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Antimicrobial Screening of the Essential Oil of Some Herbal Plants from Western Nigeria

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Abstract: The essential oil from ten herbal plants namely *Momordica charantia, Ocimum gratissimum, Senna alata, Phyllantus* reticulata, *Dissotis rotundifolia, Gossypium hirsutum, Boerhaavia diffusa, Sida acuta, Paullinia pinnata* and *Senna podocarpa* were extracted using hydro-distillation process. The oils were characterised based on their colour and UV spectroscopy. Five out of the ten oils were screened against *Klebsiella pneumonia, Bacillus megaterium, Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Aspergillus flavus* and *Trichoderma spp*. Out of the five oils screened for antimicrobial activities, only three were found active and these are *Phyllantus reticulata, Momordica charantia* and *Gossypium hirsutum.*

Key words: Antimicrobial · antifungal · essential oil · herbal plants · Nigeria

INTRODUCTION

The use of plants for healing is as ancient and universal as medicine itself. Plants act generally to stimulate and supplement the body's healing forces, they are the natural food for human beings [1, 2]. Many infectious diseases are known to be treated with herbal remedies throughout the history of man kind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [3-5, 10]. Plants still continue to be almost the exclusive source of drugs for the majority of the world's population [1-4]. The screening of essential oil has been of great interest in the discovery of drugs' effectiveness in the treatment of several diseases.

The leaf of *Momordica charantia* has been reported for the treatment of menstrual troubles and the roots used for treating syphilis, rheumatism, boils, ulcers and septic swellings even in chronic malignant ulcers. *Ocimum gratissimum* has been used as adecongestant for head-cold bronchitis and headaches [1]. The methanolic crude extract and the partially purified fractions of *Senna alata* have been found active against standard strains of *Aspergillus niger, Lieotricum candidum* and *Candida utilis* [9]. It has also been reported that its water extract exhibits higher antibacterial activity than the ethanol extract from the leaves [8]. The objective of the present study is to assess the *in vitro* antibacterial and antifungal activities of the essential oil of some plant species from Nigeria.

MATERIALS AND METHODS

Collection of plant material and extraction: *Sida acuta, Momordica charantia, Boerhaavia diffusa, Gossypium hirsutum* and *Ocimum gratissimum* were collected at the Botanical garden while *Senna alata, Senna podocarpa, Phyllantus reticulata, Paullinia pinnata* and *Dissotis rotundifolia* were collected at the Botany and Microbiology Department, all within the University of Ibadan. The samples were air dried, grinded and set for extraction. Hydro distillation method [6, 7] was used for the extraction of the oil. The macerated plants were weighed and packed into the round bottom flask. This was mixed with lots of water as the extracting solvent in the ratio of 3:1. The distillates were then transferred into a sample bottle and stored until needed for analysis.

Microorganisms and medium: The microorganisms used in this present study were representative of bacteria *Klebsiella pneumoniae*, *Bacillus megaterium*, *Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa* and *Escherichia col*i and representative of fungi, *Aspergillus favus, Aspergillus niger* and *Trichiderma spp*. They were all obtained from Medical Microbiology Unit, University College Hospital (UCH), Ibadan, Nigeria and were subsequently maintained as stock strains.

Simple susceptibility screening test using agar-well diffusion method: Each microorganism was suspended in sterile saline and diluted to 10^6 Colony Forming Unit

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(CFU) per ml. They were flood-inoculated on to the surface of agar plates which were then dried. Nine millimeter diameter wells were cut from the agar using sterile cork-borer and 0.1 ml of the oils were delivered into the wells. After incubation at 37°C over night, plates were examined for any zones of growth inhibition. Streptomycin (10 µg ml^{-1}) served as control antibiotics.

Extraction: Hydro distillation method [6, 7] was used for the extraction of the oil. The macerated plants were weighed and packed into the round bottom flask. This was mixed with lots of water as the extracting solvent. The distillates were then transferred into a sample bottle.

Micro-organisms: The slants of nine organisms, six bacteria and three fungi were collected from the laboratory stock of the Department of Medical Microbiology, University College Hospital UCH, Ibadan, Oyo state. The bacterial are *Klebsiella pneumoniae*, *Bacillus megaterium*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Eschrichia coli* and the fungi are *Aspergillus favus*, *Trichoderma spp*. and *Aspergillus niger.*

Antibacterial assay: Pour plate method was used for the assay [4, 5]. Nutrient agar was prepared by dissolving the appropriate quantity in deionised water, homogenized and sterilized. The organism was introduced into the sterile plate under aseptic conditions. 20ml of the prepared nutrient was also poured into the petri dish, swirled together and allowed to solidify. After solidifying, cork borer(9mm) was used to bore two wells in the medium or culture and the oil poured into the bored well to notice if there would be any zone of inhibition or not. A positive control with Streptomycin was used.

Antifungal assay: Known amount of Sabroud dextrose agar was prepared and 15 ml of it was poured into three separate dishes. The fungi was then inoculated and grown for three days. Agar cap diffusion method was used. 2 ml of prepared Sabroud dextrose agar was dispensed into McCartney bottles, sterilized and allowed to cool before the addition of known concentration of the oil. This was shaken together properly and poured into the plate and allowed to solidify. A portion of the fungus was placed at the centre of the solidified mixture of agar, streptomycin and oil using a cork borer. The bottom of the dish was marked and the radial growth measurement taken after every 24hrs for 4days. Two drops 5% Streptomycin Sulphate was added to the agar for fungal medium to prevent bacterial growth.

RESULTS AND DISCUSSION

The preliminary screening results of five essential of from plants are presented in Table 1. Of all the essential oils tested three were found to possess activity against at least one or more test microorganisms. *Phyllantus reticulata, Momordica charantia* and *Gossypium hirsutum* were found active. The three oils were found active against *Proteus mirabilis*, with *Gossypium hirsutum* having highest zone of inhibition.

Table 1: Characterization of essential oils from some herbal plants from South-west of Nigeria

Sample	% Yield of oil	Colour of oil	Specific gravity of oil	Solubility in water
Sida acuta	0.333	Yellow	0.714	Soluble
Momordica charantia	0.800	Colourless	0.833	Insoluble
Boerhaavia diffusa	0.333	Colourless	0.714	Insoluble
Ocimum gratissimum	1.081	Yellow	0.909	Insoluble
Phyllantus reticulata	0.450	Yellow	0.900	Soluble
Paullinia pinnata	0.300	Yellow	0.857	Insoluble
Senna podocarpa	0.375	Yellow	1.000	Insoluble
Senna alata	0.357	Colourless	0.556	Soluble
Dissotis rotundifolia	0.353	Yellow	0.500	Insoluble
Gossypium hirsutum	0.500	Colourless	0.882	Soluble

Table 2: *In vitro* antibacterial activity of essential oils from some herbal plants from South-west of Nigeria

Oil	KP	BM	BS	PM	PA	EC
Sida acuta	-	-	-	-	-	-
Sena alata	-	-	-	-	-	-
Phyllantus reticulata	-	-	-	-	-	-
Mormodica charantia	5	16	18	16	-	21
Gossypium hirsutum	17	17	-	31	-	-
Streptomycin	-	-	-	-	-	-

Key: KP = *Klebsiella pneumoniae, BM = Bacillus megatarium, BS = Bacillus subtilis, PM = Proteus mirabilis, PA = Pseudomonas aeruginosa,, EC = Escherichia coli*

Table 3: *In vitro* anti fungal activities against Aspergillus flavus

Time in hrs Oil	24 hrs	48 hrs	72 hrs	96 hrs
Sida acuta	5	20	75	90
Senna alata	6	21	60	90
Phyllantus reticulate	5	15	52	78
Mormodia charantus	5	15	50	70
Gossypium lursutum	-	5	39	56
Control	7	30	78	90

*Radial growth were measure in mm

Table 4: *In vitro* antifungal activities of essential oil against *Trichoderma spp*

Time in hrs oil	24 hrs	48 hrs	72 hrs
Sida acuta	30	34	82
Senna alata	25	30	80
Phyllantus reticulata	30	35	96
Mormodia charantus	30	46	88
Gossypium lursutum	15	20	71
Control	30	35	90

*Radial growth were measure in mm

Table 5: *In vitro* antifungal activities of essential oil against *Aspergillus niger*

Time in hrs oil	24 hrs	48 hrs	72 hrs	96 hrs
Sida acuta	10	15	68	84
Senna alata	13	28	75	90
Phyllantus reticulate	11	23	70	96
Mormodia charantus	9	15	60	80
Gossypium lursutum	5	11	58	78
control	10	20	65	96

*Radial growth were measure in mm

Only *Momordica charantia* was found to be active against *Bacillus subtilis* and *Escherichia coli. Momordica charantia* had activity against almost all the microbes except *Pseudomonas aeruginosa* but *Gossypium hirsutum* was found to be most effective by virtue of its zone of inhibition. The oils showed no activity against fungi, though only *Gossypium hirsutum* was found to be interesting within 24 hrs on *Aspergillus niger* by inhibiting its growth which later lost activity.

The activities of the oils were greater than that of the standard. This gives them an outstanding edge over the known antibiotic (Streptomycin). No literature information was found on the biological and antimicrobial activities of the essential oil of these five plants investigated. But for *Momordica charantia* the methanol extract has been worked on in Nigeria [6]. It was reported to be active against *Staphylococcus aureus*, *Ecobacterium ecoli* but not *Escherichia coli* though *Staphylococcus aureus* and *Ecobacterium* were not tested against the essential oil under this study but *Escherichia coli.*

The activity of the essential oils against *Escherichia coli* indicates that it contains compound(s) absent or not pronounced in the methanolic extract. *Ocimum gratissimum* is a well known plant whose essential oil has antimicrobial activity. It is known as one of the plants with good activity against *Pseudomonas aeruginosa* with zone of inhibition of 28 mm [7] but *Gossypium hirsutum* has proved to be better with zone of inhibition of 31mm. The yield of the *Ocimum gratissimum* gotten from this study corresponds to that reported in literature [7].

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