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Full Length Research Paper

Screening for anti-microbial activity and phytochemical constituents of some Nigerian medicinal plants

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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 the 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) 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 hydroxyl group (OH) (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, inhibitory effects.

INTRODUCTION

The use of medicinal plants in Nigeria and other countries of black Africa dates back to many centuries ago (Ojinnaka, 1998). 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 (Lown, 1993).

Information on active ingredients and curative actions of the medicinal plants was gotten by the introduction of European scientific method (Herborn, 1998). Information in the form of folklore practices showed 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 (Abayomi, 1993).

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 enabled 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. In this paper, the results of the potential of plants that are used readily by communities for curative purposes in tropical Africa were discussed (Cordell, 1981; Michael, 1990; Daiziel, 1955; Pamploma-Roger, 1999).

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MATERIALS AND METHODS

Source and extraction

The plants parts were collected from uncultivated farmlands located at southern parts of Nigeria. The Botany department of the University of Benin identified the plant samples. The samples were cleansed, dried for 3 weeks and grounded separately into powder. Each sample was extracted at a solute-solvent ratio of 1.20 for 5 h 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 (Adetuyi and Popoola, 2001; Trease and Evans, 1989; Sofowora, 1982).

Antibacterial screening

Nutrient Agar (LAB M) used for the antagonistic test was prepared according to manufacturer's instruction and sterilized in an autoclave at 121°C. Stock cultures of the 14 bacteria isolates were obtained from the Department of microbiology, University of Benin. The cultures were maintained throughout the duration of the work on agar slant.

The diluted extracts were tested for their antibacterial properties using the agar-well technique (Schillinger and Lucke, 1989). 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, served 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* contained a greater proportion by mass of the component compounds as shown in Table 1. The result of the phytochemical screening revealed that tannins, flavonoids and reducing sugar were present in the methanolic extracts of *Plumeria rubra* (flowers and leaves) and *Eucalyptus globules* (leaves). This could be responsible for their antibacterial properties (Sofowora, 1993). Pamplona Roger (1999) earlier reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections. Terpenoids were present in the methanolic extracts of *Plumeria rubra* flowers and leaves but absent in Eucalyptus globules leaves. Saponins and steroids were present in methanolic 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 (Okwu, 2001). Table 2

Table 1. The yield of methanol extract of *P. rubra* (flowers and leaves) and *E. ghobulus* (leaves).

Flower/leaves	Methanolic yield (%)
P. rubra flowers	1.5
P. rubra leaves	3.16
E. ghobulus leaves	7.15

The presence of terpenoids revealed that the plant is widely used in herbal medicine (Hayashi et al., 1993). The presence of tannins also, showed that the plants could be used as purgative. They are also used in the treatment of cough, asthma and hay fever (Gills, 1992). The antibacterial assay showed that the methanolic extract of *P. rubra* flower, *P. rubra* leaves and *E. globules* were able to inhibit the growth of the 14 indicator bacteria with a 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 Table 4 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 where O – H was present in all phenolic compounds and C – N was common to all alkaloids.

In conclusion, the phytochemical constituents (tannins, flavonoids and reducing sugar) of the methanolic extract of *Plumeria rubra* (flowers and leaves) and *Eucalyptus globules* (leaves) could be responsible for the antibacterial properties of these plants. Also, the presence of steroidal compounds in these plants necessitated its use as vegetable for hormonal balance by expectant mothers and/or breast feeding mothers. Finally, this study justifies the use of these plants as purgatives and in the treatment of cough, asthma and hay fever.

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Table 2. Phytochemical screening of methanolic extracts of *P. rubra* flower and leaf and *E. globules* leaf.

Phytochemical	Plant		
	***P. rubra* flowers**	***P. rubra* leaves**	***E. globules* leaves**
Tannins	+	+	+
Phlobatannins	-	+	-
Saponins	-	+	+
Flavonoids	+	+	+
Steroids	-	+	+
Terpenoids	+	+	-
Cardiac-glycosides	-	-	-
Reducing sugar	+	+	+
Carbonyl	-	+	-
Alkaloids	+	+	+

+ = Present; - = Absent.

Table 3. Comparative antibacterial sensitivity methanolic 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)	PF_2 (20 mg/ml)	E_1 (20 mg/ml)	S^1 (1 mg/ml)	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

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