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RESEARCH ARTICLE

Pharmacognostic Characterization and Phytochemical Screening of *Launaea procumbens*

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ABSTRACT

Launaea procumbens is not employed by the pharmacist but has a good potential in future galenicals. The present communication deals with the pharmacognostic characterization on the different parts of *Launaea procumbens* (Roxb.) Ramayya and Rajgopal, family-Asteraceae (Compositae). Macroscopic and microscopic examinations of the different organ like stomatal frequency, stomatal index, palisade ratio and macerated vessels, observations and differential microchemical test have been carried out for the authentication of the samples. Physiochemical values such as the Moisture contents, percentage of total ash, acid insoluble ash, acid soluble ash, water soluble ash, extractive values as petroleum ether-soluble extractives, ethanol-soluble extractives, methanol-soluble extractives and water-soluble extractives were calculated as well as colour reactions of powder and extract with different chemicals were performed to observe fluorescence analysis. The extracts were subjected to qualitative screening test for various constituents. This revealed the presence protein, glycosides, alkaloids, tannins and phenolic compound, steroid reducing sugars and saponin glycosides. These observations will help in the Pharmacognostical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulteration.

KEYWORDS

Launaea procumbeans, Asteraceae, Pharmacognosy, Pharmacist, Galenical

INTRODUCTION

Botanical Name : *Launaea procumbens* (Roxb.) Ramayya and Rajgopal **[**Syn. *L. nudicaulis* sensu auct. plur. non (L.) Hook.*f*., *L. fallax* (Jaub. & Spach) Kuntze, *Microrhynchus fallax* Jaub. & Spach, *Zollikoferia fallax* (Jaub. & Spach) Boiss.]

Family : Asteraceae (Compositae)

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Sunskrit Name : Galjivha

Marathi Name : Bhoi patri, Pathari

Hindi Name : Bankau

L. procumbens is a glabrous herb, with a tuft of roots at nodes. Leaves mostly radical, entire or glabrous, lower leaves obovate-oblong, pinnatifid, with rounded or very obtuse segments, spinulose on the margins with white cartilaginous teeth, cauline leaves distant, few, sessile, narrowly oblong. Heads 1.2 – 1.9 cm in long, cylindric, solitary or a few together along the branches, shortly pedicelled facicled

(sometimes 6-10 in a cluster). Supported by leaves or naked. Involve bracts all with broad white membranous margins; the outer very short, ovate, acute. Stamens five, basifixed syngenecious, Introse. Ovule single on basal placentation, unilocular, bicarpellary, style terminal, stigma bifid[1].

Figure 1: Habit of *Launaea procumbens*

Distribution

Common throughout the plains of India ascending to an altitude of 2,400m in Himalayas. Found mostly in Bengal and Punjab and Southwards through Sindh to the Deccan, Sandy of India.

Medicinal Properties and Uses

The plant *L. procumbens* is diuretic, soporific, tonic and used as fodder for goats. In Bombay, it is given to buffaloes to promote the secretion of milk. Leaves of the plant are consumed in times of scarcity considered useful as a sand binder. They are used locally in curries, the juice is applied in rheumatic affections. Plant excluding root has insecticidal activity[2-6]. It is also used in the treatment of rheumatism, inflammation and oxidative dysfunction in the kidney[7], reproductive disorders[8], hormonal imbalances[9] and liver dysfunction[10]. Nutritional analyses have shown that it is composed of synergic acid, 2-methyl-resercinol, salicylic acid, vanillic acid, and gallic acid[11], which have antioxidant, anticancer, neuroprotective and cardioprotective effects[12-14]. Some of folk uses as, leaves are employed in fever, toxiemia, cancer and swellings. Paste of leaves is applied on rheumatism, boils and swellings. The whole plant except the root, is given to treat kidney stone and body heat, juice extracted from leaves is given in jaundice. Whole plant made into ash with metal is supposed to be more efficacious for jaundice[15].

Medicinal plants are used as a source of medicine and improved human life from thousand of year. Several herbs possess bioactive constituent such as phenolic and polyphenolic compounds which regulates various immune system. The flavonoids and phenolic compounds rich herbs may also possess antioxidant and anti-inflammatory properties[16, 17]. According to the World Health Organization (WHO), herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries[18]. The work cited up to the articles can be focused on phytochemicals and there many pharmacological activities[19-24].

The pharmacognostical characters reported in this work may serve as a valuable source of information and provide suitable diagnostic tool for the standardization as well as identification of adulterants in future investigation or application. It will also be immense using carrying out further research and re-validation of its use. The work involves morphological brief descriptions, gross-anatomical diagnosis based on free- hand sections and epidermal peels. Since free-hand sections could be taken by anybody, even a small pharmacy or practitioner can taste this material for its anatomical characteristic where our authentic descriptions and photographs can serve as standard reference material. The other parameters included in the present works are the microscopic characteristic of macerated stem, histo-chemical localizations of certain phytochemicals in the hand. Sections, extractive percentage of soluble material in water, ethyl alcohol, methanol and petroleum ether, determination of ash-values are worked out.

MATERIALS AND METHODS

The fresh, healthy, mature plants were collected in winter (November-2012) from farm (At-post. Bader) of District Dhule (M.S., India) away from pollution. The plant materials were identified using the Flora of Dhule and Nandurbar District[1] at Post–graduate Department of Botany, SSVP Sansthas, L.K.Dr.P.R.Ghogrey Science College, Deopur, Dhule-(M.S) India and herbarium were also preserved. The leaves, stem and roots were washed and used for the present study. The macroscopic observations of the mature plants, leaves, inflorescences and other parts were noted down. For microscopic studies, some plant material preserved in 70% alcohol. Leaf epidermal studies were carried out on fresh specimens. Peels were removed mechanically using some chemicals. They were stained in 1% safranin mounted in glycerin and made semi-permanent by ringing with DPX solution. Stomatal index (SI), stomatal frequency, vein-islet, vein termination number and palisade ratio were calculated as defined by Salisbury[25, 26].

viz.,

$$SI = \frac{S}{E + S} \times 100$$

Where 'S' = number of stomata per unit area and 'E' = number of epidermal cells in the same area.

Stomatal index (SI), stomatal frequency, vein-islet, vein termination number and palisade ratio have been calculated out of an average of 10 readings. Palisade ratios (PR) was calculated as the average of palisade cells (P) beneath each epidermal cell (E). Vein islet number is defined as the number of vein islets per sq. mm of the leaf surface midway between the midrib and the margins.

Transections of leaf, stem and root were taken by free hand. Fresh and preserved materials were used. Sections were stained in safranin (1 %), light green (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of leaf, petiole, stem and root sections were taken by using DIGI- EYE High resolution Cameras affixed to microscope Olymphus CH 20*I*.

For study of vessels, fragments of plant organs, especially stem at nodal region and root, were macerated using nitric acid (10%) and potassium dichromate (10%) solution in equal proportions. The vessel elements were stained with aqueous saffranin (1%), dehydrated and mounted in DPX. Some vessel members were also examined in glycerine. The line and cellular sketches of the figures were drawn using a Camera Lucida. The range of length and diameter of vessel elements was determined by the measurement of 20 – 25 vessel elements.

The fresh, healthy plants were rooted out and washed with water. They were separated as root, stem and leaves and shade dried. The dried plant materials were pulverized into fine powder using a grinder (mixer). About 1 kg of powdered material was prepared. After that powder were kept into air tight bags. Physiochemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values as petroleum ether-soluble extractives, etahnol-soluble extractives, methanol-soluble extractive, and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia[27, 28].

Phytochemical studies such as qualitative examination were done on the dried powdered material for this about 5gm of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; ethanol, methanol, diethyl ether, and chloroform[29]. The respected solvents were evaporated (at 40ºC) with the help of heating mantle. Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods[30-33]. The positive tests were noted as present (+++) appreciable amount, (++) moderate amount, (+) trace amount and (-) completely absent.

RESULTS AND DISCUSSION

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance[34, 35]. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. The result of this study as follows:

Microscopic Characters

Dermal Characters

Epidermal cells of adaxial epidermis have shows anisocytic and anomocytic stomata both in equal proportion (Fig. 2: A). These cells with wavy walls. On abaxial surface anisocytic and anomocytic stomata are both in almost equal proportion. (Fig. 2: B). Epidermal cells of the abaxial epidermis are less wavy and straight walled more towards the coastal region. Epidermis is also shows non-glandular, multicellular trichomes having a single basal cell.

T. S. of Leaf of L. procumbeans (Fig 2: C, D)

Epidermis unilayered with thick cuticle. Cuticle thicker at abaxial surface. Mesophyll of the mid-rib (vein) consists of vasculature containing three vascular bundles. Central being larger. The outer most layer of the phloem contains few darkly stained idioblasts. Leaf margin mesophyll is made up of only spongy tissues, spongy mesophyll is compact.

T. S. of Stem of L. procumbeans (Fig.2: E)

The transverse section of the stem shows circular in outline, epidermis with thick cuticle, unilayered small cells compactly arranged; following the epidermis by hypodermis which is made up of single layered, non-chlorophyllous parenchyma followed by 3-5 layered chlorenchyma. Hypodermis is followed by parenchymatous endodermis, vascular cylinder is made up of about 8 vascular bundles of different sizes. All connected with sclerenchymatous sheath. Peripheral margin of vascular bundle is capped by 3-5 layered sclerenhymatous disc. Central pith is parenchymatous and star-shaped.

T. S. of Root of L. procumbeans (Fig.2: F)

The transverse section shows circular in outline. Epidermis single layered, Periderm 2-3 layered of suberised thin walled, polygonal cells. Cork cambium is hardly 2 layered. Cortex is made up of polygonal thin walled parenchyma. Cortical region having localized patches of cells containing ergastic matters. Vascular cylinder is made up of polygonal thin walled parenchyma and ring of xylem & xylem-sclerenchyma interrupted at times by incomplete 2-6 seriate medullary rays. Phloem Found in patches outside the xylem. Pith of thin walled large parenchyma.

Quantitative Microscopy

The leaf microscopic characters as stomatal frequency, stomatal index, vein islet number, vein termination number and palisade ratio were determined (Table 1).

Vessel Elements from Macerated Stem (Fig. 2)

A survey of about 40-50 vessel elements were carried out to observe various characters of vessels elements *viz.*, size, wall thickening, shape, tail, and characters of perforation plate like number, orientation and shape.

The ranges of the length and width of vessel element was determined by the measurement of 20-25 vessel elements and were classified as per Radford[36], which is reproduced here for perusal.

Sr. No.	Class	Category	Size range
1	A	Extremely short	less than 175µ
2	B	Very short	175 to 250µ
3	C	Moderately short	251 to 350µ

4	D	Medium size	351 to 800µ
5	E	Moderately long	801 to 1100µ
6	F	Very long	1101 to 1900µ
7	G	Extremely long	Over 1901µ

Dimension: Extremely short (Class-A), very short (Class-B), moderately short (Class-C), medium sized (Class –D) and moderately long (Class-E) vessel elements were observed. The frequency of medium sized (Class-D) vessel elements was higher. Moderately short (Class-C) vessel elements were observed in lower frequency (Table-2).

The width of vessel elements is 16-33 µ.

Shape: Cylindrical (Fig. 3A), linear (Fig.3B) and spindle shaped vessel elements (Fig. 3C) were common. The other types like wedge shaped (Fig.3D), fusiform (Fig.10E), barrel-shaped (Fig. 10F), conical (Fig. 3G) and spherical (fig.3H) were less frequent.

Wall Thickening: Spiral (Fig. 3A) and pitted thickenings were common (Fig. 3B, 3C) or alternate (Fig.3I).

Tail: Few perforation plates with long pointed tail were observed (Fig. 3J).

Perforation Plate: In the perforation plates, only simple perforation plates were present.

Orientation: The VES have only transverse perforation plates.

Number: Usually a vessel element has two perforation plates either on transverse or transverse-lateral end walls (Fig. 3K, 3L).

Shape of Perforation Plate: More commonly vessel elements have oval (fig.3K) or spherical (Fig.3L) perforation plates.

Results of ranges of the length and width of vessel element have been presented in Table 2. The observations show that class-D type (Range-365-747) found large quantity i.e. 31.83% while Class-C found less amount i.e. (Range-298-332) 9% (Fig.3).

Qualitative Phytochemical Screening

The microchemical screening for the phytoconstituets shows the presences of protein, alkaloids, tannins, phenolic compound, steroid reducing sugars and saponin glycosides while glycosides are total absent (Table 3).

Fluorescence Analysis

The fluorescence analysis is sensitive and enables the precise and accurate determination over a satisfactory concentration. The fluorescence colour is specific for each compound. A non fluorescent compound may fluorescence if mixed with impurities that are fluorescent. The colour of the extract from organic and inorganic solvents was observed under ordinary light (Table 4).

Physical Constants

Results of moisture contents, ash analysis and extractive values of the dried leaves, stem and root have been presented in Table 5.

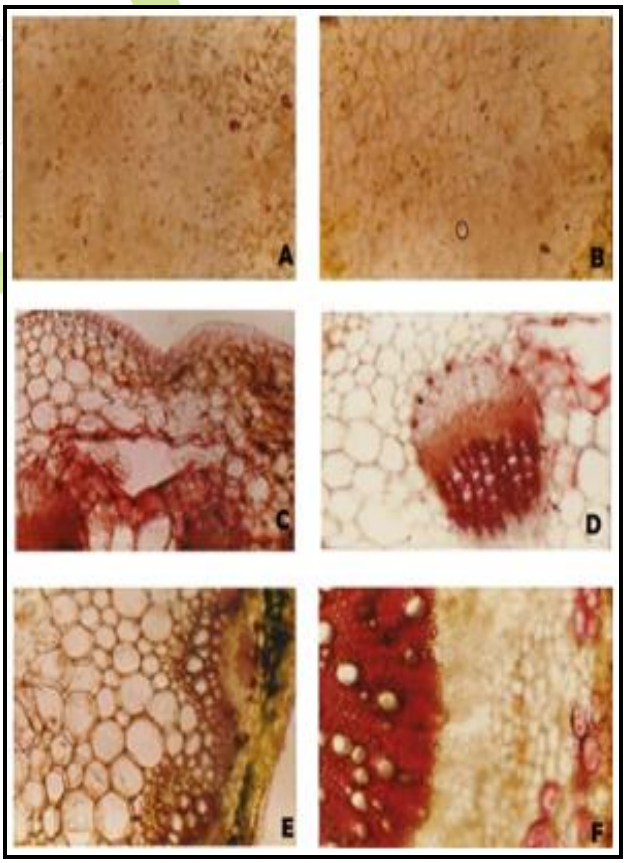

Figure 2: Anatomical feachers of *Launaea procumbens*

A: adaxial surface of leaf showing anemocytic stomata and epidermal cells with wavy walls;

B: abaxial surface of leaf showing anemocytic stomata and epidermal cells with ±straight walls;

C: T.S. of leaf with lysogenous cavity developing in the midrib mesophyll;

D: T.S. of leaf showing a midrib vascular bundle;

E: T.S. of stem showing strongly chlorophyllous cortex and large pith surrounded by angular stele;

F: T.S. of root showing secondary xylem.

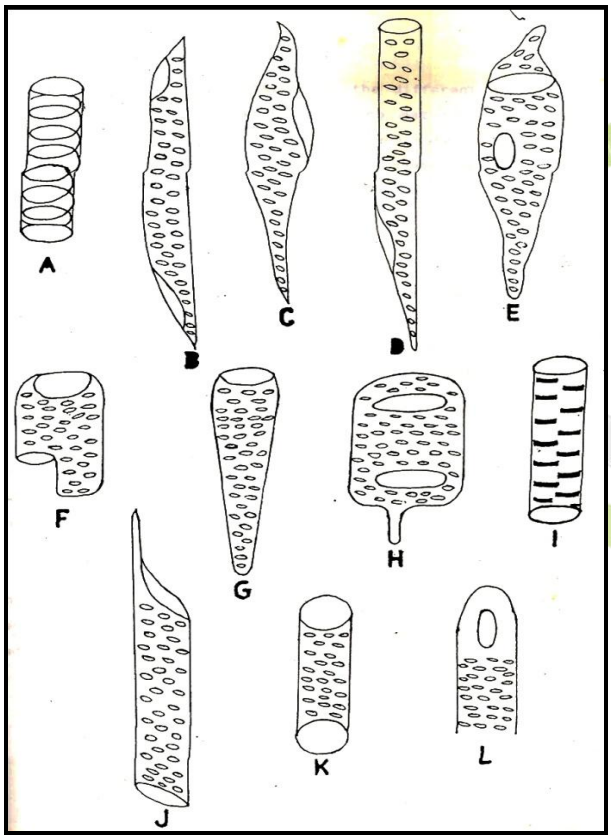

Figure 3: Vessel elements (VE) of *Launaea procumbens*

A: Cylindrical VE with spiral thickening; B: Linear VE with irregular pitted thickening; C: Spiral VE with irregular pitted thickening; D: Wedge shaped VE with irregular pitted thickening; E: Fusiform VE with irregular pitted thickening; F: Barrel shaped VE with irregular pitted thickening;

G: Conical VE with irregular pitted thickening; H: Spherical VE with irregular pitted thickening; I: Cylindrical VE with long pointed tail; J:VE with long pointed tail; K: VE with transverse end wall; L: VE with transverse-lateral end wall;

Table 1: Quantitative Microscopy of the Leaves of *L. procumbens*

Sr. No.	Parameter	Range	Mean
1	Stomatal frequency (upper surface)	242.4-296.8	269.6
2	Stomatal frequency (lower surface)	125.0-168.7	146.8
3	Stomatal index (upper surface)	24.24-29.68	26.96
4	Stomatal index (lower surface)	12.50-16.87	14.68
5	Veinislet number	11.3-14.4	12.85
6	Vein termination	9.5-13.3	10.8
7	Palisade ratio	1.22-1.26	1.24

Table 2: Classification and Relative Frequency of Vessels Found In *L. procumbens*

Sr. No.	Class	Range (µm)	Percentage (%)
1	Class -A	99-160	22.77
2	Class -B	215-249	13.63
3	Class -C	298-332	9.00
4	Class -D	365-747	31.83
5	Class -E	846-1012	22.73

Table 3: Qualitative Phytochemical of *L. procumbens*

Sr. No.	Test	Leaves Ex.in		Stem Ex. in		Root Ex. in	
		Enol	Mnol	Enol	Mnol	Enol	Mnol
1	Protein	++	++	+++	++	++	++
2	Glycosides	-	-	-	-	-	-
3	Alkaloids	++	++	++	++	++	++
4	Phenolic compound	++	++	++	++	+	+
5	Steroid	+	+	+	+	+	+
6	Saponin glycosides	+	+	+	+	+	+
7	Reducing sugar	+++	+++	++	+++	++	++
8	Fat	++	++	++	++	++	++
9	Tannins	+	+	+	+	-	-

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent; Enol= Ethanol; Mnol=Methanol; Ext=Extract

Table 4: Flourusence Analysis of *L. procumbens*

Sr. No.	Test	Leaf	Stem	Root
1	Powder	Green	Light green	Light yellow
2	Pd+Iodine	Light red	Reddish brown	Red
3	Pd+5%$FeCl_3$	Orange green	Dark orange	Yellow
4	Pd+1N NaOH	Dark green	Light green	Faint green
5	Pd+Acetic acid	Greenish yellow	Greenish yellow	Faint yellow
6	Ext+A.A.+50%H_2SO_4	Dirty green	Faint green	Orange green
7	Pd+50%H_2SO_4	Faint green	Faint yellow	Yellowish brown
8	Pd+50%Conc. HCl	Yellow	Light yellow	Light yellow
9	Pd+Ammonia	Green	Light green	Light green
10	Ext+4%NaOH+1%$CuSO_4$	Green	Green	Green
11	Ext+40%NaOH+1%Lead acetate	Dark green	Green	Yellowish green
12	Pd+50%HNO_3+Picric acid	Green	Faint green	Light green
13	Pd+Satu.Picric acid	Greenish yellow	Light green	Faint green

Abbreviations: Pd= Powder A.A=Acetic Acid, Ext=Extract

Table 5: Physical Evaluation (% W/W) of *L. procumbens*

Sr. No.	Parameter	Value (%w/w)		
		Leaves	Stem	Root
1.	Moisture content	10.10	9.50	7.30
2.	Extractive values a) Petroleum Ether b) Ethanol c) Methanol d) Water	 15.08 35.80 30.56 33.38	 20.00 26.66 23.65 25.50	 8.20 34.60 26.20 28.85
3.	Ash values a) Total ash b) Acid insoluble Ash c) Acid soluble Ash d) Water soluble Ash	 16.20 5.60 10.60 7.50	 10.50 8.90 1.60 4.27	 14.55 7.25 7.30 5.60

CONCLUSION

The morphological, microscopic and physicochemical characters reported in this work can serve as a valuable source of information for botanical study, quality control and provide suitable diagnostic tool for the authentication of the original drug, standardization as well as identification of adulterants from the drug. In the present investigation we observed the high extractive values in ethanol compared to other solvents used. The fluorescence colour is specific for each compound. The high ash contents indicate the large amount inorganic salts or extraneous matter. It is used as enrichment for Ayurvedic pharmacopoeia and photographs can serve as standard reference material.

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