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Pharmacognostic and Phytochemical Characterization of Cyanthillium cinereum Leaves

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The study investigates the pharmacognostic and phytochemical properties of Cyanthillium cinereum (L.) H. Rob., a medicinal plant widely used in traditional medicine. The leaves of the plant were collected, authenticated, and subjected to a comprehensive analysis, including physico-chemical, phytochemical, and fluorescence evaluations. Physico-chemical parameters, such as ash values, moisture content, and extractive values for various solvents, were determined, highlighting the plant's purity and potential for medicinal applications. Phytochemical screening revealed the presence of key bioactive compounds, including alkaloids, flavonoids, tannins, and glycosides, with ethanol extracts showing the highest total phenolic and flavonoid content. Fluorescence analysis further characterized the powdered leaves under various light and chemical treatments, enhancing their pharmacognostic profile. The findings demonstrate the plant's significant phytochemical richness, particularly in ethanolic extracts, which exhibited superior antioxidant properties. These results provide a foundation for the standardization and quality control of C. cinereum, underscoring its therapeutic potential in modern pharmacology.

1. Introduction

India boasts a rich heritage of traditional medicine systems, including Ayurveda, Unani, Siddha, and Homeopathy, which have been practiced since ancient times. For centuries, plants have been utilized as medicinal agents to promote and sustain human health [1]. They continue to serve as vital natural sources for bioactive compounds found in modern pharmacopoeias [2]. To harness the therapeutic potential of traditional medicines, it is essential to investigate their phytoconstituents using phytochemical screening, pharmacological evaluations, and analytical techniques [3]. A critical initial step in this process is herb characterization, which involves conducting a thorough pharmacognostic analysis to assess the crude drug's external

features, microscopic properties, and physical attributes [4].

Cyanthillium cinereum (L.) H. Rob., also known by its synonym Vernonia cinerea (L.) Less., is commonly referred to as "Sahadevi" in India and "Bach dau ong" in Vietnamese traditional medicine [5]. This plant belongs to the genus Vernonia within the family Asteraceae, which is notable for being the largest genus in the Vernoniae tribe, encompassing approximately 1,000 species. The genus was named after William Vernon, a botanist who studied and categorized these plants during the late 17th century. V. cinerea is primarily found in tropical regions, ranging from Asia and Africa to Australia. In Ayurvedic medicine, it has been traditionally used to manage conditions such as eruptive boils, parasitic worms, skin disorders, leprosy, and arthritis. In Vietnam, it is valued for its natural sedative and pain-relieving properties [6,7]. Given its extensive use in traditional practices, V. cinerea has become a focus of scientific research, with studies exploring its bioactive compounds and pharmacological potential. In spite of its numerous medicinal attributes, no published work is available till date on pharmacognostical and phytochemical characterization of its leaves. Considering this, the present study was undertaken to develop a quality standard for leaves of this plant. In the present study, investigations were attempted to record the quantitative values such as physical parameters, phytochemical screening of C. cinereum leaves. This would be a milestone in assessing the quality of the crude drug for further development.

2. Material and Methods

Plant Material Collection

The leaves of Cyanthillium cinereum were collected from the outskirt area of Mandi, Himachal Pradesh. The plant was authenticated by Himachal Pradesh State Biodiversity Board, Shimla, Himachal Pradesh. The leaves were shade-dried at room temperature, and powdered with mortar and pestle kept in an amber colour container prior to analysis.

Physico-Chemical Analysis

The physico-chemical analysis was done on the extract of the sepals by estimating the extractive values, ash values, swelling index, foaming index, moisture content, and foreign organic matter content. Total ash, acid insoluble and water-soluble ash values were estimated and extractive values of petroleum ether, 50 % ethanol and water were also determined. The foreign organic matter, swelling index and the foaming index were also analyzed [8].

Fluorescent Studies:

Fluorescence analysis of the powdered leaves was performed to determine the characteristic colour when dissolved in specific solvents. Observations were made under visible day light and UV light of short wavelength (λ 254 nm) and UV light of long wavelength (365 nm) [9].

Preliminary Phytochemical Screening:

Phytochemical screening was done to detect the presence of alkaloids, saponins, tannins, flavonoids, glycosides and terpenes [10].

Preparation of Extracts:

Firstly, the plant leaves were washed with water to remove dirt and other foreign matters and were separated and shade dried. Dried leaves were then milled to coarse powder and then passed over sieve No. 14. The obtained dried powdered leaves of C. cinereum (20 g) were placed in the tube of Soxhlet apparatus in the form of thimble and extracted with various solvents as ethyl acetate, ethanol and water (300 mL) at 60–65 °C for 3–4 h.The obtained extracts were filtered while hot and dried by evaporation using rotary vacuum evaporator and the final dried extracts samples were kept at low temperature in refrigerator for further study.

Total Polyphenols and flavonoid Contents:

The total phenolic content (TPC) and flavonoid (TFC) content of each C. cinereum leaf extracts were determined using the earlier reported method. TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g of extract, while the TFC was expressed as mg of quercetin equivalents (QE) per 100 g [11].

3. Results and Discussion

Physico-Chemical Analysis:

The results of physicochemical parameters are summarized in Table 1. Sulphated ash value (2.43%) was found lower than the total cash value (2.86%). The acid insoluble ash was found to be 0.54% and water-soluble ash value was 0.39%. The total ash indicates the amount of physiological ash, which includes mineral components such as calcium, magnesium and potassium and non-physiological ash mainly due to sand and soil that may be present in the sample [12]. The water-soluble ash value on the other hand could give an indication of previously extracted plant materials, while acid insoluble ash also gives the amount of inorganic material such as silica or metals being present. This is important in detecting the presence of excess sand and silica that could adulterate the sample. These total cash values aids in the identification of low-grade products and the establishment of purity characteristics. On further studies it was concluded that the drug contain 7.23% moisture content, foaming index and swelling index were found to be nil, while foreign organic content was reported to be less than 1%. The extractive values for various solvents such as ethanol, ethyl acetate and aqueous were found to be 39.18%, 22.34%, 13.67% respectively. No swelling index was observed for the powdered leaves of C. cinereum. This also suggests the absence of appreciable amounts of gums and mucilage.

Table 1. Physicochemical parameters of leaves of C. cinereum

Physiochemical	Results (% w/w)
Ash Value	
Total ash	2.86
Acid insoluble ash	0.54
Water soluble ash	0.39
Sulphated ash	2.43
Extractive Value	

Ethanolic Extract Ethyl Acetate Extract Aqueous Extract	39.18 22.34 13.67
Moisture Content	7.23
Foreign organic matter	Less than 1% (Presence of petiole stalks with leaves)
Foaming index	Nil
Swelling index	Nil

Preliminary Phytochemical Screening:

Various phytochemical analysis tests supported that the C. cinereum extracts contain alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and glycosides, recorded in Table 2. The aqueous extract was found to be negative for the presence of alkaloids as compared to ethanolic and ethyl acetate extract while the saponins, steroids, proteins and amino acids were absent in all three extracts.

Table 2. Phytochemical screening of C. cinereum leaves extracts

Phytoconstituents	Ethanolic Extract	Ethyl acetate Extract	Aqueous Extract
Carbohydrates	+ve	+ve	+ve
Alkaloids	+ve	+ve	−ve
Phenolic compounds	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Steroids	−ve	−ve	−ve
Proteins & amino acids	−ve	−ve	−ve
Fixed oils & fats	−ve	−ve	−ve

+ve- Present; −ve- Absent.

Fluorescence Analysis:

The fluorescence characters of powdered drug impart a valuable role in the determination of quality and purity of the drug materials. The powdered drugs when subjected to ultraviolet light and visible light in the presence of various chemical reagents, exhibit characteristic fluorescence [13]. Fluorescence report of C. cinereum powdered leaf is tabulated in Table 3.

Table 3. Fluorescence characteristics of leaves extracts

S. No.	Treatment	Visible light	UV Light	
			254 nm (Short wavelength)	366 nm (Long wavelength)
1	Leaf powder	Green	Green	Light green
2	Leaf powder rubbed on filter paper	Green	Light green	Light green

3	Leaf powder + 1N NaOH	Reddish brown	Brown	Brown
4	Leaf powder + 1N HCl	Light green	Light green	Light green
5	Leaf powder + 1N HNO_3	Light green	Light yellow	Light yellow
6	Leaf powder + 1N H_2SO_4	Green	Light green	Light green
7	Ethanolic extract of Leaf powder	Dark green	Green	Brown
8	Ethyl acetate Extract of Leaf powder	Green	Light green	Light green
9	Aqueous extract of leaf powder	Greenish brown	Brown	No fluorescence

Total Phenolic Content

TPC activity is the method for determining the phenolic level in the herbal extracts. These phenolic compounds possess redox properties and which allow them to act as potential antioxidants [14,15]. As a basis, phenolic content was measured using the Folin-Ciocalteu reagent in each extract. The results were expressed in gallic acid equivalents (GAE) per gram dry extract weight (Table 4). The results indicate that the ethanolic extractive exhibited higher TPC comparatively to the ethyl acetate and aqueous extractives which are about 62.96 ± 0.11 mg GAE/g for ethanolic extract, 35.54 ± 0.14 mg GAE/g for ethyl acetate extract, and 21.94 ± 0.23 mg GAE/g for aqueous extract. The total phenolic contents were calculated using the following linear equation based on the calibration curve of gallic acid ($y = 0.0102x + 0.4698$, $R^2 = 0.9827$). Greater phenolic level in the ethanolic extractive could suggest a potent bioactivity and potential antioxidant and antimicrobial activities.

Total Flavonoid Content

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups [16]. As a basis quantitative determination, flavonoid contents in selected plant extracts were determined using aluminium chloride in a colorimetric system. The outcomes were expressed in quercetin equivalents (QE) per gram dry extract weight (Table 4). The results showed that the ethanolic extract exhibited higher TFC as compared to the ethyl acetate and aqueous extracts which are approximately about 71.83 ± 0.19 mg GAE/g for ethanolic extract, 40.31 ± 0.17 mg QE/g for ethyl acetate extract, and 23.90 ± 0.21 mg GAE/g for aqueous extract.The total flavonoid contents were calculated using the following linear equation based on the calibration curve of quercetin ($y = 0.014x + 0.329$, $R^2 = 0.9496$).

Table 4. The total phenolic and flavonoid content.

Extracts	Phenolic content (mg/g GAE)	Flavonoid (mg/g QE)
MECC	62.96 ± 0.11	71.83 ± 0.19
EAECC	35.54 ± 0.14	40.31 ± 0.17
AECC	21.94 ± 0.23	23.90 ± 0.21

All values represent means ± SEM of three replicates. EECC: Ethanolic extract C. cinereum; EAE: Ethyl acetate extract C. cinereum; AE: Aqueous extract C. cinereum

Study reveals that ethanol is widely used to extract plant natural products Therefore, a rich

bioactive phytoconstituents level explains the widespread therapeutic use of this folklore botanical. Flavonoids are a major group of phenolic compounds and are widely distributed in plants. They play an important role in giving flavour and colour to fruits and vegetables [17]. The presence of hydroxyl groups gives flavonoids their radical scavenging ability. The same concept as for TPC was applied in the determination of the total flavonoid content (TFC), wherein the presence of a flavonoid-aluminium complex causes a change in colour. The ethanol extract of C. cinereum leaves exhibited the highest values for TPC and TFC as shown in Table 1, indicating that it contains the most phenolic compounds among the extracts. This presumption was supported by the antioxidant and cytotoxic activities determined for the ethanol extract. C. cinereum can be considered as a promising source of phenolic compounds compared with other natural sources of these compounds [18].

4. Conclusion

This study establishes a pharmacognostic and phytochemical baseline for Cyanthillium cinereum, emphasizing its suitability as a source of bioactive compounds. The high phenolic and flavonoid content in methanolic extracts suggests potential antioxidant and antimicrobial properties, validating its traditional medicinal uses. Physico-chemical analyses confirm the purity and quality of the plant material, while fluorescence characterization aids in its standardization. These findings contribute to the scientific validation of C. cinereum, supporting its integration into contemporary herbal medicine and drug development. Future research should explore the pharmacological activities of its bioactive compounds and their clinical applications to unlock its full therapeutic potential.

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