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# Qualitative and Quantitative analysis of micropropagated *Centella asiatica* L.Urb.

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***Abstract :*** Present study deals with the qualitative and quantitative analysis of ethanolic extract of root, stem & leaf of micropropagated *Centella asiatica* (L.) Urb. For micropropagation nodal explants inoculated on MS medium supplemented with various concentrations of BA 1.0, 1.5 mg/L, with combination of 0.5mg/L NAA gives maximum growth shoots. A qualitative analysis by thin layer chromatography & a quantitative analysis by standard chemical protocol of secondary metabolites in the root, stem and leaf of micropropagated *Centella asiatica* L. (URB) have been studied. Using thin layer chromatography (TLC) different components like Alkaloids, Saponin, Flavonoids, Terpenoides, Phenol & Tannin are isolated & identified. The R*f* values of the developed spots in the different solvent systems are noted. In the quantitative analysis, alkaloids, saponins, terpenoids & flavonoids are extracted by using the standard chemical protocol. These results may be helpful for rationale use of this plant in the modern system of health care.

***Keywords*** **-** *Micropropagation, Nodal explants, Qualitative analysis, secondary metabolite, Thin layer chromatography*

## I. INTRODUCTION

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [1] [2]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [3] [4]. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing [5] [6]and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables [7] [9]. *Centella asiatica* (Linn.) Urban sys. synonym Hydrocotyle asiatica Linn. It is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as "Brain food" in India [10].The scientific studies have proved a variety of biochemical components i.e. secondary metabolites have been found in *Centella asiatica*. The chemical constituents of Centella plant have a very important role in medicinal and nutraceutical applications and it is believed due to its biologically active components of triterpene saponin. [11].

## II. MATERIALS AND METHODS

### 2.1 Collection of plant material:-

The fresh parts of *Centella asiatica* (L.) Urb. were collected in flowering period from Amrutkund Tq. Basavkalyan, Dist. Bidar near Maharashtra-Karnataka border. The plant material were properly washed with tap water and then rinsed with distilled water. The nodal segment of *Centella asiatica* (L.) was used as explant for in vitro propagation.

### 2.2 Surface Sterilization:-

The explants were washed under running tap water for 15 min to remove the surface contaminants and soil particles and immersed in detergent (laboline) for 5 min and rinsed with distilled water for four times. The explants were deeped in 70% ethanol for 1 min. Then the explants were soaked in 0.1% (w/v) mercuric chloride solution for 1-2 min and thoroughly rinsed with sterile distilled water for four times. The explants were cultured on Murashige and Skoog basal medium supplemented with different concentrations of plant growth regulators.

### 2.3 Culture Media and Culture Conditions:-

The surface sterilized explants were inoculated on Murashige and Skoog [12] basal medium containing 30gm/L sucrose, and 1.5gm/L clerigel. The pH of all media was adjusted to 5.8 prior to autoclaving at $121^0$C for 20 min. MS media (Murashige and Skoog, 1962) supplemented with different concentration of phyto-hormones like, BA (Benzyl Adenine), IBA (Indol -3-butyric acid) and IAA (indol -3-acetic acid). Nodal explants

inoculated on MS medium supplemented with various concentrations of BA 1.0, 1.5 mg/L, with combination of 0.5mg/L NAA gives maximum growth shoots. The formed shoots were transferred on rooting medium i.e. 0.5 & 1.5 mg/L IAA. For quantitative analysis, plant parts were dried and grind to powder material of leaf, stem & root of micropropagated plant.

**2.4 Preparation of Ethanolic Extract (Root, Stem and Leaf)**

For preparation of ethanolic extract, a modified method of Abdulrahman et.al (2004) [13] was used. The fresh parts of the plant were dried in oven and ground to fine powder with mechanical grinder. Ten gram of each plant parts was then macerated in 100 ml of absolute ethanol for 72 hr. & properly covered with aluminum foil & labeled. After 72 hrs of extraction, each extract was filtered through Whatman's filter paper no.1 separately. The filtrate was evaporated to dryness at room temperature & store at $5^0$C in refrigerator.

**2.5 Qualitative Analysis by thin Layer Chromatography [14]**

Extract was to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel. TLC was carried out to isolate the principle components that were present in most effective extracts of plant. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

**2.5.1 Method**

The above prepared plant extracts were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under ultra violet light UV at both 254 nm and 366 nm. They were later sprayed with different spraying reagents and some were placed in hot air oven for 1 min for the development of color in separated bands. The movement of the analyze was expressed by its retention factor (R*f*). Values were calculated for different sample.

$$R\,f = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$$

(R*f*-Retention factor)

**2.5.2 Detection**

After drying the plates, they were exposed to Iodine vapours by placing in a chamber that was saturated with iodine vapours and also exposed to different spraying reagents. All plates were visualized directly after drying and with the help of UV at 254 nm and 366 nm in UV TLC viewer. The R*f* value of the different pots that were observed was calculated.

**2.6 Quantitative analysis**

**2.6.1 Alkaloid determination using Harborne (1973) method:-**

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [15].

**2.6.2 Flavonoid determination by the method of Boham and Kocipai- Abyazan (1994):-**

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [16].

**2.6.3 Saponin determination using Obadoni and Ochuko (2001) method:-**

10 g of samples powder was put into a conical flask and 50 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 100 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was

discarded. The purification process was repeated. 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the Saponin content was calculated as percentage [17].

**2.6.4 Estimation of total Terpenoides using Ferguson (1956) method:-**

10g of plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids [18].

**2.7 Result and discussion**

Explants inoculated on MS (Murashige and Skoog 1962) medium supplemented with different concentration of phyto-hormones like, BA, NAA and IAA produce maximum percentage of multiple shoots. Apical shoot and nodal explants inoculated on MS medium supplemented with various concentrations of BA 1.0, 1.5 mg/L, with combination of 0.5mg/L NAA gives maximum growth shoots. The formed shoots were transferred on rooting medium i.e. 0.5 & 1.5 mg/L IAA. Highest shoot multiplication is observed 1.5 mg/l BA in combination of 0.5 mg/l NAA. For quantitative analysis, plant parts were dried and grind to powder material of leaf, stem & root of micropropagated plant.

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TLC profiling of all extracts of micropropagated *Centella asiatica* gives an impressive result that directing towards the presence of number of phytochemicals ( As Shown in Table :-1). Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity.

**Table1: Phytochemical Analysis of different parts of Centella asiatica L. by Thin layer chromatography.**

| Chemical name | Solvent system | Plant part | Rf Value | Spray Reagent |
|---|---|---|---|---|
| Alkaloid | M:NH4OH (17:3) | R | 0.3, 0.35 | Mayer's reagent |
| | | S | 0.57 | |
| | | L | 0.68,0.72, 0.85 | |
| Flavonoid | C:M (18:2) | R | 0.23, 0.26,0.32.0.72 | UV light |
| | | S | 0.24,0.26,0.32,,0.36,0.42,0.45,0.48,0.53, 0.84,0.85 | |
| | | L | 0.18,0.19,0.23,0.32,0.42,0.48,0.65,0.74,0.79,0.85 | |
| Saponin | C:GA:M:W (6:2:1:1) | R | 0.24,0.37 | Iodine vapours |
| | | S | 0.26, 0.41, 0.70 | |
| | | L | 0.38, 0.46, 0.55, 0.64, 0.90 | |
| Terpenoid | B:EA (1:1) | R | 0.35, 0.37 0.79 | 10% $H_2SO_4$ |
| | | S | 0.37, 0.39, 0.84 | |
| | | L | 0.35, 0.39, 0.48, -.53, 0.57, 0.75, 0.87, 0.92 | |

**Note : P P-Plant part., C-chloroform, M-methanol, B-Benzene, EA- Ethyl acetate, GA- Glacial acetate, W-water, R-root, S-stem, L-leaf,**

For quantitative estimation, After micropropagation, the Plant parts (Root, Stem and Leaf) were shade dried and ground to the coarse powder. Based upon the preliminary phytochemical test, Quantitative determination phytoconstituents were carried out for the powdered plant material by various standard methods and found that alkaloid 0.05gm, 0.2 and 0.2 gm in root, stem, leaf respectively, Flavonoids 0.4gm, 0.4gm and 2.1 gm in root, stem, leaf respectively, terpenoids 0.1gm, 0.3gm and 0.9gm in root, stem, leaf resp. and Saponin 0.1gm, 0.3gm and 0.2gm in root, stem, leaf respectively.

**Table 2:- Quantitative analysis of *Centella asiatica* L**

| Plant part | Fresh wt. In gm | Dry wt. | D.M. [%] | Alkaloid In gm Extract$^n$ Each 5 gm | Alkaloid [%] | Flavonoid In gm Extract$^n$ Each 10 gm | Flavonoid [%] | Terpenoid In gm Extract$^n$ Each 10 gm | Terpenoid [%] | Saponin In gm Extract$^n$ Each 10 gm | Saponin [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Root | 39.75 | 36.9 | 92.93 | 0.05 | 0.13 | 0.4 | 1.08 | 0.1 | 0.27 | 0.1 | 0.27 |
| Stem | 57.24 | 53.68 | 95.40 | 0.2 | 0.37 | 0.4 | 0.74 | 0.3 | 0.55 | 0.3 | 0.55 |
| Leaf | 50.48 | 47.56 | 93.61 | 0.2 | 0.42 | 2.1 | 4.41 | 0.9 | 1.89 | 0.2 | 0.42 |

## III. CONCLUSION

In the present study leaf, stem and root showed the presence of bioactive compound such as alkaloids, flavonoids, terpenoids, saponins, etc. This study also leads to the further research in the way of isolation and identification of the active compound from the leaf, stem and root of *Centella asiatica L.* using chromatographic and spectroscopic techniques..

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