



Analysis of bioactive compounds in leaves extract of *Centella asiatica* by using HRLC-MS & IR techniques

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ABSTRACT

The bioactive components of leaves extract of *Centella Asiatica* have been evaluated by using HRLC-MS techniques. This technique is easy, rapid and convenient to investigate the presence of medicinally active constituents from the successive methanolic leaves extract of *Centella Asiatica*. The remarkable advantages offered by this method are showing the presence of large number of constituents in the extract, few of them are like Valine, Triparanol, Butamben, Neuraminic acid, Aesculin, Esculetin, Famciclovir, Isocitretin, Rhoifoline, Gentiopicrotin, Urocortisone, Pelargonic acid, Gabapentin, Ivermectin, sarmenoside, Khivovorin, Swietenine etc. and in negative chromatogram compounds like Xylocarpus, Losartan, Carbenicilline etc. The study is to focus the phytochemicals present in extract and their association with medicinal properties so as to find out the alternatives to the existing synthetic drugs.

Keywords: HRLC-MS, Valine, Triparanol, Esculetin, existing synthetic drugs.

INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agriculture & food industry. The use of medicinal herbs for curing diseases has been documented in history of civilization. Man in prehistoric era was probably not aware about health hazards associated with irrational therapy. With the onset of research in medicines, it was concluded that plants contain active principles which are responsible for curative action of herbs. Nature is the best doctor & herbs are the best medicines which is great gift of nature". By considering this approach, little work is carried out with the help of *Centella asiatica*.

Traditional knowledge suggest that "*Centella asiatica*" commonly known as Gota kola or Mandukparni is used as anti-bacterial, anti-inflammatory, anti-diabetic, anti-oxidant, antifungal, for wound healing, can improve blood circulation, strengthens veins, helps to decrease anxiety, stress, fatigue and can be good remedy for skin conditions such as eczema, chronic ulcers, sore, & scleroderma, for cough expectoration, also helps to increase lactation. It is one of the most valuable herbs in Ayurvedic medicine, because as per Ayurveda knowledge it is used as Medhya Rasayana, ie. brain tonic, it can revitalize nerve & brain cells, increases memory & concentration & has an overall rejuvenating effects on our body. As per Chinese traditional medicinal knowledge, it has anti-ageing properties for skin & tightens older skin, helping to prevent wrinkling and can be used in cream to get rid of cane blemishes. Most of the tribes from India use it for wound healing, for bringing down the fever & treating dysentery in children & for diarrhea. But Through the present study, these traditional beliefs have been scientifically tested with the help of "Phytochemical analysis" so as to examine the scientific evidence behind these beliefs and the knowledge, provided about it which is used in various countries from ancient times, as traditional medicinal herb and also referred as "elixir of life"¹ in Ayurveda. Also the study is aimed to make aware the people about the importance and role of

natural products for our health care as compared to synthetic drugs. With this aim and objective the above proposed work has been designed and undertaken.

EXPERIMENTAL SECTION

Preparation of sample:

The plant material was selected from "GO green" nursery, farm: Tal (Karnala), Borpada, Mumbai-Goa highway. Fresh plant materials were washed under running tap water and then with distilled water, leaves, stems and roots were separated out by scissor, air dried then homogenized to fine powder and stored in air tight bottles. Now this fine powder was treated as sample of leaves. 20gm portions of powdered plant materials were separately dispersed in 100ml Methanol. The solution was left to stand at room temp for 24hrs and was filtered with Whatsmann filter paper No.1. The filtrate was used for HRLC-MS & IR Analysis.

HRLC –MS Analysis:

The sample was prepared according to the procedure. 2ml of methanolic leaves extract of *Centella Asiatica* was sent for qualitative analysis of its chemical constituents at SAIF, IIT, Bombay by HRLC-MS technique. For this the instrument used is of Agilent technologies 6550-ifunnel, Q-TOF,LC/MS. Column type is ZORBAX RRHDSB-C18, with 100mm length, 2.1 mm diameter and 1.8 pore sizes. It was carried out with Mass Spectrometry mainly for the classes of compounds which are non-volatile like higher Terpenoids, Phenolic compounds of all types, Alkaloids, Lipids and Sugar etc so as to provide an accurate molecular weight of relevant compounds.

Test conditions are as

Table 1:

Mobile Phase	Solvent A-100% H ₂ O +0.1 % formic acid Solvent B- 100% Acetonitrile
Column Pressure	Maximum 1000bar limit
Coloumn flow	0.3ml/min
Temperature	40 ⁰ C
Injection volume	0.3ml/min
Eject Speed	100µL/min
Injection Mode	Injection with needle wash
Stop time mode	Time set 32 minutes

N	Name of Compound	Retention time (RT)	Formula	MFG formula	DB formula
1	2.Amino 3 methyl ,1-butanol	0.747	C ₅ H ₁₃ N O	C ₅ H ₁₃ N O	C ₅ H ₁₃ N O
2	3butyrl propionic acid	0.834	C ₇ H ₁₂ O ₃	C ₇ H ₁₂ O ₃	C ₇ H ₁₂ O ₃
3	5 methyl Cytidine	0.844	C ₁₀ H ₁₅ N ₃ O ₅	C ₁₀ H ₁₅ N ₃ O ₅	C ₁₀ H ₁₅ N ₃ O ₅
4	Valine	0.864	C ₅ H ₁₁ O ₂	C ₅ H ₁₁ O ₂	C ₅ H ₁₁ O ₂
5	Triparanol	0.921	C ₇ H ₁₃ NO ₂	C ₇ H ₁₃ NO ₂	C ₇ H ₁₃ NO ₂
6	Butamben	0.936	C ₇ H ₁₅ NO ₂	C ₇ H ₁₅ NO ₂	C ₇ H ₁₅ NO ₂
7	Pyroline hydroxyl carboxylic acid	1.271	C ₅ H ₇ NO ₃	C ₅ H ₇ NO ₃	C ₅ H ₇ NO ₃
8	Neuraminic acid	4.316	C ₉ H ₁₇ NO ₈	C ₉ H ₁₇ NO ₈	C ₉ H ₁₇ NO ₈
9	Aesculin	6.44	C ₁₅ H ₁₆ O ₉	C ₁₅ H ₁₆ O ₉	C ₁₅ H ₁₆ O ₉
10	Esculetin	6.424	C ₉ H ₆ O ₄	C ₉ H ₆ O ₄	C ₉ H ₆ O ₄
11	Oxyquinoline	6.629	C ₉ H ₇ NO	C ₉ H ₇ NO	C ₉ H ₇ NO
12	Famiciclovir	6.904	C ₁₄ H ₁₉ N ₅ O ₄	C ₁₄ H ₁₉ N ₅ O ₄	C ₁₄ H ₁₉ N ₅ O ₄
13	S-pyruvylglutathione	6.918	C ₁₃ H ₁₉ N ₃ O ₈ S	C ₁₃ H ₁₉ N ₃ O ₈ S	C ₁₃ H ₁₉ N ₃ O ₈ S
14	Isocitrelin	7.237	C ₂₁ H ₂₆ O ₃	C ₂₁ H ₂₆ O ₃	C ₂₁ H ₂₆ O ₃
15	2,5 Undecadienal	7.716	C ₁₁ H ₁₈ O	C ₁₁ H ₁₈ O	C ₁₁ H ₁₈ O
16	2,7 dimethyl 6 nonenoic acid	7.72	C ₁₁ H ₂₀ O ₂	C ₁₁ H ₂₀ O ₂	C ₁₁ H ₂₀ O ₂
17	Tranexamic acid	7.986	C ₈ H ₁₅ NO ₂	C ₈ H ₁₅ NO ₂	C ₈ H ₁₅ NO ₂
18	Apiin	8.19	C ₂₆ H ₂₈ O ₁₄	C ₂₆ H ₂₈ O ₁₄	C ₂₆ H ₂₈ O ₁₄
19	Hexacarboxylporphyrine	8.319	C ₃₈ H ₃₈ N ₄ O ₁₂	C ₃₈ H ₃₈ N ₄ O ₁₂	C ₃₈ H ₃₈ N ₄ O ₁₂
20	7-α (thiomethyl) spironolactone	8.347	C ₂₃ H ₃₂ O ₃ S	C ₂₃ H ₃₂ O ₃ S	C ₂₃ H ₃₂ O ₃ S
21	Cotinine methonium ion	8.35	C ₁₁ H ₁₅ N ₂ O	C ₁₁ H ₁₅ N ₂ O	C ₁₁ H ₁₅ N ₂ O
22	Rhoifoline	8.548	C ₂₇ H ₃₀ O ₁₄	C ₂₇ H ₃₀ O ₁₄	C ₂₇ H ₃₀ O ₁₄
23	Gentiopicroin	8.674	C ₁₆ H ₂₀ O ₉	C ₁₆ H ₂₀ O ₉	C ₁₆ H ₂₀ O ₉
24	5-Farmimino tetrahydrophilic acid	8.719	C ₂₀ H ₂₄ N ₈ O ₆	C ₂₀ H ₂₄ N ₈ O ₆	C ₂₀ H ₂₄ N ₈ O ₆
25	Urocortisone	8.957	C ₂₁ H ₃₂ O ₅	C ₂₁ H ₃₂ O ₅	C ₂₁ H ₃₂ O ₅
26	4 hydroxypelargonic acid	9.37	C ₉ H ₁₈ O ₃	C ₉ H ₁₈ O ₃	C ₉ H ₁₈ O ₃
27	6-β hydroxytrimecinoloneacetone	9.200	C ₂₄ H ₃₁ FO ₇	C ₂₄ H ₃₁ FO ₇	C ₂₄ H ₃₁ FO ₇
28	Oxyquinoline	9.37	C ₇ H ₁₂ O ₃	C ₇ H ₁₂ O ₃	C ₇ H ₁₂ O ₃
29	Rhoifoline	9.646	C ₂₇ H ₃₀ O ₁₄	C ₂₇ H ₃₀ O ₁₄	C ₂₇ H ₃₀ O ₁₄
30	Altretamine	10.028	C ₉ H ₁₈ N ₆	C ₉ H ₁₈ N ₆	C ₉ H ₁₈ N ₆
31	Maackiain	11.233	C ₁₆ H ₁₂ O ₅	C ₁₆ H ₁₂ O ₅	C ₁₆ H ₁₂ O ₅
32	Ginkgolide	11.677	C ₂₀ H ₂₄ O ₁₀	C ₂₀ H ₂₄ O ₁₀	C ₂₀ H ₂₄ O ₁₀

33	ValerylSalicylate	12.791	C ₁₂ H ₁₄ O ₄	C ₁₂ H ₁₄ O ₄	C ₁₂ H ₁₄ O ₄
34	Pelargonic acid	13.04	C ₉ H ₁₈ O ₄	C ₉ H ₁₈ O ₄	C ₉ H ₁₈ O ₄
35	Peridinine	14.444	C ₃₉ H ₅₀ O ₇	C ₃₉ H ₅₀ O ₇	C ₃₉ H ₅₀ O ₇
36	Sphinganine	14.491	C ₁₆ H ₃₅ N O ₂	C ₁₆ H ₃₅ N O ₂	C ₁₆ H ₃₅ N O ₂
37	Gabapentin	14.952	C ₉ H ₁₇ NO ₂	C ₉ H ₁₇ NO ₂	C ₉ H ₁₇ NO ₂
38	Ivermectin	20.057	C ₄₇ H ₇₂ O ₁₄	C ₄₇ H ₇₂ O ₁₄	C ₄₇ H ₇₂ O ₁₄
39	Sarmentoside B	20.339	C ₃₄ H ₄₈ O ₁₃	C ₃₄ H ₄₈ O ₁₃	C ₃₄ H ₄₈ O ₁₃
40	Khivorine	20.706	C ₃₂ H ₄₂ O ₁₀	C ₃₂ H ₄₂ O ₁₀	C ₃₂ H ₄₂ O ₁₀
41	Switenine	20.791	C ₃₂ H ₄₀ O ₉	C ₃₂ H ₄₀ O ₉	C ₃₂ H ₄₀ O ₉
42	Enkephaline	20.958	C ₂₉ H ₃₉ O ₇ N ₅	C ₂₉ H ₃₉ O ₇ N ₅	C ₂₉ H ₃₉ O ₇ N ₅
43	Ramiprilglucuronide	21.215	C ₂₉ H ₄₀ N ₂ O ₁₁	C ₂₉ H ₄₀ N ₂ O ₁₁	C ₂₉ H ₄₀ N ₂ O ₁₁

The important co-relations can be predicted as follows.

Around 43 compounds were identified in methanolic leaves extract of *Centella asiatica* by HRLC-MS. The active principles with their retention time, molecular feature formula, database formula are presented in table no. The prevailing compounds identified and most relevant compounds to the reported medicinal properties of *Centella asiatica* from +ve and -ve graph as Qualitative compound report are depicted below.

IR SPECTRUM

Methanolic leaves extract of *Centella asiatica* shows the presence of these constituents which may support the medicinal, pharmaceutical, chemical and industrial properties. So it becomes necessary to carry out the chemical analysis of *Centella asiatica*. It is interesting to investigate chemical studies of *Centella asiatica* in solvent like methanol. 2ml of methanolic leaves extract of *Centella asiatica* was sent for qualitative analysis of its chemical constituents at SAIF, IIT, Bombay for IR study.

Table-2

S/N	Absorption observed (cm ⁻¹)	Assignment	Absorption expected (cm ⁻¹)
1	3425.82	-OH in alcohol & Phenols	-OH stretch
2	2922.15	-CH ₃ & CH ₂ in aliphatic compounds	2990-2850 -CH antisym and sym stretching
3	2853.49	Aldehyde -C-H	2900-2800
4	1736.26	>C=O	1600-1900
5	1629.22	>C=O(Amide)	1680-1630
6	1437.36	-C=C	1400-1600
7	1374.42	-S=O	1375-1300
8	1322.00	-C-N	1350-1000
9	1245.18	-F	1400-1000
10	1055.34	Aryl / Vinyl ether -o-	1300-1000
11	893.13	Aromatic -C-H (out of plane bend)	900-690
12	779.46	-Cl	785-540
13	622.41	-Br/-I	<667

RESULTS AND DISCUSSION

The above results supports the belief of people about medicinal value of *Centella Asiatica* as it shows the presence of numerous medicinally active constituents which are further identified by HRLC-MS analysis as "Triparanol"² which is known to inhibit the reduction of desmosterol in to cholesterol (Blohm& Mackenzie in 1959). Triparanol² can block proliferation and induce apoptosis in multiple human cancer cells including lung, breast, liver, pancreatic, prostate cancer and melanoma cells and growth inhibition can be rescued by exogenous addition of cholesterol.

"Valine" is essential amino acid and electronic survey suggests that it is very important for smooth nervous system and cognitive functioning, for maintaining muscles and regulation of immune system. Studies show that it also provides improvement in Insomnia and nervousness. Valine is a branched-chain amino acid³ (BCAA) that works with the other two BCAAs, isoleucine and leucine, to promote normal growth, repair tissues, regulate blood sugar, and provide the body with energy. Natural sources of valine include meats, dairy products, mushrooms, peanuts, and soy protein. Although most people get enough valine from their diet, there have been recorded cases of valine deficiency. Maple syrup urine disease (MSUD) is caused by the inability to metabolize leucine, isoleucine, and valine. The disease is so named because urine from affected people smells like maple syrup. A deficiency of valine may also affect the myelin covering of the nerves, and cause degenerative neurological conditions.

"Esculetin"^{4,7} is phenolic compounds that possess pharmacological and biological properties including anti-cancer, anti-inflammatory, neuroprotective and anti-oxidant activity. It was also shown to have anticancer activity in human colon cancer, human leukemia and cervical cancer and to inhibit cell proliferation in human colon cancer. Electronic database survey suggest that "Aesculine/Esculin"⁸ is a coumarin glucoside -main chemical ingredient in Chinese medicine "Qinpi"-which has anti-inflammatory, anti-bacterial, a diuretic, anti-tumour effect and can be used for the

treatment of eye diseases, gout, cancer prevention study found so. “Famciclovir”⁸ has shown to be effective in suppressing recurrent genital herpes. It is an anti-viral agent against genital herpes. “Gentiopicrin”⁹ is type of glycoside, soluble in water, bitter and reported to have anti-malarial activity. “Rhoifoline” –is a type of flavone glycoside and has anti-inflammatory and anti-oxidant activity¹⁰. “Pelargonic acid” functions as skin conditioning agent in cosmetics¹¹. “Ginkgolide” is well known herbal or natural medicine and is a type of terpenoid commonly assumed to be useful for memory, prevention or treatment of Alzheimer’s diseases¹²⁻¹⁴, Erectile disinflection, Multiple Sclerosis and Tinnitus. It may have memory enhancing capacity. Its proposed mechanism of action includes anti-inflammatory, anti-oxidant, neurotransmitter regulation. Hence, it will be interesting to carry out further study.

CONCLUSION

HRLC-MS results signify the presence of around 43 phytochemical constituents. It has provided the good platform for identification of these phytochemicals which can have medicinal activity. IR also confirmed the presence of alcohol, phenol aldehyde, amide, vinyl ether, aromatic compounds and halogen compounds. These results support the traditional knowledge to use leaves of *Centella asiatica* as herbal alternative to existing synthetic drugs.

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REFERENCES

- [1] Bhavna, Dhora, and Khatri Jyoti. p *International Journal of research in Ayurvedha and Pharmacy* 2.2 (2011): 431-438. 1583S-1587S.
- [2] Wieslawa Bylka, Paulina Znajdekawizen, Elzhieta studzinsk. *Advances in dermatology and allorlogy.*, 2013, 2 , 30(1);46-49.
- [3] Shimomura, Yoshiharu, et al. *The Journal of nutrition* 134.6 (2004):
- [4] Park S.S.,Park S.K, Lim JH, Kim WJ, Moon SK, *Oncol rep.*, 2011: 25223-230.
- [5] Riberaopreto, *Brazillian journal of medical and biological research*, 2015, 48, 3.
- [6] Hecht SS, Kenney PM, Wang M, TrushinN,Agrawal S, rao AV, et. Al. *A/J mice Cancer let*, 1999:137-130,
- [7] Xiaxiao Yan, “study on thecpharmacokinetics of aesculin” Master thesis, 2008.Nanjing University of traditional chienesese medicine of pharmacy.
- [8] Wiley-liss, *S.Faro,Journal*, 1998, (1):38-43,
- [9] Richard Mabey, Anne McIntre, Lichaël McIntyre, “A book-New age Herbalist-How to use herbs for healing-nutrition bodycare”, P. No .60.
- [10] Shizo Hattori, Hittori, Hiroaki Mastuda, *Archieves of biochemistry biophysics*, Volume 37, Issue 1, Pages 85-89.
- [11] Johnson Wilbur, Heldreth, Bart, Bergfield, Wilma F, Belsito, Donald V, Klaassen, F.Alan, *International journal of Toxicology*, 2011, 30 (6), suppl.
- [12] Chung KF, Dent G, MC Cusker M et al. *Lancet*, 1987, (8527): 248-51.
- [13] “National Institute of health , National Centre for complementary alternative medicine Ginkgo, NCCAM Publication, NO D290,July 2010, NCCAM.
- [14] Usai S, Grazzi L. Bussone G. *Neuro Science.*, 2011, 5; 32 (1.1):197-9.