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Full Length Research Paper

Phytochemical and microbial screening of herbal remedies in Akwa Ibom State, South Southern Nigeria

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Ethanolic extracts of six commonly used medicinal plants (by traditional medicine practitioners in Uyo and Ikot-Ekpene), *Vernonia amygdalina del*. ("Etidod"), *Ocimum gratissimum L*. ("Nton"), *Smilax anceps* wild. ("Odufad"), *Holarrhena floribunda* ("Idid ikot"), *Parkia biglobosa* ("Epo igba"), and *Khaya senegalensis* ("Ogawo"), were analysed phytochemically and evaluated for their significant antimicrobial activity (P < 0.05) against different multiple drug resistant, MDR (pathogenic) organisms: *Neisseria gonorrhea*, *Shigella flexineri*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Mean inhibitory zones were calculated for each of the extracts. Five of the plant extracts contain saponins and tannins only; two contain salkowski, and keller-kilini only, while three extracts contain just one of alkaloids, plobatannins, liberman. The results revealed that each of the six plants extracts, at different concentrations of 80 and 40 mg/ml, exhibited various degrees of inhibition and activity index on the test organisms. The extract of the *O. graissimum* ("Nton"), had the highest mean inhibitory zone of 23 mm against *S. flexineri*. This was followed by *H. floribunda* ("Idid ikot") with a mean inhibitory zone of 22 mm against *N. gonorrhoea*, *V. amygdalina* ("Etidod") with a mean inhibitory zone of 19 mm against *S. aureus* and *S. anceps* wild ("Odufad") had a mean inhibitory zone of 17 mm against both *N. gonorrhoea* and *S. aureus*. The MIC results of *S. flexineri* in 26.24 mg/ml of *O. gratissimum* excite us to analyse for its MBC which was 47.24 mg/ml. These results provide a rationalization for the traditional use of these plants for the treatment of infectious diseases.

Key words: Ethanolic extract, phytochemical, pathogenic organisms, *Smilax anceps*, activity index.

INTRODUCTION

The global resurgence of medicinal plants in treatment occasioned by the emergence of multiple antibiotic resistances in recent years has not left Africa behind. Traditional medicine practitioners in Uyo and Ikot-Ekpene utilize *Vernonia amygdalina del*. ("Etidod"), *Ocimum gratissimum L*. ("Nton"), *Smilax anceps* wild. ("Odufad"), *Holarrhena floribunda* ("Idid ikot"), *Parkia biglobosa* ("Epo igba"), and *Khaya senegalensis* ("Ogawo") for prophylactic and therapeutic purposes. *O. gratissimum* is popular throughout Nigeria. It is called "Efinrin" by Yoruba tribe of South-Western Nigeria , Ebavbokho in Bini (Delta State), Aai doya ta gida in Hausa (Northern Nigeria) and Nchonwu in Igbo (South Eastern Nigeria) (Owulade, 2004). The six plants are qualified to be called medicinal plants by virtue of the fact that they have been in therapeutic use against various diseases like parasitic infection, URTI, Syphilis, other veneral diseases like gonorrhea, pneumonia and enteric fever respectively for over one hundred years (Lewis and Elvin-Lewis, 1977). Just a few studies in Africa are available on the phytochemistry, dosage of administration and contraindication of medicinal plants compared to the array of available medicinal plants (Gundiza, 1985; Ebana et al., 1991; Kola et al., 2002). That explains why certain less potent toxic synthetic chemicals from the west are recognized and preferred to these more potent, less toxic medicinal plants.

The potentials for multiple resistances by the isolates used in this research have been demonstrated by many researchers around the world (Diep et al., 2008). Many isolates of *Escherichia coli* (and *Staphylococcus aureus)* for instance is resistant to ampicillin, amoxicillin, tetracycline and trimethoprim-sulfamethoxazole (Aibinu et al., 2004). As far back as in the year 2000, 7.1% cases of multiple drug resistant bacterial isolates to conventional

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antibiotics was reported (Sahm et al., 2001). Umolu et al. (2006) reported that 67% of the resident isolates exhibited multiple drug resistance. The therapeutic failure of antibiotics in Nigeria, Africa and indeed all parts of the world buttresses the need for given support for the use of local medicinal plant (Oloke et al., 1988).

The aim of this study was to evaluate *in vitro* the antimicrobial potency of *V. amygdalina del.*, *O. gratissimum L.*, *S. anceps* wild., *H. floribunda*, *P. biglobosa*, and *K. senegalensis* against pathogenic bacteria, *Neisseria gonorrhea*, *Shigella flexineri*, *E. coli*, *S. aureus* and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Study period

This phytochemistry and microbial screening of herbal remedies was carried out between February and July, 2008.

Plant materials

Parts of *V. amygdalina del.* ("Etidod"), *O. gratissimum L.* ("Nton"), *S. anceps* wild. ("Odufad"), *H. floribunda* ("Idid ikot"), *P. biglobosa* ("Epo igba"), and *K. senegalensis* ("Ogawo") were collected from different medical practitioners in various localities in Akwa Ibom State, Nigeria. They were properly identified in the Pharmacognosy Laboratory of the University of Uyo.

Preparation of extracts

The plant parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 ml 80% methanol (Merck, Darmstadt) for 24 h by using Soxhlet equipment (Khan et al., 1988). The extract was filtered using Whatman filter paper No. 1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at – 20°C.

Phytochemical screening

Phytochemical analysis of the plant extracts were carried out in order to confirm the presence of Alkaloids (by Dragendorff's test and Mayer's test), Cardiac glycosides (by Liberman's test, Salkowski test and Keller-Killani test), Anthraquinones (Borntrager's test). Saponins, Tannins, and Phlobatannins were also analysed using standard phytochemical methods as described by Sofowora (1993), Culei (1982) and Trease and Evans (2002).

Microorganisms

Five samples used as test organisms (already confirmed as multiple drug resistant isolates) were collected from Standard Medical Laboratories, 12b Aka Road, Uyo. They were purified, characterized and identified as reported by Cruikshank et al. (1975) as *N. gonorrhea*, *S. flexineri*, *E. coli*, *S. aureus and* K. *pneumonia*.

Screening for antimicrobial activities

The plant extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to obtain different concentrations of the plant extracts for determining the antimicrobial activity by standard disc method. Sterile filter paper discs (5 mm diameter) were impregnated with about 5 µg/disc of different concentrations of each of the plant extracts for 30 min and the discs were overlaid on Mueller Hinton agar plates. The discs were tested in triplicate, including one with a solvent blank (DMSO) and 2 for the standard drugs. Inhibition zones were calculated as the difference between disc diameter (5 mm) and the diameters of inhibition (Hewitt and Vincent, 1989).The mean inhibition zones were used to calculate the activity index. Activity index (AI) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug (Singh et al., 2002). The plates were incubited at a temperature of 37°C for 24 h.

Standard disc (30 µg/disc) of Cefotaxime (stable to ß-lactamase) was used as the standard drug as control. This disc was placed aseptically on the prepared Mueller-Hilton agar plates along with standardized inoculums from a 24 h culture. The diameter of visible zones of inhibition was measured both in main experiment and the control after 24 h of incubation.

Minimum inhibitory and lethal concentration

The MIC was carried out by broth dilution method (Salm and Washington, 1990). This was used to achieve extract concentration of 3600, 3240, 2916 and 2624 µg/ml from the initial 40 mg/ml and to achieve 4252, 7200, 6480, 5832, 5249, 4724 µg/ml from the initial 80 mg/ml. These various concentrations were used to determine the Minimum Inhibitory and Bactericidal Concentration (MIC and MBC). To achieve this, effort were made to subculture from the broth dilution tube. The concentration with growth in the subculture was interpreted as inhibitory (MIC) while those without growth were regarded as lethal or bactericidal (MBC).

RESULTS

Phytochemistry of the plant extracts

The result of the phytochemical analysis show that the six

Table 1. Phytochemistry of the Plant Extracts

Components.>	Alkaloids	Saponins	Anthraquinones	Plobatannins	Tannins	CARDIAC GLYCOSIDES		
Plant Extracts						Liberman's	Salkowski	Keller-Kiliani
Vernonia amygdalina	-	+++	_	_	++	_	_	_
Ocimum gratissimum	-	+++	_	_	+++	_	_	_
Smilax anceps	+++	+++	_	_	_	_	_	_
Holarrhena floribunda	-	++	_	+++	+++	-	_	_
Parkia biglobosa	-	+++	_	_	++	++	+++	++
Khaya senegalensis	-	-	_	_	+++	-	++	++

+++: strongly positive, ++: moderate positive, +: trace positive
- : negative.

plants are rich in at least one of Alkaloids, Saponins, Anthraquinones, Plobatannins, Tannins and Cardiac glycosides. Table 1 shows the phytochemical screening results of ethanolic extracts of the six plants used in this study.

Antimicrobial activity

The crude extracts of the tested plants showed variable degrees of antimicrobial activities against the tested organisms. The results of the antimicrobial activity of the six crude extracts were clearly presented in Tables 3 against *N. gonorrhea*, *S. flexineri*, *E. coli*, *S. aureus* and *K. pneumonia* at concentrations of 40 and 80 mg/ml respectively. At extract concentration of 80 mg/ml, *S. anceps* and *H. floribunda* have 21 and 26 mm as mean diameter of inhibition against the dreaded *N. gonorrhea*. The latter also have the mean diameters of 22, 12, 12 and 22 mm for *S. flexineri*, *E. coli*, *S. aureus* and *K. pneumonia* respectively.

The entire six plant extracts exhibited 10, 16, 12, 16, 12 and 8 mm mean diameter of inhibition to the clinical *E. coli*. In this case, *O. gratissimum* and *H. floribunda* exhibited the highest degree of activity and activity index (16 mm and 1.1). The inhibitory activity exhibited by cefotaxime (15 mm) against *E. coli* was higher than that exhibited by some of the tested crude plant extracts at both concentrations. This is clearly reflected by the activity index, A.I with respect to the cefotaxime. Also unlike cefotaxime, none of the six crude plant extracts had any zone on inhibition for the atypical etiology of pneumonia, *K. pneumoniae*.

The extracts showed the highest degree of inhibition against *S. flexineri* at concentration of 80 mg/ml, followed by *S. anceps* and the least was observed from *K. senegalensis* while at 40 mg/ml, *O. gratissimum* had the highest inhibitory concentration against *S. flexineri*. At the crude extract concentration of 80 mg/ml, the tested plant extract showed a higher degree of inhibition against *S. flexineri* than cefotaxime except for *P. biglobosa* and *K. senegalensis*

Comparatively, the results of the mean zones of inhibition of the cefotaxime as obtained for the multi-drug clinical isolates used in this study is depicted with Table 2 and 3. Most of the crude extracts exhibited lower inhibition concentration than the initial 40 and 80 mg/ml used for sensitivity testing. MIC of the extract to *K. pneumoniae* was not carried out because it seems quite resistant to the initial 80 mg/ml out rightly. We observed that the use of higher concentration might be ridiculous as this might not be achievable in serum concentration if this extract is taken to treat a disease. *S. anceps* had the least concentration of inhibition of 3240 µg/ml and *H. floribunda* had 2916 µg/ml respectively against *N. gonorrhoea*. The most impressive of the crude extracts' least inhibition concentration was observed in *O. gratissimum* against *S. flexineri* at the concentration of 2624 µg/ml. The most prominent aetiologies in nosocomial infection, *S. aureus* and *E. coli* had the least inhibitory concentration of 3600, 3240, 3240 and 4000 µg/ml, 3600 and 3600 µg/ml to *V. amygdalina del*. ("Etidod"), *O. gratissimum L*. ("Nton"), *S. anceps* wild. ("Odufad") respectively.

The MIC results of *S. flexineri* in 2624 µg/ml of *O. gratissimum* encourage us to analyse for its MBC which was 4724 µg/ml.

DISCUSSION

The result of this study revealed that the extract of the six plant extracts exhibited antimicrobial activities against the multi-drug isolates used in this study. The result obtained from this study also revealed that the plant contained bioactive compounds known to contribute to the antimicrobial potential of plants (alkaloid, saponins, tannins, plobatannins and cardiac glycosides). These bioactive compounds have been reported to have antimicrobial potency (Sofowora, 1993). Comparatively, the zones of

Table 2. Inhibitory effect of the 5 mg/disc six crude plant extracts at concentration 40 mg/ml

Microorganisms	*N. gonorrhoea*				*S. flexineri*				*E. coli*				*S. aureus*				*K. pneumonia*			
Plant Extracts	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I
Vernonia amygdalina	_	_	_				8	0.6	4	4	4	0.3	8	8	8	0.5	_	_	_	_
Ocimum gratissimum	_	_	_		20	20	20	1.4	7	9	8	0.5	9	11	10	0.6	_	_	_	_
Smilax anceps	11	13	12	0.8	11	9	10	0.7	8	8	8	0.5	13	11	12	0.7	_	_	_	_
Holarrhena floribunda	18.5	17.5	18	1.1	4	4	4	0.3	10	10	10	0.7	_	_	_		_	_	_	_
Parkia biglobosa	_	_	_	_	6	6	6	0.4	7.5	8.5	8	0.5	_	_	_		_	_	_	_
Khaya senegalensis	_	_	_	_	3.5	4.5	4	0.3	_	_	_		_	_	_		_	_	_	_
Cefotaxime	15	17	16		13	15	14		17	13	15		15	19	17		8	12	10	

Note: Zone of inhibition, Z.I was measured in millimeters exclude the disc diameter (5 mm)
A.I means Activity Index to 1 d.p.

Table 3. Inhibitory Effect of the 5 mg/disc Six Plant Extracts at concentration 80 mg/ml

Microorganisms	*N. gonorrhea*				*S. flexineri*				*E. coli*				*S. aureus*				*K. pneumonia*			
Plant Extracts	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I	1	2	Mean Z.I	A.I
Vernonia amygdalina	_	_	_		19.5	20.5	20	1.4	8	12	10	0.7	9	11	10	0.6	_	_	_	_
Ocimum gratissimum	_	_	_		24	26	25	1.8	17	15	16	1.1	19	21	20	1.2	_	_	_	_
Smilax anceps	19	23	21	1.3	22	22	22	1.6	12.5	11.5	12	0.8	23	21	22	1.3	_	_	_	_
Holarrhena floribunda	24	28	26	1.6	17	15	16	1.1	19	13	16	1.1	8	8	8	0.5	_	_	_	_
Parkia biglobosa	_	_	_		11.5	12.5	12	0.9	11.5	12	12	0.8	6	6	6	0.4	_	_	_	_
Khaya senegalensis	_	_	_		10	6	8	0.6	8	8	8	0.5	13	11	12	0.7	_	_	_	_
Cefotaxime	15	17	16		13	15	14		17	13	15		15	19	17		8	12	10	

Note: Zone of inhibition, Z.I was measured in millimeters exclude the disc diameter (5 mm)
A.I. = Activity Index.

inhibition exhibited by the cefotaxime used as the control against the *K. pneumoniae* and *E. coli* confirmed them as extended spectrum beta lactamase producers in line with the National Committee for Clinical Laboratory Standards (NCCLS). This standard which was developed by broth microdilution and disk diffusion screening tests using selected antimicrobial agents. Each *K. pneumoniae*, *Klebsiella oxytoca*, or *E. coli* isolate is considered a potential ESBL-producer if the test results are as follows:

Disk diffusion

cefpodoxime < 22 mm
ceftazidime < 22 mm
aztreonam < 27 mm
cefotaxime < 27 mm
ceftriaxone < 25 mm (NCCLS, 1999)

It is notable that several studies have been conducted on the antimicrobial properties of herbs and spices (Khan et al., 1998; Dorman and Deans, 2000; Hsieh et al., 2001). However, not many researchers put the use of such multi drug resistant or beta lactamase producers into consideration. The beauty of these six crude extracts therefore is there display of better potency ($P < 0.05$) than the third generation parental cephalosporin, cefotaxime.

While the battle between man and microbes continues, starting with the defeat suffered by Penicillin to the emergence of vancomycin and trimethoprim resistance (Cui and Hiramatsu, 2003), there is need to consider the use of potent extracts like *V. amygdalina del*. ("Etidod"), *O. gratissimum L*. ("Nton"), *S. anceps* wild. ("Odufad"), *H. floribunda* ("Idid ikot"), *P. biglobosa* ("Epo igba"), and *K. senegalensis* ("Ogawo") that have shown some measures of antimicrobial potency judging by the significant antimicrobial activity ($P < 0.05$) (the activity index, A.I), MIC and MBC results of their crude extracts. While, it is very dangerous to used large doses of most synthetic drugs due to their toxicity, the body system can still accommodate some plant extracts at some relatively high doses. This suggests why those who have suffered therapeutic failure from orthodox medicines in this study area result to the use of these six plant extracts with better results.

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