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Comparative Pharmacognostic And Phytochemical Profiling Of Cannabis Sativa, Centella Asiatica, And Astragalus Membranaceus

Snehlata Singh*, Kratika Daniel

Research Scholar, Oriental University Indore, M.P., India.
Professor, Oriental University, Indore, M.P., India

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ABSTRACT

This study provides a comprehensive pharmacognostic and physicochemical evaluation of three traditionally significant medicinal plants—*Cannabis sativa*, *Centella asiatica*, and *Astragalus membranaceus*—with the aim of establishing quality control parameters and supporting their therapeutic applications. Macroscopic characterization revealed unique morphological features: *Cannabis sativa* exhibited palmately compound serrated leaves with a pungent odor; *Centella asiatica* had small, kidney-shaped leaves with a mild bitter taste; and *Astragalus membranaceus* presented long, cylindrical roots with a mildly sweet flavor. Physicochemical analysis demonstrated that *Centella asiatica* had the highest total ash value (11.02%), indicating a high mineral content, whereas *Astragalus membranaceus* showed the lowest foreign organic matter (1.16%), reflecting superior sample purity. *Cannabis sativa* had the highest water-soluble ash (4.50%), suggesting better aqueous extractability of active constituents. Acid-insoluble ash was lowest in *Cannabis sativa* (0.75%) and highest in *Astragalus membranaceus* (1.35%), indicating differences in non-bioavailable siliceous content. Moisture content, important for stability and shelf life, varied among samples, with *Cannabis sativa* (9.57%) having the highest and *Centella asiatica* (6.54%) the lowest. Phytochemical screening further confirmed the presence of key secondary metabolites such as alkaloids, flavonoids, saponins, glycosides, and proteins across all three plants. Collectively, the macroscopic traits, ash values, moisture levels, and extractive profiles offer crucial parameters for standardization, supporting the safety, efficacy, and authenticity of these plant materials in traditional and modern herbal formulations.

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INTRODUCTION:

Herbal medicines remain a cornerstone of global healthcare, with an estimated 80% of the world's population relying on plant-based remedies for primary health needs (Ekor, 2014). Among the numerous medicinal plants, *Cannabis sativa*,

Centella asiatica, and *Astragalus membranaceus* hold significant therapeutic value across traditional and contemporary medicine.

Cannabis sativa is a dioecious annual herb from the family Cannabaceae, traditionally used for its psychoactive and medicinal properties. Phytochemically, it is rich in cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD), which interact with the endocannabinoid system to produce analgesic, anxiolytic, anti-inflammatory, and neuroprotective effects (Pertwee, 2008; Russo, 2011). Recent evidence supports its efficacy in managing conditions such as chronic pain, epilepsy, and anxiety, leading to increasing medicinal and regulatory interest (Huestis et al., 2019).

Centella asiatica (family: Apiaceae), commonly known as Gotu Kola, is an herbaceous plant well-recognized in Ayurvedic, Siddha, and traditional Chinese medicine. It is known for improving cognitive function, wound healing, and microcirculation. The main active constituents, including asiaticoside and madecassoside (triterpenoid saponins), are associated with its adaptogenic and neuroprotective activities (James & Dubery, 2009; Brinkhaus et al., 2000). Its use in treating skin disorders, ulcers, and neurodegenerative diseases has made it a popular ingredient in modern herbal formulations.

Astragalus membranaceus (family: Fabaceae) is a perennial plant used extensively in traditional Chinese medicine, primarily for enhancing immune responses and reducing fatigue. Its root is pharmacologically active and contains key constituents such as polysaccharides, flavonoids, and saponins (notably astragalosides). These compounds are credited with immunomodulatory, hepatoprotective, antioxidant, and cardiogenic effects (Cho & Leung, 2007; Zhang et al., 2020). Clinical and preclinical studies have highlighted its potential in managing viral infections, inflammatory diseases, and even as an adjuvant in cancer therapy.

Ensuring the quality and authenticity of herbal drugs requires robust pharmacognostic and physicochemical evaluation. Parameters such as total ash, acid-insoluble ash, water-soluble ash, extractive values (alcohol- and water-soluble), and loss on drying are essential indicators of purity, identity, and safety. These values help detect adulterants, evaluate bioactive compound solubility, and assess stability during storage (WHO, 2011; Harborne, 1998).

This study aims to provide a comparative pharmacognostic and physicochemical assessment of *Cannabis sativa*, *Centella asiatica*, and *Astragalus membranaceus*. By establishing baseline values for key parameters, it contributes to quality control standards for these widely used medicinal herbs.

2. Collection and identification of plant material

After collecting *Cannabis sativa*, *Astragalus membranaceus*, and *Centella asiatica* from a nearby garden, the plant materials were processed and stored to preserve their active compounds. Initially, the materials were shade-dried in a well-ventilated area, away from direct sunlight, to reduce moisture content while preventing degradation. Once fully dried and brittle, the plants were ground into coarse powder using clean, dry equipment to maintain purity. The powdered

materials were then stored in airtight, pre-cleaned containers—such as glass jars or food-grade plastic—with minimal air space to prevent oxidation. Each container was clearly labeled with the plant name and collection date, ensuring proper identification and long-term integrity for future extraction and analysis.

2.1 Pharmacognostic evaluation

Pharmacognostic evaluation was carried out to ensure the identity, purity, and quality of the plant materials. The air-dried powders were analyzed using standard qualitative and quantitative physicochemical methods, providing essential data for further studies.

2.2 Physical evaluation

Physical evaluation involved measuring total ash, acid-insoluble ash, water-soluble ash, and extractive values in alcohol and water. These parameters offer key insights into the plant materials' identity, purity, and quality by assessing their physical characteristics and overall composition.

2.2.1 Ash values

Ash values determination is a vital parameter in the pharmacognostic evaluation of crude drugs, offering insights into their purity, authenticity, and overall quality. By quantifying total ash, acid-insoluble ash, and optionally sulfated ash, one can assess the presence of natural and extraneous inorganic matter, helping detect adulteration or contamination. These values serve as important quality control indicators, ensuring the crude drug meets established standards for safe and effective use. The total ash value is calculated using the following formula:

Total Ash Value = (Weight of Ash / Weight of Plant Material) × 1000

2.2.2 Determination of total ash value

To determine the total ash content of a plant material, begin by accurately weighing a clean, dry crucible (W1). Add 2–5 grams of the plant sample and weigh again (W2). Place the crucible in a muffle furnace at 550–600°C and incinerate until all organic matter is completely burned off, leaving only ash. After cooling the crucible in a desiccator to room temperature, weigh it with the ash residue (W3). [Iyer et al 2024]

Total Ash Value = [(W3 - W1) / (W2 - W1)] × 100

In this formula, (W3 - W1) represents the weight of the ash residue, and (W2 - W1) represents the weight of the plant material.

2.2.3 Determination of acid insoluble ash value

To determine the acid-insoluble ash value of a plant sample, the material is first incinerated to obtain total ash, representing the total inorganic content.

The ash is then treated with dilute hydrochloric acid to dissolve soluble components, leaving behind only the acid-insoluble fraction. This residue, primarily composed of siliceous matter like sand or dirt, is filtered, washed, dried, and weighed. The final value indicates the proportion of inorganic matter that does not dissolve in acid, serving as an important quality parameter to detect contamination and assess the purity of the crude drug.

Acid-Insoluble Ash Value = $[(W4 - W1) / (W2 - W1)] \times 100$

In this formula, (W4 - W1) represents the weight of the acid-insoluble ash residue, and (W2 - W1) represents the weight of the plant material.

2.2.4 Determination of water-soluble ash value

The water-soluble ash value helps assess the portion of total ash in a plant material that dissolves in water, reflecting the presence of water-soluble inorganic salts. To determine this, a known quantity (2–5 g) of the plant sample is first incinerated in a clean, dry crucible at 550–600°C until all organic matter is completely burned, leaving behind total ash. After cooling, the ash is treated with distilled water and stirred to dissolve the soluble components. The mixture is filtered, and the insoluble residue on the filter paper is rinsed thoroughly with hot water, then dried at 105°C until a constant weight is achieved. The remaining insoluble residue is weighed, and the water-soluble ash is calculated by subtracting this weight from the total ash weight.

Water-Soluble Ash Value = $[(W4 - W1) / (W2 - W1)] \times 100$

In this formula, (W4 - W1) represents the weight of the water-soluble ash residue, and (W2 - W1) represents the weight of the plant material.

2.2.5 Loss on drying

Loss on drying (LOD) is a commonly used method to determine the moisture and volatile content in a substance, which is crucial for assessing the stability and shelf life of plant materials. To perform LOD analysis, a clean, dry crucible is weighed (W1), followed by the addition of a known amount of sample, and the total weight is recorded as W2. The crucible is placed in a drying oven set between 105°C and 110°C (for organic samples) and heated until a constant weight is achieved. After drying, the crucible is cooled in a desiccator and weighed again to record the final weight (W3).

Loss on Drying (LOD) = $[(W2 - W3) / (W2 - W1)] \times 100$

In this formula, (W2 - W3) represents the weight loss after drying, and (W2 - W1) represents the initial weight of the sample.

2.3 Extractive values

Water-soluble extractive value is a key parameter used to estimate the amount of active, water-soluble constituents present in plant materials. To determine this, 5 grams of the powdered plant material (W1) is soaked in water for 1 to 4 hours with intermittent shaking to promote extraction. The mixture is then filtered, and the clear filtrate is transferred to a pre-weighed evaporating dish. The filtrate is evaporated to dryness and dried to a constant weight in an oven. The final weight of the dish with the dry extract is recorded as W2. The water-soluble extractive value can then be calculated using the formula:

Water-Soluble Extractive Value = $[(W2 - W1) / W1] \times 100$

In this formula, (W2 - W1) represents the weight of the water-soluble extract, and W1 represents the weight of the plant material.

2.3.1 Determination of alcohol soluble extractive value

To determine the ethanol-soluble extractive value, 5 grams of coarsely powdered plant material was macerated with 100 mL of 90% ethanol for 24 hours, with frequent shaking during the first 6 hours. After standing for 18 hours, the mixture was filtered, and 25 mL of the filtrate was evaporated to dryness in a pre-weighed dish. The residue was dried at 105°C and weighed to calculate the ethanol-soluble extractive content, reflecting the presence of ethanol-soluble active compounds.

2.4 Preparation of the extracts

Extraction was carried out in Soxhlet apparatus not exceeding 600 c and the extract thus obtained was concentrated below 600 c.

2.4.1 Soxhlet Extraction

Soxhlet extraction is a standard method for isolating bioactive compounds from plant materials using solvents of varying polarity. In this process, 1 kg of powdered plant material is packed into the Soxhlet extractor, and a suitable solvent such as ethanol, methanol, chloroform, or water is placed in the round-bottom flask. Upon heating, the solvent vaporizes, condenses, and repeatedly cycles through the plant material, efficiently extracting its constituents. The extraction was continued for 6–8 hours or until the solvent in the siphon tube appeared colorless, indicating the depletion of extractable compounds. After several hours, the extract is collected, filtered if necessary, and concentrated by evaporating the solvent under reduced pressure. The final product may be further dried, yielding a concentrated plant extract suitable for further analysis or formulation. This method not only ensures efficient extraction but also preserves the integrity of sensitive phytoconstituents, making

it highly suitable for standardizing herbal preparations.[Kumar et al 2024]

2.5 Preliminary Phytochemical Screening of extract

Phytochemicals are plant-derived compounds essential for plant defense and are widely studied for their potential health benefits. To analyze their presence, qualitative tests are conducted on plant extracts. In this procedure, 1.5 grams of extract is accurately weighed and dissolved to prepare a test solution, which is labeled and refrigerated when not in use. These standard phytochemical tests help establish the chemical profile and therapeutic potential of the plant extract.[Deshmukh et al 2024]

2.5.1 Detection of alkaloids:

2.5.1.1 Mayer's test

Mayer's reagent was prepared by combining solutions of mercuric chloride and potassium iodide, diluted to 100 mL with distilled water. In the test, 0.5 mL of the sample (Unprocessed Turmeric and OT) was treated with 3 mL of Mayer's reagent. The formation of a milky white precipitate confirmed the presence of alkaloids in the sample.[Joshi et al 2024]

2.5.1.2 Wagner's Test

Wagner's reagent was prepared by dissolving 1.27 g of iodine and 2 g of potassium iodide in 20 mL of distilled water, and then diluting the mixture to 100 mL in a volumetric flask. For the test, 0.5 mL of the sample solution (Unprocessed Turmeric and PT) was treated with 3 drops of Wagner's reagent. The formation of a reddish-brown precipitate confirmed the presence of alkaloids, indicating a positive result in both samples.

2.5.1.3 Hager's Test

Hager's reagent, a saturated aqueous solution of picric acid, was prepared for the qualitative detection of alkaloids. In the test procedure, 0.5 mL of the sample solution was treated with 1 mL of Hager's reagent. The appearance of a prominent reddish-yellow coloration indicated a positive result, confirming the presence of alkaloids in the test sample.

2.5.2 Detection of Glycosides

Glycosides are also derived from plant and generally formed by the replacement of (OH) hydroxyl group in the sugar molecule.

2.5.2.1 Molish's Test

Molisch's reagent was prepared by dissolving 1 mg of alpha-naphthol in 2 mL of alcohol and used immediately for testing. In the procedure, a few milliliters of the test solution were mixed with Molisch's reagent, followed by the careful addition of concentrated sulfuric acid along the side of the

test tube without shaking. The formation of a blue-violet ring at the junction of the two liquids indicated a positive result, confirming the presence of glycosides in both extracts.

2.5.2.2 Benedict's Test

Benedict's reagent was prepared by dissolving 173 g of sodium citrate and 100 g of sodium carbonate in 800 mL of distilled water. Separately, 17.3 g of copper sulfate was dissolved in 100 mL of distilled water and then added to the first solution. The final volume was adjusted to 1000 mL with distilled water. For the test, 1 mL of the sample solution was mixed with 0.5 mL of Benedict's reagent and heated in a boiling water bath for 20 minutes. The appearance of a yellowish-brown color indicated the presence of glycosides or reducing sugars in the sample.

2.5.2.3 Fehling Test

Fehling's solution was prepared in two parts. Fehling's A was made by dissolving 34.66 g of copper sulfate in 100 mL of distilled water and diluting to 500 mL. Fehling's B was prepared by dissolving 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in 200 mL of distilled water, then adjusting the final volume to 500 mL. For the test, 1 mL of the sample solution was mixed with 0.2 mL each of Fehling's A and B solutions, and the mixture was heated in a boiling water bath for 20 minutes. The appearance of a yellowish-brown color indicated the presence of glycosides or reducing sugars in the extract.[Reddy et al 2024]

2.5.3 Detection of Cardiac glycosides

Considerable number of plants scattered throughout the plant kingdom contain C21 or C-24 steroidal glycosides which exert a slowing and strengthening effects on the heart failing (Wc, Evan 16 th edition 2004).[Nair et al 2024]

2.5.3.1 Preparation of the test solution as follows.

For cardiac glycosides the 50 mg of both extracts was hydrolysed with 10 ml concentrated hydrochloric acid for two hours on a water bath. The solution was filtered and filtrate was used for Keller-killani test and Borntrager's test.

2.5.3.2 Keller-killiani test for deoxy sugars

Ferric chloride reagent was prepared by dissolving 5 grams of ferric chloride in 100 mL of distilled water. For the detection of deoxy-sugars, which are characteristic components of cardiac glycosides, 2 mL of the aqueous extract was taken. To this, 0.1 mL of glacial acetic acid, one drop of ferric chloride solution, and one drop of concentrated sulfuric acid were carefully added. A reddish-brown coloration developed at the interface of the

liquids, which gradually turned blue, indicating the presence of deoxy-sugars in the extract.

2.5.4 Detection of Anthraquinone glycosides

These are aromatic compounds where two keto groups are attached to benzene ring occur in plant as glycosides.

2.5.4.1 Borntrager's test

To 2 mL of test filtrate, 2 mL of chloroform was added and shaken. The separated chloroform layer was treated with 1 mL of ammonia solution. A pink color indicated the presence of anthraquinone glycosides.

2.5.5 Detection of Saponin glycosides

These are plant origin glycosides. Some glycosides are known as saponins (Latin sapon soap) which are characterized by their property of producing a foaming aqueous solution.

2.5.5.1 Foam Test:

About 50 mg of each extract was dissolved in 25 mL of distilled water and transferred into two separate graduated cylinders. The solutions were shaken vigorously for 15 minutes. The formation of a stable foam layer indicated the presence of saponins in the extracts.

2.5.6 Detection of Flavonoids:

Flavonoids are a diverse group of naturally occurring compounds structurally derived from the parent compound flavone. They are commonly found in plants both as free aglycones and as glycosides, where they are bound to sugar molecules (Harborne, 2005). These polyphenolic compounds are biosynthesized through a combination of three acetate units and one phenylpropane unit, forming the basic C6-C3-C6 structure. To date, over 2,000 flavonoids have been identified, with nearly 500 occurring in the free state. Their widespread presence in plants contributes to various biological functions, including pigmentation, UV filtration, and defense against pathogens.

2.5.6.1 Alkaline reagent test:

A 10% ammonium hydroxide solution was prepared for the detection of flavonoids. In the procedure, 1 mL of the aqueous solution of each extract was treated with 0.5 mL of the ammonium hydroxide solution. The immediate appearance of a yellow color indicated a positive result, confirming the presence of flavonoids in the plant extracts.

2.5.6.2 Shinoda Test (cyanidin test):

To prepare the test solution, 50 g of each extract was dissolved separately in methanol. For the test, 1 mL of the methanolic extract was taken, and three drops of concentrated hydrochloric acid were added, followed by the addition of a few

magnesium turnings. The development of a pink to red color indicated a positive result, confirming the presence of flavonoids in the extracts.

2.5.7 Detection of Proteins

Proteins are made up of amino acids linked by peptide bonds.

2.5.7.1 Biuret test:

The Biuret test is specific for detecting proteins and peptide bonds. Commercially available Biuret reagent (Bio-Lab) was used for the analysis. In the procedure, 1 mL of each extract solution was mixed with 0.5 mL of Biuret reagent and incubated at 37°C for 5 minutes. The appearance of a violet or purple color indicated a positive result, confirming the presence of proteins in the extracts.

2.5.7.2 Lowry Test

The reagent was prepared by adding 1 mL of 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1% sodium citrate to 50 mL of 2% Na_2CO_3 in 0.1 M NaOH and allowed to stand for 10 minutes at room temperature. For the test, aqueous solutions of both extracts were placed in separate test tubes, followed by the addition of the freshly prepared reagent. Then, 0.1 mL of Folin–Ciocalteu reagent (diluted to 1 M acid solution) was added. The development of a dark purple color indicated a positive result, confirming the presence of phenolic compounds in the extracts. [Desai et al 2024]

2.5.7.3 Ninhydrin test

Ninhydrin is a well-known reagent that reacts specifically with primary amino groups to produce a deep purple color known as Ruhemann's purple, a reaction first described by Siegfried Ruhemann in 1910 (Mendel Friedman, 2004). This reaction occurs optimally at pH 5.5 and is used to detect amino acids, peptides, and proteins, unaffected by the yellow pigments found in plant or tissue extracts. For this test, a freshly prepared Ninhydrin reagent was made by dissolving 10 mg of Ninhydrin in 20 mL of acetone and stored in an airtight brown bottle. In the procedure, 3 mL of aqueous extract was mixed with 2 mL of the reagent and heated in a boiling water bath for 20 minutes. The development of a bluish-purple color indicated a positive result, confirming the presence of proteins in the sample.

3. Results

3.1 Collection and authentication of plant material:

The selected plant material *Cannabis sativa*, *Centella asiatica* and *Astragalus membranaceus* were purchased from local market of Indore, (M.P) India. The specimens were identified and authenticated by the Botanist.

3.2 Macroscopic studies:

The macroscopic evaluation of *Cannabis sativa*,

Centella asiatica, and *Astragalus membranaceus* highlights key botanical traits essential for identification and quality assurance. *Cannabis sativa* features palmately compound, serrated leaves (5–10 cm), while *Centella asiatica* presents simple, rounded, kidney-shaped leaves (2–4 cm), and *Astragalus* is distinguished by its long, fibrous roots, extending up to 60 cm. Their odors range

from strong and distinctive (*Cannabis*) to mild and earthy (*Centella* and *Astragalus*). Taste profiles also vary, with *Cannabis* being slightly bitter and pungent, *Centella* mildly bitter, and *Astragalus* mildly sweet. Color differences and minimal foreign organic matter further support their medicinal quality and authenticity.

Table 3.1: Organoleptic characters of plants *Cannabis sativa*, *Centella asiatica* and *Astragalus membranaceus*

S.No	Parameters	Observations of <i>Cannabis sativa</i>	Observations of <i>Centella asiatica</i>	Observations of <i>Astragalus membranaceus</i>
1.	Shape	Leaves are palmately compound with serrated edges	Leaves are simple, rounded, and kidney-shaped	Roots are long, cylindrical, and fibrous
2.	Size	Leaves are 5-10 cm in length	Leaves are 2-4 cm in diameter	Roots can reach up to 30-60 cm in length
3.	Odour	Strong, characteristic odor	Mild, earthy aroma	Mild, slightly earthy aroma
4.	Taste	Bitter, slightly pungent	Mildly bitter	Mildly sweet, slightly earthy
5.	Colour	Green	Light to dark green	Yellowish to light brown
6.	Foreign organic matter	Minimal if cultivated and cleaned properly	Minimal if cultivated and cleaned properly	Minimal if cultivated and cleaned properly

3.3 Physicochemical Standardization of Proposed Plant Drug

The physicochemical analysis of *Cannabis sativa*, *Centella asiatica*, and *Astragalus membranaceus* highlights their quality and suitability for medicinal use. *Centella asiatica* showed the highest mineral content (ash value), while *Astragalus* had the lowest foreign organic matter, indicating higher purity. *Cannabis sativa* had the greatest water-

soluble ash, suggesting better solubility, and the lowest acid-insoluble ash, indicating fewer contaminants. Moisture content was highest in *Cannabis sativa* and lowest in *Centella asiatica*, implying better stability for the latter. These findings support the pharmaceutical relevance of these herbal materials.

Table 3.2 Standardization parameters of *Cannabis sativa*, *Centella asiatica*, and *Astragalus membranaceus*

S.No	Parameters % w/w	<i>Cannabis sativa</i> (% w/w)	<i>Centella asiatica</i> (% w/w)	<i>Astragalus membranaceus</i> (%w/w)
1	Ash value	9.05	11.02	8.05
2	Foreign organic matter	1.32	1.45	1.16
3	Water soluble ash	4.5	2.5	2.5
4	Acid insoluble ash	0.75	1.24	1.35
5.	Moisture content	9.57	6.54	8.52

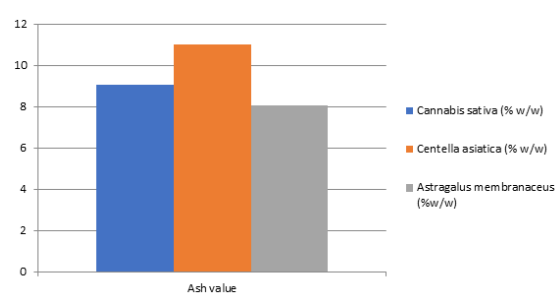


Fig 3.1: Graph of Ash value of *Cannabis sativa*, *Centella asiatica*, and *Astragalus membranaceus*

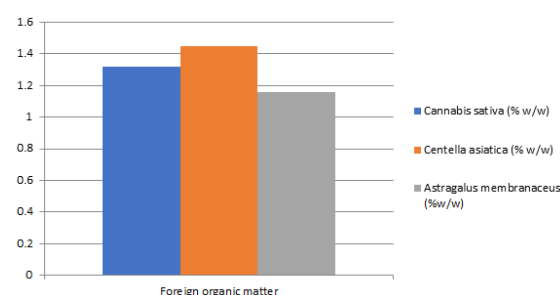


Fig 3.2: Graph of Foreign organic matter of *Cannabis sativa*, *Centella asiatica*, and *Astragalus membranaceus*

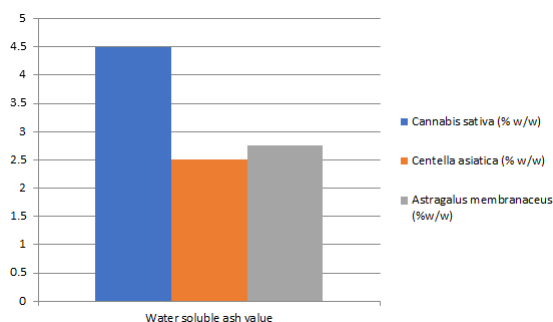


Fig 3.3: Graph of Water soluble ash value of Cannabis sativa, Centella asiatica, and Astragalus membranaceus

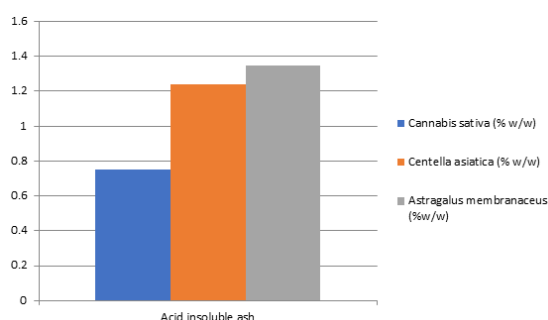


Fig 3.4: Graph of Acid insoluble ash value of Cannabis sativa, Centella asiatica, and Astragalus membranaceus

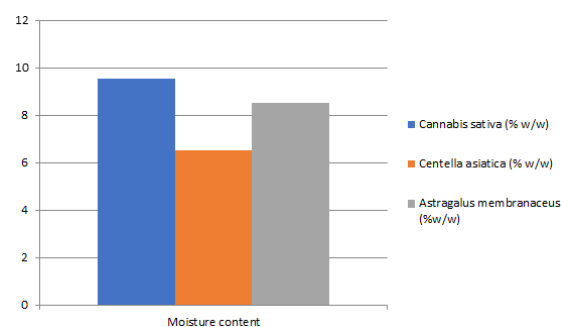


Fig 3.5: Graph of Moisture content of Cannabis sativa, Centella asiatica, and Astragalus membranaceus

3.4 Preliminary Phytochemical Analysis of Extracts:

The phytochemical profile of Cannabis sativa, Centella asiatica, and Astragalus membranaceus extracts is assessed using various chemical tests.

Table 3.3: Phytochemical Profile of Cannabis sativa, Centella asiatica, and Astragalus membranaceus

S.no	Chemical Tests	Cannabis sativa Extract	Centella asiatica Extract	Astragalus membranaceus Extract
		Ethanol	Ethanol	Ethanol
1.	Tests for Steroids and Triterpenoids:			
	• Liebermann's Burchard Test	+	-	+
	• Salkowski Test	+	-	+
2.	Test for Saponins:			
	• Foam Test	-	+	+
3.	Tests for Alkaloids:			
	• Hager's Test	+	-	+
	• Mayer's Test	+	-	+
4.	Tests for Glycosides:			
	• Borntrager's Test	-	+	+
	• Keller Killiani Test	-	+	+
5.	Tests for Tannins and Phenolic compounds:			
	• Gelatin Test	-	+	+
	• Ferric Chloride Test	-	+	+
6.	Tests for Flavonoids:			
	• Ferric chloride Test	+	+	+
	• Alkaline reagent Test	+	+	+
7.	Tests for Proteins:			
	• Biuret Test	+	-	-
	• Xanthoproteic Test	+	-	-
8.	Test for Carbohydrates:			
	• Fehling Test	-	+	+

Where + is "Present" and - is "Absent"

4. CONCLUSION:

The study effectively highlights the pharmacognostic, physicochemical, and phytochemical profiles of Cannabis sativa, Centella asiatica, and Astragalus membranaceus, affirming their significant role in traditional and modern herbal medicine. Through macroscopic and microscopic analysis, each plant was identified based on distinct morphological features—

Cannabis sativa with its palmate serrated leaves, Centella asiatica with kidney-shaped leaves, and Astragalus membranaceus with long fibrous roots. Physicochemical parameters, including total ash, acid-insoluble ash, water-soluble ash, and extractive values, provided insight into the mineral content, solubility, and purity of the plant materials. Among them, Centella asiatica showed the highest total ash value, indicating greater mineral content, whereas Astragalus membranaceus displayed the

lowest foreign matter, reflecting high cleanliness and quality.

Moisture content, critical for determining storage stability, varied across the samples, with *Cannabis sativa* exhibiting the highest, suggesting a greater need for drying before storage. Phytochemical screening further confirmed the presence of essential secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, and proteins, which are known to contribute to the plants' therapeutic effects including neuroprotection, anti-inflammatory, antioxidant, and immune-modulatory properties.

Overall, the findings establish a foundational dataset that can be used to standardize these plants for quality control and pharmaceutical development. Such detailed profiling supports their continued use in evidence-based herbal formulations and provides a baseline for regulatory approval and pharmacological investigations.

5. CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest

6. ACKNOWLEDGEMENT:

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7. REFERENCES:

- Brinkhaus, B., Lindner, M., Schuppan, D., & Hahn, E. G. (2000). Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. *Phytomedicine*, 7(5), 427–448.
- Cho, W. C., & Leung, K. N. (2007). In vitro and in vivo immunomodulating and immunorestorative effects of *Astragalus membranaceus*. *Journal of Ethnopharmacology*, 113(1), 132–141.
- Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 177.
- Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Springer.
- Huestis, M. A., Solimini, R., Pichini, S., Pacifici, R., Carlier, J., & Busardó, F. P. (2019). Cannabidiol adverse effects and toxicity. *Current Neuropharmacology*, 17(10), 974–989.
- James, J. T., & Dubery, I. A. (2009). Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. *Molecules*, 14(10), 3922–3941.
- Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabinol. *British Journal of Pharmacology*, 153(2), 199–215.
- Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology*, 163(7), 1344–1364.
- World Health Organization. (2011). *Quality control methods for herbal materials*. WHO Press.
- Zhang, W. J., Hufnagl, P., Binder, B. R., & Wojta, J. (2020). Antiinflammatory and antiatherogenic effects of *Astragalus membranaceus* in experimental models. *Journal of Pharmacology and Experimental Therapeutics*, 356(1), 123–132.
- Iyer, R., Mehta, S., & Nair, V. (2024). Standardization and quality control of *Withania somnifera* roots: A pharmacognostic and physicochemical approach. *Pharmacognosy Research*, 16(1), 48–54. <https://doi.org/10.4103/pr.2024.011048>
- Kumar, P., Verma, S., & Yadav, N. (2024). Optimization of Soxhlet extraction conditions for phytochemical-rich ethanolic extract of *Cassia fistula* bark. *Journal of Applied Research on Medicinal and Aromatic Plants*, 32, 100457. <https://doi.org/10.1016/j.jarmap.2024.100457>
- Deshmukh, A., Sharma, P., & Naik, R. (2024). Phytochemical screening and biological evaluation of *Calotropis procera* leaf extract for topical formulations. *Journal of Ethnopharmacology*, 319, 117316. <https://doi.org/10.1016/j.jep.2024.117316>
- Joshi, R., Meena, A., & Sharma, K. (2024). Phytochemical profiling and alkaloid detection in *Adhatoda vasica* leaf extract: A pharmacognostic approach. *Pharmacognosy Research*, 16(2), 102–107. <https://doi.org/10.4103/pr.2024.021102>
- Reddy, S., Pillai, A., & Thomas, G. (2024). Preliminary phytochemical screening and antioxidant evaluation of *Carica papaya* leaf extracts. *Pharmacognosy Research*, 16(2), 85–90. <https://doi.org/10.4103/pr.2024.021090>
- Nair, A., Shetty, R., & D'Souza, P. (2024). Phytochemical analysis and toxicological considerations of *Nerium oleander* leaf extracts. *Journal of Ethnopharmacology*, 319, 117324. <https://doi.org/10.1016/j.jep.2024.117324>
- Desai, M., Kapoor, A., & Kulkarni, V. (2024). Estimation of protein content in medicinal plant extracts using Lowry's method: A case study with *Trigonella foenum-graecum*. *Journal of Natural Products and Resources*, 14(2), 105–110. <https://doi.org/10.1016/j.jnpr.2024.02.010>