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PHYTOCHEMICAL EVALUATION OF *Centella asiatica* (L.) URBAN WITH SPECIAL EMPHASIS ON ITS CONSTITUENT MEDACASIC ACID AND ROTUNDIC ACID

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ABSTRACT

Centella asiatica (L.) is one of those phytochemical rich plant that has been consume for hundreds years and it is claimed that the plant possess various healing effect and antioxidant properties. For many years, a lot of commercial and medicinal researches have been focusing their resources on this plant. Hence, the profiling of this plant is vital. This study was done to investigate the behaviour of active components in two different accessions mainly roots, stems and leaves. Phenolic and Flavanoid contents were estimated and Gas Chromatography-Mass Spectroscopy (GC-MS) was performed to identify the chemical components in the extract. The essential chemical components such as Medecassic acid and Rotundic acid were found to be present in the extract.

Keywords: GC MS, Phenolic content, Flavanoid content, Medecassic acid, Rotundic acid

INTRODUCTION

Centella asiatica (L.) Urban, synonym: *Hydrocotyle asiatica* L. (Eng. Indian Pennywort, Fr. *Hydrocotyle asiatique*, Ger. Asiatischer Wassernabel), also known by common names as: Gotu kola or Tiger Grass, belongs to the Apiaceae family. It grows in Asia, mainly in India, Pakistan, Madagascar, equatorial Africa, Central America and in the tropical region of Oceania **[1]**. In traditional Asian medicine, the herb of *C. asiatica* has

been used for hundreds of years, especially in dermatological conditions, to improve small wounds, scratches, burns, hypertrophic wounds healing, and as an anti-inflammatory agent, particularly in eczema. It is also recommended as an antipyretic, diuretic, rheumatic, antibacterial, antiviral drug, in the treatment of vein insufficiency and for improving cognition, relieving anxiety and as an anti-cancer agent **[1-3]**. Formerly, *C. asiatica* was also used in epilepsy, hysteria, leprosy, and in minor itching and insect bites

Centella asiatica (L.) is a plant from peculiar districts of tropics which is in nature of therapeutic incentive under the group of *Apiaceae*, unending herb with wriggling stem, has establishing onto the hubs and amalgamates tufts of blossoms and leaves. The plant is vernacular to the nations of South East Asia, Sri Lanka, South Africa, India, Malaysia **[4]**. The medical advantages of *Centella asiatica (L.)* Urban have been exhibited by logical examinations and have prompted its utilization as a dietary enhancement or as a nourishment fixings because of its lovely scent **[5, 6]**. In Asiatic nations, *C. asiatica* (L.) Urban is utilized as a fixing in customary frameworks of medication, for example, Ayurveda, Siddha. Furthermore, in Unani. As per its latent capacity wound recuperating property, a few report depicted the wonderful defensive impact of the plant against a few infections of focal sensory system **[7, 8]**. It additionally associated with wide scope of natural exercises wanted for human wellbeing, for example, mitigating **[9],** antiulcer (hepatoprotective **[10],** anticonvulsant **[11],** cardioprotective **[12]**, cytotoxic and antitumor **[13],** antiviral **[14]** and antibacterial exercises **[15]**. The cell reinforcement action of the plant is tantamount to that of ordinarily used plant and it has been accounted for that it has excellent potential to be investigated as a wellspring of characteristic cancer prevention agents **[16, 17]**. It has been accounted for that *C. asiatica* (L.) Urban plant contains a plenty of mixes having a place with wide scope of concoction classes. Natural impacts of *C. asiatica* (L.) Urban have been ascribed to the presence of major triterpene subsidiaries including asiatic corrosive, madecassic corrosive, asiaticoside, madecassoside, and brahmic corrosive **[18, 19]**. The event of a few significant flavonoid subordinates including quercetin, kaempferol and a few significant phenolic mixes has additionally been accounted for **[20, 21]**. *C. asiatica* was also used in epilepsy, hysteria, leprosy, and in minor itching and insect bites. The healing of skin acnes and wounds of skin has been

observed in many researches proclaiming wound healing.

Madecassic acid and Rotundic acid is here targeted for future studies. This research deals with the development of usefull extraction method which could be applied so that Madecassic acid and Rotundic acid could be subsequently extracted and further their presence could be checked.

MATERIALS AND METHODS

Procurement of All Parts of *Centella asiatica* (L.) Urban.

The leaves, roots and stems were taken from the local areas like Hathwa, Bhore, Kuchaikote, *etc.* of Gopalganj. The authenticity of plant was confirmed in the Department of Botany of Gopeshwar College of Hathwa.

Extraction of *C. asiatica* (L.) Urban

The optimized parameters for extraction of essential oils from *Centella asiatica* using this Soxhlet apparatus were 100 g of leaves at a distillation rate of 2/3 ml min-1 for 75 minutes using 0.4 ml of xylene initially. It was also necessary to perform a 30 minute initial distillation with no plant matter. Steam distillation with this apparatus was found to yield the best quality oil. The extract obtained was saved in polyethylene bottles and were sealed and kept at -20^0. Several other methods were also performed before following this method likewise use of alcohols instead of xylene but the better result was obtained in this procedure and pure extraction of madecassic acid was performed. The methods were inspired by the work of Melissa, 2014.

For Rotundic acid the procedure was same but the solvent used was methanol in the same proportion.

Determination of Total Phenolic Contents in the Extracts of Methanol:

Total phenolic compounds were determined by using a modified version of the Folin-Ciocalteu Method **[22]**. One millilitre of the extract was added to 10 ml deionized water and 2.0 ml of Folin-Ciocalteu Phenol Reagent (RANKEM). The mixture was then allowed to stand for 5 min and 2.0 ml sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm.

Determination of Total Flavanoid Content in the Extracts of *C. asiatica* (L.) Urban

The concentrations of flavonoids were quantified based on a colorimetric assay method with slight modifications. Briefly, Quercetin was used as a standard to establish calibration linear with function: 0.90-1.00 g samples were weighted, and 10 ml 60% ethanol aqueous was used to extract flavonoids from these samples) for 30 min.

These samples were further centrifuged at 3000 rpm. All the supernatant was transferred to 25 ml volumetric flask and then was fixed to 25 ml with 60% ethanol aqueous. 1.5 ml of each extracts and 4.5 ml of distilled water were pipetted into a 25 mL tube and then mixed with 1 mL 5% (wv^{-1}) $NaNO_2$ solutions. After incubation for 6 min, 1 ml of the 10% (wv^{-1}) Al $(NO_3)_3$ solutions was added to the mixture. The mixture was kept for 6 min before adding 10 mL 4% (wv^{-1}) NaOH solutions and fixed to 25 mL with 60% ethanol aqueous. Finally, the mixture was reacted for 15 min and the absorbance of the mixture solution was measured with a spectrophotometer (Systronics 2202 Double Beam UV Spectrophotometer) at 510 nm against a blank containing 5 mL of extraction solvent. Samples were independently analyzed; flavonoid content was expressed as mg Quercetin equivalent per g dry weight (DW).

GCMS Analysis of Ethanolic Extract:

The phytochemical investigation of methanolic extract was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.:5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral Library Search Programme.

RESULTS

The extracts prepared and primarily observed colour change demonstrated that the extraction would have been completed.

Total Phenolic Content

For Total phenolic Content Gallic acid was used as standard and its subsequent 5-8 dilutions were formed which showed absorbance at 680 nm.

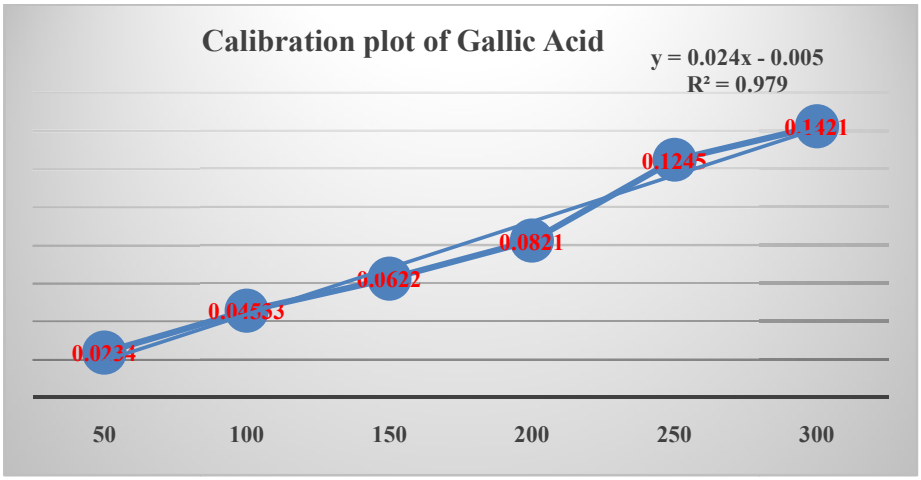
Total phenolic content gave Equation of straight line as y=0.0243x-0.0052 and R^2 Value 0.9792. The absorbance recorded for samples were *C. asiatica* root 0.1243; *C. asiatica* stem 0.1821; *C.asiatica* leaf 0.5212 the concentrations are as follows root; 5.22177 mg/ml, 7.07 mg/ml, 21.66 mg/ml equivalent of Gallic acid **(Graph 1, Figure 1)**.

Total Flavanoid Content:

Flavonoids are one class of secondary plant metabolites that are also known as

Vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities **[23]**. The total flavonoids content of *C.asiatica* (L.) Urban extract was also determined using Aluminium chloride colorimetric method. Leaves flavonoid content was higher than roots and stems **(Figure 3, 4)**.

The significant compound corresponded to Madecassic acid (MEA) and Rotundic acid (RA) are pentacyclic triterpenic acids. MEA is a major triterpenic acid present in *Centella asiatica* (L.) Urban **(Table 1, Figure 5)**.



Graph 1: The above graph depicts about the calibration plot of Gallic Acid which was considered as the standard of Total Phenolic Content

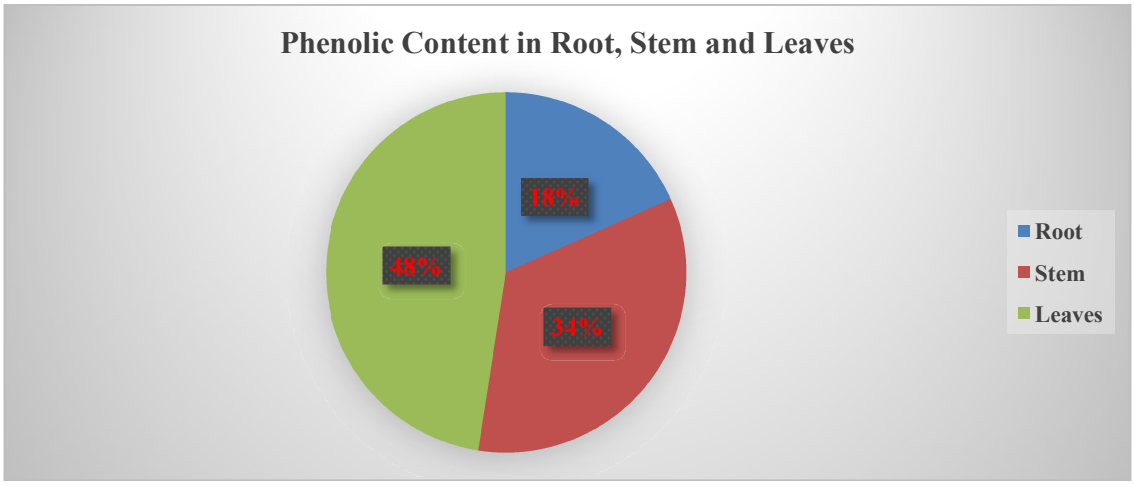


Figure 2: Complete Evaluation of Phenolic Contents in the different regions of C. asiatica plant

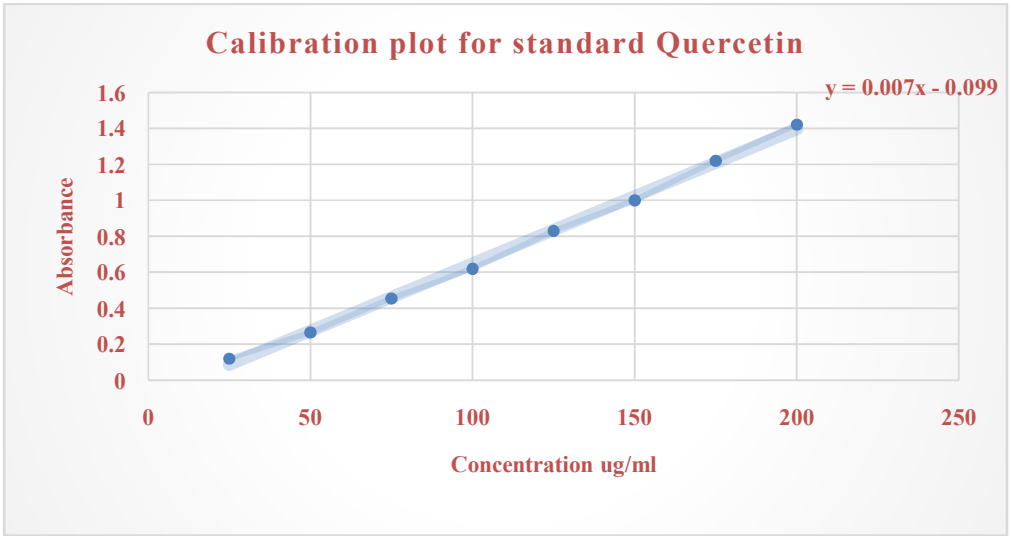


Figure 3: The above figure depicts the concentration of Flavonoid in the different dilutions of Quercetin

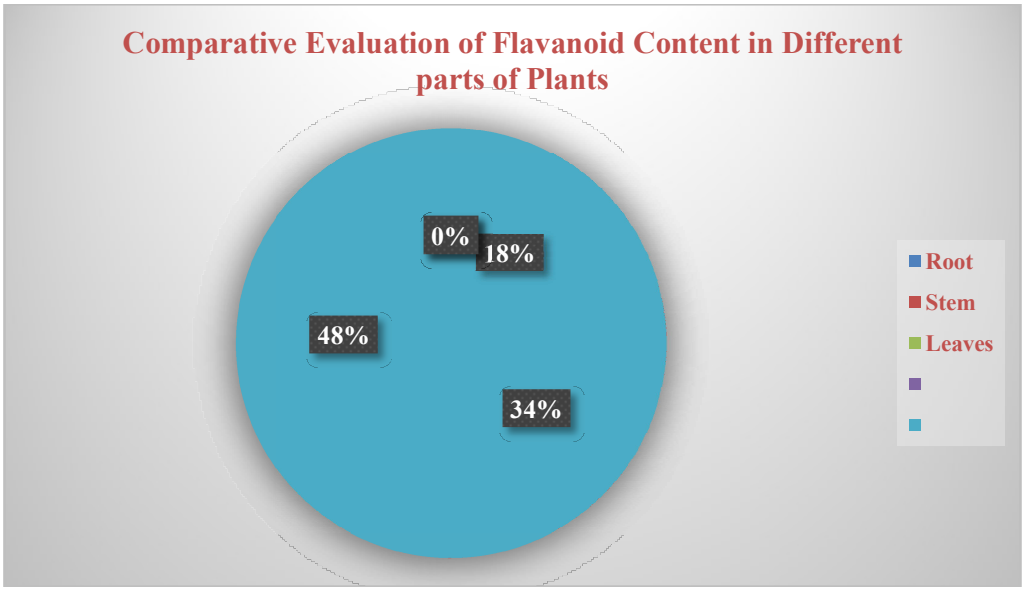


Figure 4: Depiction of comparative evaluation of Flavanoid Content in the extracts of Roots , Stem and Leaves in the above pie chart it could be observed that blue colour section represents root which shows comparatively very less amount of Flavanoid while on the other hand Leaves possesses more of it

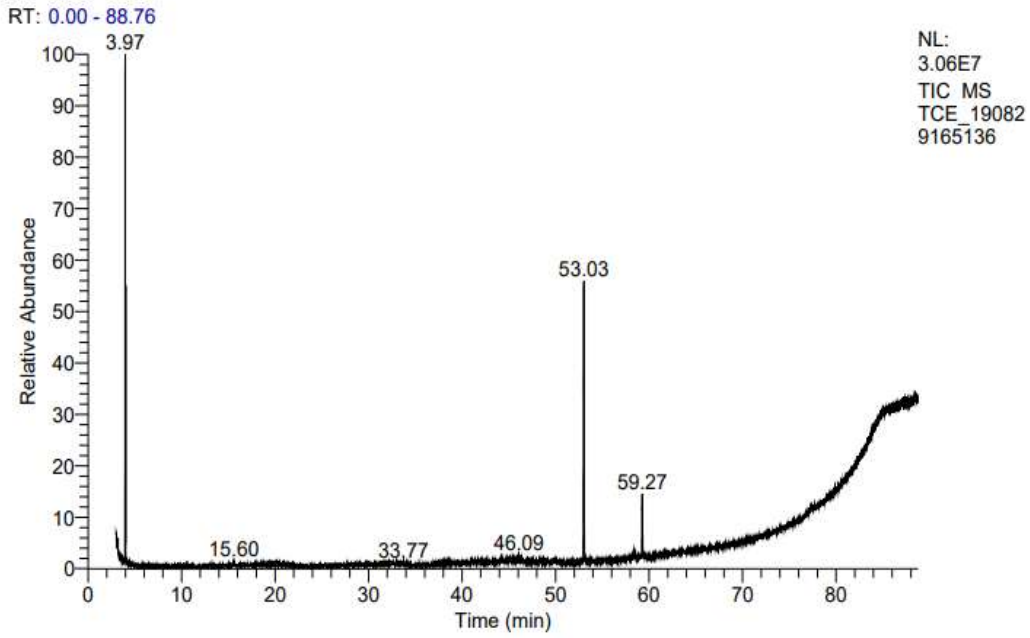
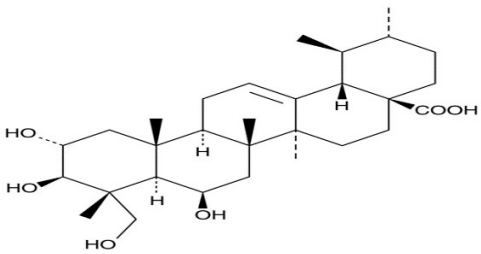
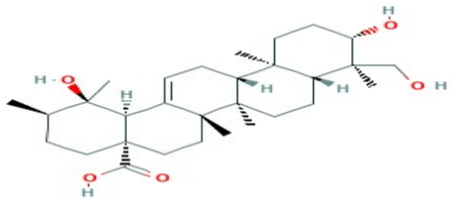


Fig 5: GC-MS Chromatogram of the plant extract

Table 1: Expected compounds in the Plant extract with their retention time, compound name, molecular formula and chemical structure

S. No	Retention time	Name of Compound	Molecular Formula	Chemical Structure
1	15.6	-	-	-
2	33.77	-	-	-
3	46.09	Medecasic Acid	$C_{30}H_{48}O_6$	
4	53.03	Rotundic Acid	$C_{30}H_{48}O_5$	
5	59.27	-	-	-

DISCUSSION AND CONCLUSION

The total phenolic content was found to be in huge ratio when compared with roots and leaf extract. The concentration variation of 18% in roots, 34% in stems and 48% in leaves were obtained. Similar variation in concentration was observed for total flavonoid content after placing the data in GC MS analysis, it was obtained that two essential compounds Medacasic Acid and Rotundic Acid was discovered which possesses antidiabetic and anticancerous properties **[24]**. *Centella asiatica* (L) Urban commonly known as Indian Pennywort is having great medicinal value and hence used as a medicinal herb in Ayurvedic medicine. The present study intends to provide an overview of the chemical constituents present in the crude leaf extracts of *C. asiatica* (L) Urban with special emphasis on presence of Medacasic acid and Rotundic acid. The results suggest that the leaves of *C. asiatica* (L) Urban are a rich source of valuable primary and secondary metabolites. Medicinal plants *Centella asiatica* based on several factors thus play a vital role in preventing various diseases. The antidiuretic, antiinflammatory, antianalgestic, anticancer, anti-viral, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Phytochemical analysis of the medicinal plants are hence commercially significant in both research institutes as well as pharmaceutical companies for the manufacturing of new drugs for the treatment of various illness. Studies conducted by previous workers and the present study show nearly similar results related to the phytochemical constituents in the leaf extracts of *C. asiatica* (L). It would not be surprising therefore to use plant samples to cure certain types of illness in humans and animals. This obtained information will therefore serve as a primary platform for further phytochemical and pharmacological studies related to the concerned plant. Hence it can be concluded that the leaves of this herb would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using leaves of the aforesaid medicinal herb as natural remedied and to protect against infectious diseases.

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