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Research Article

Chemical Composition, Antioxidant and Antibacterial Properties of Chloroform Fraction of *Platycerium Bifurcatum*

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Abstract
The study investigated the bioactive constituents, antioxidant and antibacterial activities of *Platycerium bifucartum* leaves. Chloroform fraction of *P. bifurcatum* was prepared by partitioning the ethanol extract with chloroform and water. The phytochemical analysis was carried out using standard methods. Fourier transformer-infrared (FT-IR) and Gas chromatography-mass spectroscopy (GC/MS) were used in the characterization of the bioactive compounds. The antioxidant and antibacterial activities of the chloroform fraction were evaluated using standard protocols. The fourier transformer-infrared analysis showed the presence of C=O, OH, CHO, C-F and –NH functional groups. GC/MS characterization gave benzeneethaneamine (33.3%), 2-amino-1-(4-methylphenyl) propane (17.04%), hydroxyurea (30.26%) and epinephrine (13.26). The extract inhibited the growth of the bacterial isolates. The fraction exhibited antioxidant properties that were comparable with ascorbic acid. The presence of these compounds showed that the leaves of *Platycerium bifurcatum* can be used for the treatment of some bacterial diseases.

Key words: Benzeneethaneamine, Epinephrine, Hydroxyurea, Natural Product, *Poplypodiaceae.*

Introduction

Platycerium bifurcatum belongs to the family of *Poplypodiaceae* and its common names (English) include “elkhorn fern” or “staghorn fern” [1, 2]. *Platycerium bifurcatum* is called “*akpaka iyi*” in Igbo language (especially by the natives of Owerri, Imo State Nigeria). Some species of the genus *Platycerium* like *P. bifurcatum* and *P. superbum* are commonly cultivated around homes and offices as ornamental plants. They grow on trees, rocks and gardens in tropical region. In Imo state Nigeria, the leaves of *P. bifurcatum* is used in traditional treatment of fever, premature abortion, irregular menstruation, inflammation and ulcer (oral communication). The leaves of *P. bifurcatum* are used in ethnomedical treatment of ulcer, miscarriages in women, oedema, cough and hypertension treatment [1]. The antibacterial activity of *P. bifurcatum* has been reported by previous investigators [3]. The chemical constituents responsible for these biological activities of *Platycerium bifurcatum* are yet to be established. This study investigated the bioactive constituents, antioxidant and antibacterial activities of the leaves of *Platycerium bifurcatum*.

Material and Methods

Identification and preparation of extract. The leaves of *P. bifurcatum* was identified by Dr. F. N. Mbagwu. The leaves were harvested from an oil bean tree in the University premises. The leaves samples were dried at room temperature and grinded with mortar and pestle into coarse

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powder. Three hundred gramme (300 gramme) of the ground sample was soaked in 1.5 L of ethanol for 48 h. The extract was filtered with Whatman No: 1 filter paper and concentrated with hot air oven. The crude ethanolic extract was partitioned with water and chloroform with the aid of a separating funnel. The chloroform fraction was concentrated in hot air oven and was used for the studies.

Fourier transformer infrared (FT-IR) spectroscopy. The chloroform fraction (1 mg) were mixed with potassium bromide (KBr), pulverized, and formed into a disk-shaped pellet. The spectra were recorded in the frequency region 500 – 4000 cm-1, under a resolution of 2 cm-1 and with a scanning speed of 2 mm sec-1 with Shimadzu FT-IR 8400s fourier transformer infrared.

Gas chromatography-mass spectra (GC-MS) Analysis. Analysis was conducted using GC-MS (Agilent 7890A) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 um film thickness, J & W Scientific, Folsom, CA). The initial oven temperature was 60 °C. Helium was used as the carrier gas at the rate of 1.0 mL/min. The eluent of the GC column was introduced directly into the source of the MS via a transfer line (250 °C). Ionization voltage was 70 eV and ion source temperature was 230 °C. Scan range was 41- 450 amu. The components were identified by comparing their retention times to the reference in the national institute of standards and technology (NIST, ver. 2.0, 2008) mass spectral database.

Antibacterial activity

Antibacterial sensitivity test. Agar well diffusion technique as described by Paliwal et al. [4] was used to determine the antibacterial activity of the chloroform fraction against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Ciprofloxacin was used as reference standard and the zones of inhibition were measured with metre rule to the nearest millimetre.

Minimum inhibitory concentration (MIC). Broth dilution technique was used in the evaluation of the MIC of *P. bifurcatum* [5]. Each of the organism was incubated with the extract at 10.0 to 200 µg/mL concentration.

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging Assay. The DPPH (Sigma Aldrich, USA) scavenging property of *P. bifurcatum* was evaluated as modified by Ezeja et al. [6] at 25 - 400 µg/mL concentrations in triplicate and ascorbic acid was used as standard.

Ferric reducing antioxidant power (FRAP). The FRAP of *P. bifurcatum* was evaluated as modified by Onoja et al. [7]. The FRAP of *P. bifurcatum* at 25 - 400 µg/mL concentrations were determined in triplicate.

Statistical analysis. Data were analyzed with statistical package of social science (SPSS) version 20 using one way analysis of variance (ANOVA). Least Significant Difference (LSD) was used to separate mean difference and significance was accepted at the level of $p < 0.05$.

Results and Discussions

FT-IR analysis of the chloroform fraction of *P. bifurcatum* leaves showed the presence of the following functional groups; R-OR, R-F, C=O, and –OH and two stretching (Table 1).

Table 1

Results FT-IR Analysis of *P. bifurcatum* Leaves

S/N	Functional groups	Peaks	Comments
1	R-OR	1072.46	Stretching
2	R-F	1178.55	Stretching
3	C=O	1721.53	Aldehyde
4	-NH-	3416.05	2^0 amine
5	-OH-	3735.28	Phenol

The GC/MS identified the presence of four bioactive compounds; Benzeneethamine (33.3%), 2-amino-1-(4-methylphenyl) propane (17.04%), Hydroxyurea (30.26%) and Epinephrine (13.63%) (Table 2 and Figure 1).

Table 2

Result of GC-MS Analysis

	Compound Name	Molecular weight	Molecular formula	ID	% content
1	Benzeneethanamine	185	$C_9H_{12}FNO_2$	14308	33.30
2	4-methylamphetamine	149	$C_{10}H_{15}N$	3385	17. 04
3	Hydroxyurea	76	$C_4N_2O_2$	3413	30.26
4	Epinephrine	183	$C_9H_{13}NO_3$	3438	13.63

ID=Retention index, % content=Percentage content.

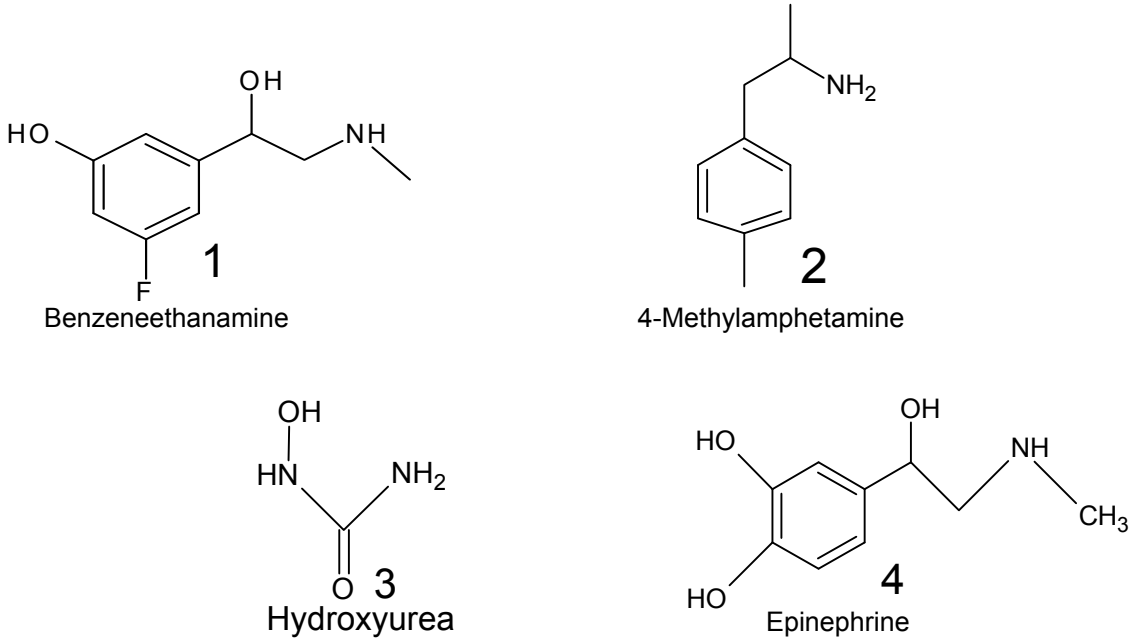

Fig. 1. Structure of identified compounds

The extract produced concentration dependent increase in zone of growth inhibition against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia* (Table 3) and the MIC of ranged from 25 – 100 µg/mL which were higher when compared with ciprofloxacin (Table 4).

Table 3

Zone of growth inhibition on test organisms

		Zone of inhibition (mm)			
Vol. (µl) of 10 mg/ml	**Drugs**	***S. aureus***	***E-coli***	***P. aeroginosa***	***K. pneumonia***
400	*P. bifurcatum.*	17	18	17	22
	Ciproflox.	20	16	17	20
300	*P. bifurcatum.*	16	16	15	20
	Ciproflox.	18	12	16	20
200	*P. bifurcatum.*	13	12	13	16
	Ciproflox.	17	15	15	18
100	*P. bifurcatum.*	-	6	-	12
	Ciprofloxacin.	12	23	13	12

Table 4

Minimum inhibition concentration

	Minimum inhibition concentration (µg/mL)			
Compound	***S. aureus***	***E-coli***	***P. aeroginosa***	***K. pneumonia***
P. *Bifurcatum*	100	50	100	25
Ciprofloxacin	>50	25	50	>25

The extract at 200 µg/mL and 400 µg/mL concentrations, caused significant reduction of DPPH free radicals of 41.17 and 75.82% respectively. The IC50 of the extract is greater than 200 µg/mL and less than 400 µg/mL of the extract (Fig. 2). The extract produced its optimum effect 1.85 µM at 400 µg/mL comparable to ascorbic acid at 125 µg/mL (Fig. 3).

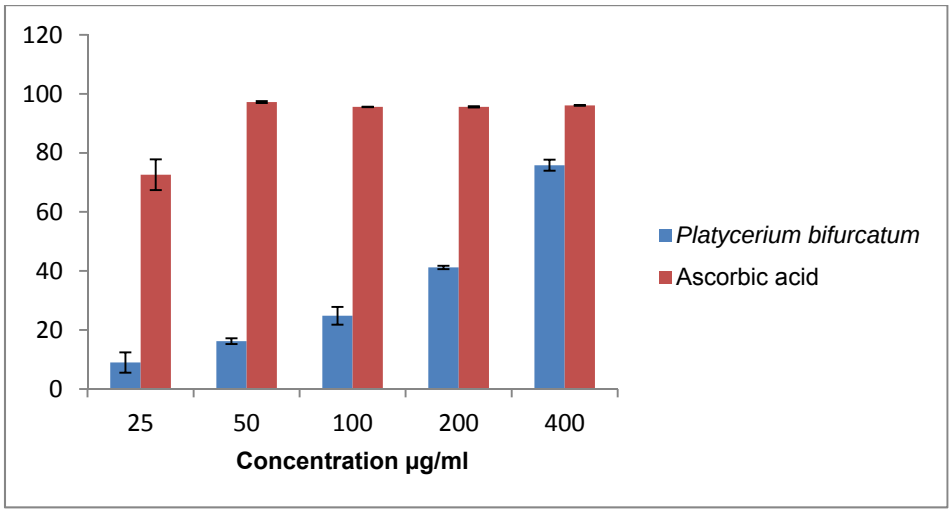

Fig. 2: DPPH radical scavenging activity of *Platycerium bifurcatum*

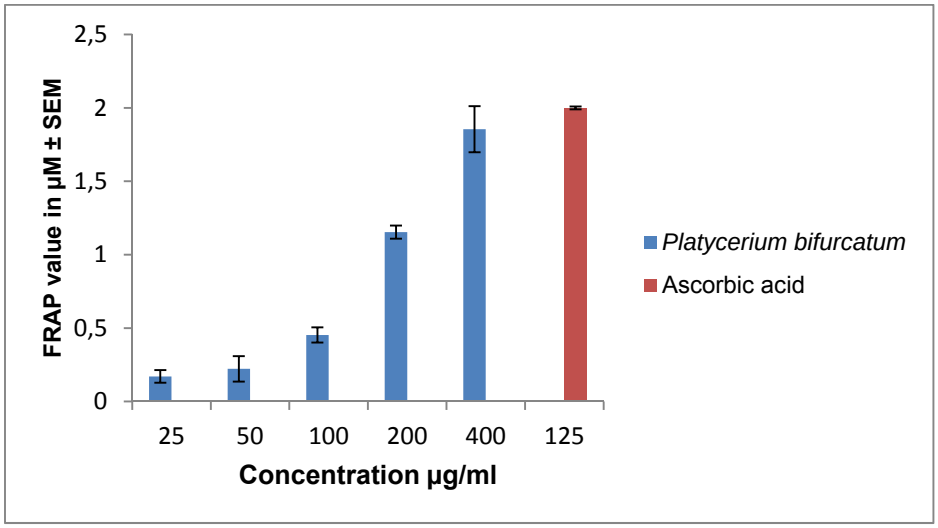

Fig. 3. The FRAP value of *Platycerium bifurcatum*

The antioxidant and antibacterial activities of *P. bifurcatum* can be linked to benzeneethanamine and hydroxyurea [8,9]. The antioxidant, antimicrobial and anti-inflammatory activities of benzeneethanamine have been reported [9,10]. Benzeneethanamine and its derivatives have been identified in several other medicinal plants such *Azardirachta indica*, *Ocimum sanctum*, *Macrotyloma uniflorum* and *Canscora perfoliata* [11-13]. 4-methyamphetamine, methylated derivative of amphetamine and epinephrine are sympathomimetic drugs that are used as central nervous system stimulant in anaphylactic shock, asthma, cardiac arrest, and as anorectic agent [14]. The major side-effect is hypertension [15]. This indicates that the plant can be used with caution in hypertensive patients [14,15]. The analgesic property of *P. bifurcatum* might be linked to epinephrine; pain neuron inhibitor [16].

Hydroxyurea is a hydroxylamine with many biological activities. The antioxidant, antibacterial, anti-sickle cell anaemia, and anticancer activities of hydroxyurea have been documented [8,17-20]. It is also used in the treatment of human

immunodeficiency virus (HIV) [8]. Hydroxyurea has been identified in *Arabidopsis*, *Cajanus cajan*, *Carica papaya* and *Zanthoxylum zanthoxyloides* leaves [21-23]. The presence of hydroxyurea in *P. bifurcatum* suggests that it can be employed in the traditional treatment of sickle cell anaemia, human immunodeficiency virus (HIV) and cancer [19,21]. The demonstrated antibacterial property against both Gram negative and Gram positive isolates, suggests that the plant would be effective in the treatment of infection caused by some of these organism such as wound contamination and enteric diseases [8].

Conclusions

The study validates the folkloric uses of platycerium bifurcatum in the treatment of infectious diseases and provided the pharmacological basis for its medicinal values.

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