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Studies on Bioactive Metabolites Constituents and Antimicrobial Evaluation of Leaf Extracts of Eucalyptus Globulus

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Abstract: Hexane, ethyl acetate and methanolic extracts of dried powdered leaves of Eucalyptus globulus were screened for basic secondary metabolites and antibacterial activity against *C. pyogenes*, *S. aureus*, *S. faecallis*, *B. stearothermophilus*, *S. epidermidis*, *B. cereus*, *B. polymyxa*, *K. pneumonia*, *P. aeruginosa*, *B. anthacis*, *B. subtilis*, *E. coli*, *P. fluorescens* and *C. sporogenes*. Phytochemical investigation of crude extracts of Eucalyptus globulus (leaf) revealed the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar in the plant. The antimicrobial sensitivity of the extracts against four species of Gram-ve and ten Gram+ve bacterial stain for Eucalyptus globulus (leaf) revealed very high antimicrobial activity. The extracts of hexane, ethylacetate and methamol of Eucalyptus globulus (leaf) were all very active on almost all the bacterial strains. The Infra Red (IR) spectra revealed the presence of different functional groups ranging from OH (3406-3338.6 cm^{-1}) stretching, C-H (2926.6 cm^{-1}, 864.1 cm^{-1}) stretching alkyl, methyl C = 0, (2162.1 cm^{-}) anhydrides, C = C (1697.6 cm^{-1}) aromatic ring sretching, C-0 (1310.6-1059 cm^{-1}) alcohol, ethers, esters, carboxalic acid bending.

Key words: Bioactive metabolites, antimicrobial evaluation, eucalyptus, globules, phytochemical screening, bacterial strain, infra-red spectra, functional groups

INTRODUCTION

Eucahyptus globules belong to the family of Myrtaceae. It is known as the blue gum and it is also called the fever tree. It is a native to Australia and Tasmania, where it is the primary food in the diet of Koala bears, it is grown in Mediterranean and subtropical regions around the world (Sharma, 1993) the leaves are leathery in texture, hang obliquely or vertically. Eucalyptus is available resource for treatment of burns, sores, ulcers and boils. It also helps relieve the pain of rheumatism, aching pains, stiffness and neuralgia. It can also work as insect repellant, especially for mosquitoes and flies (Duke, 1997).

Indigenous plants to be exploited for medicinal purposes have to undergo basic phytochemical screening and bioassay as the first step towards the ultimate development of natural drug (Odebiyi and Sofowara, 1998). Plants continue to be a major source of drugs and natural products on the basis of their therapeutics (Lown, 1993). Tropical Africa possesses a vast array of flora, which natives claimed have various curative abilities (Burkul, 2000; Dalziel, 1968). Pathogen activity within the body causes disease which requires complementary chemotherapy. Traditional medicine practitioner, who depends on the use of plant extracts, occupies a unique position in disease management in contemporary communities in Nigeria (Fasola, 2000; Sofowora, 1993).

Therefore, this research was aimed at authenticating the claims of the traditional healers which will form the basis for further research.

MATERIALS AND METHODS

Plant material collection and identification: The leaves of the plant were collected from Ihievbe, Owan East Local Government Area of Edo state, Nigeria. They were identified by Dr. S.D. Emua of the Department of Botany, Ambrose Alli University, Ekpoma Nigeria.

The samples were cleaned, air-dried and pulverized using a Thomas-Willey milling machine.

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Extraction: The hexane extract of Eucalyptus globules was prepared by soaking 100 g of dried powered samples in 200 mL of distilled hexane for 72 h (3 days). The extracts were filtered using Whatman filter paper No. 42 (125 mm). The crude extract was poured into a weighed sample bottle and left on the laboratory shelf till all the remaining solvent present in the crude evaporated and the weight of the sample determined. The same procedure was repeated for ethyl acetate extract and methanolic extract of Eucalyptus globules (leaf).

Phytochemical screening: Phytochemical screening were carried out on the extracts using standard procedures to identify the constituents as describe by Trease and Evans (1989), Harborne (1973) and Sofowora (1993) to test for tannius, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar.

Tannins: Small quantity of the extract was mixed with water and heated on water bath. The mixture was filtered and few drops of 0.1% ferric chloride ware added to the filtrate. The presence of brownish green or a blue-black colouration was observed.

Phlobatannins: The extract (about 0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate was observed.

Saponins: About 0.2 g of the extract was shaken with 5 mL distilled water and then heated to 5 mL of distilled water and then heated to boil. Frothing shows the presence of saponnins.

Flavonoids: About 0.2 g of the extract was dissolved in dilute NaOH and HCl was added. A yellow solution that turns colourless was observed.

Steroids: Two milliliter of acetic anhydride was added to 0.5 g of the extract with 2 mL H_2S0_4. The colour changed from violet to blue or green indicating the presence of steroids.

Terpenoids: About 0.5 g of the sample was dissolved in 1 mL chloroform and 1 mL concentrated H_2SO_4 carefully added to form a layer. A reddish brown or yellow colouration of the inter-phase was observed.

Cardiac glycosides: 0.5 gram of the extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring of the interphase indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Reducing sugar: The extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for 2 min. An orange red precipitate indicated the presence of reducing sugars.

Microbial test

Indicator bacteria: The micro-organisms used in this research were clinical isolates obtained from microbiology Department, University of Benin, Benin city, Nigeria. The cultures were maintained throughout the duration of the work on agar slant.

Preparation of medium: Nutrient Agar (LAB M) used for the antagonistic test was prepared according to manufacturer's instruction and sterilized to 121°C for 15 min.

Antibacterial activity test: The diluted extracts were tested for their antibacterial properties using the agar-well technique (Schillinger and Lucke, 1989). The assay for antibacterial activity was carried out with Klebsieva pneumoniae, Bacullus subtils, Staphlococais aureus, Clostridium sporogenes, Escherichia coil, Staphylococcus epiderm, Pseudomonas cerus, Bacillus anthracis, Streptococcus faecalis, Corynebacteria phyogenes, Pseudomona aeruginosa and Bacillus polymyxa.

Using a sterile 1 mL pipette, 0.2 mL of the broth culture of the test organism was put in a sterile Petri-dish and 18 mL of the sterile molten diagnostic agar was added. The plates were mixed by swirling and then allowed to sit, wells were bored into the medium using a sterile cock borer. The wells were then filled with 0.1 mL of the extracts in triplicates.

A control experiment using the solvent of extract was then set up. The plates were kept in sterile inoculation chambers for 2 h to facilitate diffusions of the solutions. The plates were then incubated for 24 h at 37% for microorganism. Zones of inhibition of microorganism for each extract were measured using a calibrated ruler.

RESULTS AND DISCUSSION

The result of the phytochemical screening of the hexane, ethyl acetate and methanolic extracts of Eucalyptus globules (dried leaves) are shown in Table 1.

Table 1: Phytochemical screening of the extracts

Test/Crude Extract	Tannius	Phlobatannius	Saponius	Fkvonoids	Steroids	Terpenoids	Cardircglycoside	Reducing Sugar
Hexane extract	-	-	-	-	+	-		-
Ethylacetate extract	+	-	-	+	+	-		-
Methanol extract	+	-	+	+	+	-	-	+

Key + = Present; - = No activity

Table 2: Zone of inhibition (mm)

Microorganism	Gram	Hexane extract	Ethylacetate extract	Methanol extract	(1mg mL^{-1})
C. pyogenese (LIO)	+	20	15	20	19
S. aureus (NCIB 8588)	+	12	12	14	21
S. faecalis (NCIB 755)	+	10	15	0	24
B. stearothermophilus (NCIB 822)	+	15	18	14	23
S. epidermidis	+	16	18	18	ND
B. cereus (NCIB 6349)	+	16	14	14	ND
B. polymyxa (LIO)	+	21	20	18	15
B. anthracis (LIO)	+	18	18	17	20
B. subtilis (NCIB 3610)	+	16	20	18	22
C. sporogenes (NCIB 523)	+	15	12	15	28
K. pneumonia (NC23418)	-	18	16	14	0
P. aeruginosa	-	12	12	10	ND
E. coli (NCIB 86)	-	12	14	17	0
P. fluorescens (NCIB 3756)	-	12	14	12	ND

S = Streptomycin; ND = Not determined; NCIB = National collection of industrial bacteria; LIO = locally isolated organism

Table 3: IR Spectroscopic Data for Eucalyptus Globulus (Leaf)

	Region cm^{-1}		
Component	Hexame extract	Ethylacetate extract	Methanol extracts
O-H	333.8	3390.1	3406
C-H	2924.3, 1447.9, 918.7-651.3	2926.6, 1453.4, 1382, 1453.4, 1382.0, 864.1-668.4	2929.7, 1453.4, 1376.2, 858-733.1
C=O	1885.6, 1727.4	2162.1	1876.2
C=C	1624.1	1697.6, 1620.0	1697.6, 1626.2
C-O	1380.9-1076.9	1310.6-1059.6	1310.6-1042.7

The results of the antimicrobial activity of the hexane, ethyl acetate and methanolic extracts of Eucalyptus globules leaves shown in Table 2.

The classes of natural product present in the plant investigated are shown in Table 1.

Phytochemical screening of the plant studies showed that the leaves were rich in steroids, flavonoids and tannins. They are known to exhibit medicinal activity as well as physiological activity (Sofowora, 1993). Steroids were found to be present in all the extracts. It has been found that the leaves of Eucalyptus globules contain steroidal compounds. 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 Eucalyptus globulus 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). The presence of flavonoids and tannins, which is known to have antibacterial and antifungal properties (Altaway, 1992; Futami, 1991) in ethyl acetate and methanol extracts but absent is hexane extracts was revealed. These finding give credence to the traditional medicinal application of the plants as remedies for measles, internal and external wounds and infections and revealed their potentials in the treatment of typhoid fever. However, methanol extract was positive for saponins; a class of compound known to be effective for the treatment syphilis and other veneral disease (Sofowora, 1993).

Hexane extracts showed anti microbial activity against all the micro organism with 4 Gram-ve and 10 Gram+ve; bacterial strain and with zone of inhibition ranging from 12-21. Ethyl acetate extracts of the plant leaves was found to have antimicrobial activity against 4 Gram-ve and 10 Gram+ve bacterial strains with inhibition zone ranging from 14-20. Methanol extracts have antimicrobial activity against 4 Gram-ve and 9 Gram+ve bacterial strain with zone of inhibition ranging from 10-20. The hexane extracts has the highest inhibition zone (21.0 mm) for Bacillus polymyxa.

Eucalyptus globules leaves extract exhibited significant activity on the pathogens. Staphylococcus aureus and Escherichia coli which is an indication that the plant can be used as remedy for gastro-enteritis and pneumonia.

Table 3 shows the Spectroscopic analysis of Eucalyptus globules extracts by infra-red spectroscopy revealed the presence of 0-H, C-H, C = 0, C = C and C-0 band stretching. This shows the similarities in the chemical nature of the component compounds of the leaves extracts (hexane, ethyl acetate and methanol) of Eucalyptus globules.

CONCLUSION

The result of this preliminary study revealed that Eucalyptus globulus (leaf) is endowed with activities gastroenteritis, pneumonia and probably veneral diseases. Research is in progress in ascertaining the chemical component of the plant extract that is responsible for these therapeutics activities.

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