RESEARCH ARTICLE

Proximate Composition, Mineral analysis and Phytochemical Characterization of an Ethnomedicinal Plant *Sphenodesme involucrata* var. *paniculata* (C.B. Clarke) Munir

Athira S. and Sreeranjini S.*

DOI: 10.18811/ijpen.v10i03.13

Abstract

Since ancient times, people have utilized plants for their therapeutic benefits. These ethnomedicinal plants acquire their therapeutic qualities from some key phytochemicals or secondary metabolites that they contain. *Sphenodesme involucrata* var. *paniculata* (C.B. Clarke) Munir is an ethnomedicinal plant that contains several bioactive components. The study focuses on the proximate composition, mineral analysis and phytochemical characterization of *S. involucrata* var. *paniculata* leaves using LC-MS and GC-MS analysis. The proximate evaluation of the leaves unveiled higher carbohydrate (60.80 ± 0.06 %) content and poor fat (0.80 ± 0.01%). The mineral analysis yielded a higher concentration of calcium (1269.00 ± 1.15 mg/100g) and a lower concentration of zinc (1.60 ± 0.12 mg/100g). The GC-MS analysis of ethanolic extract revealed the existence of several important compounds like phytol, hexadecanoic acid, phenol, 2,4-bis (1,1-dimethylethyl), squalene,1,2-Benzendicarboxylic acid, mono (2-ethyl hexyl) ester etc. Cepharanthine and isoacteoside were found to be prevailing in the LC-MS analysis. Compounds such as somniferine, grossamide, gallic acid and obaberine were also detected. Detection of diverse minerals, nutrients and secondary metabolites in this plant supports its use in traditional medicine. Additionally, it supports the claim that the plant's abundance of vital nutrients and minerals makes it safer and healthier to consume.

Keywords: Cepharanthine, Ethnomedicinal, Isoacteoside, Minerals, Proximate.

Highlights

- First-ever analysis of proximate and mineral composition of the plant.
- Presence of higher amount of carbohydrates, proteins, calcium and magnesium.
- First reporting of secondary metabolites like squalene, cepaharanthine and isoacteoside from this plant.
- Cepharanthine and isoacteoside account for the anti-inflammatory properties of the plant.

International Journal of Plant and Environment (2024); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Nowadays, the use of medicinal plants and herbal remedies is growing in popularity. Herbal medicines are regarded as cost-effective and safe compared to synthetic drugs. The majority of individuals in developing nations rely on herbal medication for several ailments (Uzoekwe & Mohammed, 2015). According to WHO (2014), these plants and herbal medicines play an essential part in the pharmaceutical industry. Medicinal plants act as a depository of various compounds and they contain enormous valuable phytochemicals that act as a precursor for many drugs. These phytochemicals, majorly secondary metabolites are found to have several important bioactivities also. These compounds contribute to the therapeutic potential of the plant. In addition, plants have a number of vital minerals, such as Fe, Ca, Cu, Mn, Mg, Zn, Na, etc. and nutrients like carbohydrates, protein, crude fiber, fat, etc. They have a significant role in the morphological, physiological, and metabolic activities of the human body.

Sphenodesme involucrata var. *paniculata* is a climbing shrub that belongs to Symphoremataceae (~Lamiaceae) family. However, it is also found in the Andaman and Nicobar Islands (Ghosh, 2014), it is native to South India and Myanmar. It is an ethnomedicinal plant used by the tribal (Malappandaram, Mala-arayans, Malaulladans, Malavedans and Malakkurava) communities of Pathanamthitta district, Kerala, India, for the treatment of body pain and rheumatism. They consume ground-pasted leaves along with rice gruels (Binu, 2011). Disorders of the central nervous system are also treated with it (Wiart, 2006). The plant is consumed for abdominal ailments, ear diseases, burns, and worms and the herb also functions as a galactagogue, antimicrobial, and vermifuge (Srivastava & Choudhary, 2008). Apart from these traditional uses, antioxidant, anti-ulcerogenic, anti-inflammatory, and antinociceptive properties of the methanol extracts of the plant have been reported (Sreeja *et al*.,2018 a). It has an interesting phytochemical profile, which includes justicidine B, austricine, gossypin, benzyl glucosinolates, etc. (Sreeja *et al*., 2018 b).

There are reports regarding the use of micro and macronutrients in curing diseases in humans and animals, such as diabetes (Patton, 2007; Farvid *et al*., 2011) and eye diseases

Department of Post Graduate Studies and Research in Botany Sanatana Dharma College Alappuzha, University of Kerala, Kerala, India.

***Corresponding author:** Sreeranjini S., Department of Post Graduate Studies and Research in Botany Sanatana Dharma College Alappuzha, University of Kerala, Kerala, India., Email: sreeranjinis80@gmail.com

How to cite this article: Athira, S. and Sreeranjini, S. (2024). Proximate Composition, Mineral analysis and Phytochemical Characterization of an Ethnomedicinal Plant *Sphenodesme involucrata* var. *paniculata* (C.B. Clarke) Munir. International Journal of Plant and Environment. 10(3), 117-123.

Submitted: 08/10/2024 **Accepted:** 14/10/2024 **Published:** 30/11/2024

(Kowluru *et al*., 2008). These nutrients give medicinal plants the ability to combat a wide range of illnesses. An idea about a plant's nutritional significance can be obtained by estimating nutrient composition and proximate values. When a plant satisfies every need for proximate composition, it is considered a food supplement or herbal medicine (Pandey *et al*.,2006). According to WHO, in order to standardize the herbs, deciding on mineral content and proximate composition is very significant (Rajani & Kanaki, 2008). Even though the plant *S. involucrata* var. *paniculata* has been consumed from earlier times, there are no published records regarding its mineral content and nutritive value. It is important to ensure the safety of consumption and confirm scientifically the health and medicinal advantages of the plant. Apart from the nutritive value the phytochemical profiling is also needed. GC-MS and LC-MS analysis will provide a detailed insight into important bioactive compounds in plant extract, and the chromatogram obtained is beneficial for the quality control of phytochemicals. The objective of this study is to assess the proximate and mineral composition along with GC-MS and LC-MS investigation of the leaves of *S. involucrata* var. *paniculata*. .

Materials And Methods

S. involucrata var. *paniculata.* leaves were collected from the Pandalam Thekkekara region of Pathanamthitta district, Kerala, India, during the month of April 2023. Following plant identification, the voucher specimens were placed in the Madras Herbarium, Botanical Survey of India Coimbatore, with accession number MH 178218. The selected leaves were washed and allowed to air dry. The dried leaves were powdered and stored for analysis.

Proximate Analysis

The proximate composition, such as total carbohydrate, protein, ash, crude fat and moisture content were analyzed by the Association of Official Analytical Chemists (AOAC) method.

Moisture content by oven method

Moisture content in the leaves was analyzed by oven method. Weighed fresh leaves were placed in the oven for 2 hours and the final weight after drying was taken. Moisture content is determined by the measurement of the mass of the sample before and after the removal of water by the evaporation process.

$$Percentage\ of\ moisture\ content = \frac{MI - MD}{MI} \times 100$$

Where MI is the initial mass of the sample before drying and MD is the mass of the specimen following drying.

Determination of protein

The amount of protein was calculated from the organic Nitrogen content by the Kjeldahl method (A.O.A.C. 2019). Sample (2 g) was transferred to a Kjeldahl flask. 0.7g of copper sulphate, 15 g of sodium sulphate anhydrous and 25 mL of concentrated H_2SO_4 were poured into the flask. The flask is kept on the stand in the digestion chamber in a tilted orientation. To prevent spurting, two or three glass beads had been placed. The flask is heated gently until the initial frothing stops and the mixture boils steadily at a moderate rate. The heating was continued till the color of the digest turned pale blue. After cooling, 200 mL of distilled water was poured into the solution in the flask. Then, a piece of granulated zinc was added to the digest. To make it more alkaline, sodium hydroxide solution was added to the digest. After that, the flask was attached to a distillation unit, and the digest was boiled until 150 mL had been distilled. 0.1 N HCl was then used to titrate it.

N HCl= 0.0014 gmN

X N HCl= Y

$$Percentage\ of\ Nitrogen = \frac{Y \times Titre\ value\ of\ sample \times 100}{weight\ of\ sample}$$

$$Total\ protein = Percentage\ of\ Nitrogen \times 6.25$$

Determination of Total fat

The Soxhlet technique of extraction was used to determine total fat using petroleum ether (A.O.A.C. 2019). In a thimble, 10 g of sample were taken. The thimble was positioned in the soxhlet extractor. A circular bottom flask is connected to the extractor and 200 mL of petroleum ether. Extraction was done for 4 hours. After that the solvent was collected and allowed to evaporate in a pre-heated and pre-weighed China dish. For one hour, it was dried at 90°C in the hot air oven. Following cooling, it was weighed.

$$Total\ fat = \frac{(Weight\ of\ dish + Fat) - (Weight\ of\ empty\ dish) \times 100}{Sample\ weight}$$

Estimation of Total ash

Estimation of total ash was done by igniting the sample in a crucible (A.O.A.C. 2016). Sample (2 g) was weighed, placed in the crucible and put in the entrance of the open muffled furnace until it turned carbonized. The carbonized specimen was then put inside the furnace at 600+20°C for 2 hours. The ash was leached with hot water and filtered. The process was continued until carbon free ash was generated. The formula used to determine total ash was:

$$Total\ ash\ percentage(\%) = \frac{Weight\ of\ ash\ (gm)}{Weight\ of\ sample\ (gm)} \times 100$$

Content of Carbohydrate

The following equation was used to determine the percentage of carbohydrates, as described in A.O.A.C. 2019.

$$Percentage\ of\ carbohydrate = 100 - (Crude\ protein\% + Crude\ fat\% + Total\ ash\% + Moisture\ content)$$

Analysis of Mineral content

Mineral content was evaluated by AAS method (A.O.A.C. 2005). In a pre-weighed crucible, 1 g sample was collected and heated at 500°C for 2 hours. It was then allowed to cool. Wet ash with 10 drops of water and 3-4 ml Nitric acid (1+1) was added carefully. A hot plate kept at 100°C was used to evaporate excess nitric acid. The crucible was then put back into the furnace and heated for 1-hour at 500°C. Ash was dissolved in 10 mL HCl (1+1), filtered to a volumetric flask, and then diluted with distilled water to make up to 10 mL. Calibration was done by aspirating standard metal solutions and noting the absorbance.

Statistical analysis

Data from chemical analysis were done in triplicate and one-way ANOVA was employed to record the relevance of each parameter using Duncan's test in SPSS.

GC-MS Analysis

The ethanolic extract of the leaves was subjected to GC-MS analysis. Ethanolic extract was prepared by the soxhelet extraction method. GC-MS (Instrument Model -7890 A GC via 5975C with triple axis detector, Column - DB 5MS 30 m x 0.250 mm Diameter x 0.25 µm Thickness) analysis was performed with the following. A 5:1 split ratio was used to inject 2 µL of the specimen for analysis. The carrier gas, helium gas (99.9995%), was used at a flow rate of 1 mL/min. The study was carried out using an ionization energy of 70 eV in the EI (Electron Impact) mode. A constant temperature of 280°C was maintained for the injector. After matching the resulting spectrum configurations with those of the mass spectral database (NIST -08 spectrum DATA), the compounds were determined.

LC-MS Analysis

Leaf extract derived through soxhlet extraction using methanol was prepared. Methanolic extract subjected to liquid chromatography electron spray ionization-mass spectrometry (LC-ESI-MS) analysis by the facility of Sophisticated Analytical Instrument Facility IIT Bombay. Dual AJS ESI was used as the ion source. A:0.1% formic acid in water and B:100% acetonitrile in water were the mobile phases utilized, having a flow rate of 300 µL/min. LC conditions were 5% at 0 to 1 minute and increased to 100% between 3 to 30 minutes and decreased to 5% at 31 and 35 minutes. The LC-MS Q-TOF Spectrometer was used for analysis.

Results

Proximate and Mineral Analysis

The results of proximate analysis carried out on the leaves of *S. involucrata* var. *paniculata* (Table 1). It implies that the plant contains a significant ($p \leq 0.001$) amount of carbohydrates (60.80 ± 0.06%), poor fat (0.80 ± 0.01%), ash (8.10 ± 0.06%) and a moderate amount of moisture (12.00 ± 0.29%) and protein (18.33 ± 0.09%). Out of the eight minerals analyzed, copper, iron and zinc come under micronutrients and magnesium comes under macronutrients. It contains high amount of Ca (1269.00 ± 1.15 mg/100 g), followed by Mg (259.00 ± 0.58 mg/100 g), Na (118.00 ± 0.58 mg/100 g), Mn (33.30 ± 0.06 mg/100 g), Fe (22.40 ± 0.06 mg/100g), Cu (10.10±0.06 mg/100 g), Zn (1.60 ± 0.12 mg/100 g) and least amount of Pb (0.20 ± 0.06 mg/100 g) From the result it is evident that the leaves have higher calcium and magnesium, a modest amount of iron, sodium, manganese, lower levels of copper and zinc and only a trace amount of lead (Table 2).

Table 1: Proximate composition of *Sphenodesme involucrata* var. *paniculata* leaves

Samples	*Percentage value*
Total fat	0.80 ± 0.01^{e}
Total protein	18.33 ± 0.09^{b}
Moisture	12.00 ± 0.29^{c}
Total ash	8.10 ± 0.06^{d}
Carbohydrates	60.80 ± 0.06^{a}
Df (n-1) = 4	
F value	28647.915***

***significant F value at p≤0.001 as determined by One-way ANOVA

GC-MS Analysis of Ethanolic Extract of leaf sample

The spectrum and data of GC-MS assessment of the ethanolic extract of the leaves were recorded (Fig. 1 and Table 3). About 28 compounds were detected from the NIIST library. The important compounds present in the leaves are found to be phytol, hexadecanoic acid, phenol,2,4-bis (1,1-dimethylethyl),1,2-Benzendicarboxylic acid, mono(2-ethyl hexyl) ester and squalene. 1,2-Benzendicarboxylic acid, mono(2-ethyl hexyl) ester is found in maximum concentration. Analysis revealed that squalene and1,2-benzendicarboxylic acid, mono (2-ethyl hexyl) ester are first ever reporting in this plant.

LCMS Analysis of Methanolic Extract of leaf sample

The LC-MS analysis was employed using both positive and negative ionization modes for better characterization of secondary metabolites present in the leaf methanolic extract. Out of the compounds detected, cepharanthine and Isoacteoside (Fig. 2 a & b) are found to be the most abundant. Isoacteoside ($C_{29}H_{36}O_{15}$) eluted at a retention time of 6.319

Table 2: Mineral content of *S. involucrata* var. *paniculata* leaves

Minerals	*Concentrations(mg/100g)*
Cu	10.10 ± 0.06^{f}
Fe	22.40 ± 0.06^{e}
Ca	1269.00 ± 1.15^{a}
Na	118.00 ± 0.58^{c}
Pb	0.20 ± 0.06^{g}
Mn	33.30 ± 0.06^{d}
Mg	259.00 ± 0.58^{b}
Zn	1.60 ± 0.12^{g}
Df (n-1) = 7	
F value	747760.23***

***significant F value at p≤0.001 as determined by One-way ANOVA

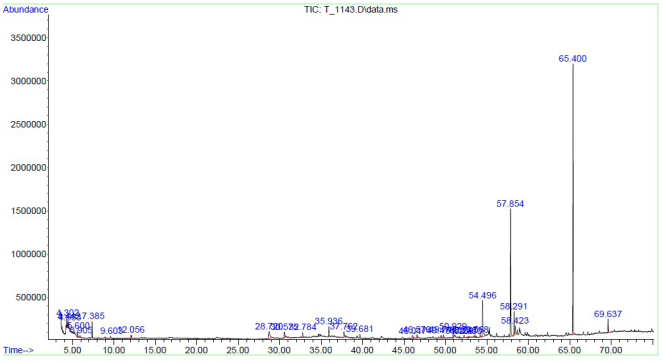

Fig. 1: Chromatogram showing the leaf ethanolic extract of *S. involucrata* var. *paniculata* leaves. Here the highest peak is obtained at retention time 65.40. The compound detected is 1,2-Benzenecarboxylic acid, mono(2-ethylhexyl) ester. Second in position is phytol which is eluted at retention time 57. 854.Here the peak height corresponds to the abundance of the compound

Table 3: List of compounds detected in GC-MS analysis

S. No.	*Retention Time(min)*	*Area (%)*	*Compound*
1	4.302	0.990	Ethane, 1,1-diethoxy-
2	4.442	0.671	Dimethoxymethane
3	4.553	0.465	Acetone alcohol
4	5.600	0.581	Ethanone,2(formyloxy)-1-phenyl
5	5.905	0.382	Pyridine
6	7.385	1.842	Glycolaldehyde dimethyl acetal
7	9.603	0.357	Isopentyl acetate
8	12.056	0.800	Butane, 1,1-diethoxy-3-methyl
9	28.720	3.633	2-Methoxy-4-vinylphenol
10	30.575	1.821	Phenol, 2,6-dimethoxy
11	32.784	0.859	Tetradecane
12	35.936	1.798	Phenol,2,4-bis(1,1dimethylethyl)-
13	37.762	1.637	Ethanone,1-(3,4-dimethoxyphenyl)
14	39.681	1.024	Hexadecane
15	46.047	0.939	Ethanol, 2- (tetradecyloxy
16	46.576	1.216	Propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-
17	49.478	0.590	5-Octadecene, (E)-
18	49.744	0.676	Heptadecane
19	50.929	1.320	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
20	51.709	0.302	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
21	52.247	0.508	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
22	52.750	0.339	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione
23	53.568	0.512	Pentadecanoic acid, 14-methyl-, methyl ester
24	54.496	9.046	n-Hexadecanoic acid
25	57.854	21.343	Phytol
26	58.291	3.601	1-Isobutylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo [3.3.3] undecane
27	58.423	2.656	cis-Vaccenic acid
28	65.400	38.052	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
29	69.637	2.037	Squalene

minutes and has a mass of 624.214. Cepharanthine ($C_{37}H_{38}N_2O_6$), having mass 606.2761, eluted at a retention time of 22.265 minutes. Apart from this, the plant contains several other compounds like somniferine, acetamidopropanal, grossamide, cancentrine, nicardipine, ryanodine, gallic acid, obabaerin, melanostatin, etc. Grossamide was found in both the positive and negative ionization modes.

Discussion

Phytochemicals and nutrients are important components present in plants. They help to attribute the therapeutic and

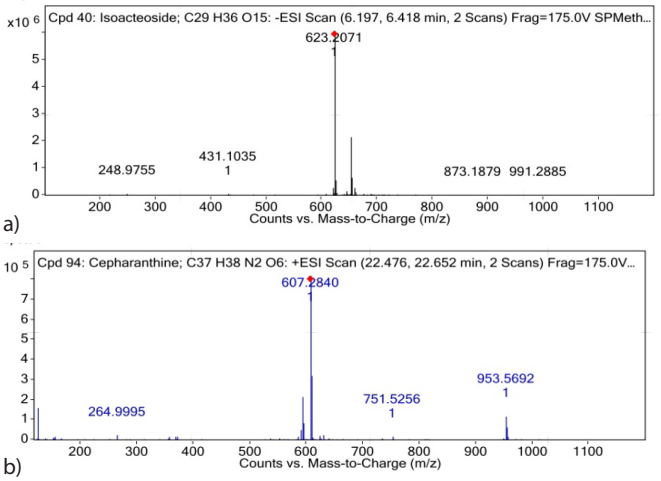

Fig. 2: Mass spectrum of a) Isoacteoside b) Cepharanthine: a) Mass spectrum of isoacteoside obtained in LC-MS characterization using negative ionization mode. The compound eluted at retention time 6.319 a with m/z value 623.2071; b) Mass spectrum of cepharanthine obtained in LC-MS characterization using positive ionization mode. The compound eluted at retention time 22.576 and m/z value 607.284

pharmacological properties of medicinal plants. Carbohydrate, protein and fat are the nutrients present in plants. Carbohydrates account for the major share of phytonutrients in leaves of this plant and it is present in significant amounts ($p \leq 0.001$). It constitutes a significant class of organic chemicals that are found in nature. The analysis revealed the leaves are good source of carbohydrates when consumed since it satisfy the RDA Recommended Dietary Allowance value (RDA value 50 to 100 g/day) (Lalitha Sree & Vijayalakshmi, 2018). Similar reports about the evident predominance of carbohydrates were reported in leaves of *Dracena* reflexa (A, Shukala. *et al*., 2015). The fundamental units of a cell are considered to be proteins. They are necessary for all vital activities. They contribute for developmental functions, fluid balance, hormone formation and maintenance of immunity (Emebu & Aniyika, 2011). *S. involucrata* var. *paniculata* leaves contain a moderate amount of protein.

The leaves contain comparatively low amounts of ash and very low fat content. The sample's total ash content portrays the amount of insoluble and soluble minerals in the specimen (Emebu & Aniyika, 2011). It also shows the level of contaminants, organic and inorganic substances, and food material quality in the sample. Lower values of ash indicate that the sample is free of foreign matter. Fat content is found to be poor. It is good since fat consumption may lead to some cardiovascular diseases.

Moisture content in leaves is found to be in moderate amounts (12%). Moisture content accounts for a high degree of food spoilage. It will speed up the growth of microorganisms and reduce the lifespan of stored food. Macro and micronutrients determine a plant's curative properties.

Calcium and magnesium are present in higher concentrations. The amount of calcium is significantly ($p \leq 0.001$) very excessive when contrasted to other minerals. They are essential for the formation and strengthening of bones and teeth, as a cofactor for enzymes, etc. (Uzoekwe & Mohammed, 2015). There are similar reports possessing high concentrations of calcium and magnesium in the leaves of *Ficus capsensis* (Uzoekwe

& Mohammed,2015). Calcium is involved in blood clotting and cellular permeability (Lalitha Sree &Vijayalakshmi, 2018). Magnesium helps in bone formation, catalyzing enzymatic activity and energy metabolism (Ilodibia *et al*., 2016). It is also known to prevent some heart diseases. Sodium is involved in maintaining electrolyte balance in the body. Iron is a necessary component required for the formation of hemoglobin, immunity and oxygen transport (Smith & Hammarsten, 1958). It also forms part of several enzymes. Mineral copper forms the structural component of several enzymes (Hambridge, 1974). It helps to stimulate antioxidant reactions. Manganese is essential for hemoglobin formation (Odhav *et al*., 2007). Lead is present only in trace amounts. A higher concentration of Pb is not desirable for the human body. It may cause toxicity. The mineral composition acts as a crucial factor in assessing the plant's stress, toxicity, and nutritional value (Indrayan *et al*., 2005).

GC-MS analysis identified a number of significant bioactive substances. Phenol 2,4-bis (1,1-dimethylethyl) has analgesic, anticancer as well as antioxidant properties (Prasad & Strzalka, 2002). It is also found to have acetylcholinestrase inhibition activity (Mujeeb *et al.,* 2014). Hexadecanoic acid has antioxidant (Kim, 2017) and anti-inflammatory activity and for rheumatism treatment, hexadecanoic acid-rich medicinal oils are used (Dandekar *et al*., 2015). Phytol possesses anti-inflammatory, antinociceptive, antimicrobial, cytotoxic and antioxidant activities (Aparna *et al*., 2012). 1,2-Benzendicarboxylic acid, mono (2-ethyl hexyl) ester was reported with cytotoxic activity (Krishnan *et al*., 2014; Islam *et al.,* 2018). The nutraceutical, pharmaceutical and cosmetic industries all have a great deal of attention on the triterpene squalene. It is essential for the biosynthesis of cholesterol, steroid hormones, vitamin D, etc., in the human body. Studies have demonstrated that squalene has anticancer, antioxidant, drug-carrying, skin-hydrating and immune-stimulating properties (A, Rani. *et al*., 2018). It aids in shielding the skin from oxidative free radical damage and is employed in alleviating skin disorders like acne, psoriasis, and seborrheic dermatitis (Huang *et al*., 2009). Nowadays, it is gaining popularity in molecular nanomedicine due to its distinctive molecular conformation and biocompatible nature (Fox, 2009). A new concept of squalenoylation has been created in which squalene was conjugated to anticancer drugs such as gemicitabine, paclitaxel, and cisplatin (Couvreur, 2016).

LC-MS analysis showed the presence of potent bioactive compounds like cepharanthine, somniferine, grossamide, acetamidopropanal, etc. Cepharanthine is an alkaloid that is known to have anti-inflammatory, analgesic, antipyretic, antioxidant, antiatherosclerosis and antiparasitic activities. It also possesses ability to prevent osteoporosis. It is reported in several *Stephania* species. It is widely employed in Japan for treating fever, leukopenia, snakebites, xerostomia, and alopecia. Recent studies suggest cepharanthine is a potent coronavirus inhibitor that is beneficial for managing the SARS-CoV-2 virus. One of the major natural sources of cepharanthine is *Stephania cepharantha Hayata* (Bailly, 2019). It is a potent molecule with immense therapeutic potential. Grossamide possesses potent anti-inflammatory and anti-neuroinflammatory effects (Rogosnitzky *et al.,* 2020; Zhuang *et al*., 2021). Isoacteoside is a phenylethanoid glycoside. It was previously isolated from *Monochasma savatieri* Franch. ex Maxim. It possesses immense anti-inflammatory activity and also have antioxidant and neuroprotective actions. It endeavors the anti-inflammatory activity by blocking the TLR-4 dimerization (Luo *et al*., 2017; Gao,2017). TLR-4 is a transmembrane protein of the toll-like receptor family whose activation leads to intracellular signaling pathway NF-Kb and inflammatory cytokine production (Li, 2018). Gallic acid is also a potent anti-inflammatory agent (Bai *et al*., 2021). These compounds are the first ever reported in this plant. Previous works suggest the presence of compounds like Justicidin B, gossysipin, benzylglucosinolates, etc. (Sreeja *et al.,* 2018 b). Obaberine is found to have hypotensive activity (Wu *et al*., 1976). Other alkaloids detected from the plant include somniferine, cancentrine, ryanodine etc. The phytochemical studies have shown promising results for bioprospecting this medicinal plant.

Conclusion

Numerous secondary metabolites with potential bioactivity are found to be present in the plant. The usage of this plant for treating a variety of ailments in traditional medical practices was justified by the presence of these metabolites. The study makes it clear that the plant has nutritional value in addition to its ethnomedicinal significance. It indicates that the presence of important nutrients and minerals make it both safe to ingest and beneficial for maintaining excellent health. It is expected that the present comprehensive documentation will provide leads to further research on the bioprospecting of *S. involucrata* var. *paniculata* .

Acknowledgment

We would like to express our sincere gratitude to the University of Kerala and the Department of Post Graduate Studies and Research in Botany, Sanatana Dharma College Alappuzha, for providing research facilities. We are thankful to CSIR (Council of Scientific & Industrial Research), India, for providing funding in the form of a JRF fellowship. We would also like to thank SAIF, IIT Mumbai, India for LC-MS characterization.

Authors Contributions

The first author designed and carried out the work and did manuscript preparations. The paper was edited and revised by second author. The final manuscript was read and approved by both the authors.

Conflict Of Interest

There is no conflict of interest in the present research work

References

A.O.A.C. (2016). Official Methods of Analysis of the Analytical Chemists. International 20th Edition, (40.1.08).

A.O.A.C. (2019). Official Methods of Analysis of the Analytical Chemists. International 21st Edition (40.01.06)

A.O.A.C. (2019). Official Methods of Analysis of the Analytical Chemists. International 21st Edition,Vol.3 (40.1.05).

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-Inflammatory Property of n-Hexadecanoic Acid:

Structural Evidence and Kinetic Assessment. *Chemical Biology & Drug Design*, *80*(3), 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x

Bai, J., Zhang, Y., Tang, C., Hou, Y., Ai, X., Chen, X., . . . Meng, X. (2021). Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. *Biomedicine & Pharmacotherapy*, *133*, 110985. https://doi.org/10.1016/j.biopha.2020.110985

Bailly, C. (2019). Cepharanthine: An update of its mode of action, pharmacological properties and medical applications. *Phytomedicine*, *62*, 152956. https://doi.org/10.1016/j.phymed.2019.152956

Binu, S. (2011). Medicinal plants used for treating body pain by the tribals in Pathanamthitta district, Kerala, India. *Indian Journal of Traditional Knowledge*,10(3), 547-549

Couvreur, P. (2016). Squalenoylation : A novel technology for anticancer and antibiotic drugs with enhanced activity. In: LourtiozJ-M,Lahmani M,HaeberlinCD, Hesto P(Eds). Nanosciences and nanotechnology, Springer, Switzerland,253-272.https://doi.org/10.1007/978-3-319-19360-1_11

Dandekar, R., Fegade, B., & Bhaskar, V.H. (2015). GC-MS analysis of phytochemicals in alcohol extract of *Epiphyllum oxipetalum* leaves. *Journal of Pharmacognosy and Phytochemistry*, 4(01),149-154.

Emebu, P. K., & Anyika, J. U. (2011). Proximate and Mineral Composition of Kale (Brassica oleracea) Grown in Delta State, Nigeria. *Pakistan Journal of Nutrition*, *10*(2), 190–194. https://doi.org/10.3923/pjn.2011.190.194

Farvid, M. S., Homayouni, F., Amiri, Z., & Adelmanesh, F. (2011). Improving neuropathy scores in type 2 diabetic patients using micronutrients supplementation. *Diabetes Research and Clinical Practice*, *93*(1), 86–94. https://doi.org/10.1016/j.diabres.2011.03.016

Fox, C.B. (2009). Squalene emulsions for parenteral vaccine and drug delivery. *Molecules*, 14(09), 3286-3312. https://doi.org/10.3390/molecules14093286

Gao, H., Cui, Y., Kang, N., Liu, X., Liu, Y., Zou, Y., … Chen, X. (2017). Isoacteoside, a dihydroxyphenylethyl glycoside, exhibits anti-inflammatory effects through blocking toll-like receptor 4 dimerization. *British Journal of Pharmacology*, *174*(17), 2880–2896. https://doi.org/10.1111/bph.13912

Ghosh, A. (2014). Endemic climbing genera of North Andaman Islands, India. *International Journal of Pharmacy & Life science*, 5(06), 3611-3616.

Hambridge, K.M. (1974).Chromium nutrition in man. *American Journal of Clinical Nutrition*, 27(05), 505-514.

Huang, Z.R., Lin,Y.K. & Fang, J.Y. (2009). Biological and Pharmacological Activities of Squalene and Related Compounds: Potential Uses in Cosmetic Dermatology. *Molecules*, *14*(1), 540–554. https://doi.org/10.3390/molecules14010540

Ilodibia, C., Ewere, F., Akachukwu, E., Adimonyemma, R., Igboabuchi, N., & Okeke, N. (2016). Proximate Composition, Vitamin and Anatomical Studies on Gomphrena celosioides. *Annual Research & Review in Biology*, *10*(3), 1–6. https://doi.org/10.9734/arrb/2016/25546

Indrayan, A.K., Sharma, S., Durgapal, D., Kumar, N. & Kumar, M. (2005). Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Science*, 89(7),1252- 1255

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., … Yeung, A. W. K. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, *121*, 82–94. https://doi.org/10.1016/j.fct.2018.08.032

Kim, C. R., Choi, S. J., Kim, J. K., Park, C. K., Gim, M. C., Kim, Y.-J., … Shin, D.-H. (2017). 2,4-Bis(1,1-dimethylethyl)phenol from Cinnamomum loureirii Improves Cognitive Deficit, Cholinergic Dysfunction, and Oxidative Damage in TMT-Treated Mice. *Biological & Pharmaceutical Bulletin*, *40*(6), 932–935. https://doi.org/10.1248/bpb.b16-00997

Kowluru, R. A., Kanwar, M., Chan, P., & Zhang, J. P. (2008). Inhibition of retinopathy and retinal metabolic abnormalities in diabetic rats with AREDS-Based micronutrients. *Archives of Ophthalmology*, *126*(9), 1266. https://doi.org/10.1001/archopht.126.9.1266

Krishnan, K., Mani, A., & Jasmine, S. (2014). Cytotoxic Activity of Bioactive Compound 1, 2- Benzene Dicarboxylic Acid, Mono 2- Ethylhexyl Ester Extracted from a Marine Derived Streptomyces sp. VITSJK8. *International Journal of Molecular and Cellular Medicine*, *3*(4), 246–254. https://pubmed.ncbi.nlm.nih.gov/25635251

Lalitha Sree, T., & Vijayalakshmi, K. (2018). Proximate Composition, Nutritional Evaluation and Mineral Analysis in the Leaves of an Indigenous Medicinal Plant ,Alternanthera Sessilis. *International Journal of Health Sciences and Research*, 8(7),55-62

Li, M., Xu, T., Zhou, F., Wang, M., Song, H., Xiao, X., & Lu, B. (2018). Neuroprotective effects of four phenylethanoid glycosides on H2O2-Induced apoptosis on PC12 cells via the NRF2/ARE pathway. *International Journal of Molecular Sciences*, *19*(4), 1135. https://doi.org/10.3390/ijms19041135

Luo, Q., Yan, X., Bobrovskaya, L., Ji, M., Yuan, H., Lou, H., & Fan, P. (2017). Anti-neuroinflammatory effects of grossamide from hemp seed via suppression of TLR-4-mediated NF-κB signaling pathways in lipopolysaccharide-stimulated BV2 microglia cells. *Molecular and Cellular Biochemistry*, *428*(1-2), 129–137. https://doi.org/10.1007/s11010-016-2923-7

Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of Aegle marmelos. *BioMed Research International*, *2014*, 1–11. https://doi.org/10.1155/2014/497606

Odhav, B., Beekrum, S., Akula, U., & Baijnath, H. (2007). Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. *Journal of Food Composition and Analysis*, *20*(5), 430–435. https://doi.org/10.1016/j.jfca.2006.04.015

Pandey, M., Abidi, A. B., Singh, S., & Singh, R. P. (2006). Nutritional Evaluation of Leafy Vegetable Paratha. *Journal of Human Ecology*, *19*(2), 155–156. https://doi.org/10.1080/09709274.2006.11905871

Patton, S. R., Dolan, L. M., & Powers, S. W. (2007). Dietary Adherence and Associated Glycemic Control in Families of Young Children with Type 1 Diabetes. *Journal of the American Dietetic Association*, *107*(1), 46–52. https://doi.org/10.1016/j.jada.2006.10.012

Prasad, M. N. V., & Strzałka, K. (2002). Physiology and biochemistry of metal Toxicity and tolerance in plants. *Springer eBooks*. Kluwer Academic Publishers. Dordrecht.Pp.460.

Rajani, M., & Kanaki, N. (2008). Phytochemical Standardization of Herbal Drugs and Polyherbal Formulations. In: Ramawat, K., Merillon, J. (eds) Bioactive Molecules and Medicinal Plants. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-74603-4_19

Rani, A., Meghana, R., & Kush, A. (2018). Squalene production in the cell suspension cultures of Indian sandalwood (Santalum album L.) in shake flasks and air lift bioreactor. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *135*(1), 155–167. https://doi.org/10.1007/s11240-018-1452-3

Rogosnitzky, M., Okediji, P., & Koman, I. (2020). Cepharanthine: a review of the antiviral potential of a Japanese-approved alopecia drug in COVID-19. *Pharmacological Reports*, 72(6), 1509–16. https://doi.org/10.1007/s43440-020-00132-z

Shukla, A., Vats, S., & Shukla, R. (2015). Phytochemical Screening, proximate analysis and antioxidant activity of Dracaena reflexa lam. leaves. *Indian Journal of Pharmaceutical Sciences*, *77*(5), 640. https://doi.org/10.4103/0250-474x.169035

Smith, W. O., & Hammarsten, J. F. (1958). Serum magnesium in clinical disorders. *Southern Medical Journal*, *51*(9), 1116–1119. https://doi.org/10.1097/00007611-195809000-00005

Sreeja, P. S., Arunachalam, K., Saikumar, S., Kasipandi, M., Dhivya, S., Murugan, R., & Parimelazhagan, T. (2018). Gastroprotective effect and mode of action of methanol extract of Sphenodesme involucrata var. paniculata (C.B. Clarke) Munir (Lamiaceae) leaves on experimental gastric ulcer models. *Biomedicine & Pharmacotherapy*, *97*(97), 1109–1118. https://doi.org/10.1016/j.biopha.2017.11.030

Sreeja, P. S., Arunachalam, K., De Oliveira Martins, D. T., Da Silva Lima, J. C., Balogun, S. O., Pavan, E., . . . Parimelazhagan, T. (2018b). Sphenodesme involucrata var. paniculata (C.B. Clarke) Munir.: Chemical characterization, anti-nociceptive and anti-inflammatory activities of methanol extract of leaves. *Journal of Ethnopharmacology*, *225*, 71–80. https://doi.org/10.1016/j.jep.2018.06.035

Srivastava, R.C., & Choudhary, R.K. (2008). Species diversity and economic importance of the family Verbenaceae in Arunachal Pradesh. *Bulletin of Arunachal Forest Research*, 24(1&2), 1-21

Uzoekwe, N.M., & Mohammed, J.J. (2015). Phytochemical, Proximate and Mineral contents of Leaves and Back of Ficus Capensis. *Journal of*

Applied Sciences and Environmental Management, *19*(4), 633. https://doi.org/10.4314/jasem.v19i4.10

Wiart, C. (2006). Medicinal Plants of Asia and the Pacific. CRC Press, Taylor and Francis, New York, pp.336. https://doi.org/10.1201/9781420006803

Wu, W. N., Beal, J. L., Mitscher, L. A., Salman, K. N., & Patil, P. (1976). Alkaloids of Thalictrum. XV. Isolation and identification of the hypotensive alkaloids of the root of Thalictrum lucidum. *Lloydia*, *39*(4), 204–212.

Zhuang, X.C., Zhang, Y.L., Chen, G.L., Liu, Y., Hu, X.L., Li, N., Wu, J.L., & Guo, M. Q. (2021). Identification of Anti-Inflammatory and Anti-Proliferative Neolignanamides from Warburgia ugandensis Employing Multi-Target Affinity Ultrafiltration and LC-MS. *Pharmaceuticals*, *14*(4), 313. https://doi.org/10.3390/ph14040313