Indian Journal of Natural Products and Resources
Vol. 12(4), December 2021, pp. 617-624

High resolution-mass spectrometry (HR-MS) analysis of *Bryonia laciniosa* L.

Shashikala M[1] and Mamta B. Shah[2*]

[1]Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Hyderabad 500090, India
[2]Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad 380009, India

Received 11 October 2020; Revised 10 November 2021

Bryonia laciniosa L. (Family: Cucurbitaceae) is commonly known as 'Shivlingi' and is a highly valued medicinal plant in Ayurveda and Homeopathy systems of medicine particularly as a remedy for infertility. In the present study, sophisticated, highly reliable, and sensitive high resolution-mass spectrometry (HR-MS) was carried out for the determination of chemical constituents present in *B. laciniosa*. Phytochemical screening of methanolic extract showed the presence of saponins, sterols, triterpenoids, flavonoids, alkaloids, phenolic compounds, and tannins. TLC of sterol-rich petroleum ether extract (SRPE) produced six major spots on precoated silica gel GF_{254}, using toluene: methanol (96:04 v/v) as mobile phase. The spots showed green, purple, light purple to violet colour spots in the Rf range of 0.2-0.91, after derivatization with the anisaldehyde-sulphuric acid reagent. Further, based on TLC, HR-MS analysis of SRPE was performed for the identification of phytoconstituents, at positive ESI (electron spray ionisation) mode. It indicated the presence of a total of 30 compounds including short fragments of peptides. The major compounds predicted in HR-MS: Q(quadrupole)-TOF(time-of-flight)-MS, as per METLIN database, were swietenine, ergosterol acetate, 4, 4, dimethyl-14α-formyl-5α-cholesta-8,24-dien-3β-ol, L-olivosyl-oleandolide, mitoxantrone, isogedunin, 3S-aminodeconoic acid, and nisoldipine.

Keywords: *Bryonia laciniosa* L., HR-MS analysis, Sterol-rich petroleum ether extract.

IPC code; Int. cl. (2015.01)- A61K 36/00

Introduction

Medicinal plants are considered biological factories as they synthesize various chemical constituents, useful for mankind for the treatment of various diseases. They are used in various countries, medicinally and as a source of many potent drugs[1]. Thus, the phytochemical analysis of such medicinal plants is very important for the identification and quantification of chemical constituents, present in them. Hyphenated techniques such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and high resolution-mass spectrometry (HR-MS) are useful tools, largely used in the identification and quantification of active components at vestige concentrations in plant extracts[2]. Presently, HR-MS technique is commonly used, which is an accurate, precise, and sensitive method that helps in the identification of compounds via spectral library matching[3]. It involves the comparison of mass spectra generated from unknown samples with a database containing spectra of known compounds (Spectral libraries) and generates high-quality data within the minimum completion time. Spectral libraries can give accurate compound identification as they incorporate additional information of the analyte and retention time[4]. HR-MS is accomplished with direct analysis of samples available with quick and simple procedures with minimal sample pre-treatment or purification, unlike traditional MS analysis which involves extensive sample preparation and chromatographic separation[5].

B. laciniosa is commonly known as 'Shivlingi' and is an annual climber with bifid tendrils. It is native to Papua, Malesia, Australia and found growing wild in India[6]. The plant shows the characteristic palmate shaped leaves, angular stem and round-shaped berry fruits with white longitudinal streaks over its surface. The fruits enclose characteristic ovate, 'Shivling-shaped' seeds ending on sharp pointed beak at the narrow end (Fig. 1). Microscopical study of various parts of the plant shows the important characters such as the presence

*Correspondent author
Email: shashimetri@rediffmail.com

Fig. 1 — Morphological features of *Bryonia laciniosa* L. a) Leaves and fruits, b) Shivling- shaped seeds.

of vascular bundle in the stem, arranged in two rings with annular and spiral thickenings, dorsiventral leaf with anomocytic stomata, abundant starch in the mesocarp of the fruit with small curved vascular strands and triangular-shaped vascular bundles in the t.s. of the root[7]. It has been used by traditional healers to treat infertility, menstrual disorders, rheumatism, stomach disorders etc[8]. The plant was reported to contain punicic acid[9], goniothalamin[10], and fibre glucomannan[11]. Many pharmacological activities have been reported from this plant, such as anti-inflammatory[12], antitumor and antioxidant[13], analgesic, antipyretic[14], antimicrobial[15], androgenic, anti-asthmatic, and anticonvulsant[16].

Considering its vast traditional use and extensive work on pharmacological activities, but only a few reports on phytochemical aspects, the HR-MS analysis of sterol-rich petroleum ether extract of *B. laciniosa* was carried out to uncover phytoconstituents present in it, which are likely to be responsible for the pharmacological activities and medicinal uses of the plant.

Materials and Methods

Plant collection, identification, and extraction

The plant of *B. laciniosa* was collected in June 2019 from the agricultural hedges of Vijayapur dist., Karnataka (India). The plant was authenticated by Dr P. V. Prasanna (Scientist G), at Botanical Survey of India (BSI), Deccan Regional Center, Hyderabad (N0. BSI/DRC/2019-20/Tech./665). The voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Hyderabad.

The plant material was washed under running tap water and shade dried and powdered. About 50 g of the powdered drug was extracted with methanol in a Soxhlet apparatus for 3 h. The resulting extract was filtered and concentrated using a rota evaporator and used to perform qualitative chemical tests and HR-MS analysis.

Phytochemical screening and proximate analysis

Qualitative chemical tests are performed to identify the presence of primary and secondary metabolites using various specific reagents which produce precipitation or specific colour reactions with metabolites[17,18]. The proximate composition such as foreign organic matter (FOM)[19], moisture content[20], ash content[21] and extractive values[22] were carried out by adopting official methods, using dried powdered material of *B. Laciniosa*[23]. For phytochemical screening, a small portion of methanolic extract of *B. laciniosa* was evaporated to dryness on a water bath and the residue was used to perform the following chemical tests.

Detection of carbohydrates

The residue was dissolved in water and treated with an alcoholic solution of α-Naphthol and conc. sulphuric acid. The formation of a reddish violet ring indicates the presence of carbohydrates. Further, a small portion of residue was hydrolysed with hydrochloric acid and treated with Fehling's A and B solution. The formation of a brick red colour after heating indicates the presence of reducing sugars[24,25].

Detection of proteins and amino acids

The residue was dissolved in 10 mL of distilled water and filtered. The filtrate was treated with a few drops of Biuret reagent. The formation of pink or purple colour indicates the presence of amino acids and proteins[25].

Detection of saponins

The residue was shaken with 5 mL of distilled water for 5 min and allowed to stand for 15 min. Persistent froth indicates the presence of saponins[25].

Detection of sterols and triterpenoids

A portion of the residue obtained after the evaporation of the extract was dissolved in chloroform and a few drops of conc. sulphuric acid was added from the side of the test tube and allowed to stand. The appearance of red colour in the lower layer indicates the presence of the sterols or the appearance of golden yellow colour indicates the presence of triterpenes.

Further, a portion was treated with a few drops of acetic anhydride and around 1 mL of conc. sulphuric acid was added from the sides of the test tube. The formation of a reddish-brown or bluish-green ring indicates the presence of sterols or triterpenes[26].

Detection of flavonoids

The extract was dissolved in alcohol and subjected to Shinoda test[27]. Few fragments of magnesium ribbon and a few drops of conc. hydrochloric acid was added to the alcoholic extract. The appearance of the magenta colour indicates the presence of flavonoids.

Detection of alkaloids

The residue was dissolved in alcohol and treated with a few drops of Dragendroff's reagent, the formation of a reddish-brown precipitate indicates the presence of alkaloids[17,28].

Detection of phenolic compounds and tannins

The extract was dissolved in alcohol and to a small portion, 10% ferric chloride (freshly prepared) solution was added. The appearance of blue or brownish-green colour indicated the presence of phenolic compounds. For the identification of tannins, a portion of the extract was dissolved in water and a 10% ferric chloride solution was added. The appearance of blue-black or blue-greenish colour/precipitate indicates the presence of tannins[29,30].

Thin layer chromatographic (TLC) analysis

The methanolic extract was fractionated with petroleum ether and applied on precoated silica gel 60 GF_{254} TLC plate (E Merck) and developed in a solvent system containing toluene:methanol (96:04). The plate was sprayed with anisaldehyde-sulphuric acid reagent and heated at 110 °C for 3-6 min to reveal the spots[31].

High resolution-mass spectrometry (HR-MS) analysis

HR-MS analysis of SRPE was carried out at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology-Bombay, Powai, Mumbai (India). Methanolic extract of the plant was fractionated with petroleum ether to get sterol-rich extract and filtered. The filtrate was subjected to the HR-MS analysis using the HR-MS: Q-TOF-MS B.05.01 version of the instrument and 100% acetonitrile with 0.1% formic acid, was used as mobile phase. The sample was injected directly via syringe pump in the +ESI (Electron spray ionization), ionization scanner to achieve the fragmentation and scanned in Full scan MS mode[32,33]. The spectra were recorded for 2 min in the range of 100-950m/z in +ve mode using 175.0 voltage, which is the optimum voltage to generate ion-source fragments. The identification of mass fragments was done by matching with spectral libraries, as per METLIN database using 6200 series, TOF/6500 series software.

Statistical analysis

The experiments were conducted in triplicate and data were analyzed as mean±SD using MS Excel 2007.

Results and Discussion

Phytochemical screening

Phytochemical screening is of special significance, it directs the presence of important chemical groups in the herbal drugs. Phytochemical screening of methanolic extract of *B. laciniosa* showed the presence of carbohydrates, proteins, saponins, phytosterols, triterpenoids flavonoids, alkaloids, phenolic compounds, and tannins.

Proximate analysis

Proximate analysis signifies the content of the crude drugs in the form of ash value which represents inorganic content, extractive values, indicating the content of chemical constituents, whereas LOD and FOM are the quality parameters[23]. The values of proximate analysis are given in Table 1.

TLC analysis

TLC analysis of SRPE showed the presence of six major spots of green, brown, purple, light purple to violet colour in the range of 0.2-0.91 Rf values (Fig. 2) after derivatization with anisaldehyde-sulphuric acid reagent[31]. It confirms the presence of sterols, triterpenoids or related compounds in the extract.

Chemical profiling of SRPE extract

High resolution-mass spectrometry (HR-MS) was carried out to identify the chemical constituents present in the SRPE extract of *B. laciniosa*. Identification of compounds was done with respect to their molecular formula, molecular mass, and molecular structure by comparing them with known spectra, available in a spectral library. Thus, the identification of compounds is based on the particular mass of ion and its match within the available database. The spectrum produced shows the presence of various peaks, characteristic of many phytochemicals[34,35]. HR-MS spectra of *B. laciniosa* with various peaks of chemical constituents is shown in Fig. 3.

Table 1 — Proximate analysis of *B. laciniosa* L.

S. No.	Parameters	Values (% w/w ± SD) (n=3)
1	FOM	0.60±0.2
2	Moisture content	5.61±0.27
3	Total ash	9.66±0.2
4	Acid soluble ash	1.33±0.12
5	Water soluble ash	3.65±0.11
6	Watersoluble extractive value	33.52 ± 0.21
7	Alcohol soluble extractive value	15.20 ± 0.18
8	Ether soluble extractive value	9.52± 0.17

n= Number of readings

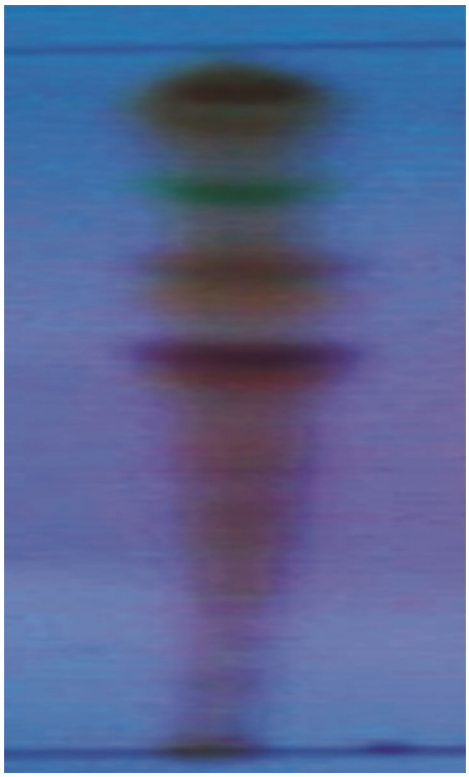

Fig. 2 — TLC profile of sterol-rich petroleum ether extract of *B. laciniosa*.

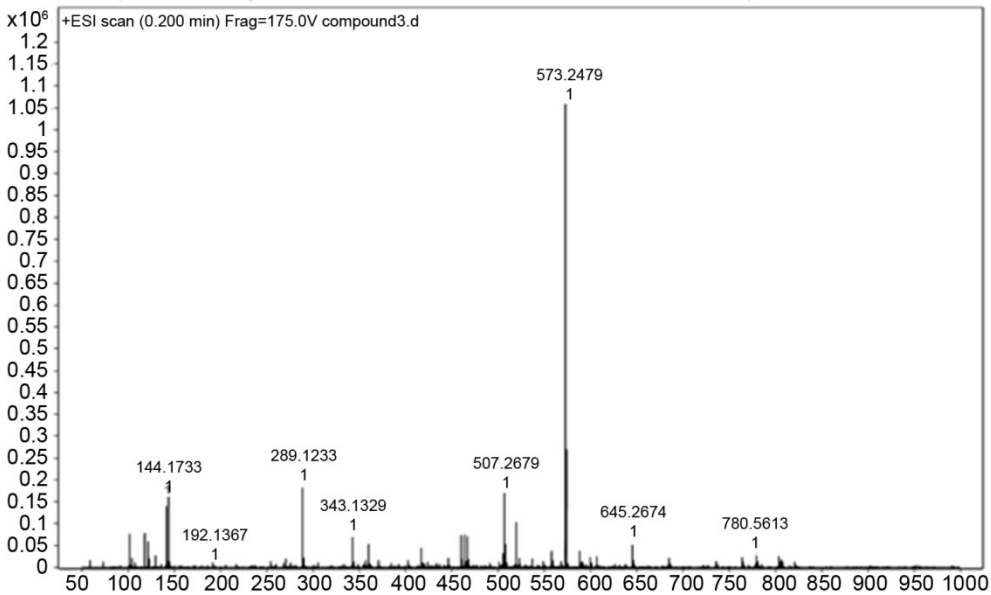

Fig. 3 — HR-MS spectra of *B. laciniosa* showing various peaks of compounds.

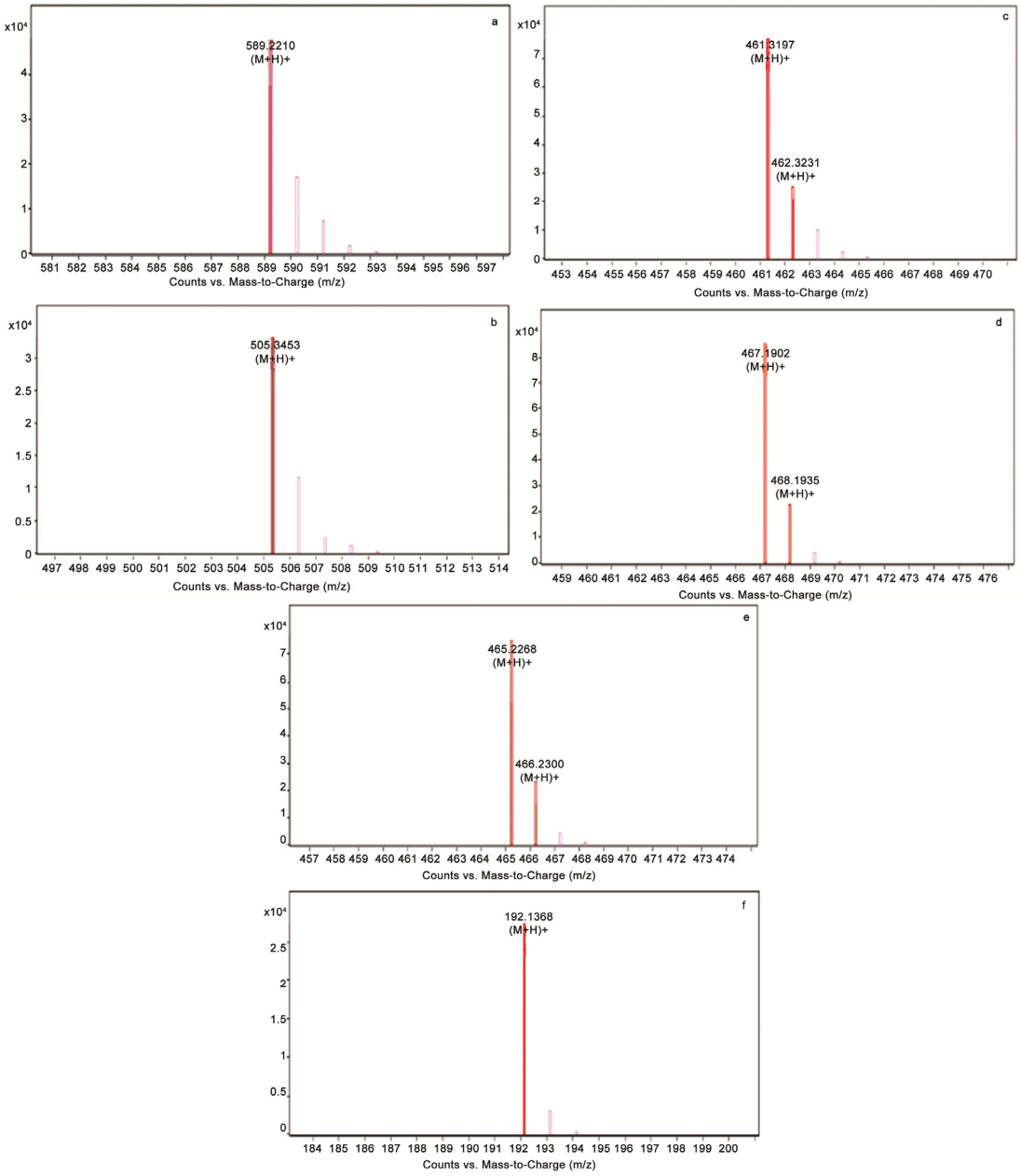

Fig. 4 — HR-MS spectra of major compounds, a) Swietenine, b) Ergosterol acetate, c) 4, 4 Dimethyl-14 α-formyl-5 α-cholesta-8, 24-dein-3β-ol, d) Mitoxantrone, e) Isogedunin, f) 3S-Aminodecanoic acid.

A Total of 30 compounds were identified in the SRPE extract of *B. laciniosa*. The major compounds identified were triterpenoids such as swietenine, ergosterol acetate,4, 4, dimethyl-14α-formyl-5α-cholesta-8,24-dien-3β-ol, mitoxantrone, isogedunin, 3S-aminodecanoic acid, and methacholine. Swietenine is a tetranortriterpenoid, isolated previously from the seeds of *Swietenia macrophylla* and shown to possess hypoglycaemic activity *in vivo* against type 2 diabetes in rats[36]. Ergosterol acetate and 4, 4, dimethyl-14α-formyl-5α-cholesta-8,24-dien-3β-ol, are triterpenoids. Ergosterol acetate is one of the active constituents, identified by GC-MS, in the pods of *Prosopis juliflora* and found to have anti-inflammatory activity[37].

The prominent peaks of major compounds identified by HR-MS in SRPE extract of *B. laciniosa*, and their structures are given in Fig. 4 and 5,

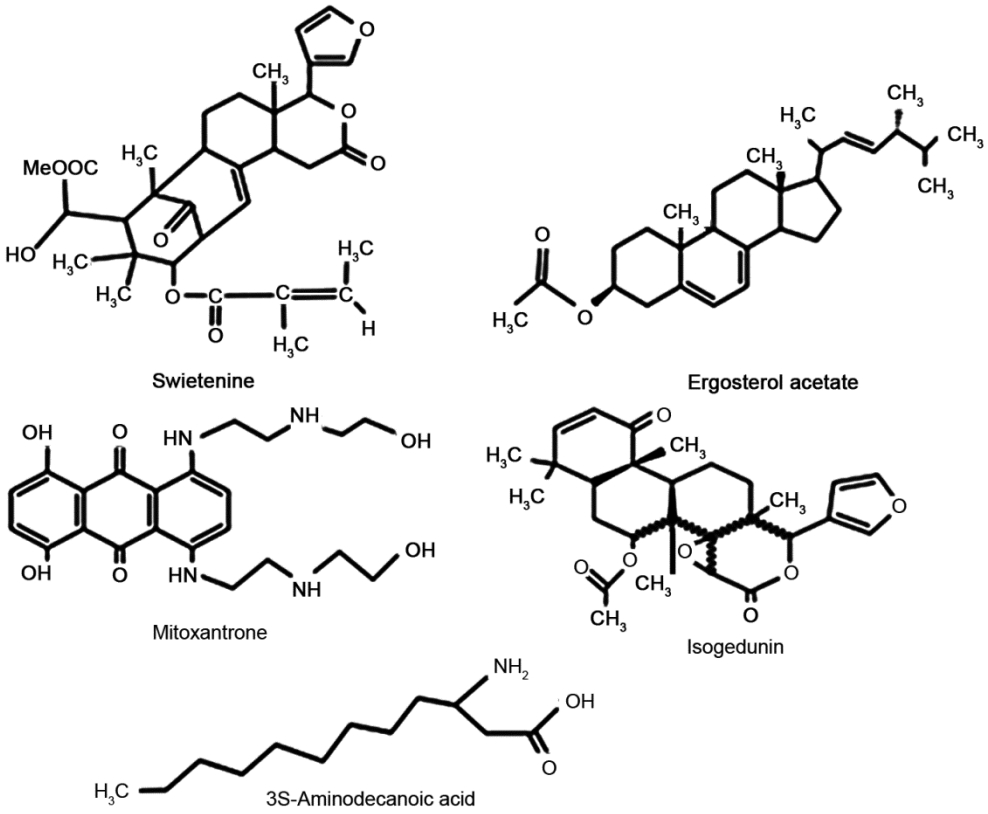

Fig. 5 — Structures of major chemical compounds identified by HR-MS technique in *B. laciniosa*.

Table 2 — Chemical compounds identified by HR-MS technique in *B. laciniosa* extract

S. No.	Name of the compound	Retention rate (tR in min)	Molecular formula	Molecular mass	Mass fragments (*m/z*)
1	Swietenine	0.19	$C_{32}H_{40}O_9$	568.2691	573.2477
2	Ergosterol acetate	0.193	$C_{32}H_{50}O_2$	466.3821	505.3453
3	4,4-Dimethyl-14a-formyl-5acholesta-8,24-dien-3b-ol	0.195	$C_{30}H_{48}O_2$	440.3671	461.3197
4	Mitoxantrone	0.191	$C_{22}H_{28}N_4O_6$	444.201	467.1902
5	Isogedunin	0.205	$C_{28}H_{34}O_7$	482.2301	465.2268
6	3S-aminodecanoic acid	0.322	$C_{10}H_{21}NO_2$	187.1581	192.1368
7	Methacholine	0.303	$C_8H_{18}NO_2$	160.1326	142.1214

respectively and details of identified compounds are given in Table 2.

Along with these 30 compounds, around four small peptide fragments were also identified by HR-MS by comparing the data of extract with the data available in the spectral library. All the four fragments are tripeptides and composed of essential and non-essential amino acids which are essentially involved in the photosynthesis, aquatic transport, and chitin-binding in the plants and some of the peptides have antimicrobial activity[4,38]. The details of identified peptides are given in Table 3.

Table 3 — Peptide fragments identified by HR-MS technique in *B. laciniosa* L. extract

Peptide fragments	Retention rate (tR in min)	Molecular formula	Molecular mass	Mass fragments (*m/z*)
Arg Glu Trp	0.189	$C_{22}H_{31}N_7O_6$	489.2341	507.2679
Lys Pro Arg	0.197	$C_{17}H_{33}N_7O_4$	399.2602	417.294
Ser Ser Arg	0.299	$C_{12}H_{24}N_6O_6$	348.1731	349.1804
Asn Asp Asn	0.306	$C_{12}H_{19}N_5O_8$	361.1209	361.144

Conclusion

Present work signifies the presence of around 30 chemical compounds in sterol-rich petroleum ether

extract of *B. laciniosa* including valuable steroidal compounds and small fragments of tripeptides. The authors are reporting the identification of these chemical constituents for the first time from *B. laciniosa* plant. HR-MS provided the basic platform for the identification of these chemical compounds.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgement

The authors are thankful to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology-Bombay, Mumbai (India) for providing HR-MS facilities to carry out our work.

References

1 Srivastava J, Lambert J and Vietmeyer N, *Medicinal plants: An expanding role in development*, (World Bank Technical Papers), 1996, 1-36.

2 Emira N, Mejdi S, El Hassane A, Mousa A, Vajid N, *et al.,* HR-LCMS-based metabolite profiling of antioxidant, and anticancer properties of *Teuriumpolium* L. methanolic extract: Computational and *in vitro* study, *Antioxidants (Basel),* 2020, **9**(11), 1089.

3 Jia X, Hu C, Zhu X, Yuan Y and Zhou Y, Simultaneous determination of 25 ginsenosides by UPLC-HRMS via quantitative analysis of multicomponents by single marker, *Int J Anal Chem*, 2021, **2021**, 1-11.

4 Subhash K T, Shriniwas P P and Hemant D U, Phytochemical analysis of *Canna indica* L. roots and rhizomes extract, *Biochem Biophys Rep*, 2018, **16**, 50-55.

5 Bertrand R, Quantitative and qualitative LC-high-resolution MS: The technological and biological reasons for a shift of paradigm, in *Recent advances in analytical chemistry*, (Intech Open), 2018.

6 Kirtikar K R and Basu B D, *Indian Medicinal Plants Plate part II*, 2nd edn, (International book distributors, Dehra Dun), 2005, Plate No. 464.

7 Shashikala M and Mamta S, Pharmacognostical and phytochemical analysis of different parts of *Bryonia laciniosa* L., *Int J Pharm Bio Sci*, 2018, **9**(4), 144-155.

8 Agharkar S P, *Medicinal plants of Bombay presidency*,(Scientific Publishers, Jodhpur), 1991, 44.

9 Gowrikumar G, Mani V V S, Rao T C, Kaimal T N B and Lakshminarayana G, *Diplocyclos palmatus* L.: A new seed source of punicic acid, *Lipids*, 1981, **16**(7), 558–559.

10 Mosaddik M A, Ekramul H M and Abdur R M, Goniothalamin from *Bryonopsis laciniosa* Linn (Cucurbiataceae), *Biochem Syst Ecol*, 2000, **28**(10), 1039–1040.

11 Singh V and Malviya T A, Non-ionic glucomannan from the seeds of an indigenous medicinal plant: *Bryonia laciniosa*, *Carbohydr Polym,* 2006, **64**(3), 481–483.

12 Gupta M, Mazumdar U K, Sivakumar T, Vamsi M L M, Karki S S, *et al*., Evaluation of anti-inflammatory activity of chloroform extract of *Bryonia laciniosa* in experimental animal models, *Biol Pharm Bull*, 2003, **26**(9), 1342-1344.

13 Sivakumar T, Kumar R S, Perumal P, Vamsi M L M, Sivakumar P, *et al*., Anti-tumour and antioxidant activities of *Bryonia laciniosa* against Ehrlisch's Ascites Carcinoma bearing Swiss albino mice, *Orient Pharm Exp Med*, 2005, **5**(4), 322-330.

14 Sivakumar T, Perumal P, Kumar R S, Vamsi M L M, Gomathi P, *et al.*, Evaluation of analgesic, antipyretic activity and toxicity study of *Bryonia laciniosa* in mice and rats, *Am J Chin Med*, 2004, **32**(4), 531-539.

15 Bonyadi R E, Awad V and Nirichan K B, Antimicrobial activity of the ethanolic extracts of *Bryonopsis laciniosa* leaf, stem, fruit and seed, *Afr J Biotechnol*, 2009, **8**(15), 3565-3567.

16 Jayaram R, Gnanasekharan D, Vijay D and Ranganathan T V, *In vitro* studies on anti-asthmatic, analgesic and anti-convulsant activities of the medicinal plant *Bryonia laciniosa* Linn, *Int J Drug Discov,* 2010, **2**(2), 1-10.

17 Parekh J and Chanda S, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants, *Afr J Biomed Res*, 2007, **10**, 175-181.

18 Kokate C K, *Practical Pharmacognosy*, (Vallabh Prakashan, New Delhi), 1999, 107-121.

19 Anonymous, *The Ayurvedic Pharmacopoeia of India*, *Part 1*, 1st edn, (Govt. of India, Ministry of Health and Welfare, Department of Indian System of Medicine and Homeopathy, New Delhi), 2001, 40-141.

20 Anonymous, *The Ayurvedic Pharmacopoeia of India, Part 1*, 1st edn, (Govt. of India, Ministry of Health and Welfare, Department of Indian System of Medicine and Homeopathy, New Delhi), 2001b, 143.

21 Anonymous, Determination of ash, in *WHO, Quality control methods for medicinal plant materials*, 1st edn, (A.I.T.B.S. Publishers and Distributors, Delhi), 2002a, 28.

22 Anonymous, Determination of extractable matter, in *WHO, Quality control methods for medicinal plant materials,* 1stedn, (A.I.T.B.S. Publishers and Distributors, Delhi), 2002b, 30.

23 Monika B, Anil B and Aakanksha B, Anatomical, physicochemical and phytochemical investigation of *Ceropegia bulbosa* var. *Lushii*, *Indian J Nat Prod Resour*, 2016, **7**(4), 314-322.

24 Trease G E and Evans W C, *Pharmacognosy*, 15th edn, (Saunder Elsevier, London), 2002, 193.

25 Mukharjee P, *Quality control of herbal drugs,* 5th edn, (Business horizons, New Delhi), 2002, 160-192.

26 Kapoor L D, Singh A, Kapoor S L and Srivastava S N, Survey of Indian medicinal plants for saponins, alkaloids and flavonoids, *Lloydia*, 1969, **32**, 297-302.

27 Farnsworth N R, Biological and phytochemical screening of plants, *J Pharm Sci*, 1966, **55**(3), 225-276.

28 Harborne J B, *Phytochemical methods: A guide to modern techniques of plant analysis,* 3rd edn, (Chapman and Hall, London),1998, 302.

29 Harborne J B, *Textbook Phytochemical methods*, *a guide to modern technique of plant analysis*, 1st edn, (Chapman and Hall Ltd, London), 1998, 21-72.

30 Wilson J A and Merrill H B, i*n analysis of leather and materials used in making it*, Ist edn, (The McGraw-Hill Book Co. Inc, New York), 1931, 290.

31 Wagner H and Bladt S, *Plant drug analysis-a thin chromatography atlas*, (Springer Verlag, Berlin, Heidelberg), 2004, 195-197.

32 Rowshanul M H, Farjana N, Matiar R, Ekramul M H and Rezaul M K, Isolation of stigmasterol and β-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn), *Pak J Biol Sci*, 2007, **10**, 4174-4176.

33 Anton K, The current role of high-resolution mass spectrometry in food analysis, *Anal Bioanal Chem,* 2012, **403**(5), 1233-1249.

34 Prashant N and Janardan L, Phytochemical profiling using HRLCMS and evaluation of antioxidant and antibacterial activities of *Nepalese* medicinal plants, *Vegetos*, 2020, **33**, 628-640.

35 Lucci P, Saurina J and Nunez O, LC-MS and LC-HRMS analysis and characterization of polyphenols in food, *TrAC-Trends Anal Chem*, 2017, **88**, 1-24.

36 Dewanjee S, Maiti A, Das A K, Mandal S C and Dev S P, Swietenine: A potential oral hypoglycemic from *Switeniamacrophyla* seed, *Fitotherapia,* 2009, **80**(4), 249-251.

37 Shazia K M, Maqsood A and Farah K, Identification of novel anticancer terpenoids from *Prosopis juliflora* (Sw) DC (Leguminosae) pods, *Trop J Pharm Res*, 2018, **17**(4), 661-668.

38 Rogozhin E A, Vasilchenko A S, Barashkova A S, Smirnov A N, Zavriev S K, *et al.*, Peptide extracts from seven medicinal plants discovered to inhibit oomycete *Phytophthora infestans,* a causative agent of potato late blight disease, *Plants,* 2020, **9**, 1294.