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Antibacterial Susceptibility of the Constituents of Ethanol Crude Extract and the Neutral Metabolite of the Root of *Curculigo pilosa* Hypoxidaceae

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Abstract

Powdered fresh rhizomes of *Curculigo pilosa* Hypoxidaceae (African Crocus) were analyzed for their phytochemicals and antibacterial activities. The crude ethanol extract showed the presence of saponins, flavonoids, steroids, alkaloids, glycosides and phenols. This crude sample was partitioned into acidic, basic and neutral metabolites. The neutral portion indicated the presence of saponin, flavonoid, steroid, glycoside, phenol and alkaloid. The antimicrobial susceptibility tests using the crude sample and the metabolites were carried out on four human pathogens; *Escherichia coli, staphylococcus aureus, pseudomonas aeruginosa and streptococcus faecalis.* At a concentration of 100 mg/mL: the crude sample showed IZD values of 22 mm for *Escherichia coli*, 29 mm for *staphylococcus aureus*, 23 mm for *pseudomonas aeruginosa* and 38 mm for *streptococcus faecalis*. The neutral metabolite at the same concentration gave IZD values of 35 mm for *Escherichia coli*, 36 mm for *staphylococcus aureus*, 35 mm for *pseudomonas aeruginosa* and 43 mm for *streptococcus faecalis*. The acidic and basic metabolites did not show any significant antibacterial activity. The control drug ampiclox at 100 mg/mL showed IZD values of 40 mm for *Escherichia coli*; 32 mm for *staphylococcus aureus*; 26 mm for *pseudomonas aeruginosa and* 22 mm for *streptococcus faecalis* respectively. The minimum inhibitory concentration (MIC) of the ethanol crude extract and the neutral metabolite were at 25 mg/mL, 50 mg/mL and100 mg/mL, the result apparently justified the use of fresh rhizomes of *Curculigo pilosa* in the treatment of infections.

Keywords: antimicrobial, neutral, phytochemicals, root, tests

1. Introduction

Plants have been major sources of medicine and plant secondary metabolites have been attributed to most plant therapeutic activities (Fabeku, 2006; Neumann & Hirsch, 2000). Phytochemicals have shown great promise in the treatment of intractable infectious diseases (Yesileda, 2005). The local uses of plants and plant products in health care are even much higher particularly in those areas with little or no access to modern health services (Saed, Arshad, M. Ahmad, E. Ahmad, & Ishaque, 2004). Traditional medicine is a source of primary health care to 80% of the world's population (Alves & Rosa, 2005; Pei, 2001) the use of herbal medicine has always been part of human culture, and African culture is one of them.

Several herbs from plants most especially have various pharmacologically active compounds that have the potential for the prevention/treatment of several cancers. The antioxidant potency of flavonoids in medicinal plants possess a variety of anticancer effects such as arrest of cell growth, inhibition of kinase activity, induction of apoptosis, reduction in tumour-invasive behavior and suppression of matrix metalloproteinase's secretion (Chau et al., 2000). Many African plants have been hypothesized to have phytochemicals that make them effective anti-inflammatory, antihypertensive, antitussive, antibiotics, anticancer agents and so on.

This article looked at one of these numerous Nigerian's medicinal plants, *Curculigo pilosa*. *Curculigo pilosa* Hypoxidaceae is known as "epakun" in Yoruba language; "orima" in Edo language, "dòòyár kùréégéé" in Hausa language and "pkeve' in Tiv languge. The plant is used in herbal or traditional medicine to treat leukemia, gonorrhea, cough, as astringent agent, aphrodisiac and demulcent in Southwestern Nigeria (Odugbemi & Akinsulire, 2006); there is a hypothesis that the rhizomes could possess anti-cancer or anti-tumour properties.

Soladoye et al. (2012) reported its use as an antidiabetic; the vasoconstictive activity of its benzylbenzoate and non-lignan glucosides were also reported (Palazzino, Galeffi, Federici, Monache, Cometa, & Palmery, 2000).

The research investigated the phytochemicals present in the crude ethanol extract of this plant species and the neutral metabolite derived from it. Also the antibacterial susceptibility of this crude sample and its neutral metabolite were verified. The root of *Curculigo pilosa* Hypoxidaceae from this work had shown itself as a highly promising source of potent antimicrobial drug. It is obviously worthy of large scale investment as the need for antimicrobial, antifungal and antiviral drugs against resistant pathogens persist around the globe.

2. Experimental

2.1 Plant Material

The fresh root of *Curculigo pilosa* (350 g) was purchased from Awolowo Market in Mushin local government of Lagos State in the month of June 2012 and identified by Applied Biology Department, Ebonyi State University Abakaliki. It was washed with distilled water to remove dirt, oven dried at moderate temperature for two days, pulverized and stored in sealed cellophane until needed. Test organisms *candida albicans*, *streptococcus faecali*, *escherichia coli, pseudomonas aeruginosa*, *staphylococcus aureus* and *coliform bacilli* were obtained from the Applied Microbiology Department of Ebonyi State University Abakaliki. All reagents used were of analytical grade.

2.1.1 Extraction

About 300 g of the ground sample was soaked in an aspirator bottle in 500 mL of ethanol and allowed to stay for 72 h. The solvent was filtered and the filtrate distilled off to obtain 50 g greenish gummy substance.

2.1.2 Phytochemical Screening

Ejele and Alinor method (2010) was used in the preparation of the acidic, basic and neutral metabolites. The crude sample and the acidic, basic and neutral metabolites were subjected to phytochemical analysis using the AOAC (2005) method and other methods (Nwokonkwo, 2009) for the presence or absence of saponin, flavonoid, alkaloid, glycoside, tannin, polyphenol and steroid.

2.1.3 Antibacterial Activity

Test organisms used were *candida albicans*, *streptococcus faecali*, *escherichia coli, pseudomonas aeruginosa*, *staphylococcus aureus* and *coliform bacilli*. Agar well diffusion method was used. A cork borer of 1 cm was used to perforate the Mueller-Hinton agar in aseptic condition. The turbidity of the sample was prepared according to Mcfarland standard; a sterile swab stick was used and rotated inside the inoculums. The swab was streaked evenly over the surface of the medium before introduction of the plant extract (Agah et al., 2011). The determination of the minimum inhibitory concentration (MIC) was carried out on the plant extracts at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL according to the method proposed by George and Roger (2002). These were prepared in agar nutrient and distributed into sterile tubes into sterile test tubes. One (1) mL of the extract was separately added to the agar plates for the bacteria and poured into petri dishes. The test organism was spotted on the surface of the solidified extract-agar mixture in increasing order of concentration. The plates were allowed to dry for 30 min and incubated at 37 ^{o}C for 18 h, after which the plates were examined for microbial growth. The lowest concentration of the extract which showed little or no visible growth of the microorganism was taken as the MIC of the extract (Roberts, Shore, Paviour, Holland, & Morris, 2006).

3. Results and Discussion

The phytochemical screening of the crude sample of plant extract is shown on Table 1. The table showed the presence of saponin, flavonoid, steroid, alkaloid and glycoside but absence of tannin (Ejele & Nwokonkwo, 2013). The phytochemical screening of the acidic, basic and neutral metabolite is shown in Table 2. In Table 2, the acidic metabolite showed the presence of saponin, flavonoid, steroid, glycoside and phenol but the absence of alkaloid and tannin. The basic metabolite showed the absence of tannin, flavonoid and steroid but contained saponin, alkaloid, glycoside and phenol. The neutral metabolite showed the presence of all the phytochemicals except tannin. Inhibition zone diameter of the crude sample, acidic, basic and neutral metabolites is shown on Table 3 while Table 4 explains the inhibition zone diameter of the control drug. The crude sample showed IZD values of 22 mm, 29 mm, 23 mm and 38 mm at 100 mg/ mL for *Escherichia coli, Pseudomonas aeruginosa, Streptococcus faecalis and Staphylococcus aureus* respectively. The IZD values obtained showed that the crude sample was potent against the organisms used in the analysis. The IZD values for the neutral metabolite at 100 mg/mL were 35 mm, 36 mm, 35 mm and 43 mm for *Escherichia coli, Pseudomonas aeruginosa, Streptococcus*

faecalis and Staphylococcus aureus, a positive result also indicating the potency of this plant species. The MIC of the crude and neutral metabolite on these microorganisms was at 25 mg/mL, 50 mg/mL and 100 mg/mL. The IZD values of the neutral metabolite were significantly high compared to the control drug except in the case of Escherichia *coli* where the control drug at 100 mg/mL gave IZD value of 40 mm. Alkaloids, phenols, glycosides and saponins have proven to have antibacterial, antifungal, astringent and such like properties and might be the reason why the crude extract gave positive result for the antimicrobial tests. The neutral metabolite which had the same constituents as the crude had higher IZD values and invariably higher inhibitory action against the pathogens. This probably indicated that these active principles were present in high concentration (s) in this medium. The concentration of the alkaloid, saponin and phenol in the basic medium might not have been significant enough to produce the required antibacterial activity. Also, the concentration of the saponin and phenol in the acidic medium might also not have been enough for the lethal dose required for it to be bacteriostatic or bactericidal. The extracts from these two media showed no appreciable MIC at 25 mg/mL, 50 mg/mL and 100 mg/mL.

Table 1. Phytochemical screening of the ethanol crude extract of the plant sample

Test	Observation	Inference
Tannin: **2 mL sample + 5 mL H_2O + 2 drops $FeCl_3$**	formation of bluish precipitate	Tanins not indicated
Saponin: **2 mL sample + 5 mL H_2O and 2 mL olive oil**	formation of frothing	saponin indicated
Flavonoid: **2 mL 10 % NaOH + 2 mL 10 % HCl**	formation of white precipitate	flavonoid ndicated
Steroid: **$CHCl_3$ + 5 mL conc. H_2SO_4**	formation of dark-brown colour	steroid indicated
Alkaloid: **2 mL Wagner's reagent + 2 mL sample**	formation of precipitate	alkaloid indicated
Glycoside: **0.5 mL $CHCl_3$ layer + 2 mL CH_3COOH + 1.0 mL conc. H_2SO_4**	formation of two layers with reddish-brown colour at interface	glycoside indicated
Phenol: **0.5 mL sample + 4 mL H_2O + 0.3 mL 1.0 M $FeCl_3$ solution**	dark-brown precipitate formed	phenol indicated

Table 2. Phytochemical screening of the acidic, basic and neutral metabolites

Phytochemical	Acidic Metabolite	Basic Metabolite	Neutral Metabolite
Tannin:	not indicated	not indicated	not indicated
Saponin:	not indicated	indicated	indicated
Flavonoid:	not indicated	not indicated	indicated
Steroid:	indicated	not indicated	indicated
Alkaloid:	not indicated	indicated	indicated
Glycoside:	indicated	indicated	indicated
Phenol:	indicated	indicated	indicated

Table 3. Inhibition zone diameter of the crude sample, acidic, basic and neutral metabolites

Test Organisms	Diameter of Zone of Inhibition(mm) Concentration (100 mg/mL)			
	Crude	Acidic	Basic	Neutral
Escherichia coli	22	-	-	35
Pseudomonas aeruginosa	29	-	-	36
Streptococcus faecalis	23	-	-	35
Staphylococcus aureus	38	-	-	43

Table 4. Inhibition Zone diameter of Ampliclox the control drug

Test Organisms	Diameter of Zone of Inhibition(mm) Concentration (100 mg/mL)
	Ampiclox
Escherichia coli	40
Pseudomonas aeruginosa	32
Streptococcus faecalis	26
Staphylococcus aureus	22

The partitioning of the crude sample into acidic, basic and neutral metabolites presented a new method of investigation as it helped in concentrating the active constituents/phytochemicals into acidic, basic and neutral media or as in this case, the neutral medium; and would make isolation through column chromatography and final characterization of the isolates easier.

Further research is still continuing; to isolate each phytochemical, test the susceptibility to microorganisms and structure elucidation.

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