



MEDFOOD'18 [1st February 2018]

National Conference on Phytochemicals in Medicinal Plants and Food

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Research Article

Outlining of Phytochemicals and GC-MS Profile of *Centella asiatica*

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Date Received: 23rd January 2018; Date accepted:

29th January 2018; Date Published: 17th February 2018

Abstract

The aim of this study is to assess the phytochemicals and bioactive compounds present in *Centella asiatica*. In the present study, qualitative phytochemical analysis of different extracts and GC-MS was carried out using aqueous extract of *Centella asiatica* for the identification of bioactive compounds. Phytochemicals like alkaloids, steroids, cardiac glycosides, terpenoids, tannins, anthraquinone, proteins, total carbohydrates, reducing sugar, flavonoids, saponins, lignins, coumarin were analysed. Ethanolic extract had nine phytochemical metabolites out of thirteen analyzed, methanol-

ic extract had seven metabolites, acetone extract had eight while aqueous extract had twelve, and henceforth aqueous extract was chosen for GC-MS analysis. The results showed the presence of phytochemicals and bioactive phytochemicals which may possess a wide range of therapeutic effects.

Keywords: *Centella asiatica*, GC-MS, bioactive compounds, phyto constituents.

INTRODUCTION

Medicinal plants have been used in healthcare since long time. Studies have been carried out worldwide to corroborate the efficacy of medicinal plants and led to the fabrication of plant-based medicines. They play a vital role in maintenance of health and healing properties. Treating of diseases through natural medicine is the most ancient method of treatment known to mankind⁽¹⁾. However, efforts need to be made to identify, recognize and standardize the products of medicinal plants.

World Health Organization (WHO) expert group defined traditional medicine as the sum of total knowledge and practices that are explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance. The traditional practices rely exclusively on practical experience and observation handed down from generation to generation, whether orally or through written records⁽²⁾.

The chemical constituents present in plants have great value in new discovery of therapeutic agents, also validates the folklore remedies scientifically⁽³⁾. Thorough validation of the herbal drugs has emerged as a new branch of science for the standardization of the natural drugs.

Plants produce a large and diverse array of organic compounds that appear to have no direct functions in growth and development⁽⁴⁾ which are called as secondary metabolites present in the plants. They have a very restricted distribution than the primary metabolites. But the secondary metabolites are recognized as the first line of chemical defense against potential pathogens.

There are innumerable potentially useful medicinal plants and herbs waiting to be evaluated and exploited for their effective therapeutic applications. One such plant is *Centella asiatica* (L.). It is a prostrate, faintly aromatic, stoloniferous, perennial, creeper herb. The leaves are orbicular-reniform, sheathing leaf base, crenate margins, glabrous on both sides.

The aim of the present study is to identify the phytochemicals of *Centella asiatica* leaves in different solvents and aqueous extract of *Centella asiatica* leaves for GC-MS Analysis.

MATERIALS AND METHODS

Endorsement of selected plant

Centella asiatica was collected in plantain field in and around Mannachanallur, Tiruchirappalli, Tamilnadu, India. The voucher specimen (*Centella asiatica*-SB001 2013/05) was identified by Rev. Dr. S. John Britto, Director, Rabinat Herbarium, St. Joseph College, Tiruchirappalli, Tamilnadu, India.

Extraction of *Centella asiatica*

Leaves of *Centella asiatica* was washed thoroughly 2-3 times with running tap water, then shade dried, ground into powder form and stored in sterilized container. The dried powder was extracted according to the procedure given by Rajamanikandan *et al.*, (2011)⁽⁶⁾ in different solvents (ethanol, methanol, acetone and water).

Phytochemical analysis and identification of bioactive compounds in *Centella asiatica*

The preliminary phytochemical analysis was carried out as per the methods and tests given by Harborne⁽⁷⁾ in different solvents. Analysis of bioactive compounds were also carried out in aqueous extract by GC-MS⁽⁸⁾.

Results and Discussion

All plants produce a diverse range of Secondary metabolites, making them a source of different types of medicines. A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharvaveda, Charaka, Sushruta etc.,⁽⁹⁾.

Centella asiatica (Table I) showed the presence of alkaloids, steroids, cardiac glycosides, terpenoids, tannins, anthroquinone, proteins, total carbohydrates and reducing sugar in ethanolic extract whereas flavonoids, saponins, lignins, coumarin were absent. In methanolic extract steroids, cardiac glycosides, terpenoids, tannins, proteins, total carbohydrates and reducing sugar were present while alkaloids, flavonoids, saponins, lignins and anthroquinones were absent. In acetone extract, alkaloids, cardiac glycosides, terpenoids, tannins, anthroquinones, proteins, total carbohydrates and reducing sugar were present while flavonoids, steroids, saponins, lignins and coumarin were absent. In aqueous extract except lignin all other phytoconstituents were present. Ethanolic extract had nine phytochemical metabolites out of thirteen analyzed, methanol had seven metabolites, acetone had eight while aqueous had twelve constituents, henceforth aqueous extract was chosen for further experiments pertaining to study.

Subashri and Justin Koil Pillai,⁽¹⁰⁾ characterized the phytochemicals of *Bacopa monnieri* using various solvents. Terpenoids and steroids were predominately found in all the five extracts, followed by saponin, phenols, coumarins which were found in four extracts and tannins and alkaloids were found in two extracts. The ethyl acetate has shown positive result for terpenoids, steroids and phenols. The results confirmed the presence of therapeutically potent compounds in the aqueous extract of *Bacopa monnieri*⁽¹⁰⁾. The results of the present were in consistent with the results of their study.

Aqueous extract of *Centella asiatica* showed the presence of thirty two bioactive compounds; of which 13 predominant compounds are shown in Table II. The bioactive compounds were identified with their retention time, molecular formula, molecular weight and % of peak area. The predominant compounds present in aqueous extract of *Centella asiatica* are n-Hexadecanoic acid (35.11%), Octade-

canoic acid (18.08%), Glycerin (10.48%), Heptadecanenitrile (7.44%), Tetradecanenitrile (4.776%), 6-Octadecenoic acid, (Z)- (4.72%), 1,6-Anhydro- α -D-glucopyranose (levoglucosan) (2.915%), Tetradecanoic acid (2.012%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (1.932%), Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester (1.558%), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.374%), Dodecanoic acid (1.166%), Phenol, 2,4-bis(1,1-dimethylethyl)- (1.062).

The remaining compounds are Pentadecanoic acid (0.9072%), Diisooctyladipate (0.7933%), Dodecanenitrile (0.6602%), Levoglucosone (5092%), Santalol, trans- α - (0.4539%), Dodecanenitrile (0.4220%), Octanoic Acid (0.4071%), 5-Caranol, trans, trans-(+)- (0.3991%), 1,4 : 3,6-Dianhydro- α -D-glucopyranose (0.3698%), 2-Methoxy-4-vinylphenol (0.3221%), 1,2-Octadecanediol (0.3146%), 3-Allyl-6-methoxyphenol (0.2938%), Sulfuric acid, diethyl ester (0.2741%), Fran, 2,5-dimethyl- (0.2122%), 4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-

one (0.2024%), 1,3,8-p-Menthatriene (0.1805%), 1,3,8-p-Menthatriene 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (0.1624%), α -Santalol (0.0827%).

Kavisa Ghosh and Indra⁽¹¹⁾ reported on the major components, identified by GC-MS analysis from ethanolic extract of *Centella asiatica* were Methylpyromeconic acid (RT: 5.183), Methoxy vinyl phenol (RT: 8.617), 3',5'-Dimethoxyacetophenone (RT: 11.867), Beta-D-Ribofuranoside (RT: 10.675), Cyclohexanecarboxylic Acid (RT: 12.825), 5-methoxy-2,2,8,8-tetramethyl-acetate (RT: 25.733) and Nobiletin (RT: 27.275).

Table II represents the presence thirteen major bioactive compounds in aqueous extract of *Centella asiatica* with the percentage of high peak area and retention time among the 32 bioactive compounds identified by GC-MS analysis. These compounds need to be further validated for its protective function against free radical formation.

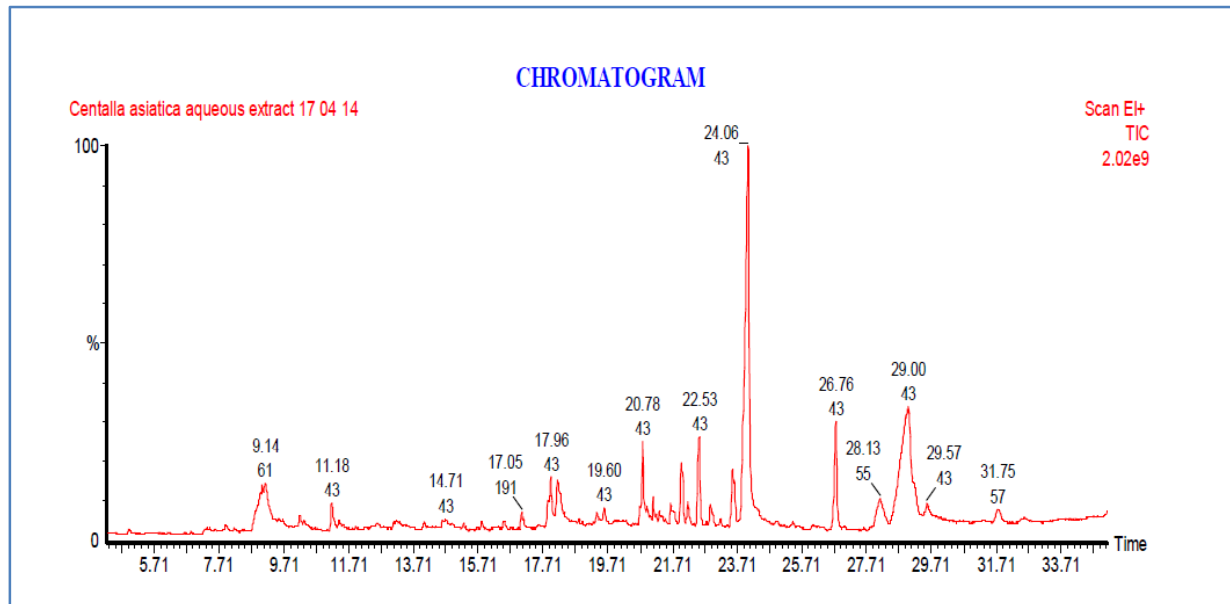


Figure I - Bioactive compounds present in *Centella asiatica*

Table (I) - Preliminary phytochemical screening of *Centella asiatica*

S.No	Name of the test	Ethanol	Methanol	Acetone	Water
1	Alkaloids				
	Marqui's tests	+	-	+++	-
2	Flavonoid	-	-	-	+
3	Steroids – libermannbuchard's test	+	+	-	+
4	Cardiac glycosides				
	Keller killani test	+	+	++	+
	Bal jet tests	++	+	+	++
5	Terpenoids – Salkowski test	++	+	+	+
6	Tannin	+	+	++	++
7	Saponin	-	-	-	+
8	Lignin	-	-	-	-
9	Anthraquinones	++	-	++	+
10	Protein	+	+	+	+
11	Total carbohydrates				
	Molisch's test	++	++	++	++
12	Coumarins	-	-	-	+
13	Reducing sugar				
	BQR test	-	-	+	+
	Fehling's test	+	++	+	+++
		9	7	8	12

Note: + indicates positive result, whereas - indicates negative result

Table II - Bioactive compounds present in *Centella asiatica*

S.No	Name of the compound	Molecular formula	Molecular weight	% of peak area
1.	n-hexadecanoic acid	C ₆ H ₃₂ O ₂	256	35.11
2.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	18.08
3.	Glycerin	C ₃ H ₈ O ₃	92	10.48
4.	Heptadecanenitrile	C ₁₇ H ₃₃ N	251	7.4
5.	Tetra decanenitrile	C ₁₄ H ₂₂ N	209	4.77
6.	6-Octadecanoic acid	C ₁₈ H ₃₄ O ₂	282	4.72
7.	1,6 anhydroαD glucopyranose	C ₆ H ₁₀ O ₅	162	2.9
8.	Tetra decanoic acid	C ₁₄ H ₂₈ O ₂	226	2.0
9.	4H-Pyranone,2,3, dihydro 3,5hydroxy 6methyl	C ₆ H ₈ O ₄	144	1.9
10.	Acetic acid, 2-(2,2,6-trimethyl-7-oxabicyclo[4,1.0]hept-1-yl) propenyl ester	C ₁₄ H ₂₂ O ₃	238	1.55
11.	7,9-Di-tert-butyl-1-oaspiro(4,5)deco-6,9-diene-2,8dione	C ₁₇ H ₂₄ O ₃	276	1.37
12.	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	1.16
13.	Phenol,2,4-bis[1,1-dimethylethyl	C ₁₄ H ₂₂ O	206	1.06

CONCLUSION

It has been widely apparent and recognized that the medicinal value of a plant lies in the bioactive components present in it. In the present study, the leaves of *Centella asiatica* Linn have been subjected to phytochemical evaluation and GC-MS analysis. The results showed the presence of many therapeutic compounds. Further studies are required to isolate and formulate the herbal drugs.

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