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PHYTOCHEMICAL SCREENING OF *Centella asiatica* L. DURING DIFFERENT STAGES OF ITS *in vitro* GROWTH

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Key words : *Centella asiatica, Phytochemicals, Shoot culture, Callus culture, Suspension culture and Methanol*

Centella asiatica L. (Syn *Hydrocotyle asiatica* Linn.) belonging to family Umbelliferae/Apiaceae of dicotyledonous angiosperm is a medicinal herb in India, China, Srilanka, Nepal and Madagascar. *Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, and hence it is known as a "Brain food" in India.

In the present investigation the important phytochemicals of *Centella asiatica* were assayed during different stages if its *in vitro* culture. The results revealed presence of tannins, flavanoids, terpenoid, saponin and steroids in all the three cultures and absence of alkaloids. The total phenol content was found to be highest in shoot culture extract followed by callus extracts and least in cell suspension extract. The total flavonoid content was maximum in shoot culture extract and minimum in cell suspension culture extract. The total tannin content was found to be maximum in shoot culture extract, followed by callus culture extract and cell suspension extracts.

The present screening experiments form a primary platform for further phytochemicals and pharmacological studies that may open the possibility of finding new clinically effective compounds. Thus, the present study has authenticated the usefulness of the *Centella asiatica* plants for medicinal purposes.

INTRODUCTION

Centella asiatica L. (Syn *Hydrocotyle asiatica* Linn.) belonging to family Umbelliferae/Apiaceae of dicotyledonous angiosperm is a medicinal herb in India, China, Srilanka, Nepal and Madagascar. *Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, and hence it is known as a "Brain food" in India. This herb is also known as Indian Pennywort,Gotu Kola, Asiatic pennywort, Spade leaf and Brahmi.

Centella asiatica (L.) is a prostrate, faintly aromatic, stoloniferous, perennial, creeper herb, attaining a height up to 15cm. Stem is glabrous, striated and, rooting at the nodes. This plant flourishes extensively in shady, marshy, damp and wet places such as paddy fields and 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 (DevkotaAnjana and Jha, 2009). The leaves, 1-3 from each node of stems, long petioled, 2- 6cm long and 1.5-5cm 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 and flowering occurs in the month of April-June. Fruits are borne throughout the growing season. It is about 6.5 cm long, oblong, globular in shape and has strongly thickened pericarp. Seeds have pendulous embryo which are laterally compressed.

Centella asiatica is used medicinally for the treatment of a wide variety of disorders such as skin diseases, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration, and diarrhoea (Yu et al., 2006). In Indian systems this plant is used as medicine for enhancing memory and for the treatment of skin diseases and nervine disorders (Jamil *et al*., 2007). The plant medicinal properties have long been utilized by the people of Java and Indonesia. In China, it is indigenously called as Gotu kola, and it was one of the documented "miracle elixirs of life" (Diwan *et al.,* 1991).

In the present investigation the important phytochemicals of *Centella asiatica* were assayed during different stages if it'sin *vitro culture*.

MATERIALS AND METHODS

Shoot culture

In vitro grown shoot was air dried at room temperature. About 0.5 gm of dried shoot culture was crushed to the fine powder and then 5 ml of 80% methanol was added and mixed properly. It was then sonicated for 20 minutes and left at room temperature for 24 hours.The extract was centrifuged for 10 minutes at 1500 g and the supernatant was collected and stored at 4°C until further use.

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Callus culture

About 0.5 g of air dried callus was soaked in 5 ml of 80% methanol and mixed properly and then sonicated for 20minutes. It was then centrifuged at 1500 g for 10 minutes and the supernatant was collected and stored at 4°C until further use.

Cell suspension culture

The *in vitro* grown cell suspension culture was transferredinto sterile falcon tubes and centrifuged at 1500 g for 15 minutes. Thesupernatant was removed so that the cells settle at the bottom as pellet. To about 0.5 g of cells 5 ml of 80%methanol was added and mixed properly. It was then sonicated for 10 minutes. The sample was centrifuged at 1500 g for 10 minutes and the supernatant was collected and stored at 4°C until further use.

Screening of Phytochemicals

(a) **Test for Terpenoids (Salkowski test) :** About 5 ml of sample was mixed with 2 ml of chloroform in a test tube to which 3 ml of concentrated sulphuric acid was carefully added through the sides to form a layer. Appearance of reddish brown colour at the interface, indicated the presence of terpenoids.

(b) **Test for Saponin :** The sample was mixed properly with 5 ml of distilled water and was vigorously shaken. The appearance of stable foam indicated presence of saponin.

(c) **Test for Tannins :** About 1 ml of the sample was added to 1 ml of Potassium ferricyanide (0.008 M) then 1 ml of Ferric chloride (0.02 M) containing 0.1 N HCl was added. Appearance of blue-black coloration indicated the presence of tannin.

(d) **Test for Alkaloids :** The sample was mixed with 2 ml of Wagner's reagent (2 g of iodine and 6 g of potassium iodide in 100 ml distilled water). Appearance of reddish brown colored precipitate indicated the presence of alkaloids.

(e) **Test for Flavonoids :** About 5 ml of dilute ammonia solution was added to small amount of the sample followed by addition of concentrated sulphuric acid. Appearance of yellow colour indicated the presence of flavonoids.

(f) **Test for Steroids : 1** ml of the sample was dissolved in 5 ml chloroform and then equal volume (5 ml) of concentrated sulphuric acid was carefully added through the sides of test tube. Appearance of red colour at upper layer and yellow colour with slight green fluorescence indicated the presence of steroid.

Flavonoid estimation : The Aluminium chloride colorimetric assay was used to estimate flav onoids. 1 ml of sample was mixed with 4 ml of distilled water. 0.3 ml of 5% sodium nitrite was added. It was mixed properly. After 5 minutes, 2 ml of sodium hydroxide (1 M) was added and made up to 10 ml using distilled water. A standard solution of quercetin of concentration 20, 40, 60, 80 and 100 µ g/ml was prepared using the above procedure. The absorbance of the sample and qurecetin standards was determined at 510 nm. The total flavonoid content obtained was expressed in terms of mg of Qurecetin Extract (QE) per gram of extract.

Phenol estimation : The Total phenol content was estimated using Folin-Ciocalteu (F-C) reagent method. The F-C reagent (10%) was prepared by 10 ml of F-C reagent added to 90 ml in water. Then sodium carbonate (5%) was prepared by dissolving 3 g of sodium carbonate in 50 ml of distilled water. About 200 µ L of was added to 1.5 ml of F-C reagent and kept at the dark condition for 5 minutes. 1.5 ml of sodium carbonate was added to it and then mixed properly. The sample was then kept in the dark for about 2 hours. The Gallic acid was used as standard whose calibration curve was plotted. About 3 mg of Gallic acid was dissolved in 10 ml of methanol to get 300 mg/L concentration. Similarly 200, 100, 50 and 25 mg/L concentrations were prepared. The absorbance of all the samples and standards was determined using UV spectrophotometer at a wavelength of 750 nm.

Tannin estimation : The tannin content was estimated by Folin-Ciocalteu method. About 0.1 ml of the sample extract was treated with7.5 ml of distilled water, with 0.5 ml of prepared F-C reagent (10%)reagent and adds 1 ml of the prepared sodium carbonate solution(35%) and then dilutes it to 10 ml using distilled water. The entire reaction mixture was mixed well and kept at room temperature for 30min. The gallic acid was used as the standard and prepared to various concentrations viz. 20, 40, 60, 80 and 100 µg/ml with methanol. The absorbance of all the samples and gallic acid standards were determined at 725 nm. The tannin content obtained was expressed as mg of gallic acid equivalent(GAE) per gram of extract.

The results obtained have been presented in Table-1 and 2; Figure-1.

Table-1

Phytochemicals in methanolic extracts of *in vitro* grown culture.

Phytochemicals	Shoot culture extract	Callus culture extract	Cell suspension culture extract
Alkaloids	–	–	–
Tannins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+

Table-2

Amount of Total Phenol, Total flavonoids and Total Tannins in different stages of culture

Stages of Culture	Total Phenol (mg/g)	Total Flavonoids (mg/g)	Total Tannins (mg/g)
Shoot culture extract	374.50 ±0.45	280.25 ±0.71	265.25 ±0.51
Callus culture extract	185.50 ±0.65	151.25 ±0.42	208.25 ±0.75
Cell suspension culture extract	71.50 ±0.55	100.00 ±0.65	65.35 ±0.71

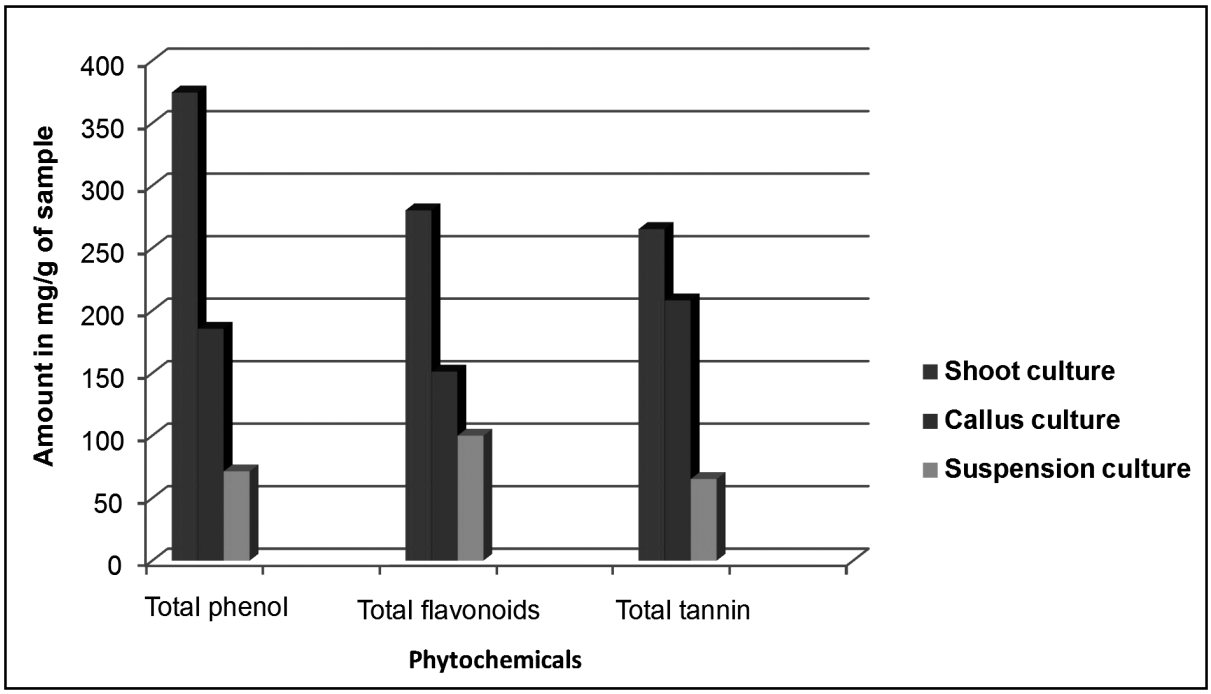

Figure-1: Amount of Total phenol, Total flavonoid and Total tannin in three different stages of *in vitro* growth of *Centella asiatica*.

RESULTS AND DISCUSSION

In the present investigation preliminary phytochemical screening was done in shoot culture, callus culture and cell suspension culture extracts. The results revealed the presence of tannins, flavanoids, terpenoid, saponin and steroids in all the three cultures and absence of alkaloids. Similar report has been obtained by Roy and Bharadvaja and Arpita Roy *et al*., (2018) (Table-1).

The total phenol content expressed in terms of mg of gallic acid (GAE) equivalent per gram of extract was found to be highest in shoot culture extract of 374.50 mg of GAE/g of extract followed by callus extracts having 185.50 mg of GAE/g of extract and least in cell suspension extract, 71.50 mg of GAE/g of extract. The total flav onoid estimation indicated interms of qurecetin equivalent(QE) per gram of extract with maximum valueof 280.25 ± 0.71 of mg QE/g of extract, following callus 151.25 ± 0.42 mg QE/gof extract and 100.00 ± 0.65QE/g of extract in cell suspension cells. TheTotal tannin content estimate using gallic acid as standard found to maximum in shoot culture extract, 265.25 ± 0.51 mg of GAE/g of extract,followed by callus culture extract 208.25 ± 0.75 mg of GAE/g of extract and 65.35 ± 0.71 mg of GAE/g of extract in cell suspension extracts (Table-2; Fig-1). The present results gain support with the work of Nithyanandan *et al*., (2014), Rahman *et al*., (2013), Gupta *et al*., (2013), Arpita Roy et al., (2018) who have also observed a more or less similar results of phytochemicals in different stages of *in vitro* growth of *Centella asiatica*.

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