RESEARCH ARTICLE

Pharmacognosy and Phytochemical Screening of *Centella asiatica*

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ABSTRACT

Background and Objective: *Centella asiatica* is a perennial herbaceous plant that is commonly used in traditional Ayurvedic medicine. The purpose of this study was to investigate its phytochemical profile and pharmacognostic properties. **Methods:** *Centella asiatica's* strong phytochemical profile supports its potential as a pharmaceutical agent. A plant's potential for antibacterial, anti-inflammatory, and antioxidant effects is indicated by the presence of tannins, phenolic compounds, and saponins. **Result:** The results of several phytochemical and physicochemical investigations offer important new information about this plant's quality control criteria and prospective medical uses. **Conclusion:** Our findings support the plant's traditional applications for healing wounds, enhancing memory, and cleansing blood. Additional in vivo and clinical research is necessary to confirm these pharmacological effects and comprehend the underlying mechanisms of action.

Keywords: Phytochemical profile, Pharmacognostic properties, HPTLC, Physiochemical screening, microscopic analysis.

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INTRODUCTION

Indian herb *Centella asiatica*, also known as "Mandokaparni," grows in moist environments and is utilized in ayurveda treatments either as a whole plant or as fresh or extractable leaves, smooth, tiny, kidney-shaped leaves are present.[1,2] Along the stem, they are placed in an alternating manner. There is a noticeable palmate venation on the leaves[3]. The thin stems root at the nodes and spread out horizontally.[4] Flowers are tiny, pink to red blooms emerge from the leaf axils in umbels and are grouped together. Every flower is radially symmetrical and has five petals.[5] The plant produces new plants at the nodes of its stolons, which are horizontal stems that grow at the soil's surface or slightly below it. The spherical, tiny fruits are filled with seeds.[6]

Geographical distribution

A perennial herbaceous creeper that is clonal and a member of the Apiceae family, *Centella asiatica* (CA) are distributed all over India. Water is the ideal habitat for this odorless and tasteless plant.[7]

Major chemical constituents

The main active ingredients are saponins, also known as triterpenoids. These include madecassoside, madasiatic acid, and asiaticosides, which are trisaccharide-linked aglycones of asiatic acid and madecassoside.[8] Due to their ability to prevent collagen formation at the wound site, these triterpene saponins and associated sapogenins are primarily responsible for the vascular effects and wound healing.[9,10] Though clinical research is yet needed to validate it, other isolated ingredients like brahmoside and brahminoside may be in charge of the CNS and uterorelaxant effects.[11,12]

Taxonomical Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Apiales
Family: Apiaceae
Genus: *Centella*
Species: *asiatica*

Pharmacology

For therapeutic uses, the entire plant is employed. Blood purification, hypertension treatment, memory improvement, and longevity promotion are among its many popular uses.

MATERIAL AND METHODS

The current study used in vitro methodology to look into *Centella asiatica* phytochemical composition.

Test Sample collection and authentication

Centella asiatica plant was collected from Galtas hills, Jaipur, India. Authentication was done in CSIR-National Institute of Science Communication And Information Resources (NISCAIR), New Delhi.

Powder Microscopy[13,14]

The specimen utilized was treated with chemical reagents since it was necessary to identify broken or powdered material by powder microscopic inspection of the medicinal plant material. Sufficient amount of the powdered sample was taken in a chemical reagent on a slide and warmed over a low flame for a short period of

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Oil Globules Vessels Leaf of *Centella asiatica*

Figure 1: Powder Microscopy

time. Few drops of glycerin was added on the slide and covered with cover slip and was observed under microscope.

Chemical reagent used for staining of powdered sample was safranin, dilute Ferric chloride, Eosin, Methylene blue.

Physicochemical study[15-17]

Physicochemical studies was done according to the Ayurvedic Pharmacopoeia of India (API).

Loss on Drying

Weighed around 10 g of the sample and put it in a tared evaporating dish. It was dried in a hot air oven at 105^0C for 5 hours. Till the difference between two successive weight readings was less than 0.25 percent, keep drying and weighing every hour. After a 30-minute drying and 30-minute cooling phase in a desiccator, the weight was deemed constant if there was a discrepancy of no more than 0.01 g between two successive weights. Ascertain the proportion of loss of a test sample of 10 grams.

Total Ash

A precisely weighed crucible containing 2 grams of crude sample burned in a muffle furnace. When free carbon is present, the temperature shouldn't go above 450oC. After that, the medication was weighted once more and kept in the desiccator to cool naturally. The ash value was calculated as a percentage based on the air-dried sample and this residue weight.

Acid Insoluble Ash

The ash value acquired from the above procedure is treated with diluted HCL to detect the silica, carbonate, and calcium oxalate content in order to obtain acid insoluble ash. For at least five minutes, the sample's ash and 25 ml of 6 N HCl were heated to boiled. Ash-less filter paper was used to gather the insoluble material.

Water Soluble Ash

25 milliliters of distilled water was added to the crucible containing all of the ash. Utilizing Whatsman 41 ashless filter paper, the insoluble material was collected. The filtrate was then heated to a neutral temperature and rinsed. Filter paper containing the insoluble material was moved and backed into the original crucible. It was then burned until it reached a constant weight after drying on a hot plate. Weigh the residue as soon as it cools in the suitable desiccator—preferably within 30 minutes. Calculate the proportion of water-soluble ash in the test sample that has been dried.

Table 1: Physiochemical Tests result of *Centella asiatica*

S. No	Name of Tests	Value	API Reference Value
1.	Loss on Drying (% w/v)	6.95	--
2.	Aqueous Extractive Value (w/w)	25.74	NLT 20 %
3.	Alcoholic Extract Value (w/w)	16.65	NLT 9 %
4.	Total Ash (w/w)	11.65	NMT 17 %
5.	Acid Insoluble Ash (w/w)	4.10	NMT 5 %
6.	Water Soluble Ash (w/w)	6.78	--

Aqueous Extractive Value

A sample of 5 g was obtained and macerated in 50 ml of distilled water. For 24 hours, this solution was set aside.It was constantly agitated, and after the 24-hour mark, the residue was filtered through Whatman filter paper with care given to avoid solvent loss. After filtering, 25 ml of the filtrates were removed, evaporated over a water bath, and then heated to 110 o C in a hot air oven until the weight remained constant.

Alcohol Soluble Extractive Value

This technique works well for raw drugs that have ingredients that dissolve in alcohol. Similar steps to determine the water soluble extractive were repeated for the alcohol soluble extractive, but 90% methanol was employed in place of distilled water.

Determination of pH value

A 10 gram sample was weighed and then placed in a sterile flask. After adding 100 ml of distilled water, shake it constantly with a glass rod for forty-five minutes. After calibrating the pH meter with a standard buffer solution with pH values of 4 and 7, the pH value was determined. The sample's pH was then determined by dipping the electrode into the sample solution.

Table 2: Phytochemical Tests results of *Bacopa monnieri*(Whole Plants)

S. No	Tests	Aqueous Extract	Ethanolic Extract
A.	Carbohydrate		
1.	Molish test	Positive	Positive
2.	Benedict test	Positive	Positive
3.	Fehling test	Positive	Positive
B.	Alkaloids		
1.	Dragendorff test	Positive	Positive
2.	Wagner's test	Positive	Positive
3.	Hager's test	Negative	Negative
C.	Amino Acid		
1.	Ninhydrine test	Positive	Absent
D.	Protein		
1.	Biuret test	Positive	Positive
2.	Xenthoprotic test	Negative	Negative
3.	Millon test	Positive	Positive
E.	Saponin		
1.	Foam test	Positive	Positive
F.	Glycosides		
1.	Borntrager's test	Positive	Positive
G.	Phenolic compound		
1.	Phenolic test	Positive	Positive
H.	Steroids		
1.	Salkowaski	Absent	Absent
I.	Tannins		
1.	$Fecl_3$	Positive	Positive
2.	Lead acetate	Negative	Negative
3.	Pot. Dichromate	Positive	Positive

Qualitative Analysis of Primary and Secondary Metabolite

Phytochemical testing[18,19] is used to identify the primary (carbohydrate, protein, and amino acid) and secondary (alkaloids, glycosides, tannin, saponin, and phenolic molecule) metabolites. The alcoholic and aqueous extracts were used to look for primary and secondary metabolites in test samples.

Carbohydrates

Molisch's Test

One milliliter of concentrated H_2SO_4 was added to the test tube, around one milliliter of test solution and two milliliters of Molisch's reagent were added, and the mixture was gently shaken. The mixture was then allowed to stand for a minute. A ring of purple color where the two layers met indicated the presence of carbohydrate.

Benedict's test

This process was used to reduce sugars, mostly using copper sulfate and sodium hydroxide. The drug's aqueous solution was heated to almost boiling point and combined with one milliliter of Benedict's solution. The test solution developed colors like green, yellow, orange, red, or brown when the concentration of simple sugar rose as a result of the formation of cuprous oxide.

Fehling solution test

This method is usually used to decrease sugar which consists of two solutions that are mixed in situ. Sodium potassium tartrate makes up Fehling solution B, whereas 0.5% copper sulfate is present in Fehling solution A. The drug's aqueous solution was added after one milliliter of Fehling A and Fehling B solutions were blended. On a water bath, the mixture was then brought to a boil for five to ten minutes.

Alkaloids

Dragondroff's Reagent

In a test tube, two milliliters of the test solution and two milliliters of the bismuth subnitrate and potassium iodide solution known as Dragondroff's reagent were added. Alkaloids were evident in the materials' capacity to produce an orange precipitate.

Wagner Test

Few drops of Wagner's reagent (diluted iodine solution) to two milliliters of test solution, the formation of a reddish-brown precipitate suggested the presence of alkaloids.

Hager test

Picric acid was dissolved in an aqueous solution that was saturated. After this reagent was applied to the test filtrate, an orange-yellow precipitate was formed, indicating the presence of alkaloids.

Amino Acid

Ninhydrin test

Ninhydrin Alpha-amino acids and proteins containing free amino groups were found using the Ninhydrin test. The development of a combination between two ninhydrin molecules and the nitrogen of free amino acids is what causes the distinctive deep blue or light

Proteins

Biuret test

Five milligrams of residue, one milliliter of 4% sodium hydroxide solution, and a drop of 1% copper sulfate solution were added to the mixture. The proteins were indicated by the emergence of a violet or pink color.

Xanthoprotic test

Two milliliters of water and five milliliters of strong nitric acid were applied to a small amount of test sample. The appearance of a yellow tint suggested the presence of proteins.

Saponin

Foam test

A small quantity of the sample and a few drops of water and sodium bicarbonate were added to a test tube, and the mixture was shaken vigorously. Saponins were characterized by a distinct, homogenous froth that looked like honeycomb.

Glycosides

Borntragor's Test

Glycoside was identified in the ethanolic extract by producing a reddish-pink color after 0.5 ml of diluted ammonia solution and 1 ml of benzene was added.

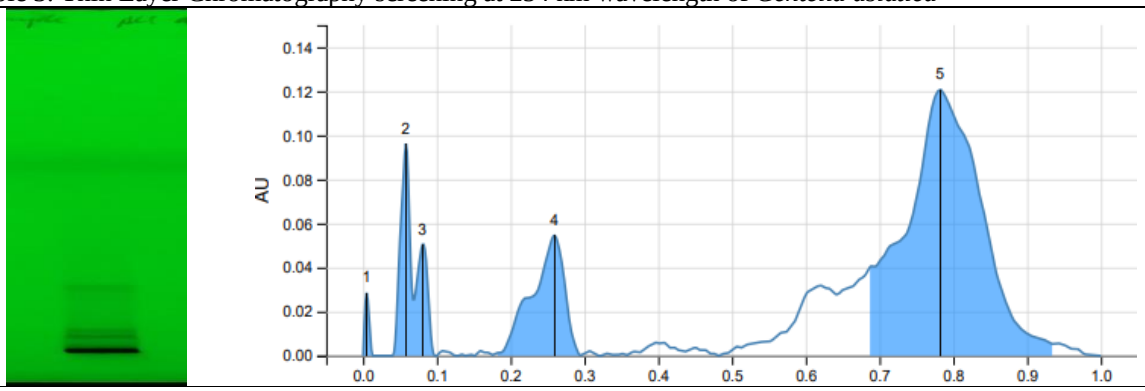
Phenolic compound test

Two milliliters of ferric chloride solution were added to the sample extract after it had been heated in water. After that, the mixture was examined to see if any blue or green color developed, signifying the presence of phenolic compounds.

Steroids

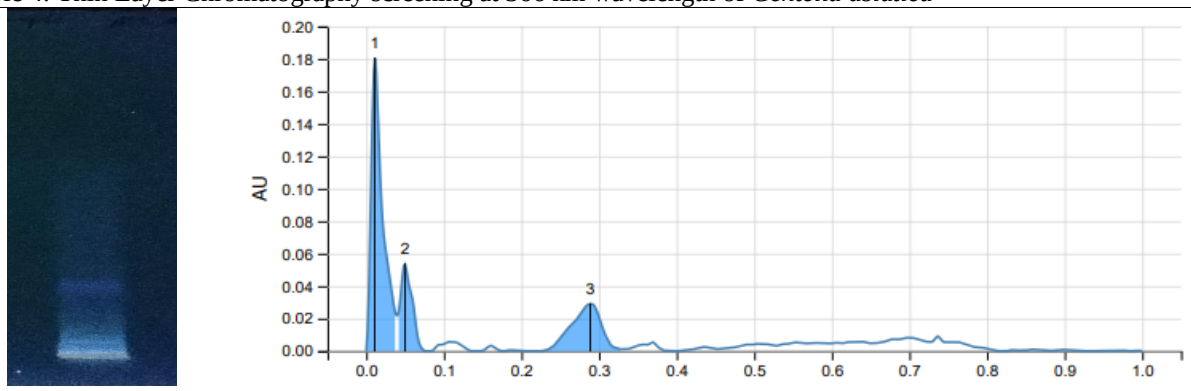
Salkoweski reaction

Table 3: Thin Layer Chromatography screening at 254 nm wavelength of *Centella asiatica*



Peak	Start		Max			End		Area	
	R_F	Height	R_F	Height	%	R_F	Height	A	%
1	0.000	0.0000	0.0050	0.0285	8.14	0.013	0.0000	0.00020	1.02
2	0.040	0.0000	0.058	0.0963	27.47	0.068	0.0256	0.00140	7.16
3	0.068	0.0256	0.081	0.0505	14.40	0.097	0.0000	0.00090	4.60
4	0.177	0.0005	0.260	0.0545	15.55	0.295	0.0002	0.00278	14.16
5	0.685	0.0395	0.782	0.1207	34.44	0.935	0.00520	0.01432	73.06

Table 4: Thin Layer Chromatography screening at 366 nm wavelength of *Centella asiatica*



Peak	Start		Max			End		Area	
	R_F	Height	R_F	Height	%	R_F	Height	A	%
1	0.000	0.0000	0.011	0.1804	68.59	0.039	0.0221	0.00314	58.34
2	0.040	0.0219	0.050	0.0534	20.31	0.077	0.0000	0.00094	17.46
3	0.224	0.0000	0.289	0.0292	11.10	0.327	0.0012	0.00130	24.20

Five milligrams of sample, two milliliters of chloroform, and two milliliters of strong sulfuric acid were added from the side of the test tube. The test tube was shook vigorously for a few minutes. The emergence of redness indicated the presence of steroids.

Tannins

Lead acetate

A 10% w/v solution of basic lead acetate in distilled water was combined with the test filtrate. The production of precipitates indicated the presence of tannins.

Potassium dichromate

For this test, the filtrate was added to a potassium dichromate solution. The dark tint suggested the presence of tannins.

Instrumentation

Spectrophotometric measurement was performed on (UV-Vis) Ultra Violet-Visible spectrophotometer equipped with 1 cm quartz cells.

Chemicals

The standards used for antioxidant assay Gallic acid monohydrate and Ethylenediaminetetraacetic acid (EDTA), ascorbic acid, butylated hydroxyanisole (BHA), quercetin, tannic acid, ferrozine. All chemicals used were of analytical grade.

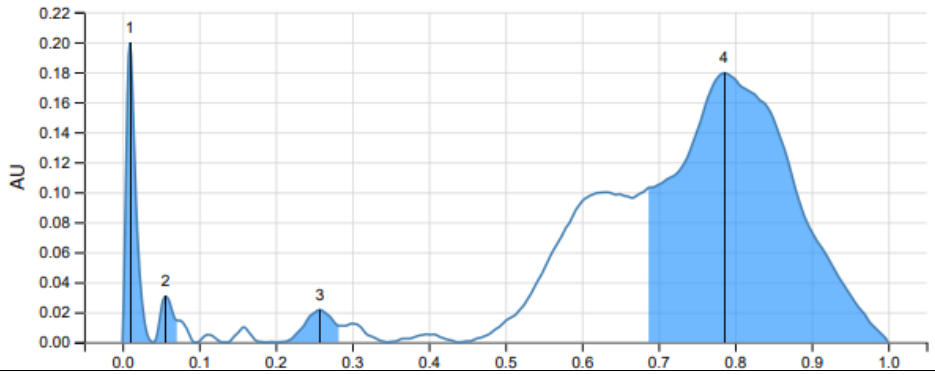
Qualitative Phytochemical screening

For the screening of phytochemical dried sample is used. Five grams of the dried sample was weighed and homogenized with 50 ml of water, HCL (1%) and ethanol separately. The extracts was boiled for 1 hour, cooled and filtered. The filtrate was used to screen for phytochemical activity.

High Performance Thin Later Chromatography[20]

Prior to extraction, the sample is incubated. Samples are centrifuged after incubation, and the supernatant is l of the material was spotted into 8mm kept in an amber tube until needed. 5 narrow bands on an HPTLC using a syringe. Following ethyl acetate:methanol:formic acid as the mobile phase, the HPTLC plates were dried and stained.

Table 5 Thin Layer Chromatography screening at 540 nm wavelengths after derivatization with p-Anisaldehyde of *Centella asiatica*



Peak	Start		Max			End		Area	
	R_F	Height	R_F	Height	%	R_F	Height	A	%
1	0.000	0.0000	0.010	0.1997	46.30	0.039	0.0001	0.00288	7.64
2	0.040	0.0000	0.056	0.0305	7.06	0.073	0.0145	0.00060	1.59
3	0.203	0.0001	0.0258	0.0217	5.03	0.290	0.0109	0.00099	2.63
4	0.682	0.1010	0.785	0.1795	41.61	1.000	0.0000	0.03318	88.15

Three distinct light sources UV light at 254 nm, 366nm and 540 nm, and white light were used to take the pictures with a TLC-visualizer camera.

RESULTS AND DISCUSSION

The plant material was examined using powder microscopy, which showed the presence of oil globules and vessels two characteristics that are specific to Centella asiatica. Safranin and methylene blue are two examples of staining reagents that were used to help clearly visualize these microscopic traits, which further aided in the plant material's authentication.

The quality and purity of the Centella asiatica samples were assessed using the physicochemical criteria, which included loss on drying, total ash, acid-insoluble ash, and water-soluble ash. After drying, the percentage of loss was 6.95%, which is within permissible bounds and suggests a low moisture content and a decreased chance of microbial contamination. The findings indicate that silica is present, as indicated by the total ash value of 11.65% and the acid-insoluble ash value of 4.10%, respectively. In order for the plant to have therapeutic potential, water-soluble minerals must be present, as indicated by the water-soluble ash value of 6.78%.

Numerous bioactive substances, including as carbohydrates, alkaloids, saponins, glycosides, phenolic compounds, and tannins, were found during the qualitative phytochemical screening. The diverse pharmacological actions of these substances are well-known. For example, it's especially significant to include triterpenoid saponins like madecassoside and asiaticoside since they have anti-inflammatory and wound-healing characteristics.

HPTLC

To further examine the phytochemical components, High-Performance Thin Layer Chromatography (HPTLC) was used. A thorough fingerprint of the chemical components found in Centella asiatica was produced by the chromatographic profiles obtained at three distinct wavelengths: 254 nm, 366 nm, and 540 nm. The chromatograms exhibit different peaks that verify the existence of numerous bioactive components. These compounds can serve as markers for quality control and standardization in herbal formulations that contain *Centella asiatica.*

CONCLUSION

As a result, *Centella asiatica's* potential as a useful medicinal plant is highlighted by the thorough pharmacognostic and phytochemical analysis that was conducted. Standards for quality assurance and uniformity can be set using the established physicochemical characteristics and chromatographic profiles. The phytochemical diversity of the plant indicates its potential for medicinal use, hence indicating the need for additional pharmacological research. *Centella asiatica* is still being used in both traditional and modern medicine, and this study adds to the increasing body of knowledge about it.

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