Research and Reviews Journal of Agriculture and Allied Sciences

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

Phytochemical Analysis of Leaves of Centella Asiatica (L)

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Brief Report

Received date: 29/09/2021

Accepted date: 01/10/2021

Published date: 05/11/2021

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Keywords: *Centella asiatica (L), Phytochemical, Antimicrobial, GC-MS, Steroid*

ABSTRACT

Centella asiatica (L), commonly known as 'Indian Pennywort' belongs to the family *Apiaceae* (Umbelliferae) have great medicinal values and therefore it is widely use in Ayurvedic medicines. All parts of the plant i.e. leave, stem and root contain numbers of secondary metabolites such as steroids, terpenoids, flavonoids, tannins, glycosides, coumarins, carbohydrates, proteins and amino acids. Because of the richness in phytochemicals, the plant is attributed to possess a number of therapeutic uses such as antimicrobial, anti-inflammatory, astringent, bronchodilator, CNS-depressant, de-toxicant, diuretic, immune stimulant, anticancer and hepato-protective etc. The present study intends to provide an overview of the chemical constituents present in the leaf, stem, and root extract of *Centella asiatica*(L). Phytochemical screening of *Centella asiatica*(L) was studied by extracting the dried powder leaves, stem, root with water solvents. The extracts were subjected to qualitative phytochemical analysis using classical method and presence of most of the phytochemicals except steroids, terpenoids, glycosides, protein and amino acid. GC-MS determination of methanol extract of the *Centella asiatica*(L) leaves to find five phytochemical constitutes have been identified by comparing the chromatogram and peak values of unknown compounds with entries in NIST database. These five phytochemical compounds are Neophytadiene, Eicosanoia Acid, Pentadecanoic acid 14-Bromo, Adipic acid Cyclo hexymethyl ethyl ester and Glycidyl palmitate. The presence of various bioactive compounds confirms the application of *Centella asiatica*(L) for various treatment.

e-ISSN: E 2347-226X
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INTRODUCTION

Centella asiatica (L) commonly known as Indian Pennywort, belongs to the family Apiaceae (previously known as Umbelliferae). This plant is listed as an important drug in the Indian Herbal Pharmacopoeia, European Pharmacopoeia, Pharmacopoeia of the People's Republic of China and German Homoeopathic Pharmacopoeia [1]. The genus *Centella* comprises of approximately 53 species worldwide [2,3]. This herbaceous perennial herb commonly grows well forming a dense green carpet in moist shady places, damp, marshy and swampy areas such as paddy fields [4,5]. The members of Apiaceae family are native to India, Sri Lanka, Iran, New Guinea, Australia, Indonesia southern and central Africa [6]. The use of *Centella* in food and beverages has increased over the years. The knowledge regarding ability of *Centella* as an alternative natural antioxidant especially of plant origin and its protection against age-related changes in brain antioxidant defense system, memory enhancing property has increased in recent years [7]. *Centella asiatica* (L) *i*s one of the chief herbs for treating skin problems, wounds, revitalizing the nerves and brain cells in India [8]. Phytochemicals are occurring naturally in medicinal plant's leaves, stem and roots that have defense mechanism and protect from various diseases. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds [9]. This plant is known to possess a number of biological activities including neuro-protective activity, anti-inflammatory, antiulcer, hepato-protective, anticonvulsant sedative immune stimulant, cardio protective, antioxidant, antimicrobial amongst others [10-17]. A numbers of phytochemicals have been isolated from the various extracts of the plant. These include terpeneoids, phenolic compounds, poly acetylenes, alkaloids, carbohydrates, vitamins, mineral and amino acid [18-20]. The present study was carried out to analyze the phytochemical components present in the leaf of the crude aqueous extracts of *Centella asatica* L. Also study GC-MS analysis of the methanolic extract of *Centella asiatica* L. leaves to identify the presence of bioactive components.

Material and Method

Collection of plant materials:

Fresh leaves of *Ipomoea aquatica* Forssk. were collected from rice field and pond of Nalanipam village situated in the Dhemaji district of Assam. These plant samples were washed with tap water in order to remove the dust. Collected plant material were air dried followed by thermostatic oven drying at a considerably low temperature not exceeding 30^0c for 24 hours. The plant material become well dried for grinding. After grinding the plant material were transferred into air tight containers with proper labeling for future use. 50 grams of powder extracted from leaves of *Ipomoea aquatica* Forssk. was taken and extracted with adequate amount of ethanol (4:1) using soxhlet apparatus. The liquid part is stored at 4^0 c in separate container.

Chemicals and reagents:

Distilled water, methanol, Di-ethyl ether hexane, Sodium phosphate buffer, DNS, Starch, DPPH, Ethanol, MH agar, Sodium acetate, Sodium hydroxide, Hydrogen peroxide, 95% ethanol, 0.1% Lead acetate, Hydro chloric acid, Sulphuric acid, Sodium carbonate, Chloroform.

Glass wares and plastic wares:

Beaker, conical flask, test tube, measuring cylinder, Pipette, Petri dish, test tube stands, plastic tray, 2 ml Syringe for filtration, glass vial etc.

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Equipment:

Some of the equipments utilized for the study include Electronic Balance (ANAMED), spectro-photometer, pipette, soxhlet apparatus, micro pipettes, centrifuge, Hot plate, water bath, Laminar air flow, -70°C Temperature Freezer, Rotary evaporator and GCMS Instrument (Perkin Elmer, USA) etc. The software used in the system was Turbo mass Ver.5.4.2.

Preparation of plant extracts:

Water extract:

The water extraction was carried out using classical method, where grinded plant material of 5gm weighed using an electronic balance and was crushed in 100 ml of sterile water. Then the mixture was boiled at 50-60°C for 30 minutes on water bath and it was filtered through what-man No.1 filter paper. Then the filtrate was centrifuged at 2500 rpm for 15 minutes. The extract was collected, labeled and stored in sterile bottles at 5° c for further different experimental use.

Qualitative analysis of phytochemicals:

Chemical test were carried by using aqueous extract to identify various phytochemicals using standard methods [23-26].The extract were subjected to qualitative analysis for presence of chemicals constituents by performing various chemical test like Steroids (Salkowski test), Terpenoids (Salkowski test), Flavonoids (Alkaline reagent test, Sulfuric acid test, Lead acetate test), Tannins (Lead acetate test), Glycosides (killer kiliani test), coumarins (Sodium chloride test), Carbohydrate (Benedict's test, Fehling's test), Protein (Xanthoproteic test) and Amino acid (Ninhydrine test).

Gas Chromatography –Mass Spectrometry Analysis:

The phytochemical composition of was analyzed by GC–MS system (Perkin Elmer, USA) make GCMS instrument, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler). For GCMS analysis, 0.1 ml of each concentrated extracts (4mg/ml) was diluted to 1ml with solvent (Methanol) and transferred to standard GCMS sample. The Software used in the system was TurboMass Ver. 5.4.2. The capillary column used was 'Elite-5MS' having dimensions—length-60 m, ID-0.25 mm, and film thickness-0.25 µm, and the stationary phase is 5% diphenyl 95% dimethylpolysilox-ane. Helium (99.99%) was used as carrier gas (i.e., mobile phase) at a flow rate of 1 ml/min. An injection volume of 2 µl was employed in splitless mode. Injector and ion-source temperatures were 280 °C and 180 °C, respectively. The oven temperature was programmed at 60 °C (for 1 min), with an increasing rate of 7 °C/min to 200 °C (hold for 3 min) then again increased at rate of 10 °C/min to 300 °C (hold for 5 min). The total run time was of 60 min. The solvent delay was kept for 8 min. MS Protocol Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e., m/z range is 50–600 amu.

Identification of Peaks:

Interpretation of the peaks that appeared in the GC Chromatogram were done by library search of the mass spectrum of the corresponding peaks using the database software of National Institute Standard and Technology-2008 (NIST-2008).

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Result

Phytochemicals analysis:

Preliminary qualitative phytochemicals screening of the aqueous extract of *Centella asiatica* leaves, stem, root to confirmed the presence of Tannin, Flavonoid, Coumarins, Carbohydrate, while the phytochemical terpenoids, Steroids, Glycoside, protein and Amino acids are absent. Summarized result tabulated in table 1.

AQUEOUS EXTRACT	STEROIDS	TERPENOIDS	FLAVONOIDS			TANNINS	GLYCOSIDES	COUMERINS TEST	CARBOHYDRATES		PROTEINS	AMINO ACIDS
	SALKOWSKI TEST	Salkowski Test	Alkaline Reagent Test	H2SO4 Test	Lead acetate Test	Lead acetate Test	Keller-Kiliani Test	Sodium chloride test	Benedict's Test	Fehling's Test	Xanthoproteic Test	Ninhydrin Test
leaves	–	–	+	+	+	+	–	+	-	+	–	–
Stem	–	–	–	+	+	–	–	–	–	+	–	–
Root	–	–	+	+	+	+	–	+	–	+	–	–

Table 1: Phytochemical analysis of *Centella asiatica.*

[+] indicate positive test result.
[-] indicate negative test result.

GC-MS analysis of *Centella asiatica* leaf:

GC-MS is one of the best techniques to identify the active phytochemical present in the plant extract. The chromatogram of the GC-MS analysis of the methanolic extract of *Centella asiatica* leaf revealed the presence of number of compounds from the GC fractions and these compounds were identified with mass spectrometry attached to GC Present study GC – MS analysis of the methanolic extracts of the *Centella asiatica* detected 5 major compounds like Neophytadiene, Eicosanoia Acid, Pentadecanoic acid 14- Bromo, Adipic acid Cyclo hexymethyl ethyl ester and Glycidyl palmitate. The identified compounds their retention time [RT], molecular weight [MW], molecular formula [MF], peak area % are given in table 2.

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

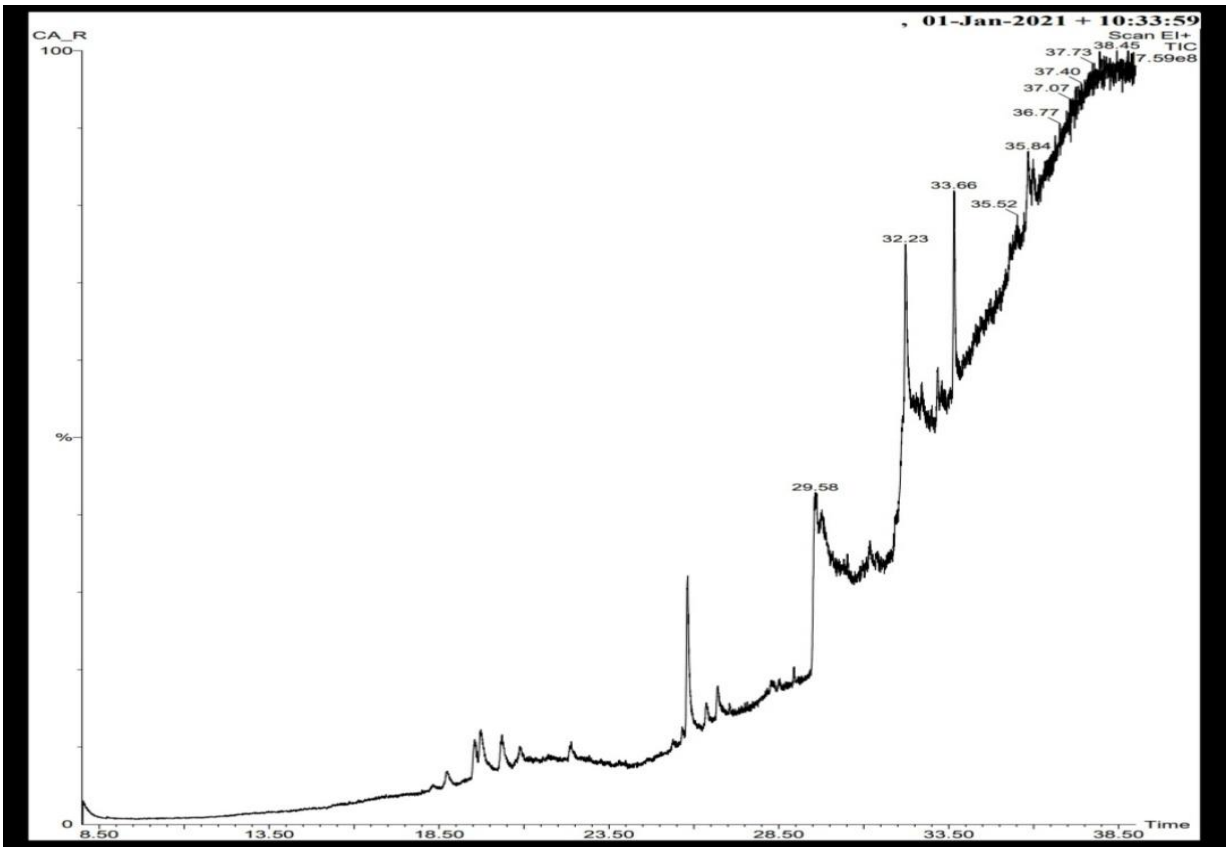

Fig.1: GC–MS chromatogram of methanolic extract of *Centella asiatica* [CA] Sample.

SL No	RT	Name of the compounds	Molecular formula	Molecular weight	Peak area %
1	25.826	Neophytadiene	$C_{20}H_{38}$	278	3.794
2	29.577	Eicosanoia Acid	$C_{20}H_{40}O_2$	312	17.888
3	32.228	Pentadecanoic acid, 14-Bromo	$C_{15}H_{29}BrO_2$	320	0.332
4	32.233	Adipic acid,Cyclo hexymethyl ethyl ester	$C_{14}H_{24}O_4$	270	15.37
5	33.659	Glycidyl palmitate	$C_{19}H_{36}O_3$	312	2.762

Table 2: Identified compounds in the methanolic extract of *Centella asiatica* leaf GC-MS peak report.

Among the identified phytochemicals Neophytadiene had the property of possess antibacterial, antioxidant, antipyretic activity as well as helping in treatment of headache, rheumatism and some skin disease, Eicosanoia Acid has a role as a plant metabolite, a food component, a daphnia magna metabolite, human blood serum metabolite and an algal metabolite, Pentadecanoic acid, 14- Bromo have no active reported, Adipic acid, Cyclo hexymethyl ethyl ester have no active reported and Glycidyl palmitate used as building blocks for the synthesis of a range of biological active natural and synthetic.

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Discussion

The preliminary phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, saponins, phenols, steroids, glycosides, Tannins, tri terpenoids and terpenoids. Bioactive secondary metabolites have been utilized as natural medicines and plants containing those Compounds have been used as medicinal plants and are prescribed in many recepies as forms of crude drugs [27]. Phytochemical assay of the plant showed the presence of various compounds such as alkaloids, flavonoids, glycosides, tannins, terpenoids, saponins, amino acids major compounds of effective medicinal property with increased potential which exhibit the anti-proliferative, antioxidant [28], Tannins are complex phenolic polymers, which will bind to the proteins and carbohydrate molecules resulting in reduction and inhibition of microbial growth [29]. The presence of tannins also aids in wound healing [30]. Our study tannins also present in leaves and roots but tannins are absent in stem of *Centella asiatica. The* various phytochemical compounds are known to have importance in medicinal sciences. Flavonoids have been referred to as nature's biological reaction transformer because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [31, 32, 33]. Certain flavonoids possess potent inhibitory activity against a wide array of enzymes. Evidence suggests that only activated cells are responding to a stimulus. So the presence of this type of phytochemical compounds in protect medicinal plants has a wide range of applications and could be certainly used for a variety of applications [34]. Flavonoids are also known for substantial antimicrobial activity [35]. Present study flavonoids are also found in all parts like leaves, stem, and roots of the plant *Centella asiatica*. Plant steroids are known to be important for their cardio tonic activities and also possess insecticidal and antimicrobial properties. They are also used in food, herbal medicine and cosmetics. Tannins were also show antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic [36]. Steroids are absent in present study.

GC–MS analysis of the methanolic extracts of the *Centella asiatica* detected 5 major compounds (Table 2) like Neophytadiene, Eicosanoia Acid, Pentadecanoic acid 14- Bromo, Adipic acid Cyclo hexymethyl ethyl ester and Glycidyl palmitate. The identified compounds their retention time [RT] molecular weight [MW], molecular formula [MF], peak area % data with those of reference standard compounds from NIST library. Some of these compounds have been reported [37-40].

Conclusion

In the present study leaves, stem and root of *Centella asiatica* contain many phytochemicals. Due to presence of wide bioactive compound, the plant has vast application. The plant can be a safer alternative for the formulation of new drugs. Qualitative phytochemical analysis showed the presence of Tannin, Flavonoid, Coumarins, Carbohydrate in aqueous extract. From GC-MS analysis five compound like Neophytadiene, Eicosanoia Acid, Pentadecanoic acid 14- Bromo, Adipic acid Cyclo hexymethyl ethyl ester and Glycidyl palmitate. Which may be responsible for anti-inflammatory, anti- microbial, anti-bacterial, anti- oxidant properties etc. Isolation of individual phytochemical constituents and subjecting it to biological activity will give fruitful results.

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p-ISSN: 2319-9857

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