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Pharmacognostic studies of leaf and stem of *Lavandula bipinnata* (Roth) Kuntze collected during Nakshatra days and normal days

Jyoti Pande, Savan Donga and Sumitra Chanda

Abstract
Nature is bestowed with unimaginable wealth of medicinal plants, the healing property of which is known from time immemorial. According to Indian Astrology there are 27 Nakshatras or Constellations and each Nakshatra is associated with one particular plant known as Aaradhya Vriksha. It is also believed that the therapeutic efficacy of plants is best exhibited when they are collected during a particular Nakshatra. It is of utmost importance to lay down quality control parameters for authentication and prevent the plant from adulteration. In the present work, leaf and stem of *Lavandula bipinnata* (Roth) Kuntze belonging to Labiatae family were collected during Magha Nakshatra days and normal days. Their pharmacognostic, physicochemical and phytochemical parameters were evaluated. The macroscopic, microscopic and powder studies of leaf and stem collected at Nakshatra days and normal days did not show any differences while physicochemical parameters (like ash values, moisture content, extractive values) and phytochemical analysis (different phytoconstituents) showed distinct differences. The extractive values and different phytoconstituents were considerably more in leaf and stem collected during Magha Nakshatra days than those collected during normal days. Finally, it can be concluded that the parameters evaluated in this study are diagnostic characteristic feature of *L. bipinnata* and they will act as reference standards for this plant and will help in its correct identification and help to maintain its therapeutic efficacy. The results also support the traditional belief that plants collected during a particular Nakshatra are therapeutically more potent than those collected during normal days.

Keywords: *Lavandula bipinnata*, pharmacognostic, physicochemical, phytochemical, magha nakshatra, nakshatra days, normal days

Introduction
Nature has been a source of vast number of drugs from time immemorial to cure diverse diseases, disorders and ailments. The main reason behind the observed biological or pharmacological activities goes for the presence of bioactive compounds or secondary metabolites present in them. The success is also because of the structural diversity present in them. The plants can be used as natural crude extracts or essential oils or they may be extracted using different extraction techniques or extracted in different solvents [1, 2]. All parts or any part of the plant is effective therapeutically. It may be leaf, stem, bud, flower, fruit, bark, pulp or even peel. They are easily available and affordable by all people [3]. Chemical based or synthetic drugs are becoming unsafe day by day and are reported for many side effects. In such a situation, when plants are used as crude drugs in various forms, it becomes very important and necessary to lay down quality control parameters and standardization becomes an essential step for every plant under investigation. This will ensure correct identification of the plant, maintain authenticity and therapeutic efficacy. Such standardization studies will help to maintain quality, safety, and efficacy of the plants in use [4]. It will also prevent adulteration and substitution intentionally or unintentionally [5].
According to Indian Astrology, human beings are always influenced by planetary structures and constellations. Indian Astrology has stated 27 Nakshatras or Constellations. Constellation means a group of specific stars in the sky. In Indian Astrology, each Nakshatra is indicated with one particular plant species known as Aaradhya Vriksha (worshipped plant). Hence the 27 Nakshatras are correspondingly correlated to 27 trees present on earth [6]. It has clearly been mentioned that the medicinal value of plants is best exhibited when they are collected during a particular Nakshatra [7]. It implies that human beings are directly related to plants and especially these 27 plants have the power to fight against the harmful effects of the planets on human lives. Plants and herbs have been found to be extremely effective in neutralizing the

detrimental influences of the astral positions of stars [8]. When plants are collected in particular Nakshatra, they have higher therapeutic potential because of increase in secondary metabolite content as compared to those collected in normal days [9]. However, there are hardly any reports for this assumption. Hence, in the present work, pharmacognostic, phytochemical and physicochemical studies have been attempted in *Lavandula bipinnata* (Roth) Kuntze plant collected during Magha Nakshatra days and normal days.

Lavandula bipinnata (Roth) Kuntze belong to Lamiaceae or Labiatae family. It is also called as mint family of flowering plants, with 264 genera and 6990 species; in fact the largest family of the order Lamiales. Lamiaceae is distributed nearly worldwide; in India itself, 64 genera and 350 species are found. Many species are cultivated for their fragrant leaves and attractive flowers. The family is particularly important to humans for herb plants are useful for flavour, fragrance, or medicinal properties. Most members of the family are perennial or annual herbs with square stems, though some species are woody shrubs or sub shrubs. The leaves are typically simple and oppositely arranged; most are fragrant and contain volatile oils. The flowers are usually arranged in clusters and feature two-lipped, open-mouthed, tubular corollas (united petals) with five-lobed bell-like calyxes (united sepals). The fruit is commonly a dry nutlet [10].

In the present work, leaf and stem of *L. bipinnata* were collected at Magha Nakshatra days and normal days. Hence forth, the leaf and stem collected during Magha Nakshatra days will be referred as Nak-leaf and Nak-stem respectively while leaf and stem collected during normal days will be referred as Nor-leaf and Nor-stem respectively. Pharmacognostic (organoleptic. Macroscopic, microscopic and powder studies), physicochemical analysis (ash values, moisture content and extractive values) and qualitative phytochemcial screening (various phytoconstituents) was done in both the leaf and stem.

Material and Methods

Plant collection

Lavandula bipinnata (Roth) Kuntze. Leaf and stem was collected during Magha Nakshatra days and during normal days (after Magha Nakshatras days) at early morning 5 o'clock in the month of August, 2017. They were collected from Pal forest located at 21.36° North and 75.90° East Jalgaon district, Maharastra, India. The plant parts, leaf and stem, were washed with tap water thoroughly; shade dried and homogenized to fine powder and stored in closed container for further studies.

Pharmacognostic study

Macroscopic, Microscopic and Powder Microscopy study

The leaf and stem of *L. bipinnata* was subjected to macroscopic studies as described in quality control method using organoleptic evaluation method [11]. The parameters evaluated were the arrangement, size, shape, base, texture, margin, apex, veination, colour, odour, taste. Photographs at different magnifications were taken by using digital camera. For microscopic evaluation of leaf and stem, thin transverse sections were made. They were washed with water and stained with safranin, fast green and mounted in glycerine for observation and confirm its lignifications (10x, 40x) [13]. The powder microscopy of dried powder of leaf and stem of *L. bipinnata* was studied using standard procedures [12]. The characteristic features observed were recorded by taking their photographs.

Physicochemical analysis

The physicochemical parameters like loss on drying, ash values (total ash, acid-insoluble ash, water-soluble ash, sulphated ash, nitrated ash and carbonated ash) and extractive values were determined as per WHO guidelines [14]. The solvents used for determining extractive values were petroleum ether, toluene, ethyl acetate, methanol and water. The procedure followed is as described earlier [15].

Phytochemical analysis

The crude powder of leaf and stem of *L. bipinnata* was subjected to qualitative phytochemical analysis [16]. The phytoconstituents analysed were alkaloids, phenols, flavonoids, tannins, phlobatanins, saponins, steroids, cardiac glycosides, triterpenes, anthocyanins, quinines, leucoanthocyanins and coumarins. The procedure followed for different phytochemical analysis is given below:

Table 1: phytochemical test and there observation

No.	Phytochemicals	Test	Observation
1	Alkaloids	The dried powder of leaf and stem was added to 2N HCl and mixture was filtered.	Formation of orange precipitate indicated the presence of alkaloids
		1) The filtrate was treated with few drops of Dragendorff 's reagent	
		2) The filtrate was treated with few drops of Mayer's reagent	Formation of Cream precipitate indicated the presence of alkaloids
		3) The filtrate was treated with few drops of Wagner's reagent	Formation of brown precipitate indicated the presence of alkaloids
2	Phenols	The dried powder of leaf and stem was dissolved in distilled water. It was filtered and the filtrate was treated with a few drops of 5% ferric chloride solution.	Formation of deep blue colour indicated the presence of phenols
3	Flavonoids	The dried powder of leaf and stem was dissolved in distilled water. It was filtered the filtrate was treated with a few drops of diluted NaOH, again add few drops of diluted HCl.	Formation of yellow orange colour to colourless indicated the presence of flavonoids
4	Saponins (Frothing test)	The crude powder of leaf and stem was vigorously shaken with distilled water and allowed to stand for 10 min.	Stable formation of froth for 1 min indicated presence of saponins
5	Tannins	The dried powder of leaf and stem was dissolved in distilled	Formation of blue colour indicated

	($FeCl_3$ test)	water. It was filtered and the filtrate was treated with alcoholic ferric chloride reagent.	the presence of tannins
6	Steroids (Liebermann – Burchard test)	The dried powder of leaf and stem was dissolved in chloroform. It was filtered and the filtrate was treated with acetic anhydride and a few drops of concentrated sulphuric acid	Formation of blue green ring indicated the presence of steroids
7	Phlobatanins	The dried powder of leaf and stem was boiled with 1% aqueous HCl	Formation of red precipitate indicated the presence of phlobatannins
8	Anthocyanins	The dried powder of leaf and stem was dissolved in methanol. It was filtered and the filtrate was treated with 2.0 ml NaOH (1 N)	The colour of the solution changed to blue indicated the presence of anthocyanins
9	Triterpenes	The dried powder of leaf and stem was dissolved in chloroform. It was filtered and the filtrate was treated with concentrated sulphuric acid	Formation of reddish brown ring indicated the presence of triterpenes
10	Cardiac Glycosides (Keller – Kiliani test)	The dried powder of leaf and stem was dissolved in distilled water. It was filtered and the filtrate was treated with 1.0 ml mixture of 5 % ferric chloride and glacial acetic acid (1.99 V/V). To this solution, few drops of concentrated sulphuric acid was added.	Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides
11	Coumarins	The dried powder of leaf and stem was dissolved in distilled water. It was filtered and the filtrate was treated with 10 % NaOH.	Formation of yellow colour of the solution indicated the presence of coumarins.
12	Leucoanthocyanins	The dried powder of leaf and stem was dissolved in distilled water. It was filtered and the filtrate was treated with isoamyl alcohol in equal proportion.	Appearance of red colour in upper layer indicated the presence of leucoanthocyanins
13	Quinones	The dried powder of leaf and stem was dissolved in methanol. It was filtered and the filtrate was treated with HCl.	Formation of yellow precipitation indicated the presence of quinones

Fluorescence analysis

Fluorescence study of leaf and stem powder was performed as per Kokoski *et al.,* [17]. A small quantity of stem/leaf powder was placed on a grease free clean microscopic slide and a few drops of freshly prepared reagent solutions were added, mixed by gentle tilting of the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colours observed were recorded.

Results

Organoleptic and macroscopic characteristic of *Lavandula bipinnata* (Roth) Kuntze

L. bipinnata is a green, woody, erect, unbranched, annual herb. The organoleptic and macroscopic characteristics of the plant is given in Table 2 and Fig. 1**.**

Leaves

L. bipinnata leaf was of decompound type (Fig. 1a). It was green in colour, phyllotaxy was opposite decussate, shape was decompound incision, margin was lobed or incised, apex was acuminate, base was symmetrical, veination was reticulate and petiole was short. Outer surface was pubescent. The odour was characteristic and taste was bitter. The average size of leaf was 8-10 cm long and 7-8 cm wide (Fig. 1b and Table 1).

Stem

The stem was green in colour, unbranched, woody, erect. Shape was square or quadrangular. Outer surface was rough and hairy. The average size of the stem was 45-55 cm height and 2-3 cm thick (Fig. 1c and Table 2).

a) Decompound leaf b) Leaf c) Stem

Fig 1: Macroscopic study of *Lavandula bipinnata* (Roth) Kuntze

Table 2: Organoleptic features of *L. bipinnata*

Parts	Observation	Observation
Part	Leaves	Stem
Arrangement	Opposite decussate	-
Size	8-10 cm long, 7-8 cm wide	2 to 3 cm thickness,45-55 cm height
Shape	Decompounds incision	Square or quadrangular
Colour	Green	Green
Odour	Characteristic	Characteristic
Taste	Bitter	Bitter
Appearance	Smooth	Rough and scabrous
Margin	Lobed or incised	-
Apex	Acuminate	-
Base	Symmetrical	-
Petiole	Short	-
Texture	Smooth hairy	Hairy
Veination	Reticulate veination	-
Outer surface	Pubescent	Dark green colour, Rough surface

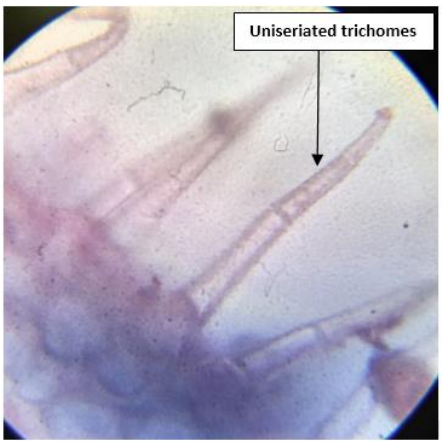
Microscopic characteristics

Petiole

The transverse section of *L. bipinnata* petiole is shown in Fig. 2. The petiole was bean shaped. The single layered upper and lower epidermis was surrounded by thick cuticle layer. The epidermis was covered with unicellular and multicellular, 2-3 celled trichomes (Fig. 2a). Ground tissue was parenchymatous, it cannot be differentiated into hypodermis and endodermis (Fig. 2b). Vascular bundles were 'arc' shape, the size of the vascular bundles varied from centre to leaf margin i.e. large too small. They were conjoint collateral open centripetal arranged (Fig. 2c). Trichomes were uniseriate, unicellular or multicellular (2-3celled) straight with pointed tips (Fig. 2d).

a) T.S. of petiole

b) T.S. of petiole with epidermis



c) Lower epidermis with multicellular trichomes

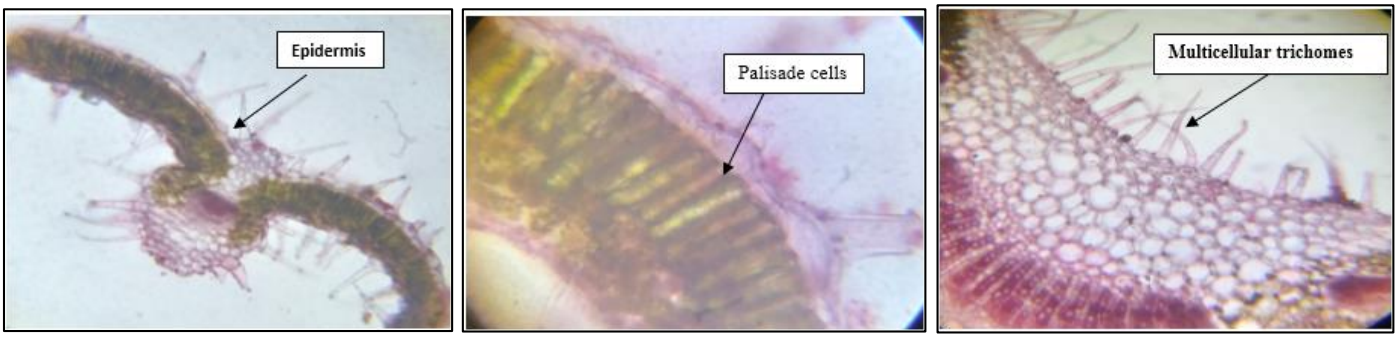
d) Vascular bundles

Fig 2: Microscopic study of leaf petiole of *L. bipinnata*

Leaf

The transverse section of *L. bipinnata* leaf is shown in Fig. 3. The leaf lamina was dorsiventral in nature. The upper epidermis and lower epidermis were single layered, surrounded by covering type of multicellular trichomes (Fig. 3a). The palisade tissue was single layered, compact elongated cells on the upper surface, it was covered with thick cuticle (Fig. 3b). The upper surface of leaf showed uniseriate,

unicellular and multicellular pointed trichomes (Fig. 3c). The mesophyll was large i.e. it consisted of 5-6 layers. T.S. passing through the mid rib region showed vascular bundles towards the ventral surface and it was surrounded by spongy parenchymatous tissues (Fig. 3d). Centrally located vascular bundles were collateral conjoint open; the xylem was surrounded by phloem (Fig. 3d). The prism or square calcium oxalate crystals were present in lower epidermis (Fig. 3e). In lower epidermis, anomocytic stomata were present and they were surrounded by small paranchymatous subsidiary wavy cells (Fig. 3f).



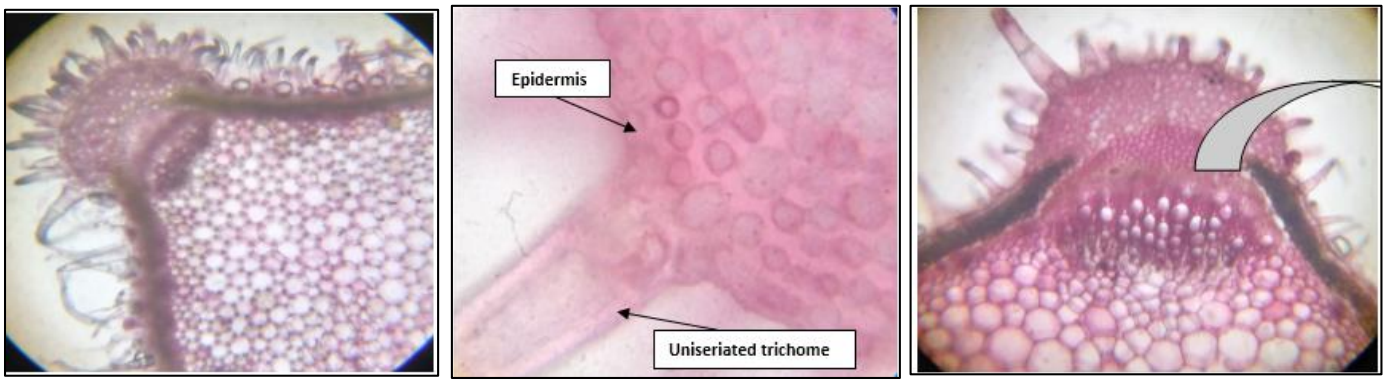
a) T.S. of leaf with epidermis b) Leaf pinna with palisade cells c) Upper epidermis with multicellular trichomes

d) T.S. of leaf with vascular bundles e) Calcium oxalate crystals f) Anomocytic stomata

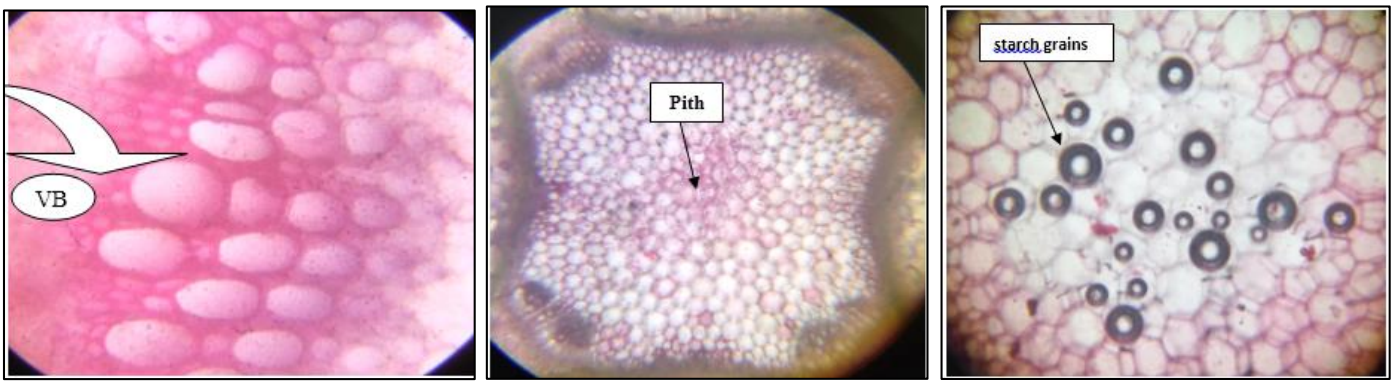
Fig 3: Microscopic study of leaf of *L. bipinnata*

Stem

The transverse section of *L. bipinnata* stem is shown in Fig. 4. The epidermis was single layered and it was surrounded by thick cuticle with covering type of trichomes (Fig. 4a). The epidermis was thick walled and consisted of cubical cells with uniseriate unicellular and multicellular trichomes (Fig. 4b). The stem consisted of four bulges at four corners, each corner consisted of vascular bundles. The vascular bundles were conjoint, collateral and open type (Fig. 4c). The xylem was lignified, well developed and consisted of vessels, fibres, metaxylem and protoxylem. Phloem was non-lignified consisted of sieve tubes and phloem parenchyma (Fig. 4d). The cortex region consisted of 6-8 layers. The vascular bundles were surrounded by polygonal lignified parenchyma and collenchyma cells (Fig. 4e). Pith was very large and consisted of spherical starch grains in thin walled sclerenchymatous cells (Fig. 4f).



a) T.S. of stem with epidermis b) T.S. of stem with trichomes c) T.S. of stem with vascular bundles

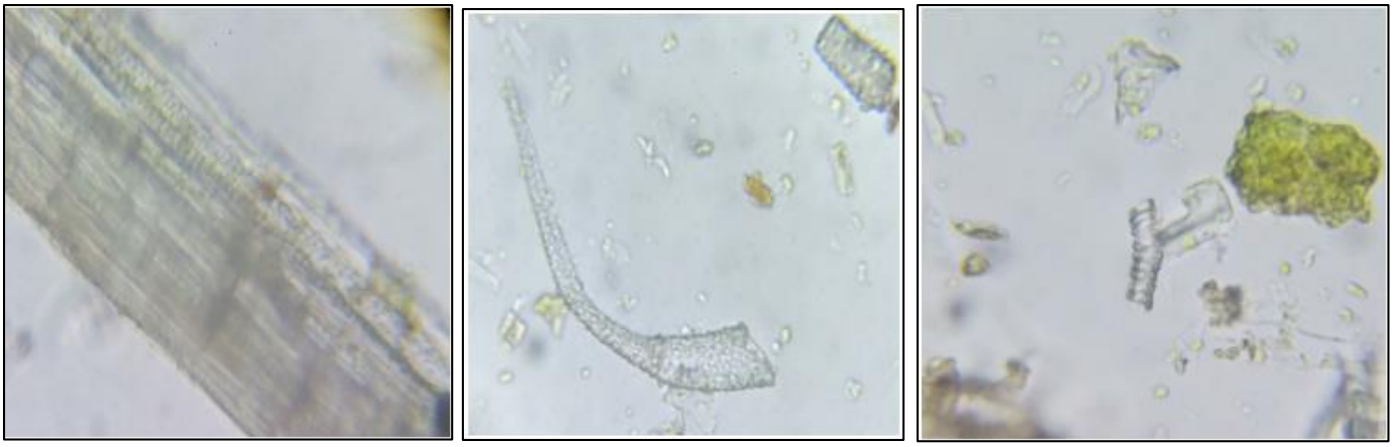


d) Metaxylem, protoxylem and phloem e) T.S of whole stem with pith f) Pith with spherical starch grains

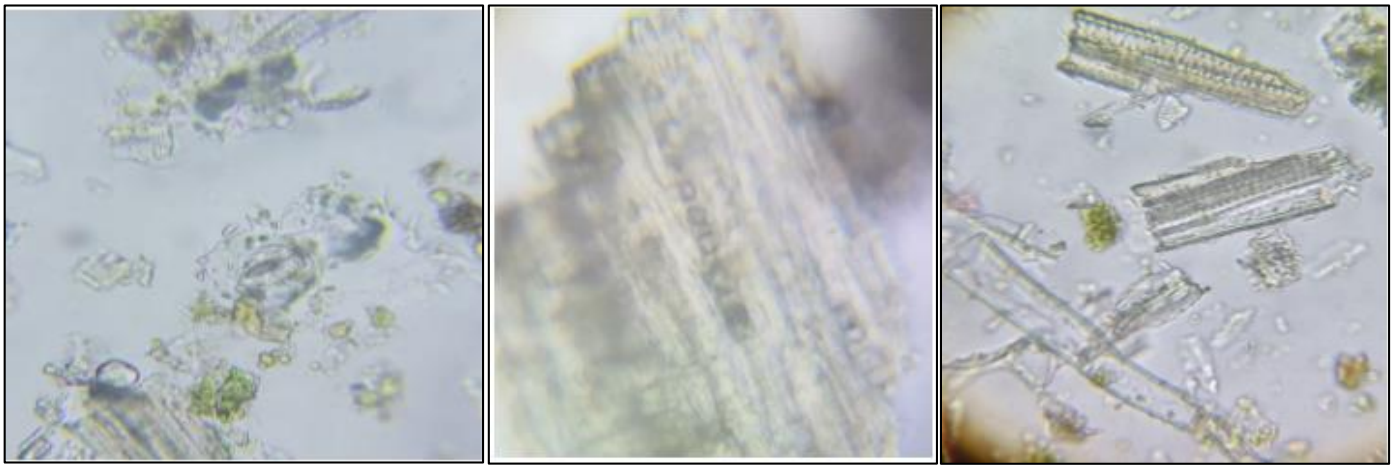
Fig 4: Microscopic study of stem of *L. bipinnata*

Powder microscopy of leaf

The crude powder of *L. bipinnata* leaf was dark green in colour, taste was bitter and odour was characteristic. The powder microscopic characteristics are shown in Fig. 5. The specific characteristics of powder determined by microscopic investigation showed spiral and reticulate vessels, multicellular trichomes, annual vessels, anomocytic stomata, square calcium oxalate crystal, scalariform and reticulate vessels, etc.



a) Spiral and reticulate vessels b) Multicellular trichome c) Annular vessels

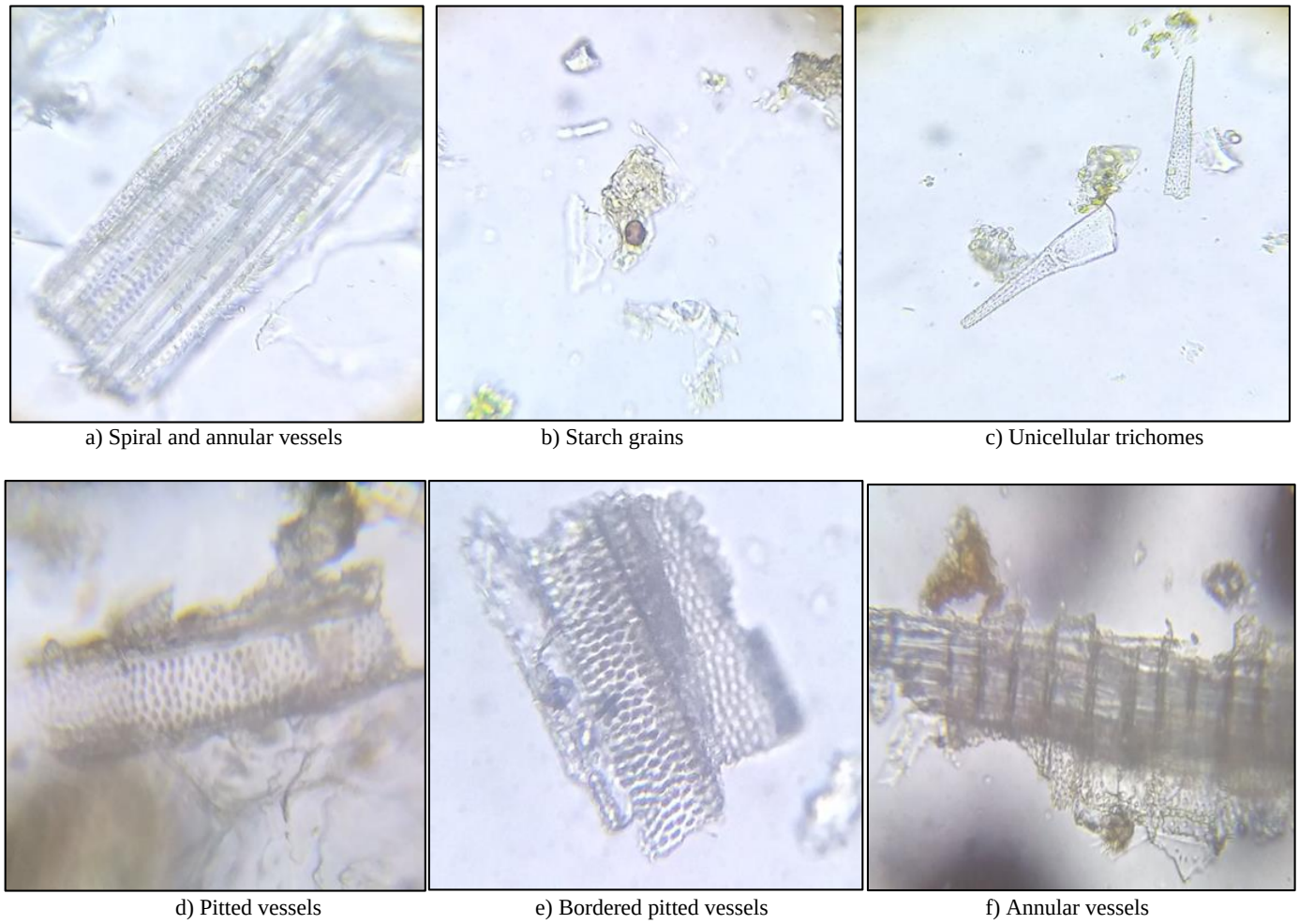


d) Anomocytic stomata e) Square calcium oxalate crystals f) Scalariform and reticulate vessels

Fig 5: Powder study of leaf of *L. bipinnata*

Powder microscopy of stem

The crude powder of *L. bipinnata* stem was green in colour, taste was bitter and odour was characteristic. The powder microscopic characteristics are shown in Fig. 6. The specific characteristics of powder determined by microscopic investigation showed spiral and annular vessels, starch grains, unicellular trichomes, pitted vessels, bordered pitted vessels, annular vessels, etc.



a) Spiral and annular vessels b) Starch grains c) Unicellular trichomes

d) Pitted vessels e) Bordered pitted vessels f) Annular vessels

Fig 6: Powder study of stem of *L. bipinnata*

Physicochemical analysis

The physicochemical analysis of Nak-leaf, Nor-leaf, Nak-stem, and Nor-stem of *L. bipinnata* is given in Table 3. The results of physicochemical analysis of leaf are as follows: The loss on drying of dry powder of Nak-leaf was 7.75 % while that of Nor-leaf was 8.21 %. The total ash in Nak-leaf was 13.32 %, water soluble ash was 5.6 % and acid insoluble ash was 1.3 % while in Nor-leaf total ash was 15.62 %, water soluble ash was 7.2 % and acid insoluble ash was 2.8 %. All the three ash values were slightly more in Nor-leaf than in Nak-leaf. Similar results were found in carbonated ash, nitrated ash and sulphated ash. In Nak-leaf, carbonated ash, nitrated ash and sulphated ash were14.5 %, 10.0 % and 15.3 % respectively while in Nor-leaf, it was 16.16 %, 12.3 % and 16.32 %. Five solvents with different polarity were used to find out the extractive values of both Nak-leaf and Nor-leaf. The extractive values of leaf powder is given in Table 3. The extractive values of Nak-leaf and Nor-leaf showed a different trend. The extractive values of Nak-leaf in all the solvents was more than that of Nor-leaf. Among organic solvents, maximum extractive value was in methanol (Nak-leaf: 13.56 % and Nor-leaf: 11.80 %) and minimum in petroleum ether (Nak-leaf: 1.75 % and Nor-leaf: 1.17%). The water soluble extractive value of Nak-leaf was 20.58 % while that of Nor-leaf was 20.09 %.

The results of physicochemical analysis of stem are as follows: The loss on drying of dry powder of Nak-stem was 9.1 % while that of Nor-stem was 10.0 %. The total ash in Nak- stem was 10.75 %, water soluble ash was 0.75 % and acid insoluble ash was 0.15 % while in Nor- stem total ash was 13.3 %, water soluble ash was 1.1 % and acid insoluble ash was 0.35 %. Like in leaf, all the three ash values were slightly more in nor- stem than in Nak- stem. When both leaf and stem are compared, irrespective of the time of collection, leaf had more ash values than stem. All the three ash values were slightly more in Nor-stem than in Nak-stem. Again these results are similar to that of leaf. In Nak- stem, carbonated ash, nitrated ash and sulphated ash were 13.0 %, 11.7 %, 14.8 % respectively while in Nor- stem, it was 14.1 %, 12.5 %, 15.0 %. The extractive values of Nak- stem in all the solvents was more than that of Nor- stem. Among organic solvents, maximum extractive value was in methanol (Nak- stem: 2.54 % and Nor- stem: 2.3 %) and minimum in petroleum ether (Nak- stem: 0.48 % and Nor- stem: 0.49%). The water soluble extractive value of Nak- stem was 19.78 % while that of Nor-stem was 18.0 %.

When both leaf and stem are compared, irrespective of the time of collection, leaf had extractive values than stem indicating that in all the solvents, the bioactive compounds were extracted more in leaf than in stem.

Table 3: Physiochemical parameters of *L. bipinnata* leaf and stem

Sr. No.	Parameters	Nakshatra days		Normal days	
		% value(w/w) Leaf	% value(w/w) Stem	% value(w/w) Leaf	% value(w/w) Stem
1	Loss on drying	7.75	9.1	8.21	10
2	Total ash	13.32	10.75	15.62	13.3
3	Water soluble ash	5.6	0.75	7.2	1.1
4	Acid insoluble ash	1.3	0.15	2.8	0.35
5	Carbonated ash	14.5	13	16.16	14.1
6	Nitrated ash	10	11.7	12.3	12.5
7	Sulphated ash	15.3	14.8	16.32	15
8	Petroleum ether soluble extractive value	1.75	0.48	1.17	0.49
9	Toluene soluble extractive value	4.74	1.15	3.67	0.90
10	Ethyl acetate soluble extractive value	4.71	1.86	4.22	1.52
11	Methanol soluble extractive value	13.56	2.54	11.80	2.30
12	Water soluble extractive value	20.58	19.78	20.09	18.00

Qualitative phytochemical analysis

The qualitative phytochemical screening of crude powder of Nak-leaf, Nor-leaf, Nak-stem and Nor-stem of *L. bipinnata* is given in Table 4.

In Nak-leaf, alkaloids, phenols, flavonoids, tannins, phlobatanins, steroids triterpenes and coumarins were present in maximum amount followed by saponins, cardiac glycosides, anthocyanins, quinones and leucoanthocyanins. In Nor-leaf, phenols and tannins were present in maximum amount followed by alkaloids, phlobatanins, steroids triterpenes, anthocyanins and coumarins (Table 4). Other phytoconstituents like, flavonoids, saponins, quinones and leucoanthocyanins were present in trace amount.

In Nak-stem, alkaloids, phenols, tannins, triterpenes and coumarins were present in maximum amount followed by flavonoids, phlobatanins, saponins, and steroids (Table 4). Other phytoconstituents like cardiac glycosides, anthocyanins and quinones were present in trace amount. In nor-stem, phenols, tannins and steroids were present in maximum amount followed by triterpenes and coumarins (Table 4). Other phytoconstituents like alkaloids, flavonoids, phlobatanins, saponins, anthocyanins and quinones were present in trace amount.

Table 4: Qualitative phytochemical analysis of *L. bipinnata* leaf and stem

Sr. No.	Phytochemicals	Nakshatra days		Normal days	
		Leaf	Stem	Leaf	Stem
1	Alkaloids				
	✓ Mayer's reagent	-	+++	-	+
	✓ Dragendorff's reagent	+++	+++	+	+
	✓ Wagner's reagent	+++	+++	++	+
2	Phenols	+++	+++	+++	+++
3	Flavonoids	+++	++	+	+
4	Tannins	+++	+++	+++	+++
5	Phlobatannins	+++	++	++	+
6	Saponins	++	++	+	+
7	Steroids	+++	++	++	+++
8	Cardiac glycosides	++	+	-	-
9	Triterpenes	+++	+++	++	++
10	Anthocyanins	++	+	++	+
11	Quinones	++	+	+	+
12	Leucoanthocyanins	++	-	+	-
13	Coumarins	+++	+++	++	++

Note: (+++) more amount, (++) moderate amount, (+) less amount, (-) absent

Fluorescence analysis

The fluorescence analysis of crude powder of *L. bipinnata* leaf and stem are shown in Tables 5 -8. In the present study dried powder of medicinal plant *L. bipinnata* leaf and stem was treated with a number of different reagents which showed characteristic fluorescence at 254 nm and 365 nm wave length. The plant powder of *L. bipinnata* showed different colors at both wave lengths. The colours observed were green, light green, dark green, yellow, brown, yellowish brown brownish black and black for leaf; and green, dark green, light green, yellow, yellowish green, red, black, brown and reddish brown for stem. The fluorescence characters of medicinal plants play an important role in the determination of quality and purity of the drug material.

Table 5: Fluorescence analysis of *L. bipinnata* Nakshatra days leaf

Sr. No.	Treatments	Visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1 N NaOH (aq)	Dark green	Black	Dark green
2	1 N NaOH (alco)	Brown	Black	Green
3	Ammonia	Green	Black	Green
4	Petroleum ether	Dark green	Black	Green

5	50% HCl	Greenish brown	Black	Dark green
6	50% H_2SO_4	Dark green	Black	Dark green
7	Ethyl acetate	Blackish green	Yellow	Green
8	Ethyl alcohol	Black	Black	Brown
9	Methanol	Dark Black	Black	Dark green
10	50% KOH	Dark green	Black	Dark green
11	50% HNO_3	Red	Black	Light black
12	Acetic acid	Dark green	Black	Brown
13	Iodine in water (1%)	Brownish black	Black	Dark green
14	$FeCl_3$	Dark green	Black	Light green
15	Picric acid	Yellowish black	Black	Black

Table 6: Fluorescence analysis of *L. bipinnata* Normal days leaf

Sr. No.	Treatments	Visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1 N NaOH (aq)	Dark green	Black	Dark green
2	1 N NaOH (alco)	Brown	Black	Green
3	Ammonia	Green	Blackish brown	Green
4	Petroleum ether	Dark green	Black	Green
5	50% HCl	Greenish brown	Black	Dark green
6	50% H_2SO_4	Dark green	Black	Dark green
7	Ethyl acetate	Blackish green	Yellow	Green
8	Ethyl alcohol	Brown	Black	Brown
9	Methanol	Dark green	Black	Dark green
10	50% KOH	Dark green	Black	Dark green
11	50% HNO_3	Red	Black	Light black
12	Acetic acid	Dark green	Black	Brown
13	Iodine in water (1%)	Brownish black	Black	Dark green
14	$FeCl_3$	Dark green	Black	Light green
15	Picric acid	Yellowish black	Black	Black

Table 7: Fluorescence analysis of *L. bipinnata* Nakshatra days stem

Sr. No.	Treatments	Visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1 N NaOH(aq)	Dark green	Black	Brown
2	1 N NaOH(alco)	Light green	Brown	Light green
3	Ammonia	Dark green	Black	Greenish black
4	Petroleum ether	Yellowish green	Black	Light green
5	50% HCl	Brown	Black	Dark green
6	50% H_2SO_4	Green	Black	Dark green
7	Ethyl acetate	Light green	Yellow	Yellowish green
8	Ethyl alcohol	Green	Dark green	Green
9	Methanol	Light green	Black	Light green
10	50% KOH	Yellowish green	Black	Brown
11	50% HNO_3	Red	Black	Black
12	Acetic acid	Dark green	Black	Light green
13	Iodine in water (1%)	Greenish brown	Brown	Dark green
14	$FeCl_3$	Dark green	Black	Black
15	Picric acid	Yellowish green	Black	Dark green

Table 8: Fluorescence analysis of *L. bipinnata* Normal days stem

Sr. No.	Treatments	Visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1 N NaOH (aq)	Dark brown	Black	Brown
2	1 N NaOH (alco)	Light green	Brown	Light green
3	Ammonia	Dark green	Black	Greenish black
4	Petroleum ether	Yellowish green	Black	Light brown
5	50% HCl	Brown	Black	Dark green
6	50% H_2SO_4	Green	Black	Dark green
7	Ethyl acetate	Light green	Yellow	Yellowish green
8	Ethyl alcohol	Yellow	Dark green	Brown
9	Methanol	Light green	Black	Light green
10	50% KOH	Yellowish green	Black	Brown
11	50% HNO_3	Red	Black	Red
12	Acetic acid	Dark green	Black	Light green
13	Iodine in water (1%)	Greenish brown	Brown	Dark green
14	$FeCl_3$	Dark green	Black	Black
15	Picric acid	Yellowish green	Black	Dark green

Discussion

Pharmacognostic studies are simple and easy but essential steps to be followed for all the medicinal plants under study so that their authenticity and efficacy can be maintained. These studies also prevent the plant from being adulterated or substituted. There are many modern methods for identification but still pharmacognostic studies are cheapest, reliable and easiest [18, 19]. In the present work, there was no difference in the macroscopic, microscopic and powder studies of Nak-leaf, Nak-stem, Nor-leaf and Nor-stem and hence only general figures of leaf and stem are presented. Organoleptic and macroscopic evaluation is a qualitative evaluation based on the study of morphological profile of the plant. Macroscopical characters of the *L. bipinnata* leaf and stem showed that the leaf was green in colour, shape of leaves was decompound type, apex was acuminate apicular, Outer surface was pubescent and base was symmetrical. The stem was green in colour, unbranched, woody, erect. Shape was square or quadrangular. Outer surface was rough and hairy. The microscopic evaluation showed leaf lamina was dorsiventral in nature, unicellular or multicellular trichomes were present. Vascular bundles were arc shape, conjoint, collateral, open type and lower epidermis showed anomocytic stomata. The stem showed single layered epidermis, uniseriate unicellular and multicellular trichomes, stem consisted of four bulges at the four corners, vascular bundles were conjoint, collateral, open type and pith was very large. The powder study of leaf and stem showed spiral and reticulate vessels, unicellular and multicellular trichomes, annual vessels, anomocytic stomata, squares calcium oxalate crystal, scalariform and reticulate vessels, starch grains, etc. Such studies are reported for other plants *Cissampelos pareira* [20], *Barleria montana* [21], *Wedelia trilobata* [22] *Andrographis echioides* and *Tridax procumbens* [23].

On the other hand, as already described in the results section, there was slight difference in physicochemical and phytochemical properties of Nak-leaf, Nak-stem and Nor-leaf and Nor-stem. The moisture content was well within limits (7 to 10%) indicating less chances for degradation or microbial contamination. Higher moisture content leads to breakdown of some constituents on storage [24]. The extractive values were definitely more in Nak-leaf and Nak-stem than in Nor-leaf and Nor-stem. Such studies will give an idea about the nature of the phytoconstituents present in the plant material. They will also give an idea about exhausted or adulterated drugs. The ash values give an idea about inorganic materials and silica present in the crude drugs. It is one of the easiest way to check contamination and adulteration as reported by other researchers in other plants [25 – 27].

Qualitative phytochemical results also showed more presence of bioactive compounds in Nak-leaf and Nak-stem than in Nor-leaf and Nor-stem. It can be concluded that Nak-leaf and Nak-stem will show better antioxidant properties than Nor-leaf and Nor-stem. Hence their therapeutic efficacy will be more than Nor-leaf and Nor-stem. The results also confirm that Nak-leaf is more efficient therapeutically than Nak-stem. Similar phytochemical screening has been reported for other plants [28 – 30].

The fluorescence analysis of dried powder of leaf and stem showed different colours with different reagents in normal light and UV light. For both leaf and stem powder, some reagents showed one colour in visible light which changed to another colour in UV light. It is characteristic of particular part and particular plant. This technique is quick and helps in qualitative assessment of crude drug. Such fluorescence studies are reported for other plants [31- 34].

Conclusions

The results of this study lays down the characteristic diagnostic features of leaf and stem of *L. bipinnata*, an important medicinal plant of Lamiaceae family. The parameters will be helpful for checking the identity and authenticity of this plant and prevent it from getting adulterated. The studies also support the traditional belief that plants collected during a particular Nakshatra are therapeutically more potent than those collected during normal days. Further studies are necessary for more concrete scientific validity. Studies in this direction are in progress.

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