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Phytochemical analysis and Antioxidation Study of Indian Pennywort

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Abstract: Indian Pennywort (*Centella asiatica)* was analysed for phytochemical studies and antioxidant analysis. The preliminary phytochemical screening was performed from leaves extract for the presence of alkaoids, saponins and tannins. Three different solvents (water, ethanol, and acetone) were used and their highest antioxidant activity was assayed by effect of phenolic content, reducing power and flavonoid for acetone concentration was 243µg/ml, 82µg/ml and 215µg/ml. 10% of acetone extract of *C.asiatica* contained significantly higher amount of phenolic content. Finally, it has been suggested that the high phytochemical content of *C. asiatica* and its antioxidant activity makes it popular and wide traditional use.

Key words: *Centella asiatica*, phytochemical, antioxidant activity phenolic content

INTRODUCTION

Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. These complex materials are referred to as secondary metabolites which

have been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing diseases due to their antioxidant effect[1]. They are valuable sources of drugs. This drugs is chemically active substance, and that the major source of medicine which plays a role in the human history[2-5] and its produce a definite physiological action on the human body[6].

C.asaitica is a prostrate stoloniferous plant that belongs to the family Apiaceae and endogenous to Bangladesh[7]. The therapeutic use of *C.asaitica* with its wide range of applications has been documented in South East Asia and Bangladesh for centuries *C.asiatica* is effectively used in the treatment of fever, jaundice, dysentery, diarrhoea, mental illness within the frame work of traditional medicines of Bangladesh[8]. The phytochemical content of *C.asiatica* has been reported to contain a vast number of chemical compounds. The major chemical compound found in this plant is triterpene saponosides[9-11].

C.asaitica has large amounts of pentacyclic triterpenoids including asiatic acid, brahmic acid, asiaticoside, madecassoside. This plant has a many phytochemicals and high antioxidant activity[12] .The phenolic content and antioxidant activity of *C.asiatica* have been studied[13-15].Synthesis of secondary metabolites including phenolic compound in plants may be stimulated by the action of different parameters like environmental factors of plants[16].The objective of the present study aims to evaluate the phytochemicals and antioxidant activity of the leaves of *C.asiatica*.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves of *Centella asiatica* were collected from Adhiyamaan Botanical Garden, Hosur, and Tamil Nadu. The leaves were properly washed with tap water and then rinsed with distilled water and dried under shade the leaves were treated with different solvents (50 g leaves powder 100 ml of each solvent) (water, acetone, and ethanol) and they were stored in the refrigerator in suitable containers for further analysis.

Phytochemical analysis (qualitative analysis): The freshly prepared diluted extract of leaves were tested for the presence or absence of phyto constituents such as alkaloids, saponins, glycosides, carbohydrates, tannin, flavonoids and steroids using standard phytochemical procedures.

Alkaloid test: 1 ml of extract was added to each solvent and Dragendroff's reagent **(**potassium bismuth iodine solution) was added to three test tubes. Orange red precipitation in any of the three test tubes indicates the presence of alkaloids.

Saponins test: Add 1 ml of diluted extract of leaves to three test tubes and add 10 ml of distilled water shake vigorously and observe for persistent froth. Formation of foam indicates the presence of saponins.

Glycosides: 1 g of powdered drug is extracted with 10 ml of 70 % alcohol for 2 minutes, filtered, added to the filtrate, 10 ml of water and 0.5 ml of strong solution of lead acetate is added and filtered and the filtrate is shaken with 5 ml of chloroform. The chloroform layer was separated in a porcelain dish and the solvent was removed by gentle evaporation. The cooled residue was dissolved in 3 ml of glacial acetic acid containing 2 drops of 5 % ferric chloride solution. This solution was carefully transferred to the surface of 2 ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Reducing sugar: 1 ml of Fehling's solution was added to 1 ml of extract and kept in boiling water bath. A brick red precipitate indicates the presence of reducing sugar.

Tannin test: 1 ml of extract was added to 0.1 g of potassium dichromate solution and shaken to dissolve presence of yellow precipitate indicates the presence of tannin.

Flavonoid test: Add 1 ml of distilled extract and 1 pellet of sodium hydroxide in three test tubes. Formation of yellow solution indicates the presence of flavonoids. Flavonoids are absent in acetone.

Steroid test: Place little quantity of sample on the 3 filter papers and allow it to stand for 15 minutes. A greasy spot was observed due to presence of fats. Flavonoid are absent in water.

Antioxidant activity: The antioxidant activity of the *C. asiatica* was measured on the basis of phenolic assay, flavonoid content and total reducing power. Antioxidant is used by aerobic organisms to protect the cells from oxidation damage during oxygen metabolism.

Total phenolic content: The amount of total phenolic in *C. asiatica* was determined by Folin-Ciocalteu phenol**.** 1 ml of sample was added to 0.5 ml of FCR, 2.5 ml sodium carbonate and vortex the test tubes were placed in dark for 40 min. The absorbance was measured at 725 nm using UV-Vis spectrophotometer. (Systronics ELICO Company, Hosur)

Total flavonoid content: A solution was prepared from 0.15 ml of sodium nitrite and after 6 minutes 0.15 ml of aluminium trichloride was added allowed to standing for 6 minutes. Immediately add 0. 4 g in 10 ml of NaoH. The volume was adjusted to 5 ml. Allow to stand for 15 minutes. The absorbance was measured at 510 nm.

Total reducing power: A solution was prepared from 1 ml of potassium ferricyanide and incubated at 50ºC for 20 min. 1 ml of trichloro acetic acid was added and volume was adjusted to 2.5 ml. Add 1 ml of ferric chloride. The absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

The qualitative analysis (phytochemical) and quantitative analysis (antioxidant) were performed. Phytochemical screening of the leaf extract of *Centella asiatica* confirmed the presence of alkaloids, glycosides, carbohydrates, tannins, flavonoids, and steroids. In this test, three solvents were used (water, ethanol, and acetone) leaf extract was treated with these solvents. In phytochemical screening the alkaloid test which indicates the presence of orange red. Glycoside test which indicates the presence of reddish yellow layer. It is a compound formed from a simple sugar and another compound by replacement of a hydroxyl group in sugar molecule.

Many drugs and poisons derived from plants are glycosides. Antioxidant is a substance that inhibits oxidation especially one used to counteract the deterioration of stored food products. Antioxidant system protects the cells from damages produced by oxidants during metabolism. Saponin is a class of steroids and terpenoids which forms foam when shaken. It is a toxic compound. Tannins are yellowish or brownish bitter tasting organic substance. The flavonoid was any of a large class of plant pigments having a structure based on or similar to that of flavone. The steroid was any of a large class of organic compounds with a characteristic molecular structure containing four rings of carbon atoms. They include many hormones, alkaloids and vitamins. (Table-1)

Table-1: Preliminary phytochemical analysis

Chemical constituents	Test	Sample 1 (Water)	Sample 2 (Ethanol)	Sample 3 (Acetone)
Test for Alkaloids	Dragendorff's test	+	+	+
Test for Glycosides	Keller Killani test	-	+	-
Test for reducing sugar	Fehling's test	-	-	-
	Benedict's test	-	-	-
Test for Tannins	Potassium dichromate test	+	+	+
	Lead acetate test	+	+	+
	Ferric chloride test	-	-	-
Test for Flavonoids	Shinoda's test	+	+	-
	Sodium hydroxide test	+	+	-
	Sulphuric acid test	-	-	-
Test for Steroids	Test for fats and oils	+	+	+
Test for Saponins	Saponins	+	+	+

Effect of phenolic content: The phenolic constituents play a vital role in antioxidant activity which is due to its oxide reduction properties and play an important role in adsoption or neutralization of free radicals. Total phenolic content was measured according to Folin-Ciocalteu method (Table-2).

Effect of reducing power content: The reducing power of extract of *Centella asiatica* was tabulated in Table 2. The effect of reducing power content of highest concentration (82 microgram/milliliter). The reducing power is often used as an indicator of electron donating activity which is an important mechanism of antioxidant activity. The increasing absorbance indicates increasing reducing ability (Table-2).

Effect of flavonoid content: Total flavonoid content was successfully analyzed in leaf samples. *Centella asiatica* demonstrated the highest flavonoid content of concentration (215 microgram/milliliter) (Table-2).

Table-2: Effect of antioxidant study from different solvent extracts

SI No	**Effect of Phenolic content**	Absorbance	Concentration (µg/ml)
1.	Water	1.83	145
2.	Ethanol	2.12	170
3.	Acetone	3.08	243
	Effect of reducing power content		
4.	Water	1.32	37
5.	Ethanol	1.59	40.5
6.	Acetone	3.0	82
	Effect of flavonoid content		
7.	Water	3.0	210
8.	Ethanol	1.7	120
9.	Acetone	2.8	215

The phytochemical analysis of *Centella asiatica* revealed the presence of medically active compounds that includes alkaloids, tannins, flavonoids, steroids and saponins. But, the phytochemical test carried out in leaves of *Terminalia bellerica* and *Tinospora cordifolia* showed absence of alkaloids[17]. The phytochemical test carried out in leaves of *Lantana camara*, *Carica papaya* and *Datura metel* showed absence of tannins[18, 19]. The antioxidant activity in *Centella asiatica* using 100% and 50% ethanol[20] is not much effective when compared to 10% ethanol and 10% acetone by total phenolic content and total flavonoid content analysis.

CONCLUSION

The results indicated that presence of medicinally important constituent in the plant *C.asiatica*. Leaves was subjected for treatment from different solvents. The present study indicated that acetone has rich phytochemical constituents and good antioxidant activity and it may be responsible for its popular and wide spread traditional use.

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