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Research Article

From Tradition to Therapy: Exploring the Pharmacological Potential of *Centella asiatica* L.

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Abstract

Medicinal plants are the great assets of drugs. There is an urgent need to explore more medicinal plants and check their pharmacological efficacy. The present study was intended to investigate the pharmacological activity of *Centella asiatica* L. that has been checked qualitatively. The plant was collected from GCU, botanical garden then it was dried, grinded, and the crude extracts were being prepared through maceration method in different solvents i.e. petroleum ether, chloroform, methanol and distilled water. Later on, different pharmacological tests were performed. After phytochemical screening, the plant found very active for the production of secondary metabolites as alkaloids, Tannins etc. and possess the strong defense system. The plant showed positive results for alkaloids, saponins, tannins, phlobatannins, cardiac glycosides, and flavonoids by producing characteristic precipitates, froth or ring productions. Whereas in some tests, it showed negative results for terpenoids, coumarins and anthraquinones. Moreover, the total antioxidant assay was also performed in which aqueous extract of all the plant parts showed very much comparable results with the available standards i.e. BHT and alpha-tocopherol.

Keywords: Pharmacological; *Centella asiatica*; *S*econdary metabolites; Medicinal plant; Antioxidant

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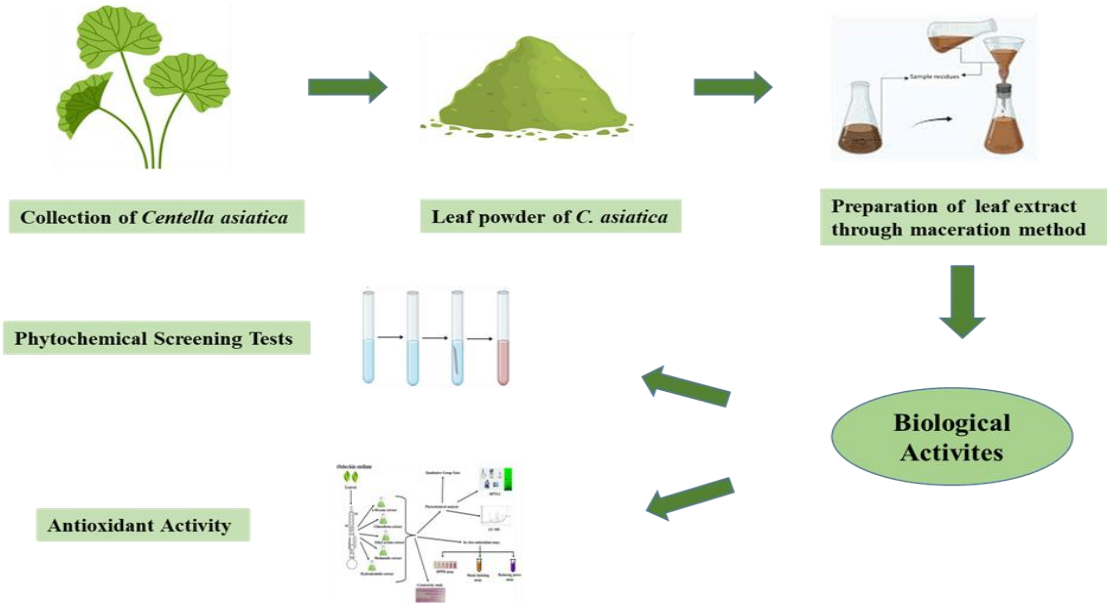
Graphical Abstract



1. Introduction

Plants are traditionally used for various purposes; particularly in the healthcare system globally, becoming the backbone of the pharmaceutical industries (Adeleye *et al*., 2021). This botanical approach for medical therapy persist in developing countries, where herbal medicines are solely source for healthcare (Hosseini *et al*., 2025). As stated by World Health Organization, approximately 80 % population of the developing countries rely upon traditional herbal practices for primary healthcare (Group, 2022). Plant possesses potent phytochemicals i.e. carotenoids, flavonoids, phenolic acids, saponins, isoflavones, phytosterols, alkaloids, etc. have strong pharmaceutical potential. These bioactive compounds of plants play vital role for curing of various human diseases. The qualitative screening of the plants are crucial step which explore the potential of plants, used in pharmaceutical companies for the production of the new pharmaceutical drugs (Thakur *et al*., 2020).

The Apiaceae family are mostly temperate herbs, umbellate inflorescences comprising about 455 genera and 3,750 species that are commonly distinguished by the presence of hollow stems and sheathing petioles. Their leaves are nearly always alternate and pinnately or palmately compound or more than one compound; stipules are generally absent. The flowers are typically small, mostly bisexual, and mostly actinomorphic except in a few instances where pseudanthia are produced and the peripheral flowers have enlarged petals directed away from the center of the

inflorescence. Their fruit is a schizocarp (Thiviya *et al.*, 2021).

Centella asiatica L. is a perennial, creeper, faintly aromatic and a valuable medicinal herb of both Old World and the New World. It is distributed throughout tropical and subtropical regions of World such as India, China, Nepal, Madagascar, Srilanka and Indonesia etc. It is a prostrate, faintly aromatic, stoloniferous, perennial, creeper herb belonging to apiaceae family, attains height up to 15 cm. Stem is glabrous, striated, rooting at the nodes. *C. asiatica* flourishes extensively in shady, marshy, damp and wet places such as paddy fields, river banks forming a dense green carpet and rather than clayey soil, the sandy loam (60 % sand) is found to be the most fertile soil for its regeneration. Their leaves, 1-3 from each node of stems, long petioled, 2-6 cm long and 1.5-5 cm wide, orbicular-renniform, sheathing leaf base, crenate margins, glabrous on both sides. Flowers are in fascicled umbels, each umbel consisting of 3-4 white to purple or pink flowers, flowering occurs in the month of April-June. Fruits are borne throughout the growing season, globular in shape and strongly thickened pericarp. Seeds have pedulous embryo which are laterally compressed (Diniz *et al*., 2023).

The objective of the study was to explore the pharmacological approaches of *C. asiatica*, aiming to achieve the good health and well-being (SDG 3)

2. Materials and Methods

Fresh rhizome, leaves and roots of *C. asiatica* were collected from botanical garden of GCU in the month of March 2014, were dried under normal conditions. Before extraction, all the dried plant material was then ground to make powder and preserved in the amber colored specimen jars, until required. A volume of 10 g of leaves, stem and roots of plant were extracted in sequence with different solvents. The extraction was carried out by soaking the powder in each of the solvent for the period of 8 days. e.g., petroleum ether, chloroform, methanol and distilled water. The residue was filtered and the filtrate was preserved in the labeled amber colored glass jars at 4 ^{o} C, whereas the residue was further soaked in the next solvent in series (Din *et al*., 2022).

2.1: Reagents and Apparatus

In this study, H_2SO_4, potassium dihydrogen phosphate, ammonium molybdate, methanol, petroleum ether, chloroform, hydrochloric acid, distilled water, Mayor's reagents, sodium hydroxide, acetic acid, ethanol, potassium hydroxide, glacial acetic acid, ferric chloride, ammonium hydroxide, iodine, benzene and DMSO were used. All the reagents were purchased from Falcon Scientific, Lahore, originating from Merck, Germany, and Sigma Aldrich, USA. The apparatus include UV illuminator, refrigerator, electric balance (GE-212, Gottingen, Germany), incubator (Model No.53114, IMERCO, Geethacht, Germany), spectrophotometer and heating oven.

2.2: Biological Activities

Phytochemical analysis

Qualitative phytochemical analysis of the four crude extracts of the *C. asiatica* was carried out by using standard procedures to identify the constituents as described by (Olufunke *et al*., 2026).

Alkaloids

To identify the presence of alkaloids, 4 mL of 1 % HCl was added to the 0.25 g of the plant powder (i.e., leaf rhizome and root) and then, it was warmed and filtered. Took 1 mL of filtrate, 6 drops of Mayor's reagents was added separately. The creamish and orange precipitates indicated the presence of respective alkaloids.

Saponins (Frothing test)

To detect saponins, 0.5 g of the plant powder (i.e., leaf rhizome and root) was boiled in 5 mL of distilled water. After cooling, it was shaken vigorously to produce stable persistent froth.

Anthraquinones

To check the presence of anthraquinones, 0.5 g of the plant powder (i.e., leaf rhizome and root) was boiled with 3 mL of 1 % HCl and was filtered. To filtrate, 2 mL of benzene was added and shaken well. The benzene layer was removed and few drops of 10 % NH_4OH were added. The formation of pink, violet or red colour indicated the presence of anthraquinones.

Coumarins

For coumarins analysis, 0.5 g of moistened plant powder (i.e., leaf rhizome and root) was taken in a test tube and covered with a filter paper moistened with 0.1 N NaOH. The test tube was placed, for few minutes, in boiling water. Then, the filter paper was removed and examined in UV light for yellow florescence to indicate the presence of coumarins.

Terpenoids (Liebermann-Burchard reaction)

To identify the presence of terpenoids, 2 mL of the plant extract (i.e., leaf rhizome and root) was dissolved in 2 mL of chloroform and was filtered. After filtrate, equal volume of acetic acid and a drop of concentrated H_2SO_4 were added. Blue-green ring indicated the presence of terpenoids.

Flavonoids

To detect flavonoids, 5 g of the plant powder (i.e., leaf rhizome and root) was washed with petroleum ether. The defatted residue was dissolved in 20 mL of 80 % of ethanol and was filtered. About 3 mL of the filtrate was mixed with 4 mL of 1% KOH. A dark yellow colour indicated the presence of flavonoids.

Tannins

To test for tannins, 0.25 g of plant powder (i.e., leaf rhizome and root) was boiled in 10 mL of distilled water and filtered. Then, 1% $FeCl_3$ was added to the filtrate. Brownish green or a blue-black colouration indicated the presence of tannins.

Phlobatannins

Deposition of a red precipitate appears, when 0.25 g of plant powder (i.e., leaf, rhizome and root) was boiled with 5 mL of 1 % aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Cardiac glycosides (Keller–Kiliani test)

To detect cardiac glycosides, 2 mL of glacial acetic acid and few drops of 1 % $FeCl_3$ were added to 0.5 g of the plant powder (i.e., leaf, rhizome and root). Then, it was underlayed with 1 mL of concentrated H_2SO_4. Green-blue color indicated the presence of cardiac glycosides.

Determination of total antioxidant capacity

The total antioxidant capacity of all the extracts was assayed according to the method of Rashid *et al*. (2023). Took 0.L ml of each solution (0.5 mg/mL) was combined with 1.9 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).

The reaction mixture was incubated at 95 °C for 60 minutes. After cooling at room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity will be expressed as the absorbance of the sample. The antioxidant activity of BHT (Beta hydroxyl toluine) (0.5 mg/mL) was also assayed for comparison.

3. Results

Biological activities analysis

Phhytochemical Analysis

Alkaloids

In the phytochemical study of *C. asiatica* leaves, rhizome and roots, presence of creamish or orange precipitate indicated the presence of their respective alkaloids as shown in figure 1.

Figure 1: Test for alkaloids

Saponins

Indication of persistent froth in the plant extract of *C. asiatica* leaves, rhizome and roots showed that saponins are present in respective plant sample as shown in figure 2.

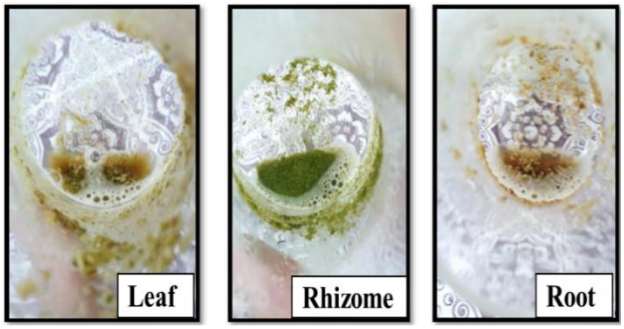


Figure 2: Test for saponins

Anthraquinones

Formation of white colour froth in the plant extract of *C. asiatica* leaves, rhizome, and roots indicated that there is no presence of anthraquinones in respective plant sample as shown in figure 3.

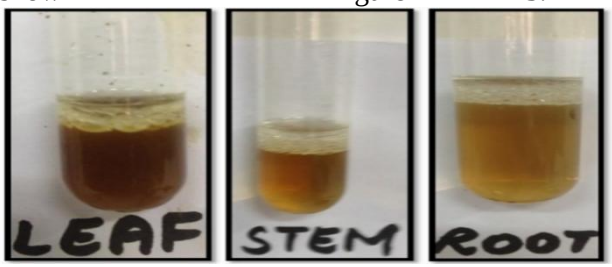


Figure 3: Test for anthraquinones

Coumarins

In *C. asiatica* leaves, rhizome and root extract there is no emission of yellow fluorescent in UV light indicated that coumarins are not present in respective plant sample, as shown in figure 4.

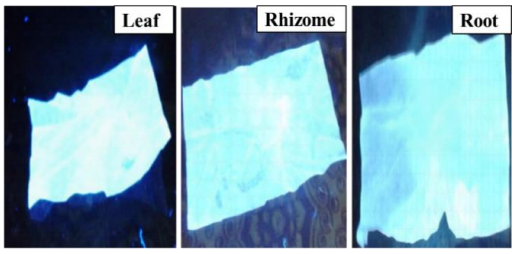


Figure 4: Test for coumarins

Terpenoids

In the analysis of *C. asiatica* leaves, rhizome and root, presence of brown rings give negative results for terpenoids, as shown in figure 5.

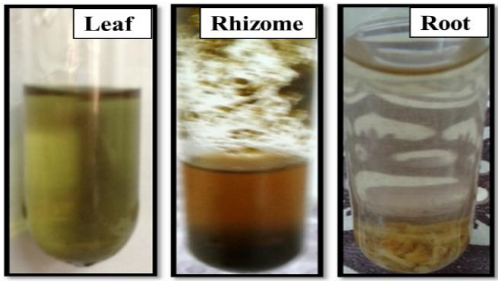


Figure 5: Test for terpenoids

Tannins

Indication of brownish green coloration in leaves, rhizome and root extract of *C. asiatica* showed that tannins are present in respective plant sample, as shown in figure 6.

Figure 6: Test for tannins

Phlobatannins

Deposition of red precipitate in the extract of *C. asiatica* leaves, rhizome and root extract indicate that phlobatannins present in the respective plant sample, as shown in figure 7.

Figure 7: Test for phylobatannins

Cardiac glycosides

Indication of blue green coloration in extract of *C. asiatica* leaves, rhizome and root seeds showed that cardiac glycosides are present in respective plant sample, as shown in figure 8.

Figure 8: Test for cardiac glycosides

Flavonoids

A dark yellow colour in the plant extract of *C. asiatica* leaf, rhizome and root indicated the presence of flavonoids as shown in figure 9.

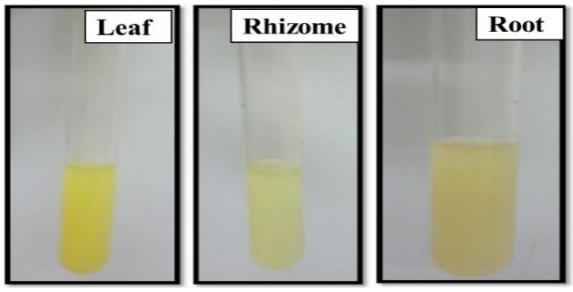


Figure 9: Test for flavonoids

Total antioxidant capacity (TAC)

TAC were performed to access the efficacy of the *C. asiatica* and evaluate their effectiveness. BHT and alpha-tocopherol were used as standard. Four different solvents i.e. petroleum ether, chloroform, methanol and distilled water were used. The results indicated that in case of *C. asiatica* leaf and rhizome extract, the metholic solvent showed highest TAC (1.73 ± 0.28) and (1.73 ± 0.5) respectively at the concentration of 100 µL, while in root extract, the distilled water solvent depicted stronger TAC (0.60 ± 0.5) at the concentration of 150 µL as shown in figure 10.

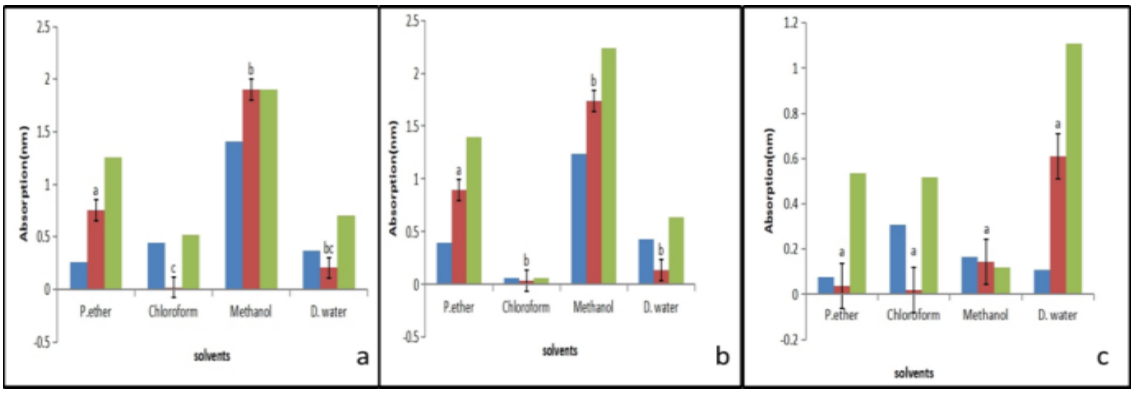


Figure 10: Total Antioxidant Capacity (a) *C. asiatica* leaf extract (b) *C. asiatica* rhizome extract and (c) *C. asiatica* root extract in different solvents i.e. petroleum ether, chloroform, methanol and distilled water.

4. Discussion

Medicinal plants are potent source of innovative drugs for primary healthcare because of their phytochemicals (Adjei *et al.*, 2021). Ethnobotanical approaches are important for discovering the pharmaceutically important drugs (Qaseem *et al.*, 2019). The present study was conducted to check the pharmacological potential of *Centella asiatica.* Quantitative screening of phytochemicals are essential to check the efficacy of *C. asiatica*. Various test were conducted. The results showed that the presence of creamish or orange precipitates, which indicated the presence of alkaloids in the extract of *C. asiatica* leaf, rhizome and root. The present results are similar to the work of Ameyaw and Duker-Eshun (2009). For the analysis of saponin test, the presence of persistent froth exhibited the presence of saponins which are confirmed by comparing the results with the work of Ezeabara *et al*. (2014).

Baştemur *et al.* (2024) investigated the separation and quantitative determination of anthraquinones is achieved by high performance liquid chromatography in mixtures of methanol, water and formic acid on a reversed-phase stationary phase. The products are identified by retention time and, more accurately, by standard additions and UV-visible spectroscopy. This methodology has been applied to extracts of plant roots and insects, commonly used in earlier times as the source of red dyestuffs for dyeing textiles. Quantitative evaluation of the anthraquinone derivatives present in ancient red dyes was

earned out after acid hydrolysis of 0.2 to 2.0 mg of textile fibre. Due to the great sensitivity of the method, important minor constituents, such as kermesic acid in cochineal, can be detected. The present investigation by maceration method, formation of white color froth gives negative results, whereas positive results are formation of pink, red or violet color froth. The results are contradict to the previous work.

(Khan *et al.,* 2011; Skalicka-Woźniak and Głowniak, 2012) reported that coumarins are nowadays an important group of organic compounds from natural sources that are useful in a number of fields. Because they possess different pharmacological properties, finding the proper extraction conditions for their separation from plant matrices is a very important step. In his report, Pressurized Liquid Extraction (PLE) under different temperature conditions and with different types of extraction solvents were tested. As a matrix, fruits of *Heracleum leskowii* have been used. A simple reverse phase high-performance liquid chromatographic method (RP-HPLC) coupled with a photodiode array detector (DAD) has been developed for separation and quantitative analysis of the main coumarins. In the present research work of *C. asiatica* extract of leaves, rhizome and root showed no emission of yellow inflorescence in UV light whereas the positive results are the emission of yellow inflorescence in UV light. Thus it gave negative results for coumarin.

The result of terpenoids showed the formation of brown rings but the work of Wadood *et al*. (2013) depicted the presence of blue-green rings. The results of terpenoids are contradicted. Khan *et al*. (2011) conducted phytochemical analysis of five different plants, to determine their tanin content. Their results indicated that the presence of brownish green color confirmed the presence of tanins in the sample. The present study results are similar from their results. Solihah *et al.* (2012) explored that Malaysian *Zea mays* hair extracts was screened for the occurrence of bioactive compounds. They showed the presence of phlobatannins in both aqueous and methanolic extract of *Zea mays* hair. In the present investigation, deposition of red precipitate in the extract of *C. asiatica* leaves, stem and root extract indicated the phlobatannins present in the respective plant sample. Khan *et al*. (2011) reported that phytochemicals are the dependable sources for the treatment of different health problems. The work reveals that phytochemical screening of twenty different medicinal plants, which were collected from the different regions of the province Khyber Pakhtunkhwa, Pakistan. In most of the samples, all the phytochemicals i.e reducing sugar, glycosides were present. In the present investigation, indication of blue green coloration in extract *of C. asiatica* leaves, stem and root showed that cardiac glycosides are present in respective plant sample. The result of present work is similar with their work. Rajeshwari and Andallu (2011) reported that the reversed-phase high performance liquid chromatography (RP-HPLC) method with UV/VIS detection was established for the separation and identification of flavonoids in the methanolic and ethanolic extracts of coriander (*Coriandrum sativum* L.) seeds. In the present research work, a dark yellow colour in the extract of *C. asiatica* leaf, rhizome and root indicated the presence of flavonoids. The result is same as the available standards set out by other scientists.

Antioxidant activity was carried out to check the potential of *C. asiatica*, either it reduces or accelerates the reactive oxygen species (ROS) formation, evaluated through TAC assay. This assay was used to examine the antioxidant compounds in the sample by following phosphomolybdate method. The results indicated that methanolic extract of *C. asiatica* leaf and rhizome depicted highest TAC potential as compared to other solvents while in the case of root extract, the distilled water exhibited greater potential as compared to other solvents. Rashid *et al.* 2023 documented in his work that *C. asiatica* have excellent antioxidant properties. In the present study, the result depicted that the *C. asiatica* exhibited strong antioxidant activity and showed similar results with the available standards as BHT and alpha-tocopherol.

5. Conclusion

C. *asiatica* showed positive results for alkaloids, saponins, tannins, phlobatannins, cardiac glycosides and flavonoids by producing characteristic precipitates, froth or ring productions. Whereas in some cases, it showed negative results for terpenoids, coumarins and anthraquinones. Total antioxidant assay was also performed in which aqueous plant extract showed very much comparable results with the available standards as BHT and alpha-tocopherol.

Author Contributions:

Methodolgy and write-up, MK; formal analysis, SM; review and supervision, MK and MA. All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest: The authors declare no conflicts of interest.

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