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Full Length Research Paper

Antimicrobial nature and use of some medicinal plants in Nigeria

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Forty eight medicinal plants in Nigeria were screened for their antimicrobial activity. Twenty three (47.91%) of the plants caused over 70% mortality of the test organism which include anopheline and culicine larva. *Bacillus* spp. and *Escherichia coli* were shown to be susceptible to the antimicrobial activity of some plants. The antimicrobial activities of plants examined in this study showed that plants are capable of producing toxic materials, which may exert some physiological effect on the target organisms.

Key words: Antimicrobial, medicinal, nature, Nigeria, plants.

INTRODUCTION

Many approaches are being intensified for the effective control of disease vectors such as mosquitoes and some etiological agents especially through the use of antimicrobial agents from natural origin based on the observation of resistance to common chemicals by certain organisms (WHO, 1973; Prescott et al., 2002; Xavier et al., 2003). Among the broad groups of insecticides or biocides used are chlorinated hydrocarbon, organophosphorus, carbomate, pyrethroids, synergists and repellents. Those from natural products include pyrethroids that are insecticide formulated from plant origin (WHO, 1973). This kind of formulation is important in this study. With recent scientific and Biotechnological development, the use of some genetically modified plants or living things in general can enhance some improvements in this area (Orvos et al., 1990; Corich et al., 1997). Generally, various researchers have also intensified the use of biocontrol agents for integrated management of specific disease in recent time (Ponmurugan and Baby, 2006).

Variety of plant species yielded products capable of exerting some physiological influence upon the human beings. This kind of toxic products (toxin) produced by higher plants against a variety of living organisms is termed phytotoxins. The substantial amounts of toxic materials that encompass a wide variety or range of groups of compounds in plants are referred to as the secondary plant substances (Pelzar et al., 1986; Adedapo, 2002).

In order to enhance adequate processing and preservation of plant parts as a guide leaves, herbs and flowers may be dried between 20 and 40°C and barks and roots between 30 and 65°C (Trease and Evans, 1972). The storage of the plant material is done by keeping them in sealed containers with dehydrating agent. In case of latex, for example, the latex of *Ficus asperifolia* the content is been stored in the refrigerator at 10°C.The commerce and quality control of this substances includes sampling, preliminary examination of the foreign matter, moisture content, extractive values for example as in the determination of water-soluble or alcohol-soluble extracts is commonly used as a means of evaluating drugs or extracts constituents (Nostro et al., 2000). The study of Kothari et al. (2005) on conservation of a typical medicinal plant gives more insight into this. Standardization of antimicrobial agents is however very important for large scale and commercial purposes. Similarly, Aburjai et al. (2001), confirms folkloric accounts in literatures on the use of different plant preparations for disease control purposes as intensified in this study.

MATERIALS AND METHODS

Plant sources

Plant materials used for this study were obtained from forest zones particularly Western Nigeria where all the processing was done. Among the plant materials used were leaf, bark/stem, root and latex as specified for the research work. Some of the plant materials that are commonly grown in the study area include the roots of *Adenia cissampelli* and *Gossypium arboreum* that were dried, grounded and processed. The collection of latex of *F. asperifolia* was done

Table 1. Antimicrobial activity of some active plants obtained in Nigerian.

Code	Plant species	Plant part / extraction solvent	Extracts activity at LD ≥ 70
1	*Adenia cissampelli* (Passifloraceae)	Stem/Methanol	--
2	*Adenia cissampelli* (Passifloraceae)	Root/Methanol	A
3	*Baphia Nitida* (Leguminosae)	Stem/ethanol	A
4	*Dennentia tripetala*	Fruit/ethanol	A
5	*Enantia polycarpa* (Enantioblastae)	Stembark/Methanol	A
6	*Ficus thonningii* (Moraceae)	Root/water	--
7	*Ficus asperifolia* (Moraceae)	Latex	A
8	*Gossypium* spp (Malraceae)	Stem/Methanol	A
9	*Gossypium* spp (Malraceae)	Root/Methanol	--
10	*Hexalobus cripsiflora*	Stembark/Water	A
11	*Hexalobus cripsiflora*	Stembark/Methanol	A
12	*Khaya grandiflora* (Meliaceae)	Stembark/Ethanol	A
13	*Khaya grandiflora* (Meliaceae)	Stembark/Methanol	A

-- = Negative, A = positive or very active plant extract, LD = lethal dosage.

by direct extraction from the plant species. The extracts of this latex were stored at 10°C to preserve them. The latex was diluted into suitable concentrations to test its larvicidal activity.

Processing of plant extracts for activity

The processing of the raw plant materials into extract samples was carried out in the laboratory with the aid of some solvents (ethanol, methanol, petroleum ether, $CHCl_3$ and water) heated at high temperature of about 100°C and passed through an extractor into which individual plant samples previously grinded was neatly packed in order to obtain different active substances. The plant extracts obtained were processed into low uniform concentration of 5 mg/ml using a cold concentrate facility that possesses a round tube inside which the extract samples were concentrated.

Microorganisms used

Pure cultures of *Escherichia coli* were obtained from the University College Hospital, University of Ibadan, Nigeria, while the *Bacillus subtilis* was obtained from wastewater in the eatery premises of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The organisms were further identified using standard microbiological measures and maintained in stock culture at 4°C.

Larvicidal activity

This was done by dissolving 100 mg of each readily prepared plant extract in 20 mls of water in a sample tube into which the test organism (about 20 mosquito larvae) were introduced. This preparation of larvae in extract solution were made in duplicates and left over for 24 h. Thus 1 ml experimental solution contains 100/20 mg/ml medium concentrate that is 5 mg/ml plant concentrate.

Lethal dosage (LD)

The plant extracts after weighing to known concentrations were diluted in 50 ml standard flasks to give lethal dosage value range at LD_{50} to LD_{100}. Samples of *Adenia cissampelli* root extracted with methanol: (a) 600 mg diluted to 50 ml with water – 600/50=12 mg/ml (b) 25 ml from A diluted to 50 ml with water – 6 mg/ml and subsequent concentrations of (c) 3 mg/ml, (d) 1.53 mg/ml, (e) 0.753 mg/ml, (f) 0.373 mg/ml, and (g) 0.183 mg/ml were obtained from this value. Similar calculations were intensified for other values.

***In vitro* antimicrobial test**

The disc agar diffusion described by Nostro et al. (2000) and Xavier et al. (2003) was used to determine growth inhibition pattern of bacteria by the plant extracts. A loopful of the test organisms, that is, *Bacillus* species and *E. coli* used for this study from broth cultures was diluted and evenly spread by streaking on the Tryptone Soy Agar plates under aseptic condition.

Paper disc impregnated with the sterilized plant extracts were placed with the aid of sterile forceps at equidistant on the plating culture. Discs dipped in sterilized distilled water served as control. The samples were incubated at 37°C for 24 h and examined for zones of growth inhibition and the diameter of these zones were measured in millimeters.

RESULTS

The result in Table 1 shows lethal effect of various Nigerian plants tested for their larvicidal activity using different solvents on the appropriate plant sources. The plants coded 1-13 with very active antimicrobial activities includes:

*Roots of *Adenia cissampelli* (Passifloraceae), and *Gossypium* spp. (Malvaceae).
*Stem of *Baphia nitida* (Leguminosae), and *Enantia polycarpa* (Enantioblastae)
*Stembark of Hexal*obus cripsiflora*, and *Khaya grandiflora* (Meliaceae).
*Latex of *Ficus asperifolia* (Moracaeae) and
*Fruit of *Dennentia tripetala*.

Table 2 shows the mortality rate of the larvae due to lethal effect of the plant extract solution at the end of 24 h culture. This test reveals the antimicrobial activity of

Table 2. Antimicrobial activity of some Nigerian plants code P 30-65.

Sample code	Plant sources	Larvae mortality (%)			Sample code	Plant sources	Larvae Mortality (%)		
		1st Trial	2nd Trial	Average			1st Trial	2nd Trial	Average
P30(a)	Ethanol extract of *Lippia adonensia* flower	-	-	-	P48 (a)	Methanol extract of *Sphenocentum jollyamum* stem			
(b)		100	100	100	(b)		95	100	97.5
P31 (a)	Water extract of *Magnifera indica* stembark	-	-	60.12	P49 (a)	Methanol extract of *Sphenocentum jollyamum* root			
(b)		95.24	25		(b)		85	85	85
P32 (a)	Ethanol extract of *Magnifera indica*	-	-	-	P50 (a)	Ethanol extract of *Sphenocentum jollyamum* root			
(b)		0	0	0	(b)				
P33 (a)	Methanol extract of *Moringa oleifera* stem	-	-	-	P51 (a)	Methanol extract of *Triplochiton scleroxylon* stembark			
(b)		0	0	0	(b)		37.5	33.33	35.42
P34 (a)	Methanol extract of *Moringa oleifera* root	-	-	-	P52 (a)	Methanol extract of *Triplochiton scleroxylon* stembark			
(b)		100	100	100	(b)		31.58	40	35.79
P35 (a)	Methanol extract of *Mallotus subutolus* root	-	-	-	P53 (a)	Methanol extract of *Trichisia subardata* stem			
(b)		100	100	100	(b)		35.29	34.78	35.04
P36 (a)	Methanol extract of *Mallotus arboreum* stembark	-	-	-	P54 (a)	Methanol extract of *Trichisia subardata* root			
(b)		100	100	100	(b)		81.82	95	88.41
P37 (a)	Methanol extract of *Mallotus myristica* rootbark	-	-	-	P55 (a)	n. BuOH extract of *Tetrapleura tetraptera* leaf			
(b)		50	60	55	(b)		20	19.05	19.52
P38 (a)	Methanol extract of *Musanga cecropioida* stembark				P56 (a)	Water extract of *Tetrapleura tetraptera* leaf			
(b)		13.64	23.08	18.36	(b)		14.29	30	22.15
P39 (a)	Methanol extract of *Pycnanthus angolensis* stem				P57 (a)	$CHCl_3$ extract of *Tetrapleura conophonium* fruit			
(b)		52.94	58.33	55.64	(b)		100	100	100
P40 (a)	Methanol extract of *Pycnanthus angolensis* root				P58 (a)	Ethanol extract of *Uvaria calophyllum* stem			
(b)		100	95	97.5	(b)		100	95.24	97.62
P41 (a)	Methanol extract of *Psidium guajava* stembark				P59 (a)	Ethanol extract of *Xylopia aethiopica* rootbark			
(b)		31.58	100	65.79	(b)				
P42 (a)	Methanol extract of *Poliphlia longifolia* seed				P60 (a)	Ethanol extract of *Z. depricum*			
(b)		30	50	40	(b)		100	82.61	91.30
P43 (a)	$CHCl_3$ extract of *Poliphlia longifolia* pericarp				P61 (a)	Methanol extract of *Crotalaria pateus*			
(b)					(b)		90.48	55	72.74
P44 (a)	Methanol extract of *Ptecocera barteri* root				P62 (a)	Methanol extract of *Piper guinences* fruit			
(b)		42.11	50	46.06	(b)		100	100	100
P45 (a)	Ethanol extract of *Palisota hirsuta* leaf				P63 (a)	Ethanol extract of *Dennetia tripetala* stembark			
(b)		38.89	37.5	38.2	(b)		100	100	100
P46 (a)	Methanol extract of *Ritichica tragnama* stem				P64 (a)	Methanol extract of *P. longifolia* fruit			
(b)					(b)		95	100	97.5
P47 (a)	Methanol extract of *Ritichica tragnama* root				P65 (a)	Control			
(b)		5.88	31.25	18.57	(b)		0	0	0

some Nigerian plants evaluated as shown below among the plants coded 30-65 including a control sample, which continued the plant samples arranged in alphabetical order. The plant samples with high larvicidal activity under this context include:

Ethanol extracts of *Lippia adonensis* flower, *Uvaria calophyllum* stem, *Z. depricum, Dennentia tripetala* stembark Methanol extracts of *Morniga oleifera* root, *Mallotus arboreum* stembark, *Pyonanthus angolensis* root, *Sphenocentum jollyamum* stem, *Sphenocentum jollyamum* root, *Trichsia sarbardata* root, *Crotolaria patens*, *Piper guineaces* fruit, *P. longifolia* fruit and $CHCl_3$ extract of *Tetrapleura conophonium* Fruit.

Table 3. Larvicidal and antimicrobial activity of methanol extract of *Adenia* root on tadpole.

Code	Antimicrobial activity of methanol extract of *Adenia* Root on Tadpole				Larvicidal activity of methanol extract of *Adenia* root			
	Larvae Mortality (%)				Larvae Mortality (%)			
	Extract conc.	1st Trial	2nd Trial	Average	Extract conc.	1st Trial	2nd Trial	Average
A (1) (2)	80 mg/ml	100	100	100	12 mg/ml	-100	100	100
B (1) (2)	40 mg/ml	100	100	100	6 mg/ml	66.67	81.82	74.25
C (1)(2)	20 mg/ml	100	100	100	3 mg/ml	69.23	90	79.62
D (1)(2)	10 mg/ml	100	100	100	1.53 mg/ml	25	25	25
E (1) (2)	5 mg/ ml	100	100	100	0.753 mg/ml	15.79	10	12.89
F (1) (2)	2.5 mg/ml	100	100	100	0.373 mg/ml	11.11	12.5	11.81
G (1)(2)	1.25 mg/ml	100	100	100	0.183 mg/ml	5.26	0	0
H (1)(2)	0.625 mg/ml	100	100	100	-	-	-	-
I (1) (2)	0.313 mg/ml	100	0	50	-	-	-	-
J (1) (2)	0.156 mg/ml	0	100	50	-	-	-	-

Table 4. Antimicrobial pattern of selected plant extract.

Plant/Extraction solvent (Conc. 5 mg/ml)	Observable zone of growth inhibition	
	Bacillus polymyxa	***Escherichia coli***
Methanol extract of *Adenia cissampelli (Passifloraceae)* root	++	++
Methanol extract of *Gossypium arboreum* root	++	++
Ficus asperifolia Latex	+	+
Methanol extract of *Adenia cissampelli (Passifloraceae)* Stem	--	--
Water extract of *Occimum gratissimum* leaf	+	++

+ = Inhibition zone between 12 and 15 mm, ++ = positive inhibition zone > 15 mm; -- = no inhibition.

The active plants sources listed above caused over 70% larval mortality at 5 mg/ml concentration in the test medium. Plants with larvicidal activity between 30 to 70% are moderately active and other samples showing lesser values have been observed as not active.

In Table 3, varied concentrations of Methanol extracts of *Adenia* root at 12 , 6 , 3 , 1.5 , 0.75 , 0.37 , and 0.18 mg/ml give an average corresponding larvae mortality in percentage (%) of, 100, 74.25, 79.62, 25, 12.89,11.81, and 0%. This shows that concentration range of 3 to 12 mg/ml is the recommended lethal dosage that can cause over 70% mortality of larva. Similarly, the antimicrobial effect of this extract on marine organisms using tadpole as a typical sample showed that concentrations range of0.625 to 40 mg/ml causes 100% mortality (LD_{100}) of the organisms, whereas concentration range of 0.156 to 0.313 mg/ml causes 50% mortality (LD_{50}) of the organisms (Table 3). The relevance of tadpole in the study is also to determine the selective activity and impact of this plant extracts on ecology.

Microbial isolates tested that is, *Bacillus polymyxa* and *E. coli* were susceptible to some active plant extracts like methanol extracts of *Adenia cissampelli* (Passifloraceace) root and *Gossypium arborium* root as shown in Table 4.

DISCUSSION

This study shows that some plant sources were very active because they cause over 70% larval mortality with the use of respective plant extracts and in addition to this, certain plant samples demonstrated antimicrobial value against some possible etiological agents used as test organisms. Plants coded 30 to 65 including a control sample were screened in this study out of which fifteen of them were found to possess high larvicidal activity (Tables 1 and 2). This attributes showed their anti-microbial property.

Both anopheline and culicines larvae were used as sample in this operation apart from the two bacterial species of *B. polymyxa* and *E. coli* tested for the plants activity. A period of 24 h was allowed for the organism exposure to the larviciding and antimicrobial agents (i.e.

plant extracts) in this experimental work. In similar work done by Nostro et al. (2000), it was indicated that the most important consideration when procedure is being chosen are the use of appropriate sample sources and facilities. The present study stresses that exposure to the larvicidal or antimicrobial agents should be for 24 h followed by transfer to clean water and that mortality counts should be made both at the end of the test period and after a further 24 h in clean water, the criterion of death being lack of movement on probing. Larvae, which pupate, are not considered in the test. Various concentrations of *Adenia* root extracted with methanol exerted corresponding physiological effect that leads to the death of both mosquito larvae and tadpoles tested for this purpose (Table 3). The relevance of tadpole in the study is also to determine the selective activity and impact of this plant extracts on ecology. Thus a lethal dosage (LD) can be deduced for this purpose. This has relevance to the study of Nwinyi et al. (2006) who assessed lethal concentration of some medicinal plants to suit specific purpose. Furthermore the active plants such as methanol extracts of Adenia cissampelli root and methanol extracts of *Gossypium arboreum* root tested inhibited the growth of cultured microorganisms to some extent (Table 4). This shows their potency over disease vectors and microbial agents in general. Modern techniques for various antimicrobial activities like this were described by Prescott et al. (2002) and Shittu et al. (2005).

Antimicrobial activity examined in this study showed that plants are capable of producing toxic materials, which may exert some physiological effect on the target organisms. Thus, some plant extracts based on their activity may be used to control infective agents and their vectors. Corich et al. (1997) and Orvos et al. (1990) study stress the significance and importance of genetic engineering as part of modern biotechnology that helps to enhance this kind of bio-control activities for environmental management. In relative terms, this study shows that most of the active plant extracts could be useful for larvicidal purpose in mosquito control. Appropriate biotechnological measures could be adapted to improve its yield and activity in future. According to Essien et al. (1983), higher plants constitute a major area of resources for us, especially in the third world and we need to mobilize fully these resources. The study of Burkill (1994) and Palembo and Semple (2001) also stresses the efficient use of our plant resources for therapeutic purposes more so some serves as alternatives for some western drugs or chemicals agents in case of resistance, which could occur naturally.

In conclusion, some of the medicinal plants commonly grown in Nigerian possess high antimicrobial activity based on the result of this study. We are putting nature on our side and development of health can be seen to be very relevant to the availability and use of these plant products as larvicides or drugs. Studies on higher plants and anti-infective agents generally give information on traditional uses of such plants in order to pursue various investigations. The antimicrobial activity of some Nigerian plants studied in this research will help tremendously to solve some infective agents and vector control problems. Similarly, it will also help in health care management scheme.

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