Phytochemical Investigation of Methanolic Extracts of *Bryophyllum Pinnatum* by Gas Chromatography-Mass Spectrometry (GC-MS) Method

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Abstract

India is a gold mine of historical medical knowledge. The central Indian region of Bundelkhand has a unique environment, distinguished by sandy sand and rocky soil, yet it is also home to a wide variety of plants. This area is home to several medicinal plants that are prized for their ability to treat a wide range of disorders and dangerous diseases. Over a year, the thorough examinations of this area uncovered about 66 therapeutic herbs and shrubs as well as 38 tree species. In the current study, the phytochemical components of the *Bryophyllum pinnatum* leaf were evaluated. GCMS is used to identify the bioactive components in the methanol-based leaf extract. A preliminary phytochemical screening showed the presence of various Phytocompounds such as glycosides, cardiac glycosides, phenol, tannins, Flavonoids, Phytosterols, Saponins, etc. In a gas chromatography-mass spectrometer examination of the extracts mentioned, a maximum of 42 compounds were identified by mass spectrum matching with the database of the National Institute Standard and Technology (NIST). *Bryophyllum pinnatum* leaves are reported for their biological activities such as Antimicrobial, antioxidant, antitumor, anti-inflammatory, antifungal, Anti-viral, Diabetes, inflammation, cancer, Antidote, coronary-dilator, and diuretic activity.

Keywords: *Bryophyllum pinnatum*, GC-MS analysis, Phytochemical Screening, Methanol, Soxhlet extraction.

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1. INTRODUCTION

Bryophyllum pinnatum (Lam.) Oken (family: Crassulaceae) (synonym: Kalanchoe pinnata, Lam.; common names: Love plant, life plant, Mexican air plant, cathedral bells, Canterbury bells, etc is a perennial herb that is widely grown and utilized in folk medicine in tropical Asia, India, China, Africa, and Australia[1]. In medicinal and aromatic plants, there is a vast potential for the discovery of novel bioactive chemicals [2]. Many of the medications that are today prescribed by doctors have a lengthy history of use as herbal cures or were created as plant-based equivalents [3]. The value of traditional medical systems and particular traditional medical practices is widely acknowledged on a global scale. Due to its ease of accessibility, availability, cultural acceptability, therapeutic potential, and affordability, some authors have recently argued that traditional medicinal systems should be incorporated into the mainstream of healthcare services[4]. The growing interest in using medicinal plants, particularly in primary healthcare, has prompted a rise in interest in plants that may be useful as medicines [5]. In traditional medicine, the leaves and bark of *B. pinnatum* are used to cure lithiasis, earaches, burns, abscesses, diarrhea, and other conditions. They are also bitter tonic, astringent, analgesic, and carminative. Leaf juice is applied topically to treat conditions such as smallpox, earaches, otitis, coughs, asthma, palpitations, headaches, convulsions, and general weakness [6].The treatment is also given to young children who are ill. It is mostly used in traditional medicine to treat hypertension and kidney stones [7]. Recent studies have emphasized positive qualities such as free radical scavenging activity [8].*B. Pinnatum* has high levels of lipids, alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, and bufadienolides. The leaves include a class of substances known as Bufadienolides, which are highly active and have antibacterial, antitumor, cancer-preventive, and insecticidal properties [9].There hasn't been any research done on the phytochemistry of the *B. Pinnatum* that is grown in the Bundelkhand region. Based on information from the literature, we are describing the phytochemical analysis of with association of these phytochemicals in the management of various illnesses in this research. This study will clarify the active principles in charge of its multiple reported therapeutic effects and offer a template for identifying, isolating, and purifying these molecules for additional research and medication development.

2. MATERIALS & METHODS

2.1 Collection of the plant material

The leaves of *B.pinnatum* were collected in October and got authenticated (Accession No. 28571) by The Central Council for Research in Ayurvedic Science

(CCRAS) - Regional Ayurveda Research Institute Gwalior Road, Jhansi Uttar Pradesh, during November 2019.

2.2. Preparation of plant extract:

The plant material was first washed with tap water and distilled water, followed by a 2-4 week period of drying in the shade. Using an electric mixer, the dried leaves (20gm) were converted into a fine powder. *B. pinnatum* leaf powder was put in a Soxhlet apparatus and extracted for 9(W/V) or 16–18 hours at 64.6°C using 200ml of Methanol AR grade solvent. Whatman filter paper No. 41 (110 mM.) and cotton wool were used to filter the pure extract of B. pinnatum leaves after it had been collected. The extract was concentrated using a Rotary Evaporator after filtering. Specifications for the model include EYELA N-12008 and EYELA-UNI TRAP UT-1000, with the water bath set to 45–50°C. The resultant solution was kept chilled at 4°C for further testing.

2.3. Preparation Phytochemical Screening:

The extract was subjected to preliminary phytochemical screening and qualitative analyses using the established techniques for determining the presence of carbohydrates, alkaloids, amino acids, phenol, tannins, flavonoids, saponins, terpenoids, quinines, cardiac glycosides, and steroids by distinctive color change [10,11].

2.4. GC-MS (Gas Chromatography-Mass spectroscopy) analysis:

On the GC-Claus 680 MS-SQ-8C PerkinElmer system, which includes an AOC-20i autosampler and a gas chromatograph attached to a mass spectrophotometer (GC-MS), GC-MS analysis was carried out. The following detection parameters were used to examine the sample: Furnace beginning temperature 40°C for 5 minutes, ramp 12°C/min to 260°C, hold 10 minutes, Inj B auto 250°C, volume 0µL, division 50:1, carrier gas He (99.999%), solvent delay 2.00 minutes, transfer temperature 1800 °C, source temperature 200°C, scan: 50 to 500 Da[12].

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical screening:

Phytochemical screening for primary metabolites (carbohydrates, Starch, proteins, amino acids, oils, and fat) and secondary metabolites (Anthraquinones, Quinines, Glycosides, cardiac, glycosides, Phenol, Tannins, Flavonoids, Phytosterols, Saponins, and Steroids) were carried out.

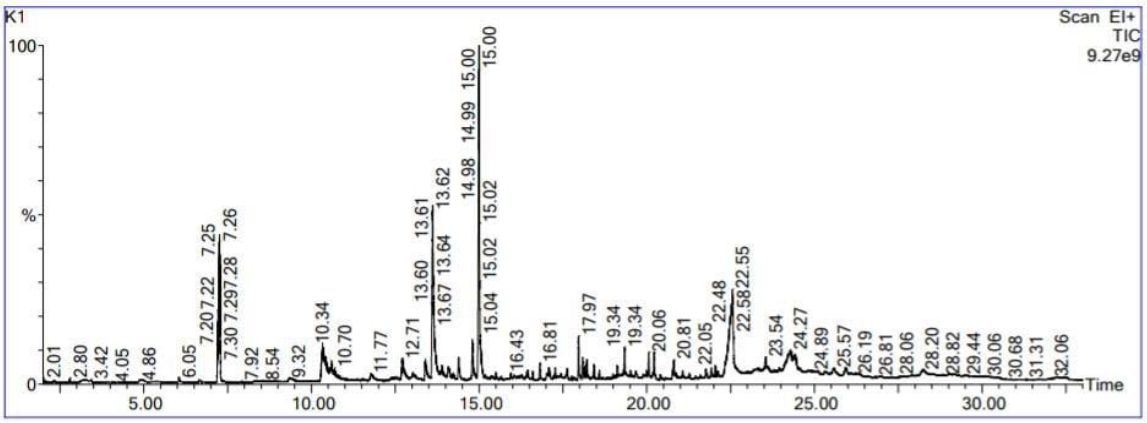
Table1: Preliminary phytochemical evaluation of methanolic extracts of *B. pinnatum*leaves. (+) = Present; (-) = Absent

Phytochemical Compounds Name	**Tests Name**	***Bryophyllum pinnatum* leaf**
Carbohydrates	Benedict's test	+
Starch	Iodine test	-
Proteins	Nitric acid test	+
Amino acids	Ninhydrin reagent test	+
Quinines	KOH test	-
Glycosides	Glacial acetic acid	-
Cardiac glycosides	Keller-Killani test	+
Phenol	Lead acetate test	+
Tannins	Ferric chloride test	+
Flavonoids	H2SO4 test	+
Phytosterols	KOH+ acetic acid+H2SO4	+
Saponins	Vigorously shaken water Test	+
Steroids	chloroform +H2SO4 test	-

3.2 Components are identified:

The National Institute of Standard and Technology (NIST) database, a private collection of more than 7,900 chemicals, was used by MS to interpret the GC chromatogram. Figure 1 provides information about the compound, such as its name, molecular weight, and the composition of its constituent parts as they are present in the test materials.

Figure 1: GC-MS spectrum of Methanol extract of *B. pinnatum*



3.3 GC-MS analysis of methanolic extract from *Bryophyllum pinnatum*:

One of the extensively used methods for identifying the components of volatile matter, including long-chain and branched-chain hydrocarbons, alcohols, acids, and esters, is gas chromatography-mass spectrometry (GC-MS) with the NIST library.

Using the GCMS-NIST library, we were able to isolate 43 main components from the methanolic extract of *B. pinnatum* (Table 2). For each, the Pubchem search was conducted. These important chemicals and details on their alleged pharmacological activities were acquired from previously published literature. tert-Hexadecanethiol, Nonahexacontanoic acid,17-Pentatriacontene, Disulfide, di-tert-dodecyl, cis-1-Chloro-9-octadecene, Tetradecanein our study through GC-MS analysis has revealed the added medicinal value such asAntibacterial and antioxidant activity, used in cosmetics, Antiinflammatory, Anticancer, Antiarthritic, Antidote, coronary-dilator, diuretic, increase superoxide dismutase activity, Skin protection and respiratory protection, and Cytotoxicity, Antipyretic, Anthelmentic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabetic, Antidiarrhoeal, etc. properties of the identified compounds have also been reported in the literature (table 2). The current report on the bioactive components of *B. pinnatum* supports the plant's application in the treatment of numerous illnesses [6]. These substances change according to the climate, soil quality, and geographic location of the plant [13]. *B. pinnatum* contains a variety of bioactive chemicals, which explains why traditional healers employ the entire plant to treat a variety of diseases. Additionally, a thorough examination of the numerous substances found in *B. pinnatum* and their pharmacological assessment may aid in the development of medications with multiple effects for the treatment of cancer and other microbial disorders

Table 2: Detail of compounds identification from GC-MS analysis of an acetone extract of *B. pinnatum.*

Sr. No	RT	Peak %	Phytochemical Compound	Formula	M.W	Activity
1.	11.289	1.505	tert-Hexadecanethiol	$C_{48}H_{99}AuS_3$	969.5	Antibacterial and antioxidant activity [14]
2.	11.289	1.505	Nonahexacontanoic acid	$C_{69}H_{138}O_2$	999.8	used in cosmetics [15]
3.	11.289	1.505	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	917.2	Antioxidant [16]
4.	11.289	1.505	2-Nonadecanone 2,4-dinitrophenylhydrazine	$C_{25}H_{42}N_4O_4$	462.6	Antimicrobial [17]
5.	11.289	1.505	17-Pentatriacontene	$C_{35}H_{70}$	490.9	Antiinflammatory Anticancer Antibacterial Antiarthritic [18]
6.	11.688	2.673	Tetradecane	$C_{14}H_{30}$	198.39	Antimicrobial [19]
7.	11.688	2.673	Nonane, 2-methyl-5-propyl-	$C_{13}H_{28}$	184.36	Anticancer [20]
8.	11.688	2.673	Pentadecane	$C_{15}H_{32}$	212.41	Antibacterial activity [21]
9.	11.870	1.093	Disulfide, di-tert-dodecyl	$C_{24}H_{50}S_2$	402.8	Antidotes, coronary-dilator, and diuretics increase superoxide dismutase activity [22]
10.	11.870	1.093	Methoxyacetic acid, 4-tetradecyl ester	$C_{17}H_{34}O_3$	286.4	Anti-microbial [23]
11.	12.011	1.826	4-Methyldocosane	$C_{23}H_{48}$	324.6	Anti-inflammatory, antimicrobial, antioxidant, diuretic activity, anticancer [24]

12.	12.011	1.826	Heneicosane, 11-(1-ethylpropyl)-	$C_{26}H_{54}$	366.7	Major component of safflower flower essential oil [25]
13.	12.389	1.483	Tetradecane	$C_{14}H_{30}$	198.39	Antifungal and Antibacterial [26]
14.	12.389	1.483	Hexadecane	$C_{16}H_{34}$	226.44	Antifungal, Antibacterial, and antioxidant activity [26]
15.	12.460	1.462	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324.6	Antifungal [27]
16.	12.460	1.462	Heptadecane, 9-octyl-	$C_{25}H_{52}$	352.7	Medical ultrasound diagnostic system [28]
17.	12.788	1.233	Butabarbital	$C_{10}H_{16}N_2O_3$	212.25	Analgesic agent [29]
18.	13.048	1.824	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	288.9	Antioxidant, antimicrobial activity [30]
19.	13.217	1.197	tert-Hexadecanethiol	$C_{48}H_{99}AuS_3$	969.5	Diabetes, inflammation, cancer [31]
20.	13.217	1.197	Geranylisovalerate	$C_{15}H_{26}O_2$	238.37	Anti-inflammatory, antioxidant, and anti-viral activities [32]
21.	13.217	1.197	17-Pentatriacontene	$C_{35}H_{70}$	490.9	Antimicrobial activity [33]
22.	13.217	1.197	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	917.2	antibacterial activity [34]
23.	13.217	1.197	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256.5	antimicrobial, antioxidant activity [35]

24.	13.217	1.197	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.4	Insect pheromone [36]
25.	13.217	1.197	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.5	antibacterial activity [37]
26.	13.217	1.197	5-Octadecenal	$C_{18}H_{34}O$	266.5	Flavoring agent, antimicrobial [38]
27.	13.355	2.562	Nonane, 2-methyl-5-propyl-	$C_{13}H_{28}$	184.36	Anti-cancer [39]
28.	13.436	1.403	tert-Hexadecanethiol	$C_{16}H_{34}S$	258.5	Antitumoral, antioxidant, insecticidal, antifungal [40]
29.	13.629	1.450	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286.9	Skin protection and respiratory protection [41]
30.	13.629	1.450	Bacteriochlorophyll-c-stearyl	$C_{52}H_{72}MgN_4O_4^{-2}$	841.5	lipoxygenaseinhibitor, pesticide [42]
31.	13.629	1.450	Acetic acid, chloro-, octadecyl ester	$C_{20}H_{39}ClO_2$	347	(odor-forming compound) [43]
32.	13.629	1.450	17-Pentatriacontene	$C_{35}H_{70}$	490.9	Antiinflammatory Anticancer Antibacterial Antiarthritic [44]
33.	13.767	1.338	8-Octadecenal	$C_{18}H_{34}O$	266.5	anti-bacterial and anti-fungal activities [45]
34.	13.767	1.338	4-Octadecenal	$C_{18}H_{34}O$	266.5	Antimicrobial, Anti-inflammatory [46]
35.	13.922	1.659	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256.5	antimicrobial, antioxidant [47]
36.	14.309	1.158	Tetradecane	$C_{14}H_{30}$	198.39	Antimicrobial, Cytotoxicity, Antipyretic, Anthelmintic, Tumour,

						Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabetic, Anti-inflammatory, Antidiarrhoeal [48]
37.	14.816	1.150	Heptadecane	$C_{17}H_{36}$	240.5	anti-oxidative [49]
38.	14.816	1.150	Eicosane, 7-hexyl-	$C_{26}H_{54}$	366.7	Anti-androgenic, aldose reductase inhibitor [50]
39.	15.005	1.447	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296.6	Flavouring agent [51]
40.	15.005	1.447	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7	anti-oxidant and antiinflammatory effect [52]
41.	15.349	1.836	Pentadecane	$C_{15}H_{32}$	212.41	Antibacterial activity [53]
42.	15.349	1.836	Dodecane	$C_{12}H_{26}$	170.33	Antibacterial activity [54]
43.	15.349	1.836	Heptadecane	$C_{17}H_{36}$	240.5	Antibacterial activity [55]

4. Conclusion

The present study has characterized the bioactive components of *B. pinnatum* leaves Methanol extract to include phytochemicals such as carbohydrates, Starch, proteins, amino acid, Anthraquinones, Quinines, Glycosides, cardiac glycosides, Phenol, Tannins, Flavonoids, Phytosterols, Saponins, andSteroids. tert-Hexadecanethiol, Eicosane, 7-hexyl-, and Octadecane, 3-ethyl-5-(2-ethylbutyl)- were found to be predominant according to the GC-MS study. Due to the existence of these bioactive substances, *B. pinnatum* leaves are now recognized as a valuable source of prospective lead compounds that have both biological and pharmacological effects, making them a promising option for drug discovery.

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