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Identification of secondary metabolites, antioxidant activity, and GC-MS analysis of *Launaea procumbens* (Roxb.) Amin (Angiosperm: Asteraceae): A neglected plant with therapeutic value

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ABSTRACT: Although *Launaea procumbens* (Roxb.) Amin is not currently used by pharmacists; it has great potential for future medicinal use. According to both Ayurvedic texts and modern research, the leaves of *Launaea procumbens* have galactagogue, diuretic, antifungal, anorexic, anti-arthritic, and hepatoprotective properties. In this communication, various sections of *L. procumbens*, a member of the Asteraceae (Compositae) family is characterized pharmacognostically. Extracts were tested qualitatively for various constituents, revealing the presence of lignans, cardiac glycosides, alkaloids, phenolic compounds, tannins, steroids, and flavonoids. The leaves and stems were extracted using four different solvents: methanol, chloroform, distilled water, and petroleum ether, with methanol and chloroform extracts showing more significant results than petroleum ether and distilled water extract. These observations will aid in identifying and standardizing the drug in its crude form and distinguishing it from adulteration. This work aims to develop standardization parameters for *L. procumbens* leaves and stems by evaluating its pharmacognostics and conducting preliminary phytochemical screening. Additionally, GC-MS fingerprinting of the leaves revealed the presence of nine different chemical constituents, which could be useful for differentiating *L. procumbens* from other species of the genus Launaea in the herbal industry.

1. INTRODUCTION

Launaea procumbens, a member of the Launaea species, is known by several names including 'Bangobhi', Jungali Gobi, Moti bhonpathari, and Country dandelion. This herb grows flat on the ground and in crevices of semi-dry areas in India. It has served as a dietary supplement and a galactagogue and has been employed in treating rheumatism. Additionally, it has been utilized to treat a number of ailments in conventional medicine such as skin problems, tumors, and dysentery, as well as kidney disorders, hormonal imbalances, and sexual diseases. Some other names for *Launaea procumbens* include *Prenanthes procumbens* Roxb., *Launaea fallax* (Jaub. & Spach) Kuntze, *Zollikoferia fallax* (Jaub. & Spach) Boiss or Microrhynchus fallax Jaub. & Spach (Chopra et al., 1956). This plant is utilized in Ayurvedic and herbal remedies for a variety of purposes, including longevity, wound healing, reproductive ailments, and relieving painful urination. Additionally, it contains antipyretic, antioxidant, insecticidal, hepatoprotective, and antifungal properties (Khan et al., 2010). A thorough pharmacognostic and phytochemical study is necessary to standardize the raw material, extract, and formulations of *Launaea procumbens* due to the controversy surrounding its nomenclature and medicinal importance. Hyphenated techniques, such as GC-MS, have the ability to identify volatile phytoconstituents and are effective in determining the molecular weight and structures of unknown organic compounds within complex mixtures (Balasundram et al., 2006; Middleton et al., 2000). The use of chromatographic techniques is highly beneficial in identifying the specific species of herbs through their GC-MS chemo profiling. Our study focused on providing essential phytochemical and chemo-profiling data for *Launaea procumbens* (Roxb.) Amin by conducting in-vitro phytochemical screening and GC-MS analysis of the extract. The results of this research can prove valuable in the identification and standardization of the plant, its extracts, and formulations.

1.1. Distribution

This species is frequently observed throughout the plains of India, and can even be sited at elevations of up to 2,400m in the Himalayas. It is primarily found in Bengal and Punjab but also

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in the southern regions of Sindh and the Deccan in India. This species typically inhabits sandy terrain.

1.2. Medicinal properties and applications of the plant

The *L. procumbens* plant possesses diuretic, soporific, and tonic properties, making it a valuable source of fodder for goats. In Bombay, it is also administered to buffaloes to enhance milk secretion. During times of scarcity, the leaves of the plant are consumed for their sand-binding properties. Locally, the leaves are added to curries, and the juice is used to alleviate rheumatic ailments. Moreover, the plant, with the exception of its roots, has insecticidal properties and is used to treat a variety of conditions, including inflammation, rheumatism, reproductive disorders, oxidative dysfunction in the kidney, and hormonal imbalances (Qureshi and Raza, 2008), and liver dysfunction (Khan et al., 2011).

Research conducted on the topic of nutrition has discovered that it is comprised of a powerful blend of 2-methyl-resorcinol, synergic acid, salicylic acid, gallic acid, and vanillic acid (Shaukat et al., 2003), which have anticancer, antioxidant, cardioprotective and neuroprotective effects (Balasundram et al., 2006; Middleton et al., 2000; Zhou et al., 2009). For centuries, people have used leaves for their numerous health benefits. They are believed to be effective in treating a variety of conditions, such as fever, toxemia, cancer, and swellings. A paste made from these leaves can be applied to rheumatism, boils, and other types of swellings. The entire plant, except for the root, is also used to treat kidney stones and body heat. In addition, the juice extracted from the leaves is known to be helpful in managing jaundice. Some people even mix the whole plant with ash and metal for even more potent effects against jaundice. Medicinal plants have been used for thousands of years as a natural source of medicine, contributing to the improvement of human life. Certain herbs, in particular, contain bioactive compounds like phenolic and polyphenolic compounds that are known to regulate the immune system. Herbs that are rich in flavonoids and phenolic content may also have anti-inflammatory and antioxidant properties (Bruneton, 1995; Tyler, 1994).

The World Health Organization (WHO) reports that herbal medicines are able to fulfill the health requirements of approximately 80% of the global population, particularly those residing in rural regions of developing nations. The literature cited in these articles focuses on the various pharmacological functions of phytochemicals (Khan et al., 2011, 2012,?). This research on the pharmacognostic characteristics of *Launaea procumbens* (Roxb.) Amin can serve as a valuable tool in the identification of potential adulterants and in the standardization of future investigations. The study thoroughly explores the plant's phytochemical profiles, antioxidant potential, and GC-MS analysis of its aerial parts, drawing on literature review and ethnopharmacological significance. Further research and re-validation of its use would be advantageous.

2. MATERIALS AND METHODS

2.1. Collection of plant sample

In July 2022, *Launaea procumbens* leaves and shoots were collected from the Banasthali Vidyapith campus and meticulously prepared both herbarium and voucher specimens. Subsequently, these specimens were officially deposited into the Department of Bioscience and Biotechnology and assigned the authentication number BURI-1629/2022. These valuable specimens are now housed within the Banasthali University Rajasthan India (BURI) Herbarium, which serves as a comprehensive resource for taxonomical and ethnobotanical information.

2.2. Preparation of plant material and extraction

The aerial parts of the plants were gathered and thoroughly washed with tap water and then autoclaved water to ensure they were free of any dirt. Next, the leaves and shoots were left to dry at room temperature for 10-15 days. Once completely dry, ground them up with an electronic grinder to create a fine plant powder. This powder was then stored in an airtight container at 4°C for future use (Mehra & De, 2017). An experiment was conducted using a Soxhlet assembly to extract compounds from 10 grams of powdered leaves and stems. Afterward, the sample was carefully placed in a Whatman filter paper thimble and inserted into the extraction chamber. Next, 150 ml of four different extraction solvents - methanol, chloroform, distilled water, and petroleum ether was added to the boiling flask and the Soxhlet apparatus was set to operate for 12 hours. Throughout the process, a constant ice-cold water flow was maintained in the condenser, and the heating mantle was set to keep the boiling flask at a temperature of 40-45 °C. Over the course of 20 to 25 cycles, the extraction solvent cyclically moved between the extraction chamber, the boiling flask, and the thimble. This movement was observed until the solvent in the extraction chamber became colorless.

2.3. Phytochemical Screening

To screen for phytochemicals, various solvents including methanol, chloroform, petroleum ether, and distilled water were used to prepare the extracts. Each solvent was evaporated at 40 °C with a heating mantle. Using standard methods, some of the extracts were screened from each solvent to identify the different types of active chemical components. The findings were categorized as (++) indicating a significant amount of phytochemicals, (+) for a small amount, and (-) for the complete absence of such a compound (Table 1).

2.4. Total phenolic content (TPC)

The estimation of Total phenolic contents (TPC) was done using the specific method of Singleton and Rossi (1965). To thoroughly evaluate each fraction, 200 microliters of it (dissolved in its respective solvent with a concentration of 1-5 mg/ml) were combined with ten milliliters of 1:10 folin-ciocalteu reagent. Following a 5-minute incubation, 7 ml

Table 1
Different tests performed for different phytochemicals

Phytochemical	Test	Procedure	Inference
Alkaloids	Dragendroff's test	Mix 0.5ml of extract with 2ml of Dragendroff's reagent diluted with HCL (provided by +1ml).	Orange precipitates
Steroids	Salkowski test	Mix 0.5ml of extract with 1ml of concentrated H_2SO_4.	Wine red color
Cardiac Glycosides	Keller – Killani test	To prepare the solution, mix 0.5ml of extract with a few drops of Glacial Acetic Acid. Boil and then cool the mixture before adding 2 drops of $FeCl_3$ solution. Slowly transfer the contents to a test tube containing 2ml of Concentrated H_2SO_4, pouring it gently down the side of the test tube.	A Reddish-Brown ring at the junction of two solvents
Phenols	Ellagic Acid test	Mix 0.5ml of extract with 2ml of a solution containing 5% (V/V) Glacial Acetic Acid and 5% (V/V) $NaNO_2$.	Muddy yellow/ Olive brown/ Niger brown/ Deep chocolate color
Tannins	Tannin test	Mix 0.5 ml of extract with 2 ml of 15% (W/V) Gelatin.	White precipitation
Lignans	Furfuraldehyde test	Mix 0.5 ml of extract with 2 ml of 2% (V/V) furfuraldehyde.	Red color
Flavonoids	Flavonoid test	Mix 0.5 ml of extract with a small amount of turnings, then add 2 ml of concentrated H_2SO_4.	Magenta color (flavonoids) / Scarlet color (flavones) / Deep cherry color (flavonoids)

of 0.115 mg/ml Na_2CO_3 was introduced, and the solution was incubated for an additional two hours. To determine the absorbance readings at 765 nm, gallic acid was implemented as the standard in the calibration curve. The results were then reported in mg gallic acid (GAE)/gram of dried plant extract, and the data for each fraction was meticulously recorded three times.

2.5. Total flavonoid content (TFC)

To determine the Total flavonoid content (TFC) method described by Sakanaka et al., 2005 was used. To prepare the samples, 0.25 ml of each fraction was combined (at a concentration of 1-5 mg/ml dissolved in its respective solvent) and Rutin standard solution (15-250 μg/ml) with 1.25 ml of distilled water in a test tube. Next, 75 μl of a 5% (w/v) sodium nitrite solution was added and waited for 6 minutes. Then added 150 μl of 10% (w/v) aluminum chloride solution and allowed the mixture to sit for another 5 minutes. Finally, we added 0.5 ml of 1 M NaOH and thoroughly mixed the contents. The mixture was adjusted to 2.5 ml with distilled water before use. The absorbance was promptly taken at a wavelength of 510 nm, and the outcomes of the samples were reported in milligrams of Rutin equivalents for the complete dried fractions. In order to ensure accuracy, each fraction was subjected to three tests.

2.6. Antioxidant Assays

2.6.1 DPPH radical scavenging

The DPPH assay was used to determine the antioxidant activity of crude extracts (Saklani et al., 2017). The reduction of the DPPH radical was indicated by a decrease in absorbance at 517 nm. Ascorbic acid was used as a point of reference. The following formula was used to determine the scavenging activity of radicals:

DPPH Scavenging activity (%) = (Absorbance of control − Absorbance of sample) / (Absorbance of control) × 100

In order to evaluate the antioxidant potential, the IC_{50} value was determined by measuring the concentration of the sample solution required to inhibit 50% of DPPH. To accomplish this, we utilized a regression equation with the extract concentration as the x-axis and the relative inhibition value as the y-axis.

2.7. Statistical analysis

The experiments were performed thrice and the average along with the standard deviation was presented. To evaluate the variations in IC_{50} of different antioxidant tests for various fractions, the ANOVA test was used with a minimum significance level (LSD) of $P < 0.01$. The experimental data was also examined for the correlation coefficient of phenolics and flavonoids with different antioxidant tests, using the student's test to establish significance ($P < 0.05$; $P < 0.01$). The graph pad prism software was used to compute the IC_{50} values.

2.8. GC-MS analysis

The analysis of the Methanol extract of the leaf of *L. procumbens* was done by performing GC-MS analysis. The carrier gas used was helium at a consistent flow rate of 3.2ml/min, and an injection volume of 1μl was employed with a split ratio of 10:1. The scan mass range was 50m/z - 650m/z. To conduct the GC-MS analysis of methanol extracts of *Launaea procumbens* (Roxb.) Amin leaves, Thermo Scientific Triple

quadruple GC-MS (trace 1300 GC, Tsq 8000 triple quadrupole MS) equipped with TG 5 MS (30 m 0.25 mm, 0.25 m) column was used. The ion source temperature was set at 230 °C, and the injector temperature was maintained at 25 °C. We kept the temperature in the oven at 50 °C. (Mehdi et al., 2020). An extensive review of the literature was conducted to identify the therapeutic benefits of phytoconstituents that were discovered through GC-MS analysis.

3. RESULTS

3.1. Phytochemical analysis

Several phytoconstituents were present in *L. procumbens* plant extracts are produced in different solvents. Methanolic plant extracts contain the highest amount of phenols, tannins, alkaloids, and flavonoids. At the same time, extracts made in chloroform had a lower quantity of tannins, flavonoids, steroids, phenols, and the least number of alkaloids. The cardiac glycosides are negligible in all the solvents. Little to no traces of selected variables were found in leaf and shoot extracts prepared with distilled water and petroleum ether (Table 2). Finally, the ideal outcome was achieved in the methanolic extract of plants.

3.2. Total phenol and flavonoid content

The presence of phenolics and flavonoid contents in different *L. procumbens* fractions are shown in Tables 3 and 4 . The maximum total phenolics concentration was found in the leaf methanolic extract (40 ± 0.0098) mg GAE/ whereas the lowest total phenolics content was found in the petroleum ether extract (14.81 ± 0.0015) mg GAE/g extract Methanolic extracts had the highest total flavonoid contents (33.1 ± 0.007) while petroleum ether extracts had the lowest concentration (16.51 ± 0.0075) mg equivalent Rutin/g of dry fraction.

Similarly in the case of shoot extracts the methanolic extracts possessed the highest total phenolics contents (37.24 ± 0.0084) mg GAE/g while petroleum ether comprised of lowest total phenolics content (8.5 ± 0.003) mg GAE/g extract. The highest amount of total flavonoids was found in methanolic extracts (27.41 ± 0.003) while the lowest concentration was found in extracts of petroleum ether (5.4 ± 0.002) mg equivalent Rutin/g of dry fraction. The extraction yield of these samples varied in descending order of methanol> chloroform > distilled water> petroleum ether fraction. For both leaves and shoots, the extraction yield with distilled water and petroleum ether was substantially lower ($P<0.01$) than that of the methanol and chloroform fractions, which produced the most significant amount of total extractable components.

3.3. DPPH assays

The evaluation of the scavenging activities of bioactive fractions in phytomedicine has frequently used the stable free radical DPPH. Using 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) free radicals, the scavenging capabilities of different fractions of leaf and shoot extracts were assessed. (Table 5). Results showed that the methanolic extract of leaf (IC_{50} 57.7 ± 0.06 μg/ml) possessed the highest antioxidant activity while extracts of petroleum ether had the lowest scavenging effect (IC_{50} 95.08 ± 0.8 μg/ml). Similarly in the case of shoot extracts the methanol extract of leaf (IC_{50} 52.74 ± 0.02 μg/ml) possessed the highest antioxidant activity while extracts of petroleum ether had the lowest scavenging effect (IC_{50} 87.05 ± 0.95 μg/ml). The extracts of leaf and shoot prepared in other solvents i.e., chloroform and distilled water also didn't show promising results. The more will be the IC_{50} value lesser the antioxidant activity. Since the IC_{50} value of methanolic extracts of leaf and shoot are less in comparison to other extracts, hence they show more antioxidant activity. The DPPH radical scavenging activities of the methanolic extracts were even less ($P < 0.01$) than those of ascorbic acid.

3.4. Correlation between total phenol and flavonoid contents, as well as the IC_{50} values for antioxidant activity

Shows the association (Pearson correlation coefficient = r) between the IC_{50} values and the extracts' total phenol and flavonoid content. TPC and TFC have a positive association, but IC_{50} values and TPC/TFC have a negative correlation, indicating that a low IC_{50} value indicates a high antioxidant capacity and vice versa.

3.5. GC-MS analysis of methanol leaf extract of *Launaea procumbens* (Roxb Amin)

Gas chromatography-mass spectrometry (GC-MS) stands as the most widely employed analytical technique for identifying organic substances in complex matrices. Chemo-profiling of leaf methanol extract of *L. procumbens* was done by GC-MS analysis. Peaks, phytoconstituents name, area %, retention time (RT), phytoconstituents class, and individual fragmentation patterns of phytoconstituents identified in methanol extract of *Launaea procumbens* by GC-MS analysis are tabulated in Table 6 . GC-MS analysis revealed the presence of a total of 9 phyto-constituents in leaf methanol extract of *Launaea procumbens*, which belongs to a different class of phytoconstituents like - aldehyde, ketone, phthalate, carbohydrate, and organic acid anhydride. Methyl stearate and trihydroxy boron were the main compounds in higher concentrations (pyridine). Hexadecanoic acid was the compound that was present in the least amount.

4. DISCUSSION

The study found that *Launaea procumbens* leaves extracted using methanol had the highest total phenolic content (TPC), while the chloroform extract had significantly lower TPC. Among the shoot extracts, the highest concentration of phenols was found in the methanolic extract, followed by chloroform. However, petroleum ether and distilled water extracts did not yield remarkable results due to the low polarity of the solvents. In terms of flavonoid content, the methanolic extracts of the leaf and shoot extracts were found to be the highest. Based on the findings, it appears that the methanolic extract exhibits significant antioxidant activity, owing to its relatively higher phenol and flavonoid content when compared to other

Table 2
Qualitative evolution of *Launaea procumbens* (Roxb.) Amin leaves and shoots in four different solvents

Phytoconstituents	Leaf Extract				Shoot Extract			
	Methanol	Chloroform	Petroleum ether	Water	Methanol	Chloroform	Petroleum ether	Water
Phenols Ellagic acid test	++	+	-	+	++	+	-	+
Alkaloids Dragendroff's test	++	+	+	+	+	+	+	+
Flavonoids Flavonoid test	++	+	+	+	+	+	+	+
Cardiac Glycosides Kellar-Killani test	-	-	-	-	-	-	-	-
Lignans Furfuraldehyde test	+	+	-	-	+	+	-	-
Tannins Tannin test	++	++	-	-	++	+	-	-
Steroids Salkowski test	-	-	+	+	-	-	+	+

Table 3
Quantitative evaluation of leaf extracts of *Launaea procumbens* (Roxb.) Amin in four different solvents

Chloroform	Water	Petroleum ether
18.41±0.011	17.51±0.0038	14.81±0.0015
24.23±0.0011	22.37±0.0010	8.54±0.0003

The data consists of the averages and standard deviations from three independent experiments.

Table 4
Quantitative evaluation of shoot extracts of *Launaea procumbens* (Roxb.) Amin in four different solvents

Variable	Methanol	Chloroform	Water	Petroleum ether
Total Phenolic content (mg/g GAE)	33.1±0.0007	23.6±.004	16.85±0.005	16.51±0.0075
Total flavonoid content (mg/g QE)	27.41±0.003	20.54±0.0013	16.04±0.0017	5.45±0.002

The data consists of the averages and standard deviations from three independent experiments.

Table 5
IC_{50} values of *Launaea procumbens* (Roxb.) Amin plant extracts for antioxidant activity

Different plant extracts of *Launaea procumbens* (Roxb.)	DPPH (IC_{50}) Leaf extracts (μg/ml)	DPPH (IC_{50}) Shoot extracts (μg/ml)
Methanol	57.7±0.06	52.4±0.02
Chloroform	85.2±0.87	74.05±0.92
Water	65.07±0.5	57.21±0.04
Petroleum ether	95.08±0.78	87.05±0.95

Three biological means SD (n=3) illustrate each value in the table.

Table 6
Correlation between total phenol, flavonoid content, and IC_{50} values

Pearson correlation coefficient (r)		
TPC and TFC	TPC and IC_{50} of DPPH	TFC and IC_{50} of DPPH
0.998	-0.999	-0.999

Correlation is significant at the 0.01 level (2-tailed).

extracts. Consequently, it is recommended that the methanolic plant extracts should be considered for further processing. Notably, the leaves methanolic extract contains higher levels of phenol and flavonoids compared to the shoots. To obtain a comprehensive phytochemical profile, the methanol-prepared leaf extract was subjected to additional processing for GC-MS analysis, building upon the aforementioned data.

According to Abdullah et al. (2020), bioactive components like n-hexadecanoic acid act as an anti-inflammatory, antimicrobial, and antioxidant agent, and antifungal properties are seen in the methyl ester of hexadecanoic acid (Beulah et al., 2018). 1, 2-Benzenedicarboxylic acid has distinct properties such as antimicrobial and antifouling (Abubacker & Deepalakshmi, 2013), whereas methyl stearate shows antibacterial activity (Tyagia & Argawak, 2017). As a result, each phytochemical found in the methanolic extract of *Launaea procumbens* (Roxb.) leaves has been determined to have its bioactivity and therapeutic value. Future herbal formulations can use these substances effectively and safely.

Table 7
GC-MS analysis of secondary metabolites present in methanolic extracts of *Launaea procumbens* (Roxb.) Amin leaves (Methanolic extract)

S. No	RT (min)	Peak Area (%)	Name of the compound	Molecular formula
1.	3.03	11.70	5-Benzyloxypyrimidine-2-carboxylic acid Thiodiglycol	$C_{12}H_{10}N_2O_3$ $C_4H_{10}O_2$
2.	3.40	15.52	Boron, trihydroxy (pyridine)-, (T-4)-	C_5H_8BN
3.	10.52	11.98	1-Chloromethyl-1-ethoxy-1-silacyclohexane	$C_8H_{17}ClOSi$
4.	13.53	6.29	2,4-Di-tert-butyl-phenol Phenol, 3,5-bis (1,1-dimethyl ethyl)-	$C_{14}H_{22}O$
5.	16.14	6.47	Octadecane, 3-ethyl-5-(2-ethylbutyl)- Heptacosane	$C_{26}H_{54}$ $C_{27}H_{56}$
6.	18.36	8.43	Stearic acid, 3-(octadecyloxy) propyl ester.	$C_{39}H_{78}O_3$
7.	18.76	10.87	1,2-Benzenedicarboxylic acid, Phthalic acid, butyl octyl ester, decyl isobutyl ester 6	$C_{20}H_{30}O_4$ $C_{22}H_{34}O_4$
8.	20.80	15.73	Methyl stearate	$C_{19}H_{38}O_2$
9.	23.54	6.18	Hexadecanoic acid, methyl ester Methyl glycocholate, 3TMS derivative	$C_{17}H_{34}O_2$ $C_{36}H_{69}NOSi_3$

5. CONCLUSION

Recent research has discovered that *L. procumbens* possesses medicinal properties, in addition to its traditional uses. Among the four different solvents tested, methanol is the most appropriate solvent for future experiments. Interestingly, the leaves of the herb contain a greater quantity of phytochemicals than the shoots. The study has identified nine phytoconstituents and their bioactivity, indicating that the herb could be a valuable antibacterial agent against drug-resistant pathogens. These promising findings suggest that further evaluation of the herb's potential use in medicine and pharmaceuticals is guaranteed.

6. CONFLICTS OF INTEREST

There are no conflicts of interest that are pertinent to this article.

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AUTHOR CONTRIBUTIONS

AA - Research concept and design, SKS - Collection and/or assembly of data, SKS - Data analysis and interpretation, SKS - Writing the article, AA - Critical revision of the article, SKS, AA - Final approval of the article.

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