



PHARMACOGNOSTICAL STANDARDIZATION OF LEAVES OF LOBELIA NICOTIANAEOFOLIA ROTH EX R. AND S.

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ABSTRACT Plants are a great source of medicines, especially in traditional medicine which are useful in the treatment of various diseases. *Lobelia nicotianaefolia* Roth ex R. and S. belonging to family Lobeliaceae is a tall, erect, much branched large herb commonly known as Ran Tambakhu and has been employed as herbal medicine in the treatment of snake bite, dog bite, asthma and arthritis. The present paper deals with pharmacognostical and phytochemical studies on leaves of *Lobelia nicotianaefolia* Roth ex R. and S. It includes macro and microscopic characters, analysis of ash, dry matter, moisture content, fluorescence analysis and analysis of powdered drug reactions with different chemical reagents. Transverse section of the leaf lamina showed the presence of lower and upper epidermis with thick cuticle and spongy parenchyma with intercellular spaces. Powder microscopy shows fragments of unicellular trichomes, starch grains, stomata and oil droplets. The powdered crude drug was extracted with different solvents and screened for their phytochemical constituents. Qualitative phytochemical analysis of these extracts revealed the presence of phenols, alkaloids, flavones, tannins, coumarins, saponins, reducing sugars and xanthoproteins. The morphological, microscopic, physicochemical and chromatographic studies would serve as standard reference for identification, authentication and distinguishing the plants from its adulterants. This is the first such study on standardization of *Lobelia nicotianaefolia* Roth ex R. and S. leaf. The study scientifically validates the use of plant in traditional medicine.

KEYWORDS : *Lobelia nicotianaefolia*, Lobeliaceae, Leaf, Pharmacognostical, Phytochemical.

INTRODUCTION

India is considered to be a store house of medicinal plants. It harbors over 2000 medicinal plant species of which 443 have been recorded for the state of Maharashtra. Traditional system of medicine has once again become a central theme of health care of modern society of modern age. There has been increasing demand for herbal medicines and the indigenous systems have now regained respectability among the scientific community all over the world. Ayurveda is gaining popularity not only in India but also abroad. The ancient knowledge of herbal medicine is a great source of information for scientific community, researchers and medical practitioners. In last couple of decades a new trend in the preparation and marketing of drugs based on medicinal plants has become increasingly apparent (Bisset, 1994). The curative properties of medicinal plants are mainly due to presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan et al., 2013). Pharmacognosy is the study of medicines derived from natural sources. It is the study of physical, chemical, biochemical and biological properties of drug substance. It is also defined as the study of crude drugs. Pharmacognostical study is the preliminary step in the standardization of crude drugs.

Herbal drugs are traditionally considered harmless and being consumed by people without prescription. It is generally believed that standardization of crude drug is not required when used by the rural community for their primary health care. However many dangerous and lethal side effects have recently been reported including direct toxic effects, allergic reactions, effects from contaminants and interactions with other drugs. In view of the above to avoid such side effects standardization of crude drug is an important step for production and manufacturing of an herbal drug. The majority of the Ayurvedic preparations in the market are spurious, adulterated or misbranded. Therefore it is essential to establish quality standards for their use as drugs and as raw material for preparation of other drug products. Herbal products cannot be considered scientifically valid if the drug has not been authenticated and characterized.

Kolhapur district has a valuable heritage of herbal remedies. The climate of the district is healthy and favorable for growth and development of plants. So district shows rich biodiversity. Its rural people living in remote forest area are till depending on the local plant resources for medical treatment. *Lobelia nicotianaefolia* Roth ex R. and S. belonging to family Lobeliaceae is a tall, erect, much branched large herb commonly known as Ran Tambakhu and has been employed as herbal medicine in the treatment of snake bite, dog bite, asthma and arthritis.

MATERIAL AND METHODS

Ethnomedicinal information was collected through interview with

traditional rural practitioners (Vaidus) as suggested by Jain, 1987. Fresh plant material was collected from Kolhapur district of Maharashtra (India). Plant was identified with the help of Flora of Kolhapur District (Yadav and Sardesai, 2002). For microscopic studies uniform, thin, free hand sections of stem bark were taken and stained as per the procedure of Johansen, 1940. Macro and microscopic character were studied as described by Trease and Evans, 2002. Ash value, dry matter and moisture content of the material were determined by following the method of AOAC, 1990. Bark material was dried in shade so as to prevent decomposition of active principles and made into fine powder for the studies of power behavior, fluorescence study and phytochemical tests as per given in Indian Pharmacopoeia. Fluorescence analysis of the powdered was examined under U.V light according to the method suggested by Chase and Pratt, 1949 and Kokoski et al., 1958.

RESULT AND DISCUSSION

Macroscopic Characters

Lobelia nicotianaefolia belonging to family Lobeliaceae is a tall, erect, much branched, large herb or undershrub which grows to 1.5 - 3m in height (Fig.1). Stem stout, hollow. Leaves alternate, sessile, oblong - lanceolate, base tapering, margin serrulate, acute apex and light green. The lower leaves being 32 x 5 cm. while the upper ones gradually become smaller and the uppermost only about 8 x 1.5cm. Leaves glabrous above and glabrous or pubescent beneath, midrib white and main nerves many, slender. Flowers white in 16 -50 cm long terminal racemes. Calyx tube pubescent. Flowers 3 - 4cm long, two lipped. Filaments connate in to a tube. Capsules subglobose. Seeds many, ellipsoid, compressed, yellowish brown.

Microscopic Characters

T.S. Of Leaf

T.S. of leaf lamina (Fig. 2) shows upper and lower epidermis. Upper epidermis was covered with thick cuticle. Mesophyll shows only spongy parenchyma with intercellular spaces. Unicellular trichomes appear only on the upper epidermis (Fig. 3). Anomocytic stomata occur only on lower surface (fig. 4). Stomatal index of lower surface was 15.21. Veinlet number was 2.3 and vein termination number was 1.05.

Ash value: 4.8%,

Dry matter: 24.41%,

Moisture content: 75.59%.

Powder Study: Leaf powder of *Lobelia nicotianaefolia* was apple green in colour and coarse in texture. Powder shows fragments of unicellular trichomes, starch grains, stomata and oil droplets.

Behavior Of Bark Powder With Different Chemical Reagents

Leaf powder indicates presence of flavonoids, alkaloids, cystein, steroids, xanthoproteins and proteins. The thick ppt obtained with picric acid for alkaloid test indicates higher concentration of alkaloids. Tests for starch, tannins and oils were negative (Table- 1) .

Fluorescence character of bark powder under visible and UV light

Leaf powder treated with conc. HCl produces green fluorescence under visible and 254nm light where as under 366nm UV light it produces brown fluorescence. Powder treated with acetone produces green fluorescence under visible and 254nm light while black fluorescence under 366nm UV light. Powder treated with FeCl₃ produces green fluorescence under visible and 254nm while under 366nm UV light powder produces black fluorescence (Table-2).

Phytochemical Screening

Leaf extract shows positive tests for phenols, tannins, coumarins, saponins, alkaloids, reducing sugars, xanthoproteins and glycosides. The tests were negative for anthraquinones and flavones (Table-3). Alkaloids are higher in methanol extract where as phenols in aqueous extract.

Prakash et al., 2010 worked on folk medicine of NR Pura taluka in Chikmangalur district of Karnataka. The study revealed the ethnomedicinal information of 59 plant species belonging to 53 genera and 34 families. *Lobelia nicotianaefolia* was one of them. *L. nicotianaefolia* leaf extract was applied on the bitten area in the treatment of snake bite. In present investigation also the similar result was found about the medicinal uses of *L. nicotianaefolia*.

Wabale et al., 2011 reported ethnomedicinal uses of *Lobelia nicotianaefolia*. Authors given the uses of root and flowers in folk medicine in Ahmadnagar district of Maharashtra. Roots of *L. nicotianaefolia* were useful in the treatment of eye diseases and in dog bite while decoction of flowers was given orally in asthma. The ethnomedicinal information of *L. nicotianaefolia* collected from Kolhapur district differs at different places. The leaves of the plant were known to be used in snake bite, arthritis and dog bite.

CONCLUSION

This plant has a great importance in Ayurvedic and Modern systems of formulations. The present study on pharmacognostical and phytochemical evaluation of leaves of *Lobelia nicotianaefolia* Roth ex R. and S. provide useful information for its correct identification. Phytochemical tests revealed the presence of alkaloids, phenols, reducing sugars, coumarins, saponins xanthoproteins and tannins. The adulterants if any in the plant material can also easily identified by these studies. The values of ash analysis, dry matter and moisture content, results of fluorescence analysis and phytochemical data will be helpful for the standardization and quality control of precious indigenous drug.



Fig. 1 Flowering Twig

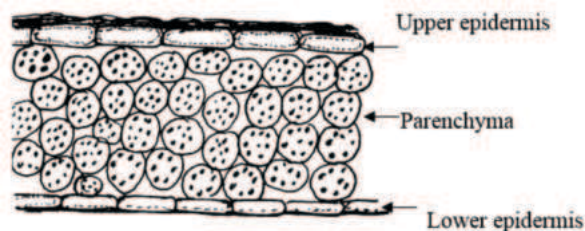


Fig.2 T. S. of Leaf lamina



Fig. 3. Trichome

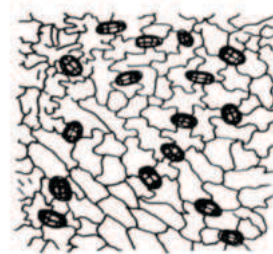


Fig. 4 Stomata

1: Behavior Of Powdered Drug With Different Chemical Reagents

SN	Treatment / Reagent	Behavior	Inference
1	Powder as such	Apple green	--
2	Powder + 1 N NaOH	Green yellow	Flavonoid
3	Powder + 5% Iodine	Apple green	--
4	Powder + 40% NaOH + Lead acetate	Green yellow	Cystein
5	Powder + Conc. H ₂ SO ₄	Brown	Steroid
6	Powder + Conc. HNO ₃ + Ammonia	Orange	Xanthoprotein
7	Powder + 5% FeCl ₃	Brown	--
8	Powder + 5% KOH	Pale yellow	Flavonoid
9	Powder + 1% AgNO ₃	Grey	Protein
10	Powder + Sudan III	Pale green	--
11	Powder + Glacial acetic acid	Saddle brown	--
12	Powder + Picric acid	Green yellow	Alkaloid

Table 2: Fluorescence Characters Of Powdered Drug Under Visible And U.V. Light

S	Treatment / Reagent	Visible	254nm	366nm
1	Powder as such	Office green	Pakistan green	Brown
2	Powder + NaOH in water	Office green	Pakistan green	Dark brown
3	Powder + NaOH in Alcohol	Forest green	Office green	Dark brown
4	Powder + Conc. HCl	Ponoma green	Pthalo green	Dark brown
5	Powder + Conc. H ₂ SO ₄	Black	Black brown	Dark brown
6	Powder + Conc. HNO ₃	Saffron yellow	Asparagus green	Black
7	Powder + 10% HCl	Ponoma green	Pthalo green	Black
8	Powder + Acetone	India green	Office green	Black
9	Powder + 5% KOH	Line green	Pakistan green	Black
10	Powder + Iodine	Asparagus green	Office green	Black
11	Powder + FeCl ₃	Dim green	Blackish green	Black
12	Powder + D.W.	Office green	Black green	Black

Table 3: Phytochemical Tests

Chem. constituents	Solvents					
	Methanol	P. ether	Acetone	Chloroform	Ethanol	Aqueous
Phenols	++	-	+	+	+	+++

Anthraquinones	-	-	-	-	-	-
Flavones	-	-	-	-	-	-
Tannins	+	-	+	-	+	-
Coumarins	-	++	-	+	-	-
Saponins	+	-	-	-	+	+
Alkaloids	+++	+	++	++	+	-
Reducing sugars	-	-	-	-	-	++
Xanthoprotein	+	-	-	+	-	++
Glycosides	-	-	-	-	-	-

(+ = Low, ++ = Medium, +++ = High, - = Absent)

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