ejpmr, 2018,5(3), 544-548

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL COMPOSITION OF ESSENTIAL OIL OF CENTELLA ASIATICA L. BY GC-MS ANALYSIS

Undrala Sushen*[1] and Awanija Chouhan[2]

[1]Department of Organic Chemistry, Dr. Sushen Research Lab, Gokul Group of Institutes, Hyderabad, India.
[2]Department of Pharmaceutical Chemistry, Bojjam Narasimhulu Pharmacy College for Women, I.S. Sadan, Hyderabad.

***Corresponding Author: Dr. Undrala Sushen**

Department of Organic Chemistry, Dr. Sushen Research Lab, Gokul Group of Institutes, Hyderabad, India.

Article Received on 17/01/2018 **Article Revised on 07/02/2018** **Article Accepted on 28/02/2018**

1. ABSTRACT

Centella asiatica (L.) (Umbelliferae), commonly known as gotukola or jalbrahmi in india. *Centella asiatica* (L.) is widely used for many pharmacological purposes in folk medicine, Ayurvedic, siddha, unani and now a days the compounds of *Centella asiatica* (L.) are also using in modern medicine to treat diseases related to nervous system, muscular system and blood vascular system etc. *Centella asiatica* (L.) is also used for treating Alzheimer's diseases, Parkinson's disease and Huntington's disease. The extract was found to have neuroprotective ability through modifying a key signaling pathways and events concerned with neuro-degeneration. The essential oil of *Ocimum gratissimum* (L) exihibits considerable antibacterial, antifungal, anti tumor, antioxidant and many more activities. The present investigation has been carried out for the phytochemical study of leaf extract of *Centella asiatica* (L.). The essential oil is extracted by hydro distillation using Clevenger apparatus. The oil yield is observed to be 0.7 mL in 1 Kg. The essential oil is analyzed by GC and GC-MS instrumentation. Around 64 volatile compounds were identified from the essential oil of *Centella asiatica* (L.) P-Cymene (35%) is the predominant compound in the leaf essential oil of *Centella asiatica* (L.).

KEYWORDS: *Centella asiatica* (L.), Essential oil, Clevenger apparatus, GC-MS analysis.

2. INTRODUCTION

Centella asiatica is a perennial herbaceous plant having pantropical distribution. This species is well known for its therapeutic and cosmetic applications. The plant is used as a major ingredient of formulations of Ayurveda, Siddha, Homeopathy and Unani systems of medicine. In Indian pharmacopoeia, the plant is listed as useful for the treatment of skin malconditions such as leprosy, lupus, varicose veins, ulcers, eczema, psoriasis and wounds. In addition it is also used in the treatment of diarrhoea, dysentery, fever, amenorrhea and female genitourinary tract infections, filariasis and tumour. It is also used as a brain tonic for the mentally retarded. Pharmacological studies revealed its usefulness in inducing human collagen-1 synthesis, accelerating nerve regeneration, cardio-protection, improving age-related neurological antioxidant status, protection of critical neurons, treatment of venous hypertension and elevation of antioxidant level etc. The plant contains essential oil in which terpenoidal constituents such as β-caryophyllene, β-cymene, trans-β-farnesene, germacrene-D, α-terpineol, limonene, and linaloolwere identified. Among these components, α-terpineol is known for myorelaxant and antispasmodic effects. Linalool, the main compound of this oil, is a very important substance used in foodstuffs as additive and in pharmacology for its different properties. Another terpenoid, farnesol, have shown anti cancer effects and antibacterial activity in several different studies. Among the hydrocarbon, some compounds such as β-elemene, limonene and β-cymene are important compounds finding application in fragrance, pharmaceutical and agrochemical fields. Limonene has also been studied for its possible anti cancer activity. In the present study, detailed chemical profile of the essential oil of leaf of *Centella asiatica* (L.) was carried out and the plant was collected from the surroundings of Hyderabad.

3. EXPERIMENTAL

3.1. Collection of the plant materials

Centella asiatica (L.) leaf was collected from surroundings of Hyderabad during the month of December 2017. The plant material was identified by the authors and its herbarium sheet was deposited at the post graduate laboratory, Department of Chemistry, Gokul Group of institutions, Hyderabad. The collected aerial parts of plant, *Centella asiatica* (L.) was brought to the laboratory at Gokul Group Of institutions, Hyderabad, washed thoroughly with detergent to remove the dirt present on the aerial parts and separated the leaves and shade dried for one day.

3.2. Chemical Reagents
All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

3.3. Essential oil extraction
The shade dried aerial parts of *Centella asiatica* (L.) plant collected (1Kg) was subjected to hydrodistillation (1500 mL distilled water +500 g plant material, in 3000 mL round bottom flask) in a Clevenger apparatus for 4hrs using distilled water. The round bottom flask was kept on a heating mantel, the heat maintained is between 85^0C and 100^0C and the heating system is monitored by thermometer arranged to the round bottom flask. The heating and condensation process repeated continuously for four hours. The essential oil was traped by n-hexane and separated from the aqueous layer using a 100mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was stored at 4^0C in dark brown 5mL capacity sample vial until analysis. The yield of the oil was found to be 0.7% (w/w) in relation to the dry weight.

3.4. GC and GC-MS analysis
GC analysis was carried out in Agilent Technology 6890N Gas Chromatograph data handling system equipped with a spilt/splitless injector using N_2 as carrier gas. The column was HP-5 capillary column (30m x 0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60^0C (hold: 2min) programmed at a rate of 3^0C/min to a final temperature of 220^0 C (hold: 5min). The temperature of injector was maintained at 210^0C.

The GC-MS analysis was performed by Perkin Elmer Clarus 500 Gas Chromatograph equipped with a spilt/splitless injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary column (60 mm x 0.32 mm, 0.25µm film thickness). Helium was used as carrier gas at a flow rate of 1.0mL/min. The GC was interfaced with Perkin Elmer 500 Mass Detector operating in EI+ mode. The mass spectra was recorded over 40-500amu and revealed the Total Ion Current chromatograms. The temperature program remained the same as in GC. The temperatures of injector and transfer line were kept at 210^0C & that of the ion source at 200^0C.

Identification of constituents was done on the basis of retention index (RI, determined with reference to homologous series of n-alkanes C_8–C_{25} under identical experimental conditions on BP-1 column), MS library search NIST 08 MS Library (Version 2.0 f; Thermo Fisher Scientific Austria), and WILEY MS 9th Edition (Thermo Fisher Scientific Austria), and by comparing with the MS literature data. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

4. RESULTS AND DISCUSSION
Table 1. Composition of the essential oil of *Centella asiatica* (L).

S.No	Retention time	Name of compound	RRI Cal	RRI Lit	Percentage
1	6.3	α -Thujene	935	934	0.6
2	6.7	α - Pinene	940	939	0.3
3	7.2	Camphene	962	968	<0.1
4	8.3	β - Pinene	984	979	0.5
5	8.7	Myrcene	994	992	0.2
6	8.9	γ - 2- Carene	1003	1002	0.6
7	9.2	α-Phellandrene	1006	1005	<0.1
8	9.5	α-Terpene	1012	1015	0.3
9	9.6	P - Cymene	1018	1024	35.0
10	9.7	Limonene	1025	1029	3.0
11	12.0	P - Menth,3,8 - diene	1076	1072	<0.1
12	12.4	C-Terpinene	1085	1085	<0.1
13	12.6	Terpinolene	1091	1088	<0.1
14	13.2	Linalool	1125	1126	0.4
15	14.5	Allo - Ocimene	1134	1132	<0.1
16	14.9	3-None-2-one	1150	1149	0.4
17	15.6	Menthone	1161	1160	0.3
18	15.9	4 - Terpineol	1165	1166	<0.1
19	16.6	Meta cymen - 8 - ol	1179	1179	0.2
20	17.2	Cis dihydro carvone	1194	1192	0.5
21	17.6	Verbenone	1205	1205	<0.1
22	18.2	Trans carveol	1216	1216	0.3
23	18.8	Methyl thymol	1235	1236	<1.0
24	19.2	Carvone	1243	1243	<0.1
25	19.4	Pulegone	1248	1249	0.3

26	19.7	Mthyl cavacrol	1250	1252	<0.1
27	19.9	Trans myrtenol	1262	1261	0.7
28	20.5	Bornyl acetate	1291	1290	0.2
29	21.1	P - Cymen 7 - ol	1292	1290	0.4
30	22.3	Myrtenyl acetate	1324	1326	0.5
31	22.7	Neo iso verbenol acetate	1330	1330	0.3
32	22.9	α - Elemene	1335	1338	6.3
33	23.7	Bicycloelemene	1341	1342	0.5
34	25.2	Nonanal	1374	1378	<0.1
35	26.4	E - Caryophyllene	1419	1419	5.3
36	26.9	Guaiene	1439	1439	<0.1
37	27.1	B - Caryophyllene	1442	1440	3.6
38	27.5	6,9 - Guaiadiene	1445	1444	<0.1
39	27.8	Cis muurola 3,5 - diene	1453	1450	0.6
40	27.9	α-Humulene	1455	1454	4.8
41	28.6	Dauca 5,8 - diene	1473	1472	3.0
42	28.8	Trans cadina - 1,6,4 - diene	1475	1476	2.3
43	29.0	Amorpha - 4,7 (11) - diene	1481	1481	3.8
44	29.2	C-Curcumene	1485	1484	<0.1
45	29.5	Aristolochene	1488	1488	0.4
46	30.4	Bicyclogermacrene	1494	1493	<0.1
47	31.1	Germacrene A	1495	1495	<0.1
48	31.7	γ-Cadinene	1528	1525	1.4
49	32.1	Germacrene B	1562	1561	2.4
50	32.8	Spathulenol	1578	1578	0.5
51	33.0	Caryophyllene oxide	1582	1583	0.5
52	33.2	Globulol	1590	1590	<0.1
53	33.8	Humulene epoxide I	1595	1596	<0.1
54	34.0	Humulene epoxide II	1611	1608	0.6
55	34.2	10 - epi - γ - Eudesmol	1625	1623	1.0
56	34.4	Cubenol	1626	1627	<0.1
57	34.6	1- epi - Cubenol	1629	1628	3.5
58	34.7	α - Muurolol	1631	1631	6.2
59	35.8	Valerianol	1658	1658	0.3
60	35.9	7 - epi - α - Eudesmol	1663	1663	0.6
61	36.5	Elemol acetate	1678	1680	2.5
62	36.9	Z - α - trans Bergamotol	1690	1690	0.9
63	39.0	8,α - 11 - Elemodiol	1748	1747	<0.1
64	40.1	Torilenol	1756	1759	<0.1
				Total	95.2

RRI Cal: Relative retention index calculated on HP-5 column, with respect to homologous of n - alkanes (C6-C30, Aldrich Chem. Co. Inc.). RRI Lit: Relative retention index from literature (Adams 2007).

The GC-MS analysis of essential oil of *Centella asiatica* (L.) showed the presence of 64 components and the identified components are presented in Table-1.The essential oil yield of *Centella asiatica* (L.) was 0.07% (v/w). 95.2% of the total oil constituents were identified. Monoterpenoids dominated over sesquiterpenoids. p-cymene (35.0%), α - Muurolol (6.2%), α-elemene (6.2%), E-Caryophyllene (5.2%), α-Humulene (4.0%), Limonene (3.6%) were the major major components present in the oil. Characteristic aroma compounds such as γ-2-carene, limonene, myrtenol and carvone may be responsible for the peculiar aroma of the plant.

Ali (2008) reported 23 compounds and α-pinene, α-humulene are same compounds seen in essential oil of *Centella asiatica* (L.), Oyedeji and Afolayan (2005) identified 40 compounds and they reported that the oil contain monoterpenoid hydrocarbon 20.20%, oxygenated monoterpenoid is 5.46%, sesquiterpenoid hydrocarbon 68.89% and oxygenated sesquiterpenoid 3.9%. Kanchan and Preeti (2013) reported 40 compounds. Humulene epoxide II, caryophyllene oxide, Germacrene B, α-humulene, δ-Terpinolene, limonene, p-Cymene, β-pinene, α-pinene, α-Thujene etc are same. As like that Supawan and Patchanee (2014) identified 87 compounds and similar compounds are Humulene epoxide II, caryophyllene oxide, Germacrene B, α-humulene, δ-Terpinolene, limonene, p- Cymene, β-pinene, α-pinene, α-Thujene etc. From Malaysia. Santhi C. (2016) identified 43 compounds monoterpenoid hydrocarbon is 49%, oxygenated monoterpenoid is 2.4%, sesquiterpenoid hydrocarbon is 26.9% and oxygenated sesquiterpenoid is 15%. In the present study we have found 64 components and Monoterpenoids dominated over sesquiterpenoids as agreement with the literature. p-cymene (35.0%), β - Muurolol (6.2%), elemene (6.2%), E-Caryophyllene (5.2%), α-Humulene (4.0%), Limonene (3.4%), Dauca 5,8-diene (3.0%) were the major major components present in the oil.

The quantitative and qualitative divergence may be due to the geographical, climatic, and soil conditions, which in turn may affect the composition and other secondary metabolites of the plants.[42,43] The composition of the essential oil often changes between different plant parts. The difference in the complex composition of essential oils of one kind may sometimes be difficult to assign to specific chemotypes. The formation of essential oils depends on the tissue differentiation (secretory cells and excretion cavities, etc.) and on ontogenetic phase of the respective plant.[44] Individual plants also showed variation in the percentage of chemical components depending on the part of the plant from which the oil was extracted.[45] Moreover, oil constituent was extremely variable, and individual constituents were not affected by intralant location of the leaves, plant age, or geographic site.[46] This limits their taxonomic value but possibly enhances their ecological significance as a defense adaptation to herbivores.[47]

5. CONCLUSION

In conclusion, nevertheless, there are an almost uncountable number of single substances and a tremendous variation in the composition of essential oils. In our present study all the components identified with the percentage is compared to the earlier investigations according to literature. Essential oil yield of *Centella asiatica* (L.) is 0.07%. 95.2% of the total oil constituents were identified. There are 64 compounds identified in the essential oil. Monoterpenoids dominated over sesquiterpenoids. Major compounds in essential oil are p-cymene (35.0%), α - Muurolol (6.2%), α-elemene (6.2%), E-Caryophyllene (5.2%), α-Humulene (4.0%), Limonene (3.4%). Characteristic aroma compounds such as γ-2-carene, limonene, myrtenol and carvone may be responsible for the peculiar aroma of the plant.

Centella asiatica (L.) is one of the important plants in Indian society traditionally. The plant is used as a major ingredient of formulations of Ayurveda, Siddha, Homeopathy and Unani systems of medicine. In Indian pharmacopoeia, it is used as mainly for brain tonic for memory, mental stability and for the mentally retarded people. The plant is listed as useful for the treatment of skin malconditions such as leprosy, lupus, varicose veins, ulcers, eczema, psoriasis and wounds in addition to treatment of diarrhoea, dysentery, fever, amenorrhea and female genitourinary tract infections, filariasis and tumour. Other therapeutic properties of *Centella asiatica* (L.) include anticancer, anti-fungal, anti-bacterial, antidepressant, wound healing etc. *Centella asiatica* (L.) contains several bioactive constituents, of which the most important are the triterpinoid saponins such as asiaticoside, madecassoside and centelloside. In addition, the species possesses other components including flavonoids, tannins, phytosterols, aminoacids and sugars. A large store of research findings are available highlighting the potential therapeutic effects of *Centella asiatica* (L.).

6. ACKNOWLEDGEMENT

The Authors and the research group are very much thankful for financial support from Mother Terisa's Educational Society, I.S.Sadan, Hyderabad, And providing the lab for research work. The authors are specially thank full to Mr. Swamy Rajan Garu, Mrs. Kowsalya (Ayurvedic medicine specialists) and Mrs. S. Subbalakshmi Garu (Principal Padmavathi Degree and P.G College), Mrs. P. Manichandrika Garu (Principal Bhojireddy college of pharmacy) for spending their valuable time and advising the group with suggestions and preparative methods. And also Test House, Banglore, India, for furnishing the spectral information.

7. REFERENCES

1. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th edn. Allured Publ. Corp., Carol Stream. IL, 2007.
2. Ali MSM. Analysis of phenolics and other phytochemicals in selected Malaysian traditional vegetables and their activities *in vitro*. PhD Thesis. University of Glasgow. UK, 2008.
3. Jain P, Guiquing K, Chuanxun Y, Beibei Z, Risheng J, Yuan Y. Separation and determination of madecassic acid extracts of *Centella asiatica* using HPTLC with β-cyclodextrin as mobile phase additive. *Chinese Journal of Chromatography*, 2007; 25: 316-318.
4. Kanchan J, Preeti C. Therapeutic Efficiency of *Centella asiatica* (L.) Urb. An Underutilized green leafy vegetable: an overview. *International Journal of Pharma and Biosciences*, 2013; l4(1): 135-149.
5. Lee J, Jung E, Kim Y, Park J, Hong S, Kim J, Hyun C, Kim YS, Park D. Asiaticoside induces human collagen I synthesis through TGF beta receptor I kinase (Tbeta RI Kinase)-independent Smad signalling. *Planta Medica*, 2006; 72(4): 324-328.
6. Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. *Pharmaceutical Biology*, 2006; 43(3): 249-252.
7. Subathra M, Shila S, Devi MA, Panneerselvam C. Emerging role of *Centella asiatica* In improving age-related neurological antioxidant status. *Experimental Gerontology*, 2005; 57(9): 707-715.
8. Supawan R, Patchanee Y. Antibacterial, antioxidant and chemical profile of *Centella asiatica*. *Biomedical and Pharmacology Journal*, 2014; 7(2): 445-451.

9. Van den Dool H , Kratz PD. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *Chromatography*, 1963; 11: 463-471.
10. Wong KC, Tan GL. Essential oil of *Centella asiatica* (L.) Urb. *Journal of Essential Oil Research*, 1994; 6(3): 307-309.
11. Kokate CK, Purohit AP, Gokhale SBT. EXT book of Pharmacognosy, Nirali Prakashans, Forty ninth edition, Pune, 2014: 9: 54-55. http://www.drugs.com/npp/gotu-kola.html
12. The Weaith of India. A Dictionary of Raw materials and Industrial Products-Raw material series, Publications and Information Directorate, CSIR, New Delhi, Rev Ser., 1992; 3(Ca-Ci): 428-430.
13. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, 2005; 5: 188-189.
14. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, 2001; 3: 159-160.
15. Suguna L, Sivakumar P, Chandrakasan G. Effects of C.asiatica extracts on dermal wounds healing in rats J Experimental Biology, 1996; 34: 1208-1211.
16. Shakir SC. Asiatica (linn.) Urban-A Review, J Natural Product Radiance, 2007; 6(2): 158-170.
17. Jamil SS, Nizami Q, Salam M. *Centella asiatica* (L) Urban - A review, Natural products radiance, 2007; 6(2): 158-170.
18. Bhavana D, Jyoti KC. Entella asiatica the elixir of life International, Journal of Research in Ayurveda and Pharmacy, 2011; 2(2): 431-438.
19. Cheng LC, Guo JS, Luk J, Koo MW. The healing effects of Centella extract and asiaticoside on acetic acid induced gaastric ulsers in rats Life Sci., 2004; 74(18): 2237-49.
20. Jayashree G, Jacob VB, Sudarslal S. Antioxidant activity of C.asiatica on lymphoma bearing mice Fitoterapia, 2003; 74: 431-434.
21. Evans WC, Trease. Pharmacognosy. Fifteenth edition WB. Saunders, 2002; 303-304.
22. Mukherjee PK. Quality control of herbal drugs, first edition, Business horizon pharmaceutical publishers, New Delhi, 2002; 677.
23. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian Plants for biological activity 1, Ind J of Exp Biology, 1968; 6-232.
24. Yoosook C, Bunyapraphatsara N, Boonyakiat Y, Kantasuk C. Anti-herpes simplex virus activity of crude water extract of Thai medicinal plants, Phytomedicine, 2000; 6(6): 411-419.
25. Mohd SMA, Analysis of phenolics and other phytochemicals in selected Malaysian traditional vegetables and their activities in vitro, PhD diss., University of Glasgow, 2008.
26. Lee TK, Vairappan CS. Antioxidant, Antibacterial and Cytotoxic Activities of Essential Oils and Ethanol Extracts of Selected South East Asian Herbs, J Med Plants Res., 2011; 5: 5284–90.
27. Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa, Pharm Biol., 2005; 43: 249–52.
28. Wongfhun P, Gordon MH, Apichartsrangkoon A. Flavour characterisation of fresh and processed pennywort (C*entella asiatica* L.) juices, Food Chem., 2010; 119: 69–74.
29. Govarthanan M, Rajinikanth R, Kamala-Kannan S, Selvankumar T, A comparative study on bioactive constituents between wild and in vitro propagated C*entella asiatica*. J Genet Eng Biotechnol, 2015; 13: 25–9.
30. Arumugam T, Ayyanar M, Koil Pillai YJ, Sekar T, Phytochemical screening and antibacterial activity of leaf and callus extracts of C*entella asiatica*. Bangladesh J Pharmacol, 2011; 6: 55–60.