for pharmacological investigations, development of screening techniques and utilization of chemical diversity of phytochemicals as endless source of new drugs; to biotechnology as the basis for the selection of species for micropropagation and for monitoring infochemicals produced by cell cultures (Borokini and Ayodele, 2012). Kon*éet al*, (2007) investigated the *in vitro* activity of 90% ethanol stem/leaf extract of *U. togoensis* against *Streptococcus pneumoniae* and *Staphylococcusaureus* and found thatthe extracts are potent against these pathogenic microbes. Despite the importance of *U. togoensis* to traditional medicine, pharmacology and other disciplines, no phytochemical screening of its extracts has been carried out to discover the metabolites and bioactive agents responsible for its antimicrobial activities.

Therefore, this study was carried out to discover the phytochemicals responsible for the antimicrobial (antimicrobial and antifungal) activity of the extracts

MATERIALS AND METHODS

Collection and identification: The fresh leaves of *U. togoensis* collected from the open field at Inye in Ankpa Local Government Area of Kogi State, Nigeria were identified in the herbarium of the Department of Biological Sciences, Faculty of Science, Kogi state University, Anyigba.

Preparation of the plant material: The stem bark of *U. togoensis* tree were collected in February 2013 and cleared of the rhytidome. The inner bark (phloem or non-collapsed secondary phloem) was air-dried and later pulverized with the aid of mortar and pestle into the powder. The powder was packed into a clean plastic container with screw cap for subsequent work.

Extraction: One kilogram of the powdered stem bark was successively extracted using the three selective solvents: n-hexane, ethyl acetate and methanol with the aid of Soxhlet extractor. Each extract was dried to a constant mass for use in phytochemical screening and antimicrobial assays.

Test Organisms: The microorganismsused in study were clinical isolates obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. All isolates were checked for purity and the bacteria and fungi were maintained in slants of Nutrient agar and Sabouraud Dextrose agar respectively. The test microbes were Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin – Resistant Enterococci (VRE), standard strains of *Staphyloccusaureus*, *Corynebacteriumulcerans*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi*, *Shigelladysenteriae*, *Psueomonasaeruginosa*, *Proteus mirabilis*, *Neisseria gonorrhea*, *Candida albicans*, *Candida stellatoidea*, *Candida krusei* and *Candida tropicalis*.

Antimicrobial Assays: This include agar well diffusion method to determine the zones of inhibition (ZI) of microbial growth and broth microdilution method to determine the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC).

Well diffusion method: Blood and sabouraud dextrose agars were used as the growth media for the bacteria and fungi respectively. Each media was prepared according to the manufacturer's instruction: dissolved and sterilized at 121^0C for 15 minutes. The media were allowed to cool to 45^0C and 20mL of the media was dispensed into each petridish and then covered to cool and solidify. The blood agar media was seeded with 0.1mL standard inoculums of the bacterium and spread evenly over the surface of the medium with the use of sterile swabs. The plates were dried at 37^0C for 30 minutes. By the use of a standard sterile cork borer of 6mm in diameter, a well was cut at the centre of each inoculated plate and 0.1mL of the solution of the extract was filled into each well. The plates were then incubated at 37^0C for 24 hours for the bacteria and at 25^0C for 48 hours for the fungi. After the incubation, the plates were immediately observed for the zones of inhibition of growth. The diameters of the zones of inhibition were measured with a transparent ruler and the results recorded in millimeters (mm).

Broth Microdilution method:

A. Determination of the MIC.

Nutrient and Sabouraud dextrose broth was prepared according to the manufacturer's instruction. 10mL of the broth was dispensed into each test tube, sterilized at 121^0C for 15 minutes and then allowed to cool. Normal saline solution was prepared and dispensed into test tubes and inoculated with the test organism. Dilution of the test microorganisms in the normal saline was performed until the turbidity matched that of Mc-Farland's scale number 0.5 by visual comparison at the point the test microorganisms has concentration of about 1.5×10^8cfu/mL. Incubation was done at 37^0C for 6 hours for bacteria and 26-28^0C for fungi. The solution of the extract was prepared by dissolving 0.1g of the extract in 10mL of the sterile nutrient broth. Two-fold serial dilution of each extract in the broth was performed to obtain the concentrations of 10mg/mL, 5mg/mL, 2.5mg/mL, 1.25mg/mL and 0.625mg/mL. Then 0.1mL of the standard inoculums of the test organisms in the normal saline was then inoculated into the different concentrations of the extract in the broth and incubated at 37^0C for 24 hours for bacteria and at 26-28^0C for 48hours for the fungi. Afterwards, the broth in test tubes were checked for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was taken as the MIC.

B. Determination of the MBC and MFC.

Blood agar and sabouraud dextrose agar were prepared according to manufacturer's instruction: they were boiled and sterilized at 121^0C for 15 minutes, cooled to 45^0C and poured into sterile petri dishes. The plates were covered and the media were allowed to cool and solidify. The contents of the MIC test tubes were then sub-cultured on the prepared media and incubated at 37^0C for 24 hours for bacteria and at 26-28^0C for fungi, after which the media was observed for colony growth. The lowest concentration of the extracts without colony growth was taken as the MBC for bacteria and MFC for fungi.

Preliminary Phytochemical Screening

This was carried out using standard procedure described by Trease and Evans, (1996). The assays include Carbohydrate, Glycosides, Tannins, Flavonoids, Saponins, Cardiac glycosides, Anthraquinones, Steroids, Triterpenes and Alkaloids.

II. RESULTS

Table 1: Extracts yields from *U. togoensis* stem bark

Solvent	Mass of extract(g)	% recovery
n-Hexane	4.4076	0.44
ethyl acetate	18.5046	1.85
methanol	176.3600	17.64

Table 2: Phytochemical groups in *U. togoensis* stem bark.

Test	Methanol extract	Ethyl acetate extract	n-hexane extract
1. **Carbohydrate**			
a. Molisch test	+	+	+
b. Fehlings test	+	-	+
2. Glycosides			
a. Fehlings test	+	+	-
b. Ferric chloride test	+	+	-
3. Tannins			
a. Ferric chloride test	+	+	-
b. Bromine water test	-	-	-
c. Lead sub-acetate test	+	+	+
4. Flavonoids			
a. Shinoda test	+	-	-
b. Sodium hydroxide test	+	-	-
c. Ferric chloride test	+	+	-
5. Saponins			
a. Frothing test	+	-	-
b. Haemolysis test	+	-	-
6. Cardiac glycoside			
a. Keller-killiani test	+	+	+
b. Kedde's test	+	+	-
7. Anthraquinones			
a. Free anthracene derivatives	-	-	-
b. Combined anthracene	-	-	-
8. Steroids and Triterpenes			
a. For steroids	+	+	+
b. For triterpenes	+	+	+
9. Alkaloids			
a. Dragendoff's test	+	-	-
b. Wagners test	+	-	-
c. Meyers test	+	-	-

Key: + = Present, - = Absent

Table 3: Diameter of Zone of inhibition of U. togoensisstem bark extracts and controls.

Test organism	n-Hexane extract (mm)	ETAC extract (mm)	MET extract (mm)	Erythromycin	Fluconazole
Methicillin resistant *Staphylococcusaureaus* (MRSA)	20	27	23	35	0
Vancomycin resistant *enterococci* (VRE)	0	0	0	0	0
Staphylococcusaureaus	21	32	24	37	0
Corynebacteriumulcerans	18	25	22	40	0
Streptococcuspyogenes	0	0	0	38	0
Escherichia coli	18	24	22	39	0
Salmonella typhi	21	27	24	37	0
Shigelladysenteriae	22	30	25	40	0
Pseudomonas aeruginosa	0	0	0	0	0
Proteus mirabilis	18	23	20	35	0
Neisseria gonorrhoae	18	24	21	37	0
Candida albicans	17	22	20	0	35
Candida stellatoidea	0	0	0	0	37
Candida krusei	20	27	23	0	38
Candida tropicalis	18	24	21	0	35

Key: ETAC = Ethyl acetate. MET = Methanol

Table 4: The MIC and MBC/MFC of *U. togoensis* stem bark extracts.

Test organism	Antimicrobial Parameter	n-Hexane extract (mg/mL)	ETAC extract (mg/mL)	MET extract (mg/mL)
Methicillin resistant *Staphylococcus aureus* (MRSA)	MIC	5	2.5	5
	MMC	20	10	20
Vancomycin resistant enterococci (VRE)	MIC	-	-	-
	MMC	-	-	-
Staphylococcus aureus	MIC	5	2.5	5
	MMC	20	5	10
Corynebacteriumulcerans	MIC	10	5	5
	MMC	20	10	10
Streptococcus pyogenes	MIC	-	-	-
	MMC	-	-	-
Escherichia coli	MIC	10	5	5
	MMC	20	10	20
Salmonella typhi	MIC	5	2.5	5
	MMC	20	5	10
Shigelladysenteriae	MIC	5	2.5	5
	MMC	20	5	10
Pseudomonas aeruginosa	MIC	-	-	-
	MMC	-	-	-
Proteus mirabilis	MIC	10	5	5
	MMC	20	20	20
Neisseria gonorrhoae	MIC	10	5	5
	MMC	20	10	20
Candida albicans	MIC	10	5	5
	MMC	20	10	20
Candida stellatodea	MIC	-	-	-
	MMC	-	-	-
Candida krusei	MIC	5	2.5	5
	MMC	20	10	10
Candida tropicalis	MIC	10	5	5
	MMC	20	10	20

III. DISCUSSION OF RESULTS

Extraction yields:n-hexane being a non-polar solvent extracted the least (0.44%) of the plant components followed by ethyl acetate which is a solvent of medium polarity and extracted 0.85% of the plant component. It was found that methanol was the most effective, and extracted up to 17.64% of the plant material(Seidel *etal*, 2006). The extraction by these solvents is according to their respective polarity. Thus, n-hexane extracts essentially nonpolar substances (fatty substances), ethyl acetate extracts substances of medium polarity and methanol extracts essentially polar substances.

Phytochemical groups present: There was an even distribution of phytochemicals with all the solvents. Thus, n-hexane extract was composed of tannins, cardiac glycosides, steroids, triterpenes and glycosides while the major phytochemicals in the ethyl acetate extract was composed of carbohydrates, glycosides, tannins, cardiac glycosides, steroids and terpenes. The methanol extract is composed of carbohydrates, glycosides, alkaloids, steroids and triterpenes. This means the *U. togoensis* stem bark extract is composed of carbohydrates, glycosides, tannins, flavonoids, saponins, cardiac glycoside, alkaloids, steroids and triterpenes.

Antimicrobial activities: The production of zone of inhibition by the extracts in the agar well diffusion assay is indicative of the activity of the extract. Therefore, from Table 3, it is seen that the n-hexane extract is active against MRSA, *S. aureus, C. ulcerans, E. coli, S. typhi, S. dysenteriae, P. mirabilis, N. gonorrhea, C. albicans, C. kursei* and *C. tropicalis* but not active against VRE, *S. pyogenes, P. aeruginosa* and *C. stellatoidea*. The activity of ethyl acetate and methanol are of similar pattern. The extracts have activity against both bacteria and fungi. This indicates that the *U. togoensis* stem bark extracts of n-hexane, ethyl acetate and methanol are both antibacterial and antifungal. The ZI of n-hexane ranges from 18 to 22mm, that of ethyl acetate ranges from 23 to 32mm and that of methanol ranges from 20 to 25mm but that of erythromycin ranges from 35 to 40mm for the sensitive bacteria indicating that the erythromycin drug is more active than the extracts of *U. togoensis.* Also, the ZI of n-hexane ranges from 17 to 20mm, ethyl acetate ranges from 22 to 24mm and methanol extract ranges from 20 to 23mm for the sensitive fungi but the ZI of fluconazole ranges from 25 to 38mm meaning that fluconazole is more active than the *U. togoensis* stem bark extracts. But whereas the *U. togoensis* extracts are both antibacterial and antifungal, erythromycin is only antibacterial and fluconazole is only antifungal. From table 4, it is seen that the MIC is generally lower than the MBC/MFC. This means at the MIC the extracts are neither bactericidal nor fungicidal. In most cases, the MIC of the extract is 10mg/mL and the MBC/MFC is 20mg/mL.Hence we conclude that the MIC of the extracts is 10mg/mL and the MBC/MFC is 20mg/mL.

IV. CONCLUSIONS

The extracts of *U. togoensis* demonstrated activities against certain bacteria and fungi. This means the extract possess both antibacterial and antifungal activity. As the extract have activityagainst MRSA but not VRE, it means the extract can be used to treat antibiotic resistant diseases due to MRSA but not VRE. The activity of the extracts against *S. aureus* justifies its use in ethnomedicine to treat respiratory diseases, and vomiting. As the extracts are active against many more bacteria than *S. aureus* only it is concluded that the extracts of *U. togoensis* can treat many more microbial infections than is presently used to treat. It is recommended that the activity of the extracts against many more antibiotic resistant strains of pathogenic microbes studied to discover the full spectrum of activity of *U. togoensis* extract so gain full knowledge of the potential of this plant as an antibacterial and antifungal agent.

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