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Evaluation of Antimicrobial Activities of Extracts of Five Plants Used in Traditional Medicine in Nigeria

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Abstract: Ten extracts from five Nigerian medicinal plants were screened for antimicrobial activity using disc agar diffusion and micro dilution broth assays. Methanol and Dichloromethane extracts of each of the plants were tested against five collection culture microorganisms consisting of two Gram positive (*Staphylococcus aureus* ATCC 12600, *Bacillus subtilis* ATCC 6051) and two Gram negative (*Escherichia coli* ATCC 11775 and *Pseudomonas aeruginosa* ATCC 10145) bacteria and the pathogenic yeast, *Candida albicans*(ATCC 18804). All the extracts exhibited moderate to high level of broad spectrum antimicrobial activities against these microorganisms. The antimicrobial activity was measured by the diameter of zone of inhibition and Minimum Inhibitory Concentration (MIC). The diameters of zones of inhibition range from 7mm-30mm at 2000μg mL of crude extract per disc. The MIC values were between 31.5μg mL-500μg mL or greater. The activities of some of the crude extracts were comparable to the reference antibiotics at standard concentrations used per disc. The potential of these Nigerian medicinal plants for development of cheap, culturally acceptable standardized herbal medicines and as sources of novel molecules for broad spectrum antimicrobial agents is discussed.

Key words: Medicinal plants, antimicrobial activities, methanol extracts

INTRODUCTION

Infectious diseases in form of gastrointestinal, respiratory, urinary and skin infections caused by various groups of microorganisms remain the common health problems in the developing countries. Some of these infections can become serious generalized illness and life threatening.

In Nigeria as in other parts of Africa, traditional system of medicine based mainly on medicines from medicinal plants remain the mainstay of primary healthcare for majority of the rural populace. This system of medicine is rich in ethno medical knowledge of the use of medicinal plants in treating the category of diseases that affect the people most. This is reflected in the large number of plants reported to be in use in treating infectious conditions in Africa[1]. These medicinal plants employed in traditional medicine represent potential sources of cheap and effective standardized herbal medicines (phytomedicines) and novel molecules for the development of new chemotherapeutic agents[2,3].

This study presents the results of the evaluation of the antimicrobial activities of extracts from five Nigerian medicinal plants against strains of *S. aureus*, *B. substilis*, *E. coli*, *P. aeruginosa* and *C. albicans*.

MATERIALS AND METHODS

Plant materials: The plant materials were collected from various rural communities in Southeastern Nigeria. Documented reports of folklore use in African traditional medicine[1] and the traditional practices by herbal medicine practitioners in Southeastern Nigeria provided the basis for selecting the parts of the plants collected and tested. Authentication of plant materials was done by Mr. Alfred Ozioko of the Department of Botany, University of Nigeria. Voucher Specimens were deposited at the herbarium of the International Centre for Ethno Medicine and Drug Development, Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria.

Preparation of extracts: Air-dried plant materials were powdered and extracted by successive maceration in Dichloromethane (CH_2Cl_2) and Methanol for 24 h in glass percolators. The process was repeated once. The CH_2Cl_2 and Methanol extracts were filtered and the solvent removed under reduced pressure in a rotary evaporator. The dried extracts were stored at 2-4°C until tested. A stock solution of 100 mg mL and further dilutions of each of the extracts were made in Dimethylsulphoxide (DMSO) or distilled water. The

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extracts solutions were filtered sterilized using cellulose nitrate filter with a pore size of 0.2 μm (Nalgene Company, Rochester, NY). Solutions were used immediately.

Microorganisms: The strains of microorganisms used (*Staphylococcus aureus* ATCC 12600, *Bacillus substilis* ATCC 6051, *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC11775 and *Candida albicans* ATCC18804) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The bacteria were maintained on Nutrient agar and the yeast on Sabouraud Dextrose agar slants after reactivation from the freeze- dried materials according to the instruction in product leaflet insert. All culture media used were from Difco Laboratories, Detroit, USA.) A cell suspension of each microorganism was prepared by transferring 2-3 isolated colonies on Nutrient agar plate to a sterile vial containing sterile physiological saline. The turbidity was adjusted to McFarland turbidity standard 0.5 with sterile physiological saline. About 65ml of Nutrient agar or Sabouraud Dextrose agar was poured into each of 150mm plastic Petri dishes. The agar was left to set and 0.5 mL of diluted (1:100) cells suspension was aseptically spread on the agar surface to give an inoculum size of 10^5-10^6 colony forming units (CFUs).

Antimicrobial assays: Disc Diffusion assays: This was done according to Kirby-Bauer method[4] with slight modification. Filter paper disc (Whatman No.3, 6mm diameter) were placed in glass Petri dishes and sterilized in hot air oven. Each disc received 20 μL portion of extract stock solution (100 mg mL) to give a concentration of 2000μg of crude extract per disc. The discs were placed in an incubator at 35-37°C for 2 hours to dry. They were used immediately or kept in sterile vials at 4°C until used, usually within 3 days.

Discs of Ampicillin, Streptomycin, Tetracycline, Gentamicine and Nystatin were similarly prepared. Ten (10) μL portions of appropriately diluted antibiotics were placed on each paper disc.

The discs of the extracts and antibiotics were placed on the seeded agar plates which, were then incubated at 37°C for 24 h. The diameter of any clear zone of inhibition around the discs was measured manually with a transparent ruler. The experiments were replicated three times for each extracts and twice for reference antibiotics.

Microdilution broth assay: Stock solutions of the extracts (100 mg mL) in DMSO were diluted with Nutrient broth or Sabouraud Dextrose broth. To obtain concentration of 100 mg mL, 100 μL of each diluted extract solutions and 2x100 μL negative controls were brought unto a microtitre plate(96 well, round bottom) to obtain 6 serial dilution wells with final concentrations of 15.63, 31.25, 62.5, 125, 250, 500 μg mL. For each test organisms, 2 columns were used in parallel experiments. Standardized Suspensions of the test organisms were made as previously described and 1:10 dilution was made in nutrient broth just before the dilution wells were inoculated with 100 μL of suspension of the test organism. The negative control wells received 100 μL of Nutrient broth. Sabouraud Dextrose broth was used for *Candida albicans.* The inoculated microtitre plates were then incubated at 37°C for 12-18 h under aerobic conditions. The MIC was defined as the lowest extract concentration that prevented visible growth as detected by unaided eyes. Where there was a difference of one step between parallel experiments, the higher concentration was taken as the MIC. No case of difference more than one titer step between parallel experiments was encountered. The MIC of standard antibiotics against the test bacteria were obtained from BIOMIC Computer program (Giles Scientific, Inc, Santa Barbara, CA) by manual entry of the diameters of zone of inhibition against each organisms.

RESULTS

Ten extracts obtained from five Nigerian Medicinal plants were screened for antimicrobial activities against four bacteria species and the yeast *Candida albicans*. The antimicrobial activities of the extracts were measured by the diameter of the zone of inhibition using the disc agar diffusion assay and by the microdilution broth assay to determine the potency of the extracts by measuring the Minimum Inhibitory Concentration (MIC) of the extracts. The summary of the diameters of zone of inhibition produced by the plant extracts against the test organisms is shown in Table 1. All the five extracts exhibited moderate to high level of antimicrobial activity against all the test organisms. Generally, the extracts appeared to be more active against the Gram Positive bacteria than the Gram Negative species. The antimicrobial activities of the Methanol extracts of the five plants against *S. aureus* are presented in Table 2. The methanol extract of the root of *Cryptolepis sanguinolenta* manifested the highest antimicrobial activity against the test organisms as shown by the mean diameter zone of inhibition of 22 mm. In comparison with the reference antibiotics, 2000 μg mL of the crude extract of this plant produced diameters of zone of inhibition comparable to 10 μg of Gentamicin and Ampicillin against *S.aureus* and *B. subtilis*. It produced a larger zone of inhibition against *E. coli* than 10 μg of Ampicillin. It was more active against *Pseudomonas aeruginosa* than 10 μg of Ampicillin. The activity of this

Table 1: Antimicrobial activities of extracts by diameter of zone of inhibition (mm)

Test microorganisms							
Plant species and (Family)	Part tested	Extract type	*S.aureus*	*B.subtilis*	*E.coli*	*P.aeruginosa*	*C.albicans*
Cryptolepis sanguinolenta	Root	Methanol	22.0[a]	30.0	14.0	8.0	8.0
(Asclepiadaceae)		Dichloromethane	9.0	9.0	7.0	7.0	11.0
Erythrina senegalensis	Root	Methanol					
(Papilionaceae)		(Chloroform fraction)	10.0	8.0	9.0	7.0	8.0
		Dichloromethane	9.0	7.0	9.0	8.0	7.0
Gossypium arboretum	Leave	Methanol	11.0	10.0	10.0	7.0	7.0
(Malvaceae)		Dichloromethane	7.0	7.0	7.0	7.0	7.0
Holarrhena floribunda	Leave	Methanol	9.0	10.0	7.0	7.0	8.0
(Apocynaceae)		Dichloromethane	7.0	10.0	8.0	7.0	7.0
Solenostemon monostachys	Aerial parts	Methanol	8.0	8.0	9.0	7.0	7.0
(Labiatae)		Dichloromethane	9.0	15.0	7.0	7.0	7.0
Antibiotics		Ampicillin 10 µg	30.0[b]	28.0	11.0	7.0	NT
		Gentamicin 10 µg	22.0	37.0	25.0	18.0	NT
		Tetracycline 30 µg	25.0	18.0	18.0	8.0	NT
		Streptomycin 10 µg	12.0	11.0	18.0	8.0	NT

Mean of 3 values to the nearest millimeter Mean of 2 values to the nearest mm NT-Not tested

Table 2: Means diameter of zone of inhibition and MIC values of methanol extracts of the plants against *S. aureus*

Plant species	Part tested	Diameter zone of inhibition (mm)	MIC (µg mL)
Cryptolepsis sanguinolenta	Root	22.0	125
Erythrina senegalensis	Root	10.0	12.5
Gossypium arboretum	Leaves	11.0	62.5
Holarrhena floribunda	Leaves	9.0	>500
Solenostemon monostachys	Aerial parts	8.0	500
Antibiotics			
	Ampicillin 10 µg	30.0	0.25
	Gentamicin 10 µg	22.0	0.50
	Tetracycline 30 µg	25.0	1.80
	Streptomycin 10 µg	12.0	28.0

extract against *P. aeruginosa* is similar to those of 30 µg of Tetracycline and 10 µg of Streptomycin against the organism.

Microdilution broth assay: Table 2 shows the MIC values of the ten plant extracts against the test organisms. The MIC values range between 31.25 µg mL -500 µg mL of crude extract. The methanol extract of the leave of *Gossypium arboreum* showed the highest potency against *S.aureus* and *B. subtilis*. Other extracts with noticeable MIC values were *Cryptolepis sanguinolenta* and *Erythrina senegalensis*.

DISCUSSION

The antimicrobial activities of some of the plants studied have been reported. Boakye-Yiadom[5] found weak antimicrobial activity in the aqueous extract of *Cryptolepis sanguinolenta*. In our study reported here, the methanol extract from the root of the plant produced the largest diameters of inhibition zone of 22mm and 30mm against *S. aureus* and *B. subtilis*, respectively. The antimicrobial activity of the plant has been linked with some of the indole alkaloids isolated from the plant, of which Cryptolepine seems to be the most important[6]. Biyiti, Pesando and Puiseux-Dao[7] reported the antimicrobial activity of the chloroform extracts and two flavanones isolated from the bark of the Cameroonian *Erythrina sigmoidea*. The flavanones were not active against all gram negative bacteria tested. In our study, we found broad spectrum activity in the chloroform fraction of the methanol extracts of the root of *Erythrina senegalensis s*. The extract was both active against *E. coli* and *P. aeruginosa* producing inhibition zones comparable to those produced by 10 µg of Streptomycin sulphate against these organisms (Table 1). It is possible that the root of *E. senegalensis s* contains high concentration of the antibacterial compounds or different antibacterial agents. Based on the MIC values and the zones of inhibition, the methanol extracts of *Gossypium arboreum* and *Cryptolepis sanguinolenta* were the most active in this study.

The results presented here contribute to the scientific validation for the use of these medicinal plants in traditional medicine and serve as a guide for selection of plants with antimicrobial activity for further phytochemical work on the isolation and identification of the active compounds. Furthermore, these results show the potential of some of these medicinal plants for development of standardized culturally acceptable herbal medicines for local use as broad spectrum antimicrobial agents.

REFERENCES

1. Iwu, M.M., 1993. Handbook of African Medicinal plants, C R C press, Boca Raton, FL.
2. Farnsworth, N.R., O. Akerele, A.S. Bingel, D.D. Soejarto and Z. Guo, 1985. Medicinal plants in therapy. Bull. Wld. Health Organ., 63: 965-981.
3. Iwu, M.M., 1997. Production of Phytomedicines and Cosmetics from indigenous Genetic Resources: from Lab to market: In: Iwu, M. M., Sokomba, E. N., Okunji, C. O., Obijiofor, C. and Akubue, P. I (eds): Commercial Production Of Indigenous Plants as Phytomedicines And Cosmetics. BDCP press. Lagos, Nigeria, pp: 1-5.
4. Bauer, A.W., M.D.K. Kirby, J.C. Sheries and M. Turck, 1966. Antibiotic susceptibility testing: Standard single disc diffusion method. Am. J. Clinical Pathol., 45: 493-496.
5. Boakye-Yiadom, K., 1979. Antimicrobial properties of West African medicinal plants: ll Antimicrobial activity of aqueous extracts of *Crytolepis sanguinolenta* Q. J. Crude Drug Res., 17: 78-80.
6. Cimanga, K., T. De Bruyne, L. Pieters, J. Tohe, L. Tona, K. Kambu, D. Vanden Berghe and A.J. Vlietink, 1998. Antibacterial and antifungal activities of neocryptolepine, biscryptolepine and cryptoquindoline alkaloids isolated from Cryptolepis sanguinolenta Phytomedicine, 5: 209-214.
7. Biyiti, L., D. Pesando and S. Puiseux-Dao, 1988. Antimicrobial activity of two flavanones isolated from the Cameroonian plants Erythrina sigmoidea Planta Medica, pp: 126-128.