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Screening for Anti-Microbial Activity and Phytochemical Constituents of Some Nigerian Medicinal Plants

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Abstract: Anti-microbial activity and phytochemical constituents of methanol extract of Plumeria rubra (flower and leaf) and *Eucalyptus globules* (leaf) was investigated. Phytochemical screening of the crude extract revealed the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar in the plants investigated. Phlobatanins were found to be absent in methanol extract of Plumeria rubra (flower) and Eucalyptus globulus (leaf) except the methanol extracts of Plumeria rubra (leaf). All the crude extract displayed higher inhibitory effects at the tested concentration (20 mg ml^{-1}) except on Corynebacterium pyogenese and Bacilhis anthracis of Plumeria rubra leaf and Streptococcus faecalis of *Eucalyptus globules* leaf. The infra-red (IR) spectra of the crude extract revealed the presence of different functional group ranging from OH stretching for hydroxyl group (3406-3338.6 cm^{-1}), C-Hstr., alkyl group (2926.6 cm^{-1}), C=O stretching for carbonyls (2162.1 cm^{-1}), C-O bending for alcohols, ethers, esters, carboxylic acid and anhydrides (1310.6-1059.6 cm^{-1}), C-H bending alkyl (1453.4-1376.2 cm^{-1}) and C-H bending for methyl group (864.1-668.4 cm^{-1}).

Key words: Anti-microbial activity · phytochemical constituents · phytochemical screening · functional groups · infrared spectra and inhibitory effects

INTRODUCTION

The use of medicinal plants in Nigeria other countries of black Africa dates back to many centuries ago [1]. Medicinal plants were used by people of ancient cultures, without knowledge of their active ingredients. The common practice of taking crude extracts orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever-increasing need to limit toxic clinical drugs [2].

Information on active ingredients and curative actions of the medicinal plants was got by introduction of European scientific method [3]. Information in the form of folklore and practices shows that, the aborigines used many plants materials for curative purposes, long before the conquest by the Europeans. Many of the reported medicinal plants came under scrutiny, leading to extraction and characterization of their active ingredients. Plants are found to be sources of many chemical compounds, most of which account for their various uses by man. The most important of these compounds are alkaloids, terpenoids, steroids phenols, glycosides and tannins [4].

Characterization of extracts of medicinal plants is necessary, due to its numerous benefits to science and society. The information obtained, makes pharmacological studies possible. It also enables structure-related activity studies to be carried out, leading to the possible synthesis of more potent drug with reduced toxicity. The mode of action of the plants producing the therapeutic effect can also be better investigated if the active ingredients are characterized. We report in this paper, the results of potential of plants that used readily by communities for curative purposes in tropical Africa [5-8].

MATERIALS AND METHODS

Source and Extraction: The plants parts were collected from uncultivated farmlands located at southern parts of Nigeria. Botany department, University of Benin,

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identified the plant samples. The samples were cleansed, dried for 3 weeks and grounded separately into powder. Each samples was extracted at a solute-solvent ratio of 1.20 for 5 hours in a soxhlet extractor.

Phytochemical Analysis: The extracts were analysed for the presence of alkaloids, glycosides, terpenoids reducing sugars, saponins, tannins, carbonyls flavonoids phlobatannis and steroid [9-11].

Alkaloids: 0.2 g of the extract were warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragencloff's reagent were added. Oranges red precipitate indicates the presence of alkaloids.

Cardiac glycoside

Keller-killani Test: About 0.5 g of each extracts was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was underlayed with 1ml of concentrated tetraoxosulphate (vi) acid to give the brown ring formation at the interface [12].

Terpenoids (Salkowski Method): About 0.5 g of each extracts in 2 ml of chloroform. Concentrated H_2SO_4 carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the present of terpenoids.

Reducing Sugars: The crude extract of each plant was shaken with 5 ml of distilled water and filtered. The filtrate was boiled with drops Fehling's solution A and B for 2 minutes. An orange red precipitate indicates the presence of reducing sugar.

Saponins: About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponin.

Tannins: Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Carbonyl: 2 ml of each plant extract was added few drops of 2,4 dinitrophenyl hydrazine solution and shaken. Yellow crystals were observed immediately indicating the presence of an aldehyde.

Flavonoids: About 0.2 g of each plant extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

Phlobatanins: About 0.5 g of each plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

Steroids: 2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Antibacterial screening

Preparation of medium: Nutrient Agar (LAB M) used for the antagonistic test was prepared according to manufacturer's instruction and sterilized in an autoclave at 121°C.

Indicator Bacteria: Stock cultures of the 14 bacteria isolates were obtained from the microbiology Department of University of Benin. The cultures were maintained throughout the duration of the work on agar slant.

Antibacterial Assay: The diluted extracts were tested for their antibacterial properties using the agar-well technique [13]. Overnight broth culture of the indicator bacteria were used to seed agar before pouring into plates. This was done in triplicate for each of the indicator bacteria. Two wells were made on the seeded agar plate with the aid of sterile cork borer of diameter 12 mm. One well, which contain sterile methanol, serves as control while the other was filled with the methenol extract of the plants.

Infrared (IR) spectroscopy analysis: This was done using infrared spectrophotometer of Shimadzu Corporation of model IR prestige 21. The extracts were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the FT IR and scanned through the IR region.

RESULTS AND DISCUSSION

The crude extract of the leaf of Eucalyptus globulus contain a greater proportion by mass of the component compounds as shown in Table 1.

The result of the phytochemical screening reveals that tannins flavonoids and reducing sugar are present in methanol extracts Plumeria rubra (flowers and leaves) and *Eucalyptus globules* (leaves).

Table 1: The yield of methanol extract of Plumeria rubra (flowers and leaves) and Eucalyptus ghobulus (leaves)

Plumeris rubra flowers	1.5%
Plumeria rubra leaves	3.16%
Eucalyptus ghobulus leaves	7.15%

Table 2: Phytochemical Screening of methanol extracts of *P. rubra* flower and leaf and *E. globules* leaf

Plants	Tannins	Phloba-tannins	Saponins	Flavo-noids	Steroids	Terpenoids	Cardiac-glycosides	Reducing sugar	Carbony	Alkaloids
P. rubra flower	+	-	-	+	-	+	-	+	-	+
P. rubra leaf	+	+	+	+	+	+	-	+	+	+
E. globules leaf	+	-	+	+	+	-	-	+	-	+

Key: + = Present -= Absent

Table 3: Comparative Antibacterial sensitivity methanol extracts of *P. rubra* (flower and leaf) and *E. globules* (leaf)

Zone of inhibition (mm)					
Micro organism PF_1 L_{10}	PF_1 20 mg ml^{-1}	PF_2 20 mg ml^{-1}	E_1 20 mg m^{-1}	S^1 1 mg m^{-1}	Gram
Corynebacterium pyogenes	25	0	20	19	+
Staphylococus aureus (NC1B8588)	23	15	14	21	+
Streptococcus faecalis (NC1B822)	22	12	0	24	+
Bacillus stearothermophelus (NC1B822)	25	20	14	23	+
Staphylococcsu epidermides	26	14	18	ND	+
Bacillus cereus (NC1B 6349)	28	18	14	ND	+
Bacillus polymyxa (L10)	24	14	18	15	+
Klebsiella pneumonia (NC23418)	23	0	17	0	-
Pseudomonas aeruginosa (NCIB)	21	14	18	ND	-
Bacillus anthacis (L10)	22	13	15	20	+
Bacillus subtilis (NCIB 3610)	20	15	14	22	+
Eschericha coli (NCIB 3610)	18	12	10	0	-
Pseudomonas fluorescens (NCIB 3756)	21	12	17	ND	-
Clostridrum sporogenes (NCIB 523)	20	12	12	28	+

PF_1 = *P. rubra* flower, PF_2 = *P. rubra* leaf, E_1 = *E. globules* leaf, S^* Steptomycin

Table 4: The 1R spectroscopic analysis gave the following characteristic absorption peaks for all the crude extracts in methanol

Component	*P. rubra* flower	*P. rubra* leaf	*E. globulus* leaf
0-H			340.6
C-H	851.6-780.8	864.1, 7747	858-733.1
C=O	1727.4	1858.3 and 1727.4	1876.2
C=C	1608.3-1453.4	1614.4, 1459.5	1697.6, 1626.2
C-O	1382-1036.6	1239.2	1310.6-1042.7
C-N	2322.8/-2138.1		
C-H	2954-2918.2	2918.2	2929.7, 1453.4, 1376.2

This may be responsible for their antibacterial properties [14], Pamplona Roger [8] and earlier reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections. Terpenoids are present, in methanol extracts of plumeria rubra flowers and leaves but absent in *Eucalyptus globules* leaves. Saponins and steroids are present in methanol extracts of *Eucalyptus globules* leaves and plumeria rubra leaves but absent in plumeria rubra flower extracts. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones. This may be the reason the leaves of *P. rubra* and *E. globules* are used as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones [15].

The presence of terpenoids revealed that the plant is widely used in herbal medicine [16]. The presence of tannins also, shows that the plants can be used as purgative. They are also used in the treatment of cough, asthma and hay fever [17].

The antibacterial assay showed that the methanol extract of *P. rubra* flower, *P. rubra* leaves and *E. globules* were able to inhibit the growth of the 14 indicator bacteria

with the zone of inhibition between 12-28 mm. The extracts of *P. rubra* leaf and *E. globules* leaf could not inhibit all the bacteria. The extract of *P. rubra* flower was found to be more active against Bacillus cereus with zone of inhibition of 28 mm as shown in Table 3.

Spectroscopic analysis of the plant extract with infrared spectroscopy as shown in Tables 4 and revealed the presence of 0-H, C = 0, C-H, C = C, C-N and C-0 bond stretching. This agrees with the result of the phytochemical analysis: O-H present in all phenolic compounds and C-N is common to all alkaloids.

REFERENCES

1. Ojinnaka, C.M., 1998. A Forest of Novel Drugs: Natural Products Chemical, University of P.H. Inaugural Lecture Series No. 19.
2. Lown, J.W., 1993. "Discovery and Development of Anthracycline, Antitumor, Antibiotic" Royal Society of Chemistry, 165.
3. Herborn, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 2nd Edition Hall, New York, pp: 5-11.
4. Abayomi, S., 1993. Historical Review of Traditional Medicine in Africa. Spectrum Book Ltd. Ibadan, pp: 9-25.
5. Cordell, G.A., 1981. Introduction to Alkaloids Wiley Interscience. New York, 1055.
6. Michael, J.B., 1990. Ethnobotany and the Identification of Therapeutic Agents from Plants. John Wiley Chichester, pp: 22-39.
7. Daiziel, J.M., 1955. The useful plant of West Tropical Africa. Grown Agent for Overseas Govt. and Admin. London, pp: 135-203.
8. Pamploma-Roger, G.D., 1999. The Encychopedia of Medicinal Plants. Education and Health Library, 2nd Edn. Spain, pp: 76-97.
9. Adetuyi, A.O. and A.V. Popoola, 2001. Extraction and Dye ability Potential Studies of the Colourant in Zanthoxylum Zanthoxyloides Plant on Cotton Fabric. Journal of Science Engineering Technology, 8(2): 3291-3299.
10. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy 11th Edn. Brailliar Tiridal can Macmillian Publishers.
11. Sofowora, A., 1982. Medicinal Plants and Traditional Medicine in West Africa John Wiley and Sons, New York, 256.
12. Finar, I.L., 1983. Organic Chemistry: Vol 2 5th Edition. Longman London, pp: 696-765.
13. Schillinger, U. and F. Lucke, 1989. Antibacterial Activity of Lactobacillus Sake from meat. Applied and Environmental Microbiology, 55: 1901-1906.
14. Sofowora, A., 1993. Medicinal Plant and Traditional Medicine in Africa 2nd Edn. John Wiley and Sons. New York, pp: 6-65.
15. Okwu, D.E., 2001. Evaluation of the Chemical Composition of Indigenous Spices and Flavouring Agents. Global J. Pure Appl. Sci., 7(3): 455-459.
16. Hayashi, T., K. Okamuka, M. Kawasaki and N. Morita, 1993. Production of Diterpenoids by Cultured Cells from two Chemotypes Scoparia Dulcis Phytochemistry, 35(2): 353-356.
17. Gills, L.S., 1992. Ethnomedical uses of Plants in Nigeria University of Benin Press Nigeria, 276.