PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EVALUATION OF THREE MEDICINAL PLANTS USED IN NIGERIA.

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Abstract

Methanol extract of three Nigerian medicinal plants were screened for antimicrobial activity using modified Kirby-Bauer disc diffusion and agar dilution techniques to determine the diameters of zone of inhibition and minimum inhibitory concentrations (MIC) of the extracts respectively. The extract of each of the plants were tested against five clinical bacterial isolates comprising of two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and three Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*) organisms. All the extracts exhibited moderate to high level of antimicrobial activities against these microorganisms. Phytochemical screening of powdered plant material revealed the presence of some secondary metabolites such as alkaloids, saponins, tannins, anthraquinones and flavonoids. These Nigerian medicinal plants could be developed into cheap, safe and culturally acceptable standardized herbal products and may serve as a source of new molecules for broad-spectrum antimicrobial agents.

Key words: *Dissotis rotundifolia, Costus lucanusianus, Solenostemon monostachys*, Methanol extract, Antimicrobial activity

Introduction

Plants form an integral part in traditional medicinal practices in all cultures worldwide and a sizeable portion of the world population uses plant for prevention and management of different kinds of ailments. The rural population in particular who do not have access to primary health care, either as a result of non-availability or inability to afford it depends solely on plant remedies for their health problems (Johanna *et al* 2005). Many of the plants used in ethnomedicine have been found to contain useful therapeutic substances and a good number of them have found their way into orthodox medical practice. For instance, morphine, digoxine, quinine etc which are very useful drugs commonly prescribed by clinicians until date are derived from plants. The search for new compounds which can be useful in the management of diseases that have defied current therapeutic options focuses majorly on plants as a reliable source of lead substances (Kong *et al* 2003). The high incidence of résistance organisms has made it mandatory for a continuous search for a more potent and safe therapeutic agents. Many plants, which are used in ethnomedicine, have shown promising activity against a host of disease causing microorganisms and they have been documented. *Dissotis rotundifolia, Costus lucanusianus, Solenostemon monostachys* are medicinal plants used for the prevention and management of different kinds of disease conditions in Nigeria and some countries in West African sub region.

Dissotis rotundifolia Triana, (Melastomataceae), which is one of the 140 species in the genus Dissotis is a native of tropical West Africa (Loigier, 1994; Wagner et al., 1990) and common names include Pink lady (English), Ebafo (Bini), and Awede (Yoruba). It is a versatile perennial slender creeping herb with prostate or ascending stems up to 40 cm high, rooting at the nodes and producing from seeds and stolons (Abere *et al* 2009). Traditionally, in various parts of tropical Africa, it has various uses. In Nigeria, the plant is used mainly for the treatment of rheumatism and painful swellings, and the leaves decoction is used to relieve stomach ache, diarrhoea, dysentery, cough, stop abortion, conjunctivitis, circulatory problems and veneral diseases. It is used in East Africa for the treatment of bilharzias (Kokwaro, 1976), and in Cameroun, the leaves are used for dysentery (Noumi and Yomi, 2001).

Costus lucanusianus J. Braun & K. Schum (Costaceae) is an herbaceous plant of the forested areas of Africa. It is well known in the southern Ivory Coast for its antiabortive activities (Sawadogo, 1986). Indeed, the juice of the stem has been shown to exhibit tocolytic activity (Komenan, 1986; Foungbe et al., 1987).

The Lamiaceae are mostly herbs or shrubs comprising about 200 genera and 3,200 species, commonly with aromatic, herbage, quadrangular stems, and verticillate inflorescences. The leaves are opposite or whorled, and are simple or occasionally pinnately compound *S.monostachys* is repotedly used in the treatment of type II diabetes, tuberculosis and magagement of treatened abortion (Erah , et al 1996; Idu et al 2006 and Folu. et al 2009).

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Materials and Methods

Plant materials

The Plants were collected in Ugbowo area of Benin City, South- South Nigeria between January and June 2009. Documented folklore use and oral information from the residents formed the basis for the selection of the parts of the plants collected and tested. The authentication of the plant material was done by Dr. B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. The voucher specimen numbers for *S. monostachys*, *D. rotundifolia* and *C. lucanusianus* are 1023, 1143 and1098 respetively.

Phytochemical screening

The powdered material of the plants were subjected to different kinds of chemical tests to investigate the presence of secondary metabolites such as saponins, tannins, flavonoids anthraquinones cyanogenic glycosides, cardiac glycosides and alkaloids using standard procedures (Evans, 1996; Brain and Turner, 1975;Ciulei, 1981; Harborne, 1992).

Preparation of the extracts

The air-dried plant materials were pulverized into fine powder. A weighed portion (200g) of each of the plant powder was extracted with aqueous methanol by cold maceration for 48 hours. The extracts were filtered and the solvent removed at low temperature (40 - 45^{0}C). Stock solutions of 100mg/mL and 400mg/mL were prepared for the disc agar diffusion and micro dilution broth assays respectively.

Antimicrobial Screening of Plants' Extracts

Agar diffusion method (zone of inhibition measurement)

Five clinical bacterial isolates (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*) were obtained from the microbial bank of department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy Niger Delta University, Wilberforce Island, Nigeria; for the antimicrobial screening of the plants' extracts. The bacterial isolates were standardized using colony suspension method and matching the strain's suspension with 0.5 McFarland standard to give a resultant concentration of 1.5 x 10^8 cfu/ml. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer diffusion technique by swabbing the Mueller–Hinton agar (MHA) (Oxoids U.K) plates with the resultant saline suspension of each strain and six wells were made in the agar with aid of cork borer (No. 4, the diameter of the borer is 6mm). The wells were sealed at the bottom with molten sterilized agar, 0.1ml and 0.2ml of each of the plants extract representing 10mg/ml, and 20mg/ml respectively were aseptically dispensed into the labeled wells while antibiotic disc (ciprofloxacin 5µg) used as control was placed on the agar aseptically. The plates were then incubated at 37°C for 24 hours. The zone diameters of inhibition produced by each concentration of the plants extracts and that of the antibiotic disc was measured and recorded (CLSI, 2008).

Agar dilution method (MIC measurement)

Each of the plants extracts was used to prepare Mueller–Hinton agar plates of varying concentrations from 5mg/ml to 40mg/ml and the plates were all spot-inoculated with each organism's standardized suspension and incubated for growth at 37^{o}C for 24h. The plates that showed no growth were observed, while those with lowest inhibitory concentrations were taken as the minimum inhibitory concentrations (MIC) of the extract (mg/ml) against each tested organisms (CLSI, 2008).

Results

The methanol extracts of the three plants showed considerable antimicrobial activities at concentration of 20mg/ml against most of the tested organisms (Table 2). Their antimicrobial activities were comparable to the standard antibiotic (ciprofloxacin 5µg) used. The minimum inhibitory concentration (MIC) of the plants' extracts showed that the extract of *S. monostachys* exhibited the most antimicrobial activity by its low MIC (5mg/ml to 20mg/ml) on all the tested organisms (Table 3). The phytochemical screening (Table 1) indicates the presence of secondary metabolites such as Tannins, Flavonoids, Saponins, Anthraquinones, Alkaloids.

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Table 1: Phytochemical Screening of the Powdered Plant Material

Constituents	*S. monostachys*	*D. rotundifolia*	*C. lucanusianus*
Alkaloids	-	+	-
Glycosides	+	+	+
Flavonoids	+	-	-
Tannins	+	-	+
Saponins	+	+	+
Anthraquinones	+	-	-

\+ Present
\- Absent

Table 2. Zone diameter of Inhibition (mm) of the Methanol extracts of *C. lucanusianus, D. rotundifolia and S. monostachys* against five selected organisms

Organisms	**The Diameter of Zone of Inhibition of Plants Extracts (mm)**						
	C. lucanusianus Concn. (mg/ml)		*D. rotundifolia* Concn. (mg/ml)		*S. monostachys* Concn. (mg/ml)		Control (ciprofloxacin 5µg)
	10	20	10	20	10	20	
B. subtilis	-	**12**	-	-	-	**16**	**12**
S. aureus	-	**9**	**13**	**16**	**24**	**26**	**15**
Ps. aeruginosa	-	**14**	-	**11**	-	**15**	**23**
E. coli	-	**9**	-	**12**	-	**11**	**21**
K. pneumonia	-	-	-	**11**	-	**13**	-

\- No activity

Table 3: Minimum Inhibitory Concentration (MIC) in $mgml^{-1}$ of the Methanol extracts of *C. lucanusianus, D. rotundifolia and S. monostachys* against five selected organisms

Organisms	The MIC of the Plants' Extracts (mg/ml)		
	C. lucanusianus	*D. rotundifolia*	*S. monostachys*
B. subtilis	15	>30	15
S. aureus	30	30	5
P. aeruginosa	20	30	20
E. coli	30	>30	20
K. pneumonia	-	>30	20

Discussion

The three methanol extracts generally exhibited antimicrobial activities on all the tested organisms at concentration of 20mg/ml. Ekundayo and Ezeogu (2006) reported a weak antimicrobial activity of the dichloromethane and methanol extract of the aerial part of *S. monostachys.* In our study reported here, the methanol extract of the leaves of *S. monostachys* exhibited the most pronounced activity on the Gram-positive organisms (*Bacillus subtilis* and *Staphylococcus aureus*) by its wide zone diameter of inhibition and low minimum inhibitory concentration. The reasons for the discrepancy in their report and our own are; the aerial part of the plant which they used comprises of the stem and leaves; if the activity resides only in the leaves, the stem which may not have any activity would weaken the activity of the leaves. Secondly, they extracted the plant material with dichloromethane and methanol in succession thus distributing the active principles into the dichloromethane and methanol; if the active compounds act in synergy, the extraction process would weaken the activity. *S. monostachys* showed greater activity against the tested micro-organism compared with the other plants. The control antibiotic (ciprofloxacin- a broad-spectrum antibiotic) had very little significant activity against these strains of bacteria. The presence of copious amounts of Tannins and Flavonoids in *S. monostachys* is responsible for the high antimicrobial activity (Scalbert, 1991). The result justifies the use of these plants in traditional medicine for the treatment of various kinds of diseases including infectious disease (Idu *et al.*, 2006).

S. monostachys' extract could be a possible choice of treatment of infections caused by resistant strains of Gram-positive bacteria especially *Bacillus subtilis* and *Staphylococcus aureus*, which other known antimicrobial agents like ciprofloxacin are not able to treat.

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