ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 1, 2015

ISSN - 0974-2441

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF PHYTOCOMPONENTS IN THE ETHANOLIC EXTRACT FROM WHOLE PLANT OF *LACTUCA RUNCINATA* DC.

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Received: 22 September 2014, Revised and Accepted: 31 October 2014

ABSTRACT

Objective: The objective was to characterize the phytochemical constituents in ethanolic extract from the whole plant of *Lactuca runcinata* DC. by gas chromatography-mass spectrometry (GC-MS).

Methods: The shade-dried whole plant powder was extracted with ethanol using Soxhlet extractor and crude extract was used for GC-MS.

Results: The GC-MS analysis was used to determine the presence of 20 different phytochemical compounds in the ethanolic whole plant extract of *L. runcinata,* showed that they were different types of high and low molecular weight compounds.

Conclusions: This GC-MS study helps to predict the formula and structure of phytoconstituents and which can be used as drugs, and further investigation may lead to the development of drug formulation.

Keywords: Ethanolic extract, Gas chromatography-mass spectrometry analysis, *Lactuca runcinata* DC., Phytocomponents.

INTRODUCTION

Lactuca runcinata DC.; synonyms, *L. heyneana* DC. Family: *Compositae; Asteraceae*. This occurs in many parts of India, as a common weed. It is considered as valuable medicinal herb in traditional systems of medicine in India. Action diuretic, slightly aperient. It is used as a diuretic in calculous affections, also for chronic obstruction of liver and bowels [1]. A smaller var., found in western Uttar Pradesh, Rajasthan, Saurashtra and the Deccan Penninsula, is equated with *Lactuca remotiflora* DC.

And nobody has analyzed ethanolic extract of *L. runcinata* by gas chromatography-mass spectrometry (GC-MS). This work is the first-time report for the preparation of crude extract and analysis by GC-MS. For this reason, the aim of this work was to investigate and characterize the bioactive chemical constituents in the ethanolic crude extract by using GC-MS from *L. runcinata*. There is growing the awareness in correlating the bioactive constituents of a medicinal plant with its pharmacological activity [2-8].

METHODS

Collection and preparation of plant material

The fresh whole plants of *L. runcinata* DC were collected from the natural habitats of Kayathar, Thoothukkudi district, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into a fine powder. The powdered materials were stored in airtight polythene bags until use.

Plant sample extraction

The powder samples of *L. runcinata* were extracted with ethanol at temperature between 60°C and 65°C by hot continuous percolation method in Soxhlet apparatus [9] for 24 hrs. The extract was concentrated using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

GC-MS analysis

GC-MS analysis of ethanolic extract of *L. runcinata* was performed using a Perkin-Elmer GC clauses 500 system and GC-MS employed a fused silica capillary column packed with Elite-1 (100% dimethyl polysiloxane, 30 nm × 0.25 nm ID × 1µm df) For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/minute and an injection volume of 2 µl was employed (split ratio of 10:1) injector temperature was set at 250°C; ion-source temperature was set at 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes) with an increase of 10°C/minutes to 200°C, then 5°C/minutes to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC detection time was completed in 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatogram was a Turbo mass.

Identification of components

Interpretation on a mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULTS

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanolic extract of *L. runcinata*. These compounds were identified through MS attached with GC. The results of the present study were tabulated in Table 1. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of 3-nonen- 1- ol, (Z)- (2.57), E-7- tetradecenol (0.40), 3- decyn- 2- ol (0.57), octadecanoic acid, ethyl ester (3.32), 6- octen- 1- ol, 3,7- dimethyl-, (ń)- (2.06), Z- 1,9-hexadecadiene (0.97), 9,12,15-octadecatrienoicc acid, methyl ester,(Z,Z,Z)- (3.47), pentadecanoic acid, 2,6,10,14-tetramethyl-,methyl ester (0.52), 3,trans-(1,1-dimethylethyl)-4,trans-methoxycyclohexanol (0.58), 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl- (0.89), 1b,5,5.6a-tetamethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one(0.26),Z,Z,Z-4,6,9-nonadecatriene (0.61), hexadecanal,2-methyl- (4.81), vitamin E (1.74), 1,6,10,14-hexadecatetraen-3-ol,3,7,11,15-tetramethyl-,(E,E)- (4.02),

ledol (10.88), 2(1H) naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (14.61), humulane-1,6-dien-3-ol (6.45), 2,6,10,14-hexadecatetraen-1-ol,3,7,11,15-tetramethyl-,acetate,(E,E,E)- (35.26), 1,6,10-dodecatrien-3-ol,3,7,11-trimethyl- (nerolidol) (6.02). The spectrum profile of GC-MS (Fig. 1) confirmed the presence of 20 compounds with retention time 11.01, 11.26, 11.46, 12.75, 14.19, 14.78, 14.88, 15.19, 19.02, 23.53, 26.32, 26.83, 27.19, 27.76, 31.18, 32.15, 33.00, 33.18, 34.18, and 34.37, respectively. The individual fragmentation of the components is illustrated in Fig. 2a-n.

CONCLUSION

In recent times, in addition morphological markers, anatomical, cytological, biochemical, and molecular markers are also being used to categorize the organisms [10]. GC-MS is a valuable tool for reliable identification for phytocompounds [11,12]. In the current study, 20 compounds have been identified from the ethanolic extract of the whole plant of *L. runcinata* by GC-MS analysis. Among the identified phytochemicals, 9,12,15-octadecatrienoicc acid, methyl ester,(Z,Z,Z)- to be a polyenoic compound and it may be acts as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, 5-alpha reductase inhibitor and antiandrogenic. 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl-; 1,6,10,14-hexadecatetraen-3-ol,3,7,11,15-tetramethyl-,(E,E)-; ledol; 1,6,10-dodecatrien-3-ol,3,7,11-trimethyl- (nerolidol) are suggested to be a sesquiterpene alcohol and 2(1H)-naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- to be a sesquiterpene compound both may be employed as an anti-tumor, analgesic, anti-bacterial, anti-inflammatory, sedative, fungicide. Vitamin E is suggested to be a vitamin compound and it may be acts as an anti-ageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, anti dermatitic, antileukemic, antitumour, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchiti and anticoronary. 2,6,10,14-hexadecatetraen-1-ol,3,7,11,15-tetramethyl-, acetate,(E,E,E)- is a terpene alcohol compound it may act as an anti-microbial, anti-inflammatory, anticancer, diuretic activity. The activity of phytocomponents

Table 1: Chemical composition of ethanolic extract of *L. runcinata* DC.

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	11.01	3-Nonen- 1- ol, (Z)-	$C_9H_{18}O$	142	2.57
2	11.26	E-7- Tetradecenol	$C_{14}H_{28}O$	212	0.40
3	11.46	3- Decyn- 2- ol	$C_{10}H_{18}O$	154	0.57
4	12.75	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	3.32
5	14.19	6-Octen-1-ol, 3,7-dimethyl-, ń)-	$C_{10}H_{20}O$	156	2.06
6	14.78	Z- 1,9-Hexadecadiene	$C_{16}H_{30}$	222	0.97
7	14.88	9,12,15-octadecatrienoicc acid, methyl ester,(Z, Z, Z)-	$C_{19}H_{32}O_2$	292	3.47
8	15.19	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	$C_{20}H_{40}O_2$	312	0.52
9	19.02	3, trans-(1,1-dimethylethyl)-4, trans-methoxycyclohexanol	$C_{11}H_{22}O_2$	186	0.58
10	23.53	2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	0.89
11	26.32	1b, 5,5.6a-tetamethyl-octahydro-1-oxa-cyclopropa[a] inden-6-one	$C_{13}H_{20}O_2$	208	0.26
12	26.83	Z, Z, Z-4,6,9-nonadecatriene	$C_{19}H_{34}$	262	0.61
13	27.19	Hexadecanal, 2-methyl-	$C_{17}H_{34}O$	254	4.81
14	27.76	Vitamin E	$C_{29}H_{50}O_2$	430	1.74
15	31.18	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-,(E, E)-	$C_{20}H_{34}O$	290	4.02
16	32.15	Ledol	$C_{15}H_{26}O$	222	10.88
17	33.00	2 (1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	218	14.61
18	33.18	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	222	6.45
19	34.18	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate,(E, E, E)-	$C_{22}H_{36}O_2$	332	35.26
20	34.37	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (Nerolidol)	$C_{15}H_{26}O$	222	6.02

MW: Molecular weight, *L. runcinata: Lactuca runcinata*

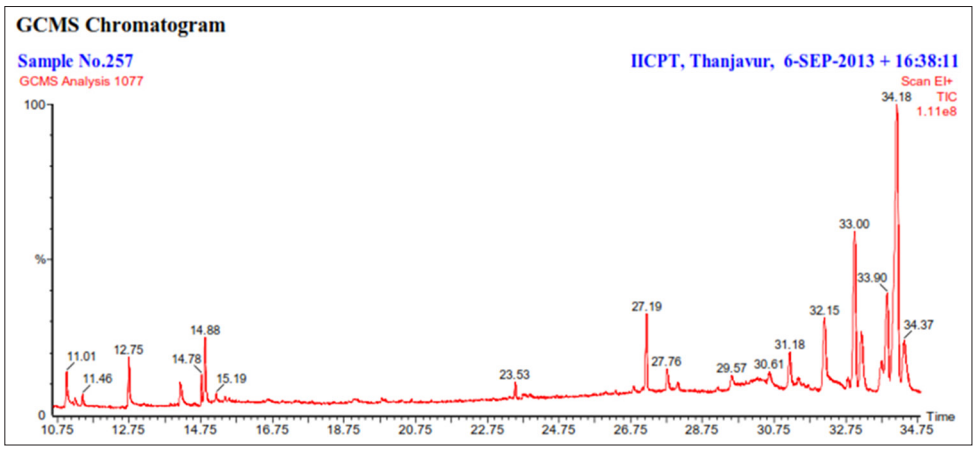

Fig. 1: Gas chromatography-mass spectrometry chromatogram of ethanolic extract of *Lactuca runcinata* DC.

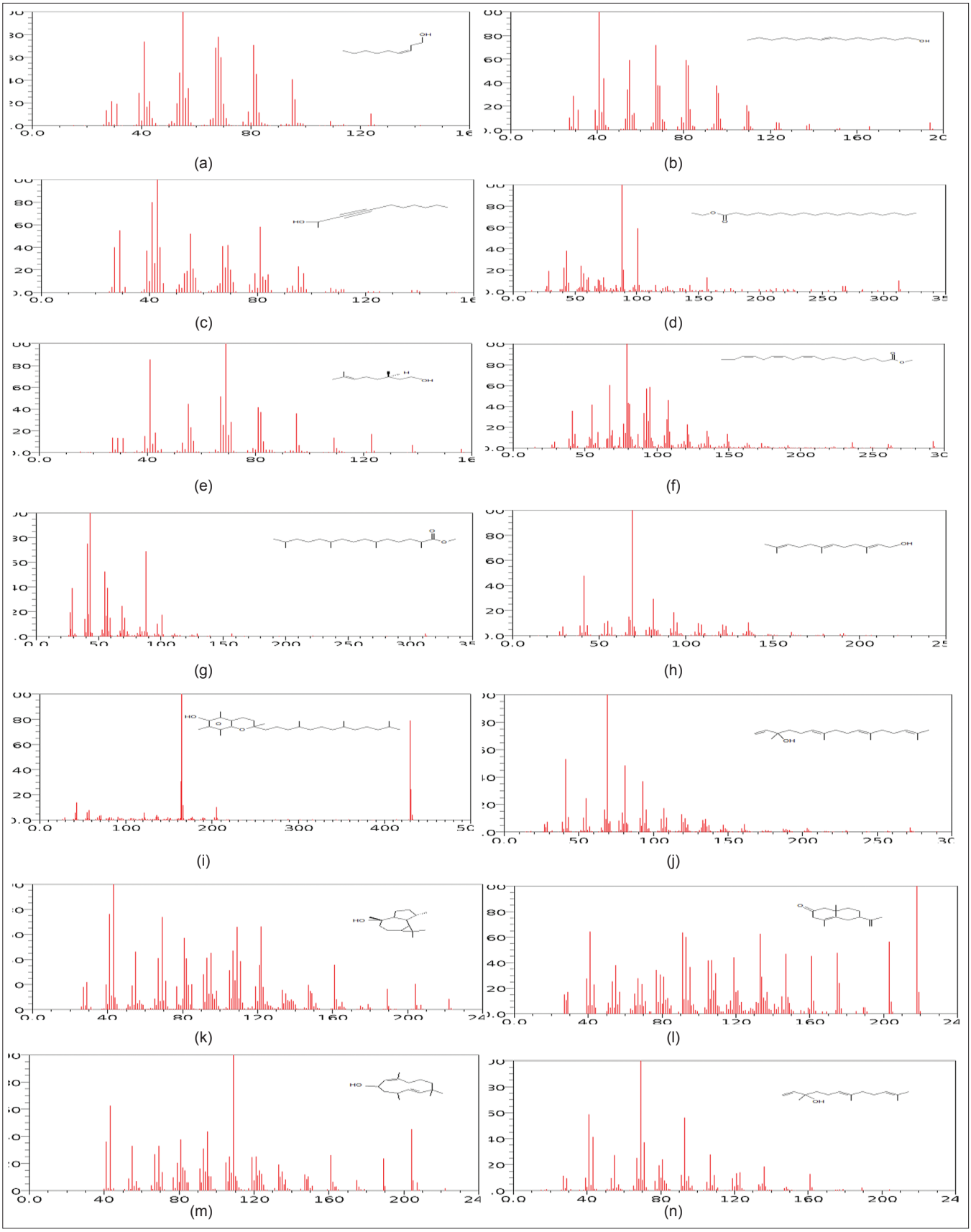

Fig. 2: (a) 3-Nonen- 1- ol, (Z)- (b) E-7- Tetradecenol (c) 3- Decyn- 2- ol (d) Octadecanoic acid, ethyl ester (e) 6- Octen- 1- ol, 3,7-dimethyl-, (ń)- (f) 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (g) Pentadecanoic acid,2,6,10,14-tetramethyl-,methyl ester (h) 2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl- (i) Vitamin E (j) 1,6,10,14-Hexadecatetraen-3-ol,3,7,11,15-tetramethyl-,(E,E)- (k) Ledol (l) 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (m) Humulane-1,6-dien-3-ol (n) 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl- (Nerolidol)

Table 2: Activity of phytocomponents identified in *L. runcinata* DC. by GC-MS

RT	Name of the compound	Nature of compound	Activity
11.01	3-Nonen- 1- ol, (Z)-	Alcohol	No activity reported
11.26	E-7- tetradecenol	Alcohol	No activity reported
11.46	3- Decyn- 2- ol	Alcohol	No activity reported
12.75	Octadecanoic acid, ethyl ester	Fatty ester	No activity reported
14.19	6- Octen- 1- ol, 3,7- dimethyl-, (ń)-	Alcohol	No activity reported
14.78	Z- 1,9-Hexadecadiene	Alkene	No activity reported
14.88	9,12,15-Octadecatrienoicc acid, methyl ester,(Z, Z, Z)-	Polyenoic fatty acid	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anticoronary, antieczemic antiacne, 5-alpha reductase inhibitor, antiandrogenic
15.19	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	Ester	No activity reported
19.02	3, trans-(1,1-dimethylethyl)-4, trans-methoxycyclohexanol	Alcohol	No activity reported
23.53	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	Sesquiterpene alcohol	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
26.32	1b, 5,5.6a-tetamethyl-octahydro-1-oxa-cyclopropa[a] inden-6-one	Ketone	No activity reported
26.83	Z, Z, Z-4,6,9-Nonadecatriene	Alkene	No activity reported
27.19	Hexadecanal, 2-methyl-	Aldehyde	No activity reported
27.76	Vitamin E	Vitamin compound	Antiageing, analgesic, antidiabatic anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumour, anticancer, hepatoprotective, hypocholesterolemic antiulcerogenic, vasodilator, antispasmodic, antibronchiti and anticoronary
31.18	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-,(E, E)-	Sesquiterpene alcohol	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
32.15	Ledol	Sesquiterpene alcohol	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
33.00	2 (1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	Sesquiterpene	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
33.18	Humulane-1,6-dien-3-ol	Alcohol	No activity reported
34.18	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E, E, E)-	Terpene alcohol	Anti-microbial, anti-inflammatory, anticancer, diuretic
34.37	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (Nerolidol)	Sesquiterpene alcohol	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide

L. runcinata: Lactuca runcinata, GC-MS: Gas chromatography-mass spectrometry

identified in *L. runcinata* is tabulated in Table 2. Several phytochemical screening studies have been carried out in different parts of the world using GC-MS [13-15]. In the present study, we characterized the chemical profile of *L. runcinata* using GC-MS. The GC shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the comparative concentrations of the components present in the plant. The MS analyzes the compounds eluted at different times to recognize the nature and structure of the compounds. The large compound fragments into small compounds giving rise to the appearance of the peak at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *L. runcinata* using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the current study supported and supplemented the prior observations [13-16].

GC-MS analysis revealed the existence of different compounds with various chemical structures. The presence of various bioactive compounds confirms the application of *L. runcinata* for various ailments by traditional practitioners and their diversity and detail phytochemistry may add new knowledge to the information in the traditional medical systems. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

ACKNOWLEDGMENTS

The authors are grateful to Dr. S. Kumaravel, Quality Manager, Food Testing Laboratory and the Director, Indian Institute of Crop Processing Technology (IICPT), Thanjavur, for providing the laboratory facilities (GC-MS) and support to carry out the work.

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