GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC

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ABSTRACT

Background: The presence of phytochemical constitutes has been reported from species of the Compositae (Asteraceae). Hitherto no reports exist on the phytochemical components and biological activity of *Lactuca runcinata* DC. **Objective:** The present study was designed to determine the bioactive compounds in the whole plant methanol extract of *Lactuca runcinata.* **Materials and Methods:** Phytochemical screening of the entire herb of *Lactuca runcinata* DC revealed the presence of some bio-active components. Gas chromatography-mass spectrometry (GC-MS) analysis of the whole plant methanol extract of *Lactuca runcinata* was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. **Results:** The phytochemical tests showed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, phlobatannin, reducing sugars, saponins, steroids, tannins, terpenoids, volatile oils, carbohydrates, and protein/amino acids in methanolic extract of *L. runcinata*. The GC-MS analysis has shown the presence of different phytochemical compounds in the methanolic extract of *Lactuca runcinata*. A total of 21 compounds were identified representing 84.49% of total methanolic extract composition. **Conclusion:** From the results, it is evident that *Lactuca runcinata* contains various phytocomponents and is recommended as a plant of phytopharmaceutical importance.

Key words: GC-MS analysis, *Lactuca runcinata*, phytochemical screening, whole plant methanol extract

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INTRODUCTION

Use of plants as a source of medicine has been inherited and is an important component of the health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world.[1] Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[2] *Lactuca runcinata* DC [*L. runcinata*, Synonym-*Lactuca heyneana* DC.] commonly known as Undirachakam[3] or Atheli is an annual erect herb belonging to the family Compositae (Asteraceae). Traditionally this plant finds its wide applicability as diuretic and in chronic obstruction of liver and bowel.[3] *Lactuca runcinata* DC also has been reported to be a valuable source of essential nutrients, such as carbohydrate, protein, fat etc., and micro-nutrients like calcium, iron, phosphorous etc., Chewing *Lactuca runcinata* with betel leaf is useful to cure the blisters of mouth and tongue.

In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species.[4-6] A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide, related to the possible chemical components of "*Lactuca runcinata*". So, the present study was aimed to investigate the possible chemical components by first preparing the methanolic extract and separation and identification of the compounds by subjecting it to GC-MS analysis.

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MATERIALS AND METHODS

Collection of plant material

The entire herb of *Lactuca runcinata* DC plant (healthy and disease free plant samples) was collected from the

nearby area of Thoothukudi District fields (Tamil Nadu) in December 2011, identified and authenticated by Dr. V. Chelladurai (Retired Research Officer-Botany, Central Council for Research in Ayurveda and Sidda, Govt. of India), Tiruneveli, Tamil Nadu, India. Herberium of the plant, *Lactuca runcinata,* was prepared and preserved in the Department of Pharmacognosy, Koringa College of Pharmacy, Korangi, East Godavari District, Andhra Pradesh, India.

Preparation of extracts

A portion of dried aerial parts (100 g) of *Lactuca runcinata* DC was placed in a soxhlet apparatus. Extraction was performed with 750 ml of methanol for 48 h at a temperature not exceeding the boiling point of the solvent. Extract was filtered through a 45 µm filter.[7] The resulting solution was concentrated in vacuum to dryness to give methanol extract (9 g). The extract was stored in a refrigerator at 4°C for further use.

Preliminary phytochemical screening

The methanol extract was tested for alkaloids, anthroquinones, flavonoids, phenols, steroids, tannins, terpenoids,[8] cardiac glycosides, saponins,[9] phlobatannin,[10] reducing sugars,[11] volatile oils,[12] carbohydrates and protein/amino acids.[13,14]

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

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 AND DISCUSSION

After the successful conventional hot soxhlet extraction of the whole part of the plant in investigation, the preliminary phytochemical study revealed that methanolic extract of *Lactuca runcinata* contains alkaloids, cardiac glycosides, flavonoids, phenols, phlobatannin, reducing sugars, saponins, steroids, tannins, terpenoids, volatile oils, carbohydrates, and protein/amino acids. Anthraquinones were absent in the *L. runcinata* methanolic extract, as summarized in [Table 1].

Table 1: Preliminary phytochemical evaluation of methanol extracts of *Lactuca runcinata*

Phytochemical constituents	Test/Reagents	Result
Alkaloids	DragendorfFs test	+
	Wagner test	+
Anthraquinones	Borntrager's test	–
Cardiac glycosides	Kellar-Kiliani test	+
Flavonoids	Shinoda test	+
Phenols	Phenol test	+
Phlobatannin	1% HCl	+
Reducing sugars	Fehling test	+
Saponins	Frothing test/Foam test	+
Steroids	Liebermann-Burchardt test	+
Tannins	Braemer's test	+
Terpenoids	Liebermann-Burchardt	+
	Salkowski test	+
Volatile oils	Strin test	+
Carbohydrates	Molish's test	+
Protein/Amino acids	Biuret test	+
	Ninhydrin test	+

+ =Present; – =Absent

The results pertaining to GC-MS analysis of the methanolic extract of *Lactuca runcinata* DC lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The various components present in the entire herb of *Lactuca runcinata* DC that were detected by the GC-MS are shown in [Table 2]. Carbetapentane, 2-Propenoic acid, 2-methylpropyl ester, Bornyl Acetate, Anti (9, 10)-tricyclo [4.2.1.1 (2, 5)] dec-3-en-9-endo-ol, Methanone (1-hydroxycyclohexyl) phenyl, 1 (3H)-Isobenzofuranone, 3-ethoxy, 4-Hydroxy-6-(4-methoxyphenyl)-2-pyranone, 1-Benzoyl-3-(4'-nitrophenyl) pyrrolo [2,1-a] phthalazine, Pyridine-3-carboxamide, 2,4,6-trichloro-N-(4-methoxyphenyl)- 5-nitro, Ethyl1-benzyl-4,6-dibromo-3-methyl-2-oxoindoline-3-carboxylate, 5à-Cholestan-19-oic acid, 2à- hydroxy-, acetate, (5á) Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate, O, O-Diethyl (1 formylpentyl) selenophosphate, 3-n-Pentadecyl-2,4-dinitrophenol, 1,3-Bis (4-nitrobenzyl)-5,6 dihydrobenzo[f] quinazoline, 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester, Dibenzoxazabicycloundecane, 4-[p-Bis (2-hydroxyethyl) amino] phenyl-1-bromoisoquinoline, Ergost-25-ene-3,6-dione, 5,12-dihydroxy-, (5à,12á), Lup-20 (29)-en-3-ol, acetate, (3á)-, E-Ethyl (Z)-3-(4- Acetylphenylthio) cinnamate were present in the methanolic extracts of *Lactuca runcinata*. The composition determined for this methanolic extract corresponds to 86.63% of the entire GC-MS chromatogram.

Table 2: Compounds identified in the methanolic extract of *Lactuca runcinata* In GC-MS

RT	Name of the compound	Molecular formula	Molec ular weight	Peak area %	Compound nature
3.13	Carbetapentane	$C_{20}H_{31}NO_3$	333	0.45	Ester
8.10	2-Propenoic acid, 2-methylpropyl ester	$C_7H_{12}O_2$	128	0.83	Ester
14.34	Bornyl Acetate	$C_{12}H_{20}O_2$	196	0.89	Ester
17.77	anti (9,10)-tricyclo [4.2.1.1 (2,5)] dec-3-en-9-endo-ol	$C_{10}H_{14}O$	150	1.24	Alcohol
20.60	Methanone (l -hydro xycyclohexyl) phenyl	$C_{13}H_{16}O_2$	204	3.28	Ketone
23.00	1 (3H)-Isobenzofuranone, 3-ethoxy	$C_{10}H_{10}O_3$	178	7.79	Ketone
29.06	4-Hydroxy-6-(4-methoxyphenyl)-2-pyranone	$C_{12}H_{10}O_4$	218	0.62	Ketone
33.40	l-Benzoyl-3-(4'-nitrophenyl) pyrrolo [2,1-a] phthalazine	$C_{24}H_{15}N_3O_3$	393	0.64	Ketone
33.82	Pyridine-3-carboxamide, 2,4,6-trichloro-N-(4-methoxyphenyl)-5-nitro	$C_{13}H_8Cl_3N_3O_4$	375	0.55	Amide
34.56	Ethyl 1-benzyl-4,6-dibromo-3-methyl-2-oxoindoline-3-carboxylate	$Ci_9H_{17}Br_2N_{03}$	465	0.68	Ester
34.89	5à-Cholestan-19-oic acid, 2à-hydroxy-, acetate	$C_{29}H_{48}O_4$	460	0.63	Steroidal ester
36.01	(5a) Pregnane-3,20a-diol, 14à, 18à- [4-methyl-3-oxo- (1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}N_{06}$	489	0.78	Steroidal ester
36.56	0,0-Diethyl (l-formylpentyl) selenophosphate	$Ci_0H_2iO_4PSe$	316	0.72	Phosphate ester
36.87	3-n-Pentadecyl-2,4-dinitrophenol	$C_{21}H_{34}N_2O_5$	394	0.93	Phenol
37.16	l, 3-Bis (4-nitrobenzyl)-5,6 dihydrobenzo [f] quinazoline	$C_{26}H_{20}N_4O_4$	452	1.54	Aromatic
38.00	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	1.41	Ester
38.59	Dibenzoxazabicycloundecane	$C_{20}H_{23}NO_4$	341	2.39	Hydrocarbon
41.66	4- [p-Bis (2-hydroxy ethyl) amino] phenyl-1 -bromoisoquinoline	$Ci_9Hi_9BrN_{202}$	386	0.54	Iso-quinoline
43.20	Ergost-25-ene-3,6-dione, 5,12-dihydroxy-, (5a, 12a)	$C_{28}H_{44}O_4$	444	10.46	Steroid
43.84	Lup-20 (29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468	15.11	Steroidal ester
45.13	E-Ethyl (Z)-3-(4-Acetylphenylthio) cinnamate	$C_{19}H_{18}O_3S$	326	33.01	Ester

RT=Retention time

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in [Figure 1]. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

The present study helps to predict the formula and structure of 21 biomolecules. Further investigation may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development.

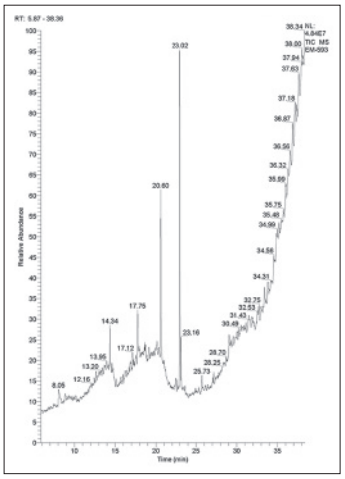

Figure 1: GC-MS chromatogram of methanolic extract of *Lactuca runcinata*

CONCLUSION

The presence of various bio-active compounds detected after GC-MS analysis using the methanolic extract of *Lactuca runcinata* DC justifies the use of whole plant for various elements by traditional practitioner.

However, isolation of individual phytochemical constituents and subjecting it to the biological activity will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency. From these results, it could be concluded that "*Lactuca runcinata*" contains various bio-active compounds. Evaluation of pharmacological activity is under progress. Therefore, it is recommended as a plant of phytopharmaceutical importance.

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