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Botanical Standardization of *Leea macrophylla* Roxb. (Leeaceae)

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Abstract

Worldwide various systems of medicines make use of drugs from plant, animal and mineral origin. Majority of medicines utilized globally are from plant origin. *Leea macrophylla* Roxb. is one amongst these being used as medicine worldwide. It belongs to family Leeaceae. Leaves, stem, roots as well as seeds possess medicinal properties. Tuberous roots along with the other herbal combinations are effectively used to cure bone fracture in various parts of India. It is well recognized in India as one of the wound healing plants. It is used as drug in various indigenous systems of medicines like Ayurveda, Siddha, Unani and Tibbi. Standardization of such drug is of prior importance for quality control with respect to commerce and trade. In the present research work various Botanical and Phytochemical techniques are used to standardize this unique novel drug.

Keywords: *Leea macrophylla*, Botanical and Phytochemical techniques.

1. Introduction

India is prosperous in diversity of plants. Traditional medicinal practioners of country make use of medicinal plant resources to cure various diseases. The plant wealth for medicine was collected from various biodiversity rich regions. It is used as drug in indigenous systems of medicine viz., Ayurveda, Siddha, Unani and Tibbi. The present investigation deals with the standardization of plant resource, *Leea macrophylla* Roxb. (Leeaceae). The plant is commonly found distributed within the hotter parts of the Indian peninsula. It is found growing in Assam, Bengal, Eastern Himalayas and Western-ghats of Maharashtra. It is called as Gajkarni or Hattikaan in vernacular language. According to Indian Mythology, Gaj/Hatthi means the animal Elephant, Karna means Ears, the leaves of the plant are as long as the ears of Elephant. Dhola Samudra and Dinda are yet other vernacular names. Local tribal peoples often harvest leaves of the plant to prepare vegetables. Root tubers are also utilized for the same purpose. It also finds broad application in various medications. Root tubers are taken internally as medicine to cure sexual debility and applied externally on wounds to stop bleeding. It is also in fact used as traditional medicine to cure guinea worm. The root tubers along with the other herbs are used to cure bone fracture. Due to wide-ranging applications the plant is often harvested by conventional medicine men. Review of literature reveals that the plant possesses nutritional as well as medicinal properties. The attempt is made to standardize the plant with the help of Botanical, Pharmacognostic and Phytochemical techniques. The research work evaluates the plant with respect to appearance, distribution, locality, medicinal plant parts and therapeutically active chemical constituents.

2. Review of Literature

Leea macrophylla Roxb. is found distributed throughout the hotter parts of the India. It found well growing in Assam, Bengal and West Indian Peninsula (Hooker, 1875). In Maharashtra (India) it is located in Amboli region of Konkan (Almedia, 1990). It is referred by various vernacular names like Dholsamudra in Bengali, Samudraka in Hindi and Dinda in Marathi. It is the herbaceous erect plant and attains the height about 1ft. – 3 ft. (Cooke, 1967). Leaves are broadly ovate 9 in. – 2 ft. long and coarsely serrate. Flowering period is found within August to October (Singh, 2000). Leaves are eaten as vegetable (Watt, 2014). Root tubers are deep red in color and applied to cure wounds and sores (Annonymous, 1962). Roots are used as remedy for ringworm and also to cure guinea worm. In addition these are used to relieve pain (Chopra *et al*., 1956). These are further used to stop effusion of blood (Kirtikar and Basu, 2005). Raphides, mucilage cells and acicular crystals are present internally within the roots (Metcalfe and Chalk, 1950). Roots show presence of vitamins, Thiamine, Riboflavin and Ascorbic acid along with Vitamin B12 (Jadhao *et al*., 2009). Leaves, roots and stem are rich in minerals (Jadhao *et al*., 2010). The root tubers also possesses antioxidant and antibacterial activity and showed good results against gram positive and gram negative bacteria (Joshi *et al*., 2016). Seeds possesses phytochemicals, imparts antibacterial activity to the drug

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(Islam *et al*., 2013). Analgesic and cytotoxic activity of ethanolic extract of roots was checked by Mahmud *et al*., (2011).

3. Tables and Figures

Table I: Macroscopic study of *Leea macrophylla* Root Tubers

S.N.	Macroscopic Characters	Observation
1	Color (Outer)	Red
2	Color (Inner)	Faint Red
3	Odor	Characteristic
4	Fracture	Hard
5	Taste	Astringent

Table II: Histochemical study of *Leea* Root Tubers

S.N.	Histochemical Test	Reagent	Location	Color Observed
1	Starch	I_2KI	Cortex Pith	Dark Blue
2	Proteins	K_3FeCN_6	Pith	Blue
3	Red. Sugars	Fluikger's Reagent	Cortex, Pith	Orange yellow
4	Tannins	10% $FeCl_3$ (Acidic)	Pith	Yellowish Green
5	Saponins	$Ba(OH)_2$, $CaCl_2$, $K_2Cr_2O_7$	Cortex Pith	Yellowish Red
6	Fats	Sudan III	Cortex	Pinkish purple
7	Alkaloids	Dragendorff's	Cortex, Pith	Brick Red

Table III: Qualitative Phytochemical Study of Leea macrophylla Root Tubers

S.N.	Phytochemical Tests	Reagents Used	Color Observed
1	Starch	I_2KI	Sky-Blue
2	Proteins	Millons	Red
3	Sugars (glucose)	Benedicts	Orange
4	Tannins	10%Aq.$FeCl_3$	Green
5	Glycosides	Alcohol(Warm), Benzene	White
6	Flavanoids	Conc.HCl, Mg Turnings	Dark Pink
7	Alkaloids	Dragendorff's	Brick Red
		Wagner's	Dark Brown
		Mayer's	Grayish

Table IV: Quantitative Phytochemical Study of *Leea macrophylla* Root Tubers

S.N.	Phytochemicals	Values Obtained
1	Carbohydrates	41.10±0.4
2	Proteins	20.36±0.70
3	Phenols	0.050±0.006

[The values are the mean of triplicates (S.E.)]

Table V: Percentage extractive an Ash Analysis of *Leea macrophylla* Root Tubers

S.N.	Solvents	Weight (mg) %
1	Water	0.40±0.014
2	Alcohol	0.52±0.17
3	Petroleum Ether	0.45±0.041
4	Solvent Ether	0.37±0.08
5	Total Ash	0.22±0.017
6	Acid insoluble ash	0.084±0.004

[The values are the mean of triplicates (S.E.)]

Table VI: Fluorescence analysis of *Leea*

Treatment	Powdered root observed under U.V.	
	254 nm	365 nm
Powder as Such	Blue	Black
Powder as Such Mounted in Nitrocellulose	Fluorescent Blue	Violet
Powder + 1N NaOH in CH_3OH after drying for ½ hour and mounted in Nitrocellulose	Purple	Black
Powder + 1N NaOH in CH_3OH	Black	Bluish Black

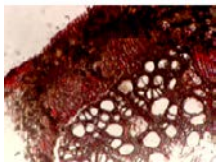
Fig. 1.

***Leea* root tuber**

Fig. 2.

***Leea* root tuber Internal**

Fig. 3.

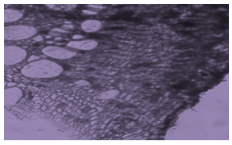


Microscopy

Fig. 4.

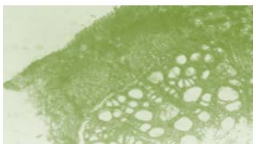
Tracheid

Fig. 5.



Starch in *Leea*

Fig. 6.



Tannins in *Leea*

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Fig. 7. **Fig. 8.**
Fig. 7 **Fig. 8**

Fig. 7 **Fig. 8**

Saponins in *Leea*

Alkaloids in *Leea*

4. Experimental work:

4.1 Identification (Fig. 1 and Fig. 2): Frequent field visits were organized. Herbarium was prepared. It was further compared with the standard herbarium specimens of *Leea macrophylla* from Botanical Survey of India, Pune Maharashtra State. Photographs of the experimental plant were taken in flowering and fruiting season.

4.2 Collection: Collection spots of the phytogeographic regions were marked as per the standard Indian floristic literature. Collection was done from Lonawala and Khandala regions of Maharashtra state (India). Root tubers were uprooted. These were brought in laboratory for further study.

4.3 Preservation: The collected root tubers were divided into three parts. First part was utilized in fresh condition for detailed Botanical and Histochemical study. Second part was dried in shade and the dried pieces of the root tubers were used for analysis. Third part included fine powder made out of shade dried root tubers. It was further used for phytochemical analysis.

4.4 Botanical Study: This included Macroscopic and Microscopic Study of the drug.

4.4.1 Macroscopic (Organoleptic) study (Table 1): This included study of the drug part with respect to color internal and external, fracture, taste, odor, markings internal and external (Gatercoal and Writh, 1949; Wallis, 1967; Trease and Evans, 1980).

4.4.2 Microscopic study (Fig. 3): Microscopic examination was done by taking the cross section of roots. Thin sections were stained in safranin and light green. These were used to reveal the anatomical details (Johansen, 1940).

4.5 Histochemistry (Table II and Fig's. 5, 6, 7 and 8): The study included detection and localization of active chemical constituents within drug. Cross section of the fresh plant materials were taken. These were treated with respective chemical reagents and observed under the microscope. Starch, Proteins, Reducing sugars, Tannins, Saponins, Fats, and Alkaloids, were detected. Photographs of the slides were taken (Johansen, 1940 and Krishnamurthy, 1988).

4.6 Phytochemistry: Powder prepared from the collected plant material was used to study presence of phytochemicals **(Table III)**. Water extract made out of the drugs was used for detection of Starch, Proteins, Sugars, Tannins and alcoholic extract for detection of Glycosides, Flavanoids and Alkaloids (Wallis, 1967; Peach and Tracy, 1955; Trease and Evans 1972). Powder of the root tubers was further used for quantitative estimation of Carbohydrates (Nelson, 1941); Proteins (Lowry *et al*., 1951) and Phenols (Malick *et al*., 1980). Percentage extractives and ash analysis was done to estimate quality standards (Annonymous, 1955). Fluorescence analysis of powdered drug was carried as stated by Chase and Pratt, (1949).

Results: Underground roots have tubers at the certain interval these are swollen and hollow with longitudinal linings of ridges. These are reddish and shiny in appearance. In transverse section microscopic observations shows wavy outline. Pericle parenchymatous, vascular bundles with massive metaxylem. Pith small at the solid constrictions. Needles of raphides are present in parenchymatous cells. Quantification values of Starch, Proteins and Carbohydrates are depicted in **table IV**. Values of Percentage extractives with respect to solubility are given in **table V** along with ash analysis. Observations with respect to fluorescence test depicted in **table VI** marks the characteristic features with respect to standardization and quality control. These may find its use to check identity as well as adulteration of the drug with spurious materials or similar looking other plant parts.

Conclusions

It is of prime importance to exhibit the use of drug *Leea macrophylla* in front of the society. The data available in the present research work may help to check potency as well as efficacy of the drug. With the help of macroscopic study one can easily locate the plant observing larger broad leaves and swollen underground reddish root tubers. Histochemical observations provide the exact location of therapeutically active chemical constituents like Alkaloids and Glycosides within the specific plant tissues. Positive results obtained from Phytochemical tests throw light on therapeutic activity of the drug. Percentage extractives and ash analysis provides standards helpful for quality control. Fluorescence analysis may help to check and verify identity of the drug. So far the data provided in the present research work may find its importance in drug identification and standardization. It will be beneficial for commerce and trade of the drug *Leea macrophylla*.

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