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Analysis of the Phytochemical Content and Anti-microbial Activity of *Plectranthus glandulosis* Whole Plant

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Abstract: The extracts of Plectranthus glandulosus by using hexane, ethyl acetate and ethanol as solvents were screened for secondary metabolites and antibacterial activity. The extracts revealed the presence of alkaloids, tannins, anthraquinones, glycosides, reducing sugars, saponins, flavonoids, phlobatanins, steroids and terpenoids. Steroids were present in hexane and ethyl acetate extract but absent in ethanol extract. The ethyl acetate extract of Plectranthus glandulosis is the most active, showing activity against 3 Gram-ve and 3 Gram-ve bacterial stain and thus displayed highest inhibitory zone of (26:0 mm) at the tested concentration (20 mg ml^{-1}). Infrared spectroscopic analysis of the hexane ethyl acetate and crude extract of Plecthrathus glandulous revealed the presence of O-H, C=O, C-H, C=O, C-N and C-O bond stretching. The medicinal values of this plant could be attributed to the presence of one or more of the detected metabolites.

Key words: Secondary metabolites · antibacterial activity · bacterial strain · inhibitory zone

INTRODUCTION

Tropical Africa possesses a vast array of plants, which natives claimed have various curative abilities [1]. Plants are natures "chemical factories" providing the richest source of organic chemicals on earth. Nigeria is blessed with a great variety of natural vegetation, some of which are used in traditional medicine to cure various sicknesses and diseases. They do not serve as source of medicinal importance only; some are also useful for ornamental purposes, while many due to their odoriferous nature are useful in flavouring or as food additives and preservatives [2-6].

The use of plants whether herbs, shrubs or trees in parts or in whole in the treatment and management of diseases and disorders date back to pre-historic days [7]. Plant extracts have been used in folk medical practices for the treatment of various ailments since antiquity [8]. The medicinal properties of various plant material and extracts have been recognized since the beginning of the 5th century [9]. A rich store house of medicinal plants exist everywhere especially in Africa which offers a vast reservoir of plants that have been categorized [10]. World Health Organization (WHO) [11] described plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The results of investigation performed in the late 19th and 20th century and the advent of streptomycin and other antibiotics provide the ground for experimentation of a vast number of plants for antibiotic or antimicrobial activities that are useful to man.

The study is aimed at investigating the antimicrobial properties Plectranthus glandulosus whole plant as well as to identify the active ingredients of the plant.

MATERIALS AND METHODS

Plant material: Plectranthus glandulosus whole plant was obtained from a forest near the University and identified in the Department of Botany, University of Benin, Benin City, Nigeria.

Extraction: The whole plant (leaves, stem and root) was thoroughly washed. Each parts cut into pieces, dried in an oven at 60°C for 9hr and pulverized together. The sample was extracted at a solute-solvent ratio of 1:10 for 6 h in a soxhlet extractor. The crude extracts were kept in a sample bottles and stored in the refrigerator.

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Micro-organisms collection and maintenance: The microorganisms used in the study: *Streptococcus faecalis, Bacillus authracis, Bacillus cereus, Pseudomonas aerugenosa, Escherichia coli, Pseudomonas fluorescence, Staphylococcus aureus, Clostridium sporogenes, Klebsiella pneumoniae, Bacillus polymyxa, Ballcillus subtilis* and *Bacillus stearothermophilus* were obtained from stock culture in the Department of Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria. The organisms were stored on agar slant in McCartney bottles and kept in the refrigerator, prior to subculture.

Antimicrobial sensitivity testing of the extract against selected bacterial isolates: Susceptibility test were carried out. The modified agar well diffusion method [12,13] to test the antimicrobial activity of the extracts. The medium employed was diagnostic sentivity agar (Lab M, Ltd).

The cultures were prepared in triplicates and incubated at 37°C for 24 to 72 h. 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 sensitivity agar, was added. Wells were bored into the medium using 0.1 ml of the extracts (hexane, ethyl acetate and ethanol extracts). Streptomycin was used as the standard antimicrobial agent at a concentration of 1 mg ml^{-1}. The plates were kept in sterilized inoculation chambers for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37°C fro 24 h and the diameters of the zones of inhibition of microbial growth were measured in the plates in millimeters.

Phytochemical screening: Chemical tests were carried out on the hexane, ethyl acetate and ethanol extracts of Plectranthus glandulosus using standard procedures to identify the constituents as described by Sofowora [1], Trease and Evans [14] and Harborne [15].

Alkaloids: About 0.2 g of the extracts was warned with 2% H_2S0_4 for two minutes. It was filtered and few drops of Dragencloffs reagent were added. Orange red precipitate indicates the presence of alkaloids.

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.

Anthraquinones: About 0.5 g of the extracts was boiled with 10% Hcl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour indicates the presence of authraquinones.

Glycosides: The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

Reducing sugars: The extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for minutes. An orange red precipitate indicates the presence of reducing sugars.

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

Flavonoids: Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

Phlobatanins: The extract (0.5 g) 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 the extract of each with 2 ml of H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski test): 0.2 g of the extract of the whole plant sample was mixed with 2ml of chloroform ($CHCl_3$) and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

IR spectroscopy analysis: Infrared Spectroscopy of Shimadzu Corporation of model IR prestige 21 was used. 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

The weight percentage yield of the crude extracts of hexane, ethyl acetate and ethanol extracts of Plectranthus glandulosus whole plant are shown in Table 1.

Table 1: Weight of crude extract and percentage yield of crude extract of plectranthus glandulosus whole plant

Solvent	Weight of crude extract	Percentage yield
n-Hexane extract(g)	2.68	0.69
Ethyl acetate extract(g)	3.29	0.86
Ethanol extract(g)	5.84	1.26

Table 2: Antimicrobial sensitivity testing of hexane, ethyl acetate and ethanol extracts of plectranthus glandulosus

Microorganism	Gram	S	Hexane extract 20 mg ml^{-1}	Ethyl acetate 20 mg ml^{-1}	Ethanol extract 20 mg ml^{-1}
K. pneumoniae (NCIB 418)	-	0	13	12	15
C. sporogenes (NCIB 532)	-	28	10	0	13
P. fluorescence (NCIB 756)	-	N1	0	0	0
E. coli (NCIB 86)	-	0	0	0	0
P. aerugenosa	-	N1	10	12	10
B. stearothermophihus (NCIB8222)	+	23	18	10	26
B. subtilis (NCIB 610)	+	22	20	23	21
B. polymysa (LIO)	+	15	0	0	0
S. aureus (NCIB 8588)	+	21			
B. cereus (NCIB 69)	+	N1			
B. authracis (LIO)	+	20			
S. faecalis (NCIB 755)	+	24			

Key: NCIB=National Collection of Industrial Bacteria
LIO = Locally Isolated Organism
S = Streptomycin at 1 mg ml^{-1}
N1=No Inhibition

Table 3: Phytochemical screening of hexane, ethanol and ethyl acetate crude extracts plectranthus glandulosus

Chemical components	n-Hexane extract	Ethylacetate extract	Ethanol extract
Alkaloids	-	+	+
Tannins	-	-	-
Anthraquinones	-	-	-
Glycosides	-	-	-
Reducing sugars	-	+	-
Saponins	-	-	+
Flavonoids	-	+	-
Phlobatanins	-	-	-
Steroids	+	+	-
Terpenoids	-	-	+

Key + = Present
- = Absent

Table 4: IR Spectroscopic data

Component	Region (cm^{-1}) Hexane extract	Ethyl acetate extract	Ethanol extract
0-H	3472.7	3362.6	343.6
C=O	1923.7, 1871.8 -1817.7, 1721.3	1912.1, 1733.4	1889.9
C=O	1667.3-1594.9	1638.1-1453.4	1643.1-1414.4
C-H	2932.7-1450.6	2924.2, 923.6	2924.3, 842.8-716.5
C-O	1374.2-1127.6	1382-1048.7	1270.1-1041.4
C-N	1352.9	1234.6	14054.2

The susceptibility of test organisms to the crude extracts of Plectranthus glandulosus whole plant is shown in Table 2.

The phytochemical screening of Plectranthus glandulosus whole plant is shown in Table 3.

The IR spectroscopic analysis of hexane, ethyl acetate and ethanol crude extracts of Plectranthus glandulosus gave the following characteristic absorption peaks as shown in Table 4.

DISCUSSION

The result of the whole plant extracts of Plectranthus glandulosus showed that ethanol extracts contain a greater proportion by mass of the component compounds.

The medicinal properties of the plant could be attributed to the presence of one or more of the detected plant natural products. Ethyl acetate extract of Plectranthus glandulosus contain flavonoids such a quercetin which has antioxidant properties.

These findings give credence to the traditional medicinal application of the plant as remedies for measles, internal and external wounds and infections.

Ethanol extracts were positive for saponin and alkaloids; class of compounds that are known to be effective for the treatment of syphilis and other venereal disease [1].

Hexane and ethyl acetate extracts were positive for steroids. It should be noted that steroidal compounds are of importance and interest in pharmacy due to sex hormones [16]. Phytochemical screening of the extracts revealed the presence of different functional groups.

However, the pharmacological actions of the plant cannot be ascertained by the result of the phytochemical analysis only. Hexane extracts showed antimicrobial activity against *Klebsiella pneumoniae, Clostridium sporogenes, Pseudomonas aerugenosa, Bacillus stearothermophilus* and *Bacillus substilis* with zones of inhibition ranging from 10-20 mm. Ethyl acetate

extracts was found to have antimicrobial activity on *K. pneumoniae, P. aerugenosa, B. stearothermophilus, B. substilis* and *S. aureus* with zones of inhibitions ranging from 10-26 mm, revealing its great medicinal potentials for the treatment of gastroenteritis and pneumonia. The inhibitory activity of these extracts confirmed the potential use of the plant in the treatment of microbial induced ailments.

The IR analysis gave results that suggest the presence of different functional groups ranging from O-H stretching, hydroxyl (3472.7-3362.6cm^{-1}), C-H stretching, alkyl (2933.7-1460.6 cm^{-1}), C=C stretching aromatic ring (1667.3-1414.4 cm^{-1}), C-O bending, alcohols, ethers, esters, carboxylic acid and anhydrides (1382-1041.4 cm^{-1}), C=O stretching, carboxylic, carbonyl (1923.7-1721.3 cm^{-1}) and C-N bending, alkaloids (1405.2-1234.6 cm^{-1}).

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