Pharmacogn. Res., 2024; 16(2):414-422.
https://www.phcogres.com

Original Article

Micromorphology, Physiognomies and Bio-element Analysis of *Launaea nudicaulis* (L.) Hook. f.

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ABSTRACT

Background: *Launaea nudicaulis* (L.) Hook. f. is a glabrous perennial herb having simple, sessile leaf and yellow flower. In Ayurveda it is used as a botanical source of *Gojihva*. The herb is said to be useful as a *Shaka* (vegetable) and medicine for various diseases like *Raktapitta* (hemorrhagic disorders), *Shwaasa* (breathing problems), *Kasa* (cough), *Kushtha* (skin diseases), *Vrana* (wounds), *Visarpa* (erysipelas), *Prameha* (diabetes mellitus), and *Aruchi* (anorexia). Few researches have also been published on its analgesic, anti-inflammatory activity, anti-diabetic and wound healing but its micromorphology, pharmacognosy, physiochemical and bio-element analysis is not reported yet. **Objectives:** The present study was carried out to study the micromorphology, pharmacognosy, physiochemical and bio-element analysis for proper identification and authentication of the herb. **Materials and Methods:** The macroscopy, microscopy, physicochemical, phytochemical parameters and bio-element analysis were done using different chemicals and reagents with standard procedures. **Results:** The presence of golden yellow latex in the root of the herb is identification characteristic. Calcium oxalate crystals and stone cells were seen in powder study. The GC-MS analysis identified 25 metabolites which comprised of fatty acids, sugars, amino acids etc. HPLC analysis revealed presence of 10 metabolites among which Cichoric acid was found in maximum concentration. **Conclusion:** The results of the study will help for the proper identification and authentication of the herb.

Keywords: Ayurveda, GC-MS, *Gojihva*, HPLC, Pharmacognosy.

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Received: 01-12-2023;
Revised: 18-01-2024;
Accepted: 20-02-2024.

INTRODUCTION

A scientific review suggests that 30% plants mentioned in Ayurveda have controversial identity and *Gojihva* is one among them.[1] In recent researches various source plants have been used as *Gojihva* particularly *Launaea* species like *pinattifeda, procumbens*, and *nudicaulis* of Asteraceae family.[2,3] Asteraceae family is one of the largest flowering family which includes 25000 plant species.[4] Adulteration and substitution is a major issue in herbal raw material which hampers the quality. Pharmacognostical studies are one of the most reliable sources to solve the controversy as well as to detect adulteration and substitution. Pharmacognosy also includes identification, authentication and standardization on the basis of physicochemical and phytochemical analysis of medicinal plants.[5]

In Ayurveda classics the herb *Gojihva* is mentioned for internal and external use for prevention and treatment of various diseases.[3] Studies also suggests that *Launaea nudicaulis* (L.) Hook.f. [L. N] has anti-diabetic,[6] anti-bacterial, anti-fungal[7] and wound healing activity.[3] In folk medicine leaves of the herb are used for the treatment of fever, skin problems like itching, eczema, swelling, cuts and ulcers.[8] The chemical profile of ethanolic extract of L. N leaves has been reported[8] but the definitive morphological, pharmacognostical, physicochemical, phytochemical, and bio-element analysis is not reported. Thus, in present study pharmacognostical, physiochemical, phytochemical, bio-element analysis of the L. N is carried out with standard procedures which may be helpful for further reference standard for identification and authentication of L. N.

MATERIALS AND METHODS

Collection and authentication of herb

The herb *Gojihva* [*Launaea nudicaulis* (L.) Hook. f.] was collected from natural habitat of south Delhi in the month of October-November 2018. The plant was authenticated by Botanical Survey of India, Dehradun with Accession number - 118605 (Annexure 1).

DOI: 10.5530/pres.16.2.51

Macroscopic Evaluation

Various macroscopic observations of the entire plant such as size, shape, color, surface appearance, texture, margin, fracture and venation were recorded.

Microscopic Evaluation

Fresh herb was used for microscopic evaluation, the sections were stained in safranine, fast green solution and pure Tetra Butyl Alcohol (TBA) respectively. The sections were mounted in canada balsam and observed under Axiocam ICc5 camera microscope.

For powder study, the whole herbal material was dried and crushed to attain a fine powder. It was soaked in water overnight and centrifuged at 6000 rpm for 5 min. 8-10 slides were prepared taking different layers from the centrifuged matter and observed under microscope.

Physiochemical Evaluation

The shaded dried powder of the herb was used for quantitative determination of physiochemical values such as total ash, acid insoluble ash, moisture content (loss on drying), alcohol extractive and water extractive values as per guidelines mentioned by Ayurvedic Pharmacopoeia of India.[9]

Phytochemical Investigation

All the chemicals used were of analytical grade procured from Merck and Co. Inc. HPLC-grade methanol, acetonitrile, trifluoracetic acid, and acetone were purchased from SRL chemicals (Mumbai, India). All standard reference metabolites, N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA), and methoxyamine hydrochloride were purchased from Sigma-Aldrich Chemical Co. Ltd., (Bangalore, India).

Phytochemical investigation of hydroalcoholic extract of whole plant was carried out according to standard procedure C.K. Kokate *et al.*[10]

Metabolite profile by GC-MS

Sample preparation

The sample preparation for the GC-MS analyses of major primary and secondary metabolites are carried out by recently reported method.[11] One-gram dry powdered herbal tissue was crushed in liquid nitrogen and then macerated with 10 mL of acidified methanol (0.1% HCl). 2-phenylphenol was added (7 µL from 1 mg/mL stock) as internal standard. The sample was kept under shaking for ~12 hr in a shaker at the room temperature (26 ± 2°C). Thereafter, the sample was centrifuged at 10,000 x g for 15 min. The supernatant was collected, passed through a 0.45 µm membrane filter and then used as source for GC-MS analyses of metabolites. The supernatant was either used freshly for GC-MS analyses or stored at -20°C. An aliquoted supernatant (500 µL) was collected in a new 1.5 mL microcentrifuge tube and then dried in a vacuum concentrator (Eppendorf Concentrator plus, Germany). Finally, dried material was subjected to double derivatization for GC-MS analyses. The double derivatization was carried out through first derivatization by adding 35 µL MeOX (20 mg/mL methoxyamine hydrochloride (MeOX) dissolved in pyridine) for 120 min at 37°C and followed by second derivatization with 50 µL of N-methyl-N-(trimethylsilyl)-trifuoroacetamide (MSTFA) for 30 min at 37°C. Derivatized mixture was then centrifuged at 12000 x g for 15 min and supernatant was used for GC-MS injection.

GC-MS analyser and technique

GC-MS analysis was carried out in an Agilent 7890B gas chromatograph coupled with an Agilent 5975B mass detector (Agilent technologies, CA, USA). A HP-5 ms column (5% phenyl methyl polysiloxane: 30 m length x 0.25 mm i.d. x 0.25 µm thickness; Agilent technologies, CA, USA) was used for the separation of metabolites with helium as a carrier gas with flow rate of 1 mL/min. 1µL sample was injected in a split less mode. The injector and transfer line temperatures were operated at 280°C and 280°C, respectively. The oven temperature programmed with an initial temperature of 70 to 270°C at a heating rate of 4°C/min, after one-minute hold at 270°C, temperature was increased to 300°C with ramp rate of 10°C/min, finally a hold for 15 min at 300°C followed by a 7 min solvent delay. The mass detector was operated at the ionization energy of 70 eV with scanning range at m/z 28-750 amu and scanning speed of 0.34 s. MS Source and MS Quad temperature programmed with 230°C and 150°C respectively. The data acquisition, instrument control and peak quantitation were performed using the Agilent Mass Hunter Worktation (B.09.00 Software) and Agilent Chemstation software (F.01.03.2357) respectively. Metabolites were identified on the basis of a comparison of retention time and mass spectra with those of authentic standards and also by matching with mass-spectral fragmentation pattern of NIST17 (National Institute of Standards and Technology, Gaithersburg, MD, USA) library. Sample was analysed in triplicate.

HPLC analyses of major secondary metabolites

HPLC analysis was carried out on a Waters HPLC system (Milford, MA, USA) consisting of 1525 binary pump and 2998 photodiode array detector (PDA). The separation was achieved by using a reverse-phase column (4.6x150 mm; Symmetry® C_{18} 5 µm) using a gradient method. The mobile phase consisting of gradient elution with solvent-A (1% aqueous acetic acid) and solvent-B (50% methanol and 50% acetonitrile). The gradient elution conditions were: from 5 to 10% B in 3 min (flow rate 1 mL/min), 10 to 50 % B in 77 min (with flow rate 0.5 mL/min), 50 to 100 % B in 2 min (with flow rate 1 mL/min), 100 % B for 10 min (with flow rate 1 mL/min), 100 to 5 % B in 2 min (with flow rate 1 mL/min), 5 % B for 5 min (flow rate 1 mL/min), then finally 100 to 5% B in 97 min (with flow rate 1 mL/min). The injection volume was 20 µL. Data acquisition and analyses were performed

by Empower-3™ software from Waters. The identification of metabolites was confirmed by comparing the UV-spectra and retention time of each peak with authentic standards or available spectrum data in the literature. The detection wavelength of the scan was set at 210 to 800 nm and the chromatogram was monitored at 280nm.

OBSERVATIONS AND RESULTS

Macroscopic evaluation

Root

The root was cylindrical, tortuous, and nodular divided at the top to give rise to separate bunch of leaves. It was approximately 7-10 cm in length with 0.5-0.7 cm in diameter. The external surface was very thin and was dark brown in color. The internal surface of root was pale yellow in color (Figure 1).

Stem

The stem of L.N. was not differentiated (Figure 1).

Leaf

The leaves were simple, sessile, lobate with serrated margins. The ventral side was slightly dark green in color and dorsal side was bright green in color with prominent venation at dorsal side. It had a main central vein with reticular venation. The length of leaves was approximately 10-20 cm and its width was 1-2.5 cm (Figure 1).

Flower

The flower was yellow in color when fresh and white when dried; with persistent calyx having a total of 8 sepals. Pappus hairs were soft, white, and copious. Sepals were combined and of green in color. Petals were whitish yellow in color and they were fused with each other. Base of anther was seen fused with petals (Figure 1).

Fruit

Fruit is Achene type.

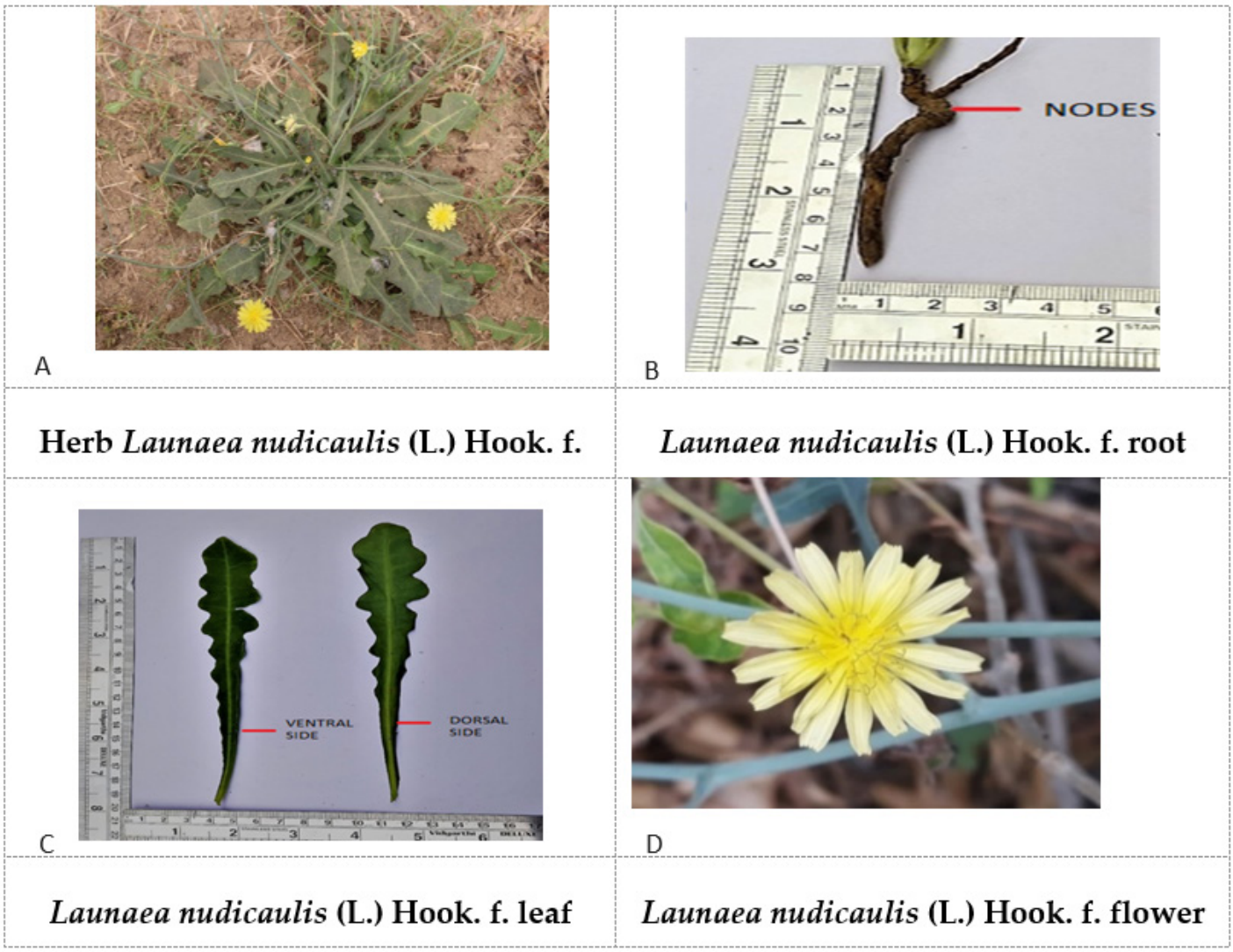

Figure 1: (a-d) Macroscopic features of the herb *Launaea nudicaulis* (L.) Hook. f.

Organoleptic Evaluation

The leaves and roots of *Launaea nudicaulis* (L.) Hook. f. has bitter and astringent taste with yellow latex secretion. The outer covering of the root was dark brown in color while it's inside structure was pale yellow in color. The fracture of the root was fibrous. The plant did not possess any specific odour.

Microscopic Evaluation

Transverse Section (T.S.) of root of L. N

The T.S. showed a circular outline. Epidermis was single layered. Cortex was made up of 8-10 layers of thin walled parenchymatous cells with limited patches of cells having ergastic matter. Vascular bundle consisted of polygonal thin-walled parenchyma and ring of xylem. The xylem vessels sclerenchyma was interrupted with many seriate medullary rays. Phloem found in patches outside the xylary rays. Pith consisted of thin-walled large parenchyma (Figure 2).

T.S. of stem of L. N

The T.S. of the stem showed a circular outline with cuticle layer. Epidermis was single layered with compactly arranged cells, followed by 5-7 layered hypodermis having parenchymatous cells. It was followed by parenchymatous endodermis and 6-8 vascular bundles of different sizes. The cortex had parenchymatous cells followed by irregular central pith. The vascular bundle was collateral and the protoxylem was 3-4 cell layered (Figure 2).

Transverse section of leaf of L. N

The T.S. of leaf showed single layer epidermis with thick cuticle in the abaxial surface. Mesophyll of midrib consisted of vasculature containing three to five vascular bundles. Central bundle was relatively larger. The outer most layer of the phloem comprised of few darkly stained idioblasts. Xylem consisted of net like vessels. Leaf margin mesophyll was made up of compact spongy tissues (Figure 2).

Powder microscopy

The powder microscopy of the herb L.N showed pitted vessels which signified the presence of root part. Spongy mesophyll with stomata embedded in it denoted towards leaf part along with trichome with numerous cells and single larger base cell. Fibers were also found in the sample which represented the stem part. Group of sclerenchyma, Sclerieds and Stone cells were largely observed. Brachy Sclerieds and scleroids were distinctively seen (Figure 3).

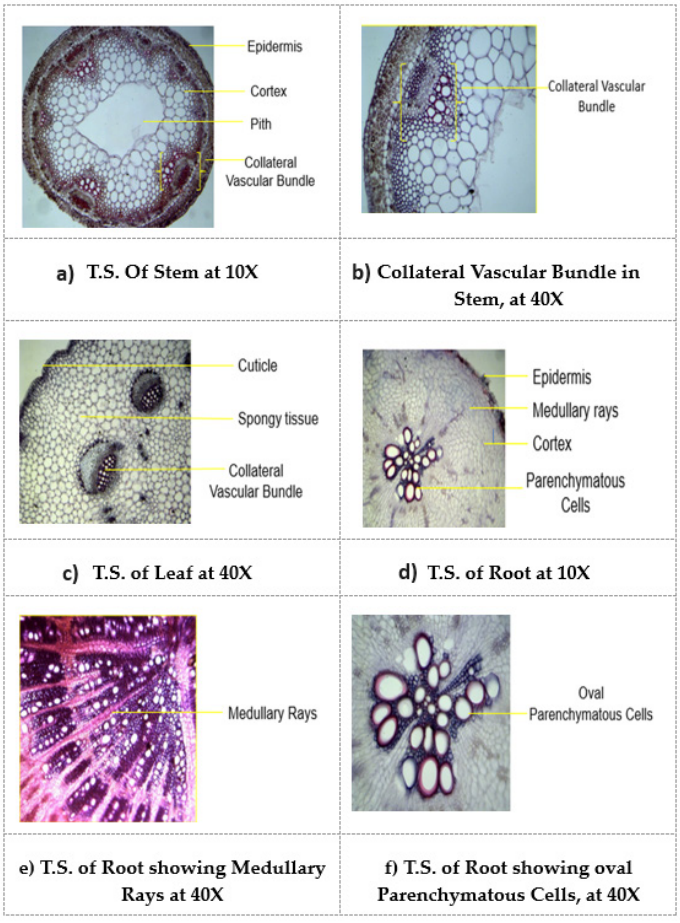

Figure 2: (a-f) Microscopic characteristics of the plant *Launaea nudicaulis* (L.) Hook. f.

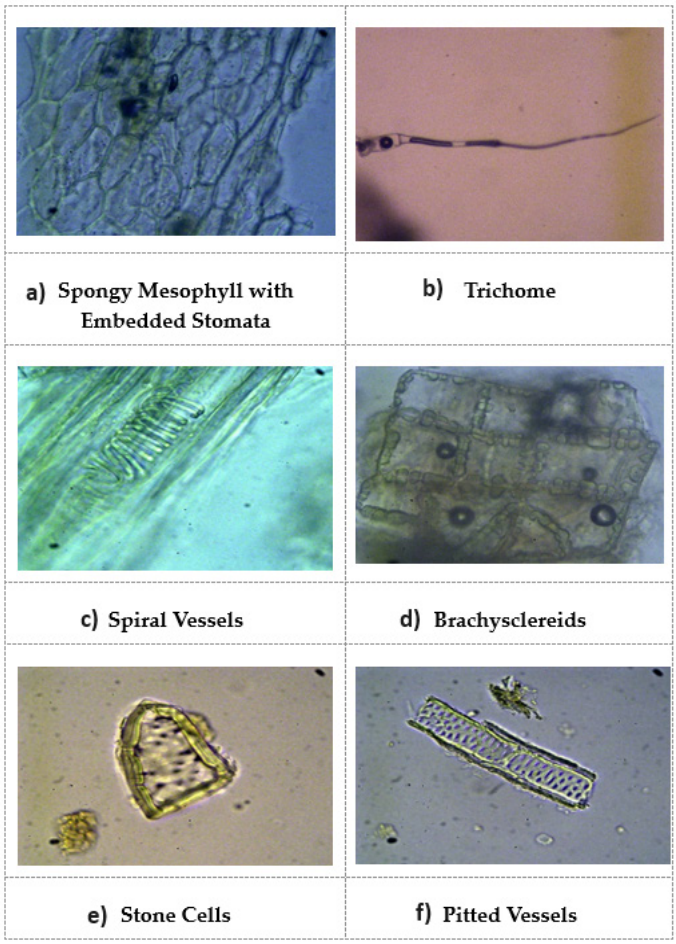

Figure 3: (a-f) Powder Microscopic characteristics of the plant L. N.

Physiochemical Study

The Physiochemical parameters are mentioned in (Table 1).

Phytochemical Study

The Phytochemical Study was done of Hydro-alcoholic extract of *Launaea nudicaulis* (L.) Hook. f., showed that the plant is rich in Steroids and Glycosides (Table 2).

GC-MS analysis of primary and secondary metabolites

Using GC-MS analyses, total 25 metabolites were detected in the L. N whole plant dried powder. (Figure 4) Out of 25 metabolites, six organic acids, four amino acids, three sugars, four phenolic acids, two fatty acids and seven sugar alcohols were detected as shown in Table 3 and Figure 5.

HPLC analysis of major secondary metabolites

HPLC analyses of the L. N whole plant dried powder showed six phenolic acids (gallic acid, chlorogenic acid, caffeic acid, ferulic acid, Cichoric acid, and sinapic acid), two flavonoids (rutin hydrate and vitexin) and two coumarins (scopolin and scopoletin) (Figures 6 and 7). Among phenolic acids, ferulic- and chlorogenic acids were present in higher amount, whereas rutin hydrate and cichoric acid was found to be predominant flavonoids as shown in Table 4.

Table 1: Physicochemical characters of dried powder.

Sl. No.	Parameters	Result
i.	Loss on Drying at 110°C	10.24% w/w
ii.	Total Ash	13.96% w/w
iv.	Acid Insoluble Ash	1.06% w/w
v.	Alcohol Soluble Extractive	9.50% w/v
vi.	Water Soluble Extractive	22.95% w/v

Table 2: Phytochemical characters of hydro-alcoholic extract of *Launaea nudicaulis* (L.) Hook. f.

Sl. No.	Qualitative Phytochemical Screening		Presence
i.	Alkaloids	Mayer's test	-
		Wagner's Tests	
		Dragendroff's test	
ii.	Flavonoids	Ferric chloride test	-
iii.	Steroids (Triterpenes)	Salkowski Tests	++++
		Lieberman Burchardt tests	
iv.	Phenol	Ferric chloride test	-
v.	Tannins	Ferric chloride test	-
vi.	Glycosides	Kellar Killani's test	++++
vii.	Saponins	Foam test	-

DISCUSSION

Plants are useful as dietary supplements for maintaining good health.[11] *Launaea nudicaulis* (L.) Hook. f. known as *Gojihva* in Ayurveda is the herb ascribed as medicine and food for the management of variety of ailments i.e. fever, diabetes, wound etc.[3,8] but the results are expected if the herb is genuine.

Organoleptic characteristics, microscopy, physicochemical analysis and phytochemical studies are important as they are tool for drug identification and authentication. Thus, all these parameters are considered in our study. The yellow latex is main characteristic features for organoleptic evaluation of the plant. In microscopic study presence of multicellular trichomes, stone cells, spiral and pitted vessels can be appreciated and these characteristics of microscopic study of the herb may be used to identify the plant (Figure 3).

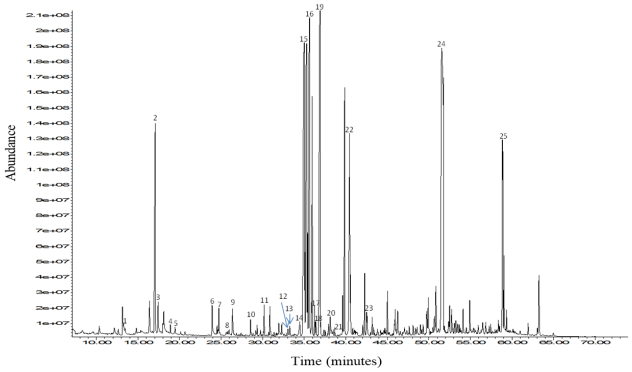

Figure 4: Typical GC-MS chromatogram (TIC) of *Launaea nudicaulis* (L.) Hook. f. sample. Key to peak identity: 1: Butanedioic acid; 2: Glyceric acid; 3: Malic acid: 4: Tartaric acid; 5: Shikimic acid; 6: L-Leucine; 7: L-Proline; 8: Phenylalanine; 9: L-Valine; 10: D-Fructose; 11: D-Glucose; 12: Sucrose; 13: Quininic acid; 14: Ferulic acid: 15: Caffeic acid; 16: Chlorogenic acid; 17: Palmitic Acid; 18: Stearic acid; 19: Glycerol; 20: Erythritol; 21: Xylitol; 22: D-Pinitol; 23: D-Mannitol; 24: D-Sorbitol; 25: Myo-Inositol.

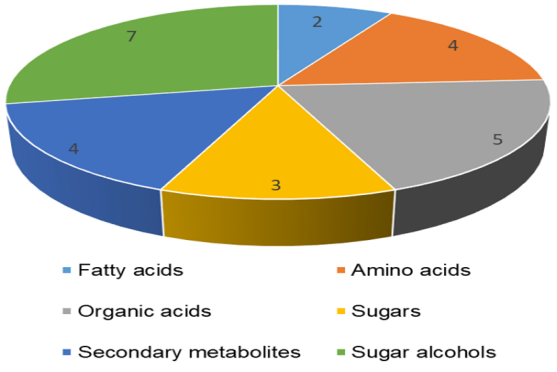

Figure 5: Number of identified metabolites within each class.

Table 3: List of 25 identified major metabolites from *Launaea nudicaulis* (L.) Hook. f. sample by GC-MS analyses. The peak area of the quantification ion was used for comparative quantification. Data are mean of three independent runs.

Sl. No.	Retention time (min)	Metabolites	Type of metabolites	Quantification (µg/g DW)
1.	13.2	Butanedioic acid, 2TMS derivative	Organic acids	4.2
2.	18.9	Glyceric acid, 3TMS derivative		3.8
3.	23.916	Malic acid, 3TMS derivative		4.6
4.	28.544	Tartaric acid, 4TMS derivative		3.6
5.	33.054	Shikimic acid, 4TMS derivative		3.9
6.	13.294	L-Leucine, TMS derivative	Amino acids	2.8
7.	17.404	L-Proline, 2TMS derivative		5.4
8.	25.657	DL-Phenylalanine, TMS derivative		2.4
9.	26.479	L-Valine, 2TMS derivative		2.7
10.	35.006	D-Fructose	Sugar	12.4
11.	35.578	D-Glucose		7.8
12.	51.462	Sucrose		10.6
13.	34.455	Quininic acid (5TMS)	Secondary metabolites	2.2
14.	39.35	Ferulic acid, 2TMS derivative		1.8
15.	40.462	Caffeic acid, 3TMS derivative		2.8
16.	58.843	Chlorogenic acid (6TMS)		8.6
17.	38.132	Palmitic Acid, TMS derivative	Fatty acids	4.4
18.	42.514	Stearic acid, TMS derivative		2.8
19.	17.065	Glycerol, 3TMS derivative	Sugar alcohols	10.8
20.	24.734	Erythritol, 4TMS derivative		2.4
21.	30.179	Xylitol, 5TMS derivative		1.8
22.	33.694	D-Pinitol		2.2
23.	36.252	D-Mannitol, 6TMS derivative		0.9
24.	36.407	D-Sorbitol, 6TMS derivative		0.8
25.	37.058	Myo-Inositol, 6TMS derivative		6.8

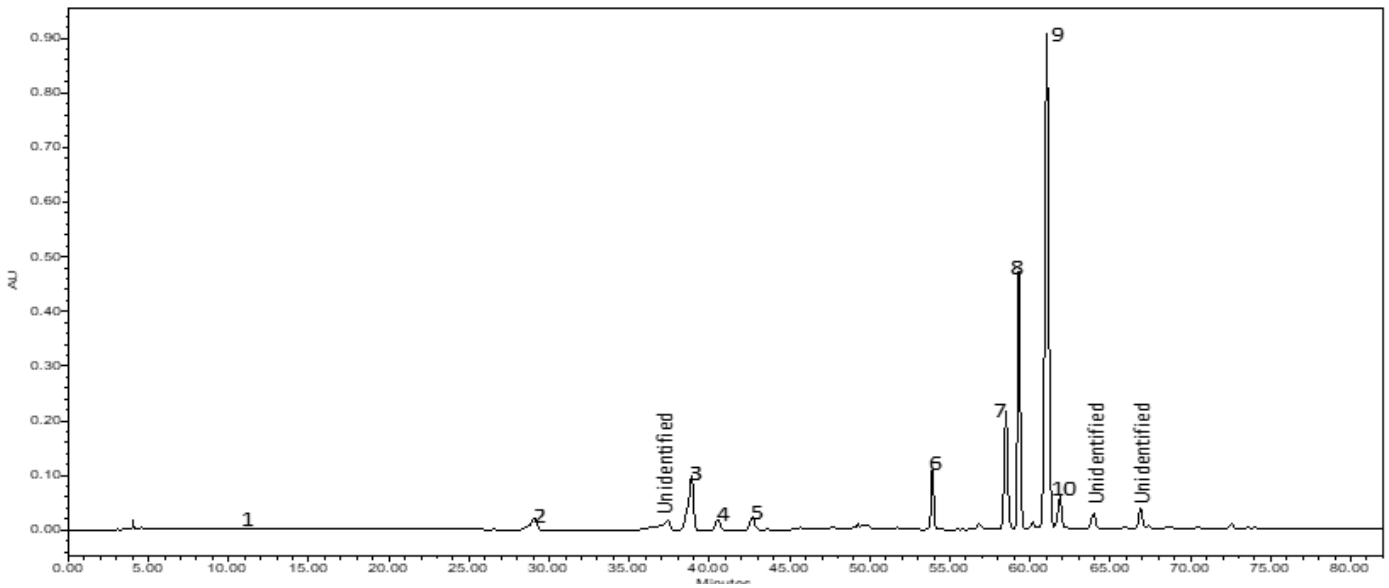

Figure 6: A representative HPLC chromatogram showing the detection of secondary metabolites from *Launaea nudicaulis* (L.) Hook. f.

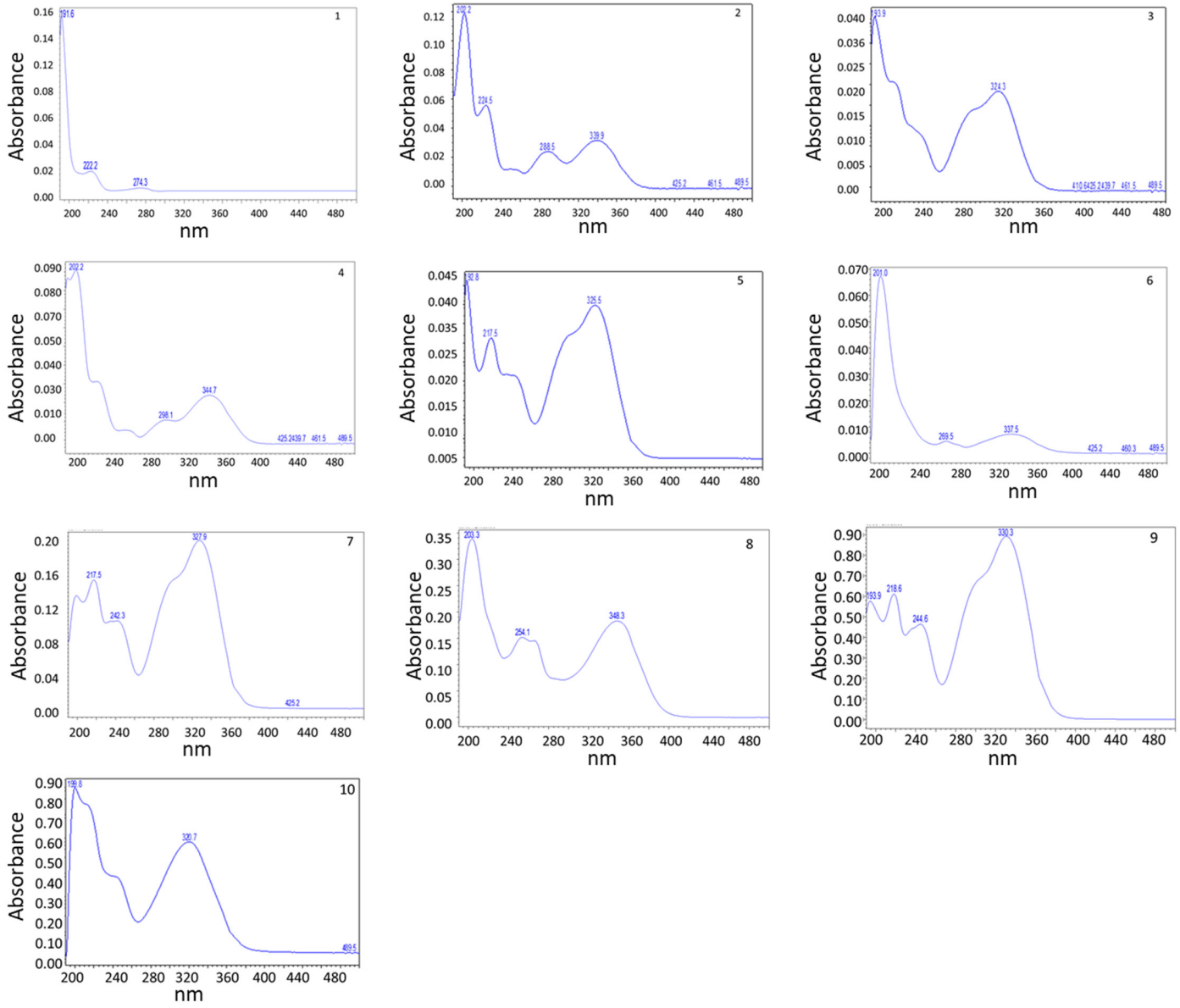

Figure 7: HPLC-DAD spectrum (1-10) of identified metabolites from sample *Launaea nudicaulis* (L.) Hook. f.

Less amount of moisture content in physio-chemical analysis indicates less hygroscopic nature of the drug and high-water soluble extractive indicates that the active constituents of the plant are water soluble and aqueous extract may be used for further quantification of chemical constituents.

Various organic acids, amino-acids, sugars, secondary metabolites, fatty acids and sugar alcohol has been identified in GC-MS analysis including malic acid, L-proline, D-fructose, chlorogenic acid, pamatic acid, glycerol in maximum concentration respectively in each category.

Several phenolic acids, flavonoids and coumarins could not be detected in GC-MS analyses, possibly due to their thermal instability, or due to low abundance of those metabolites.[12] Therefore, in addition to GC-MS analyses, secondary metabolites are analysed by HPLC. Among analytical tools HPLC is a powerful tool used to separate the compounds of a mixture and to identify, quantify and purify the single components of plant materials and their medicinal preparations.[13]

Among these metabolites, primary metabolites such as organic acids, amino acids, sugar and sugar alcohols were most prevalent, followed by the four phenolic acids. Primary metabolites are important nutritional constituents especially sugar alcohols which are known to prevent onset of several metabolic disorders, such as obesity.[14] Sugar alcohols are recently shown to be potential candidates for anti-viral molecule.[15] Among secondary metabolites, caffeic acid and chlorogenic acid is known to exhibit an array of biological activities.[16]

Table 4: Metabolites detected from sample *Launaea nudicaulis* (L.) Hook. f.

Sl. No.	Metabolite	Retention time (min)	Concentration (µg/g DW)	Remarks
1.	Gallic acid	9.50	4.71	
2.	Scopolin	29.08	297.39	
3.	Chlorogenic acid	38.87	1344.31	
4.	Scopoletin*	40.52	185.15	*Tentative
5.	Caffiec acid	42.67	91.04	
6.	Vitexin*	51.70	14.00	*Tentative
7.	Ferulic acid	59.07	1140.13	
8.	Rutin hydrate	59.28	679.85	
9.	Cichoric acid	61.03	6185.57	
10.	Sinapic acid	61.85	104.13	

The plant is found having rich triterpenoids and glycosides. Terpenoids are responsible for number of biological activities including antibiotics, anti-inflammatory, anti-HIV and anti-tumor effects; hypotensive agents; sweeteners; insecticides; anti-feedants; phytotoxic agents; perfumery intermediates; and plant growth hormones.[17]

Glycosides (molecules made up of carbohydrate) are also reported for having wide range of pharmacological activities such as antioxidant, anti-inflammatory, antihypertensive, and antidiabetic activities.[18]

CONCLUSION

Gojihva [*Launaea nudicaulis* (L.) Hook. f.] is a small herb, used as a medicine and wholesome vegetable in various disease conditions. Therefore, to establish the standardization parameters for authentication and identification of this herb is needed and the present study is first it's kind of attempt. The present study may serve as a base for further evaluation and establishment of this herb for benefit of mankind.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Bhargava Bhide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **HPLC:** High Performance Liquid Chromatography; **T.S.:** Transverse Section; **L.N:** *Launaea nudicaulis* (L.) Hook. f.

SUMMARY

Launaea nudicaulis (L.) Hook. f. is a glabrous perennial herb having yellow flower known as a source plant for *Gojihva* in Ayurveda. The herb is said to be useful as a *Shaka* (vegetable) and medicine for various diseases like *Raktapitta* (hemorrhagic disorders), *Shwaasa* (breathing problems), *Kasa* (cough), *Kushtha* (skin diseases), *Vrana* (wounds), *Prameha* (diabetes mellitus), and *Aruchi* (anorexia). Various pharmacological activities of the herb i.e. analgesic, anti-inflammatory, anti-diabetic and wound healing are reported but its analytical profile is not reported yet. Thus, the present study was carried out to study the micromorphology, pharmacognosy, physiochemical and bio-element analysis for proper identification and authentication of the herb. The presence of golden yellow latex in the root of the herb is identification characteristic. Calcium oxalate crystals and stone cells were seen in powder study. The GC-MS analysis identified 25 metabolites which comprised of fatty acids, sugars, amino acids etc. HPLC analysis revealed presence of 10 metabolites among which Cichoric acid was found in maximum concentration.

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Cite this article: Ghildiyal S, Kumar V, Bidhuri Y, Nesari TM, Sircar D. Micromorphology, Physiognomies and Bio-Element Analysis of *Launaea nudicaulis* (L.) Hook. f. Pharmacog Res. 2024;16(2):414-22.