Asian Resonance

Phytochemical and Anatomical Studies of the Medicinal Plant *Barleria Lupulina* Lindl

Abstract

The present work is undertaken to produce some pharmacognostic studies of *Barleria lupulina* Lindl. The paper deals with the studies on leaf epidermal micromorphology, wood element characters and phytochemicals identification of the medicinal plant *Barleria lupulina* Lindl. of the family Acanthaceae. The epidermal cells in leaf are irregular in shape and the outline is wavy. Stomata are present on the lower surface only and are of anisocytic type. Multicellular trichomes are present on leaf. Vessels have helical and pitted thickenings. Fibres are typical libriform. Methanolic (90%) extracts of leaf, root and stem powders have been screened for qualitative determination of different phytochemical groups by specific chemical colour reaction tests. The preliminary phytochemical analysis indicated the presence of alkaloids, starch, tannin, reducing sugar, protein, flavonoid, amino acids and lignin present in the methanolic extract of *Barleria lupulina* Lindl.

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Introduction

Herbal drugs play an important role in health care programs especially in developing countries. Use of micromorphology and anatomy is now recognised tool in the field of plant systematics. Importance of epidermal characters in general and those of trichomes in particular as well as comparative wood anatomy are widely recognised in taxonomic consideration of angiosperms (Ogundipe and Olatunji, 1991, Mukherjee *et al.,* 2000; Banerjee *et al.,*2002; Choudhury *et al.,*2009; Banu *et al.,*2012). Ontogeny and structure of stomata are now also considered as an important taxonomic characters of many of the angiospermic taxa (Choudhury *et al.,*2009; Banu *et al.,* 2012). Different angiosperms have been studied anatomically by various workers with special emphasis on leaf epidermal micro-morphology (Metcalfe and Chalk, 1950; Hossian andKhan,1994; Choudhury *et al,* 2009; Banu *et al.,* 2012). The genus *Barleria* of the family Acanthaceae is a pantropical genus of herbs and shrubs comprising of 300 species and is represented in India by 25 species. *Barleria lupulina* Lindl. commonly known as Bishalyakarani is an introduced species from Mauritius. It is also used for its medicinal importance as the leaf juice is given to stop bleeding when cut and the leaf-paste is used as poultice to relief pain. It is also used as an anti-inflammatory against insect bites, snake bites, herpes simplex virus. (Kanchanapoom *et al.,* 2001). Compounds found in the leaves of *Barleria lupulina* Lindl include barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester, ipolamiidoside and iridoidglucosides (Lans *et al*., 2001)In the present investigation an attempt was made to study the anatomy of stem, root, leaf and petiole of *B. lupulina* and identification of crude drugs from this taxon.

Material and Method

Plant materials for the present study were collected from the medicinal plant garden of Rampurhat college, Rampurhat, Birbhum, located in the lateritic belt of West Bengal.

Anatomical Study

Plant materials (leaf, petiole, stem and root) were collected from the garden and hand sections (transverse) were made and stained it diluted saffranin (50%) and fast green solutions (1%) for anatomical study (Johansen, 1940). To study the fibre-tracheids and vessel elements, small pieces of stem and root tissues were macerated with 10% nitric acids and 10% KOH solutions separately for 10 minutes each. The treated samples

were washed thoroughly with distilled water, macerated separately and stained with dilute saffranin solution for microscopic study. Photomicrographs were taken using light microscope with the hand section and observed under 10x X 10x microscopic lens.

Micromorphological study

Peelings were made from the fresh leaf materials, mounted with 10% glycerine and observed under light microscope.

Pharmacognostic study

Powdered drug samples were prepared from all the parts (stem, root and leaf) of the plant separately by drying the fresh materials at 35^{o} C in a hot air oven for 48 hrs. Powdered drug was boiled with saturated chloral hydrate solution, kept for 24hrs and studied under microscope.

Microchemical study

Microchemical tests were performed following the method of Jana *et al.,*(2009). One gram crude drug from stem, root and leaf were dissolved in 10ml methanol and kept for 25 days. The extract was then filtered and the filtrate was evaporated to concentrate the extract. The concentrated extract was redissolved in methanol to obtain pure extract. The extract was finally subjected to microchemical tests using the reagents Wagner's, $KI+I_2$, 10% lead acetate, Benedict's, Lugol's, 10 % NaOH, Fehling's (A + B), Millon's, 0.2% Ninhydrin and 1% Phloroglucinol.

Result and Discussion

Leaf

The transverse section of leaf shows (Fig. 2 a) single layered epidermis on both surface. The upper and lower epidermal cells are irregular and the outline of the cell wall is wavy (Fig.1 A). Leaf epidermis is glabrous. Multicellular, cylindrical covering trichomes are abundant on both the epidermis (Fig. 1B). Mid-rib showed 4-5 layered thick walled closely packed collenchymas on both the surfaces. Stomata are present on the lower surface only. They are anisocytic type (Fig. 1C). Each stomatal complex is having 4 subsidiary cells.

Petiole

T.S. of the leaf petiole is more or less triangular in outline (Fig. 2b). Epidermis is uniseriate and cutinized. Hypodermis is collenchymatous, 5-7 layered thick. Just beneath the hypodermis ground tissue is found. It consist of thin walled parenchyma cells having well defined inter cellular spaces among them. Vascular bundles are arranged in a half ring in ground tissue. A thick continuous zone of sclerenchyma presents encircling the vascular bundles. Xylem is located towards the upper side of the petiole, where as phloem towards the lower side of the petiole. Only one vascular strand is found on ground tissue. Two leaf traces are present on both side of the petiole.

Stem

Transverse section of the mature stem is quadrangular with 4 ridges and shows single layered epidermis (Fig. 3a). At the ridged zone 4-6 cell thick sclerenchyma patch is found just below the epidermis. Hypodermis is collenchymatous, 4-6 celled thick present encircling the vascular bundle. Bellow hypodermis there is parenchymatous layers of cortex encircling the stem also. The inner most layer of the cortex is endodermis consisting of barrel shaped, elongated, compact cells having no intercellular spaces among them. The pericycle present after endodermis. Vascular bundles arranged in a ring. The cambium separates the secondary phloem from secondary xylem. Vascular bundles are conjoint, collateral, endarch and open. Annual rings are not distinguishable. Xylem consists of vessel elements with pitted thickening (Fig. 1F) and tracheids show annular and helical type of thickenings (Fig. 1E). Thick walled parenchymatous cells are observed in the outer layer of pith, while thin walled cells occupy the central part of the stem. Long libriform stem fibres are also found (Fig. 1D).

Root

Transverse section of root shows (Fig. 3b) periderm in the outer region consists of phellem, phellogen and phelloderm. Cortical zone is narrow consisting of thin walled parenchymatous cells. The secondary vascular tissues form a continuous cylinder. Exarch primary xylem is located in the centre. Broad vascular rays i.e. medulary rays are also found traversing the xylem and phloem. Pith is short and consist of thick walled parenchymatous cells. Lenticels are also present. The vessels have pitted walls.

Trichome feature is very important in proper identification of the plants and considered as one of the valuable taxonomic marker now (Leelavathi and Ramayya, 1983; Dinc and Ozturk, 2008). Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants (Pant and Mehra, 1963; Krishnamurthy and Kannabiran, 1970; Treas and Evans, 1983 and Jana *et al*., 2009). The features of stomatal character found in this taxon may have a role in identifying the species. The taxonomic value of petiolar anatomy have been demonstrated for Saxifragaceae, Proteaceae, Ericaceae, Rhamnaceae at the generic level (Hare, 1943) and also in Plumbaginaceae (Jana *et al.,* 2009). Anatomical characters of the petiole in several species of *Jatropha* (Euphorbiaceae) are used to establish in intra-generic relationship and the sectional lines of this genus have been drawn on the basis of numerical constancy and relative uniformity in the arrangement of petiolar traces (Dehgan,1982). The present study on petiole character of *Barleria lupulina* can be used as a tool for the taxonomic discrimination of the species.

Anatomical characters are considered extremely important for identifying markers at intergeneric and interspecific levels (Chaturvedi,1995). Particularly the wood anatomical features seem to have taxonomic significance in distinguishing different plant species (Chanda and Mukherjee,1969;Ozdemir and Senel,2001). The correlation between a variety of wood anatomical features, vessels specialization and need to study the variability of vessel elements in individual genera and

species have long been established (Carlquist,1961; Burggraaf, 1973 ; Zakrzewski, 1983 ; Naik and Bhojaonkar, 2002 ; Jana *et al.,* 2009) Greater length and width of fibres may be correlated with faster growth rate as indicated by Bisset *et al.,* (1950).

Chemical constituents

The methanolic extracts of the leaf, stem and root of *B. lupulina* shows similarity in respect to chemical constituents. The methanolic extract of leaf, stem and root show positive tests for alkaloid, starch, protein, reducing sugar and lignin. The leaf and stem extract give positive result for tannin and flavonoid whereas, amino acid present only in leaf extract (Table-1). From the result it is found that the amount of chemical constituents present leaf is lower than stem and root.

Chemical analysis is very important aspect in pharmacognostic evaluation of medicinal plant (Trease and Evans, 1983; Harborne *et al*., 2000; Choudhury *et al*., 2009; Jana *et al*., 2009; Banu *et al*., 2012; Venkateswarlu *et al*., 2013). Through the chemical test in methanolic extract of leaf stem and root of *B. lupulina* it is revealed that the important phytochemical groups (alkaloids, starch, tannin, reducing sugar, protein, flavonoid, amino acid and lignin) are present in the plant confirming their medicinal properties. Similar type of result was obtained by Moin *et al*., 2012 in *B.lupulina* under in vitro callus production.

Conclusion

Microscopic analysis and qualitative parameters carried out on the plant of *Barleria lupulina* Lindl., may substantive as an essential data for identification of raw material and also used to differentiate the plant from its allied species and adulterants of controversy. These are the standard pharmacognostic parameters that can be used to differentiate closely related plant species.

Table 1.
Phytochemical analysis of methanol extract of stem, root and leaf of *Barleria lupulina* Lindl.

Sl. No.	Reagents	Colouration	Secondary metabolites	Plant parts		
				Stem	Root	Leaf
1.	Wagner's	Dark Brown	Alkaloid	+++	+++	+
2	KI + I_2	Blue Black	Starch	+	++	+
3	10 % aqueous lead acetate	Yellow	Tannin	+	-	+
4	Benedict's	Brick Red	Reducing sugar	+	-	+
5	Lugol's	Dark Brown	Protein	++	++	+
6	10 % NaOH	Yellowish Brown	Flavonoid	+	-	+
7	Millon's	Yellowish Brown	Protein	++	-	-
8	Fehling's (A+B)	Brick Red	Reducing sugar	+	+	+
9	0.2 % Ninhydrin	Lemon Yellow	Amino acids	-	-	+
10	1% phloroglucinol in 50% HCL	Reddish Brown	Lignin	+	++	+

(+,++,+++ represent degree of intensity of colour change i.e. presence of phytochemical groups and – represents no change of colour i.e. absence of phytochemical groups)

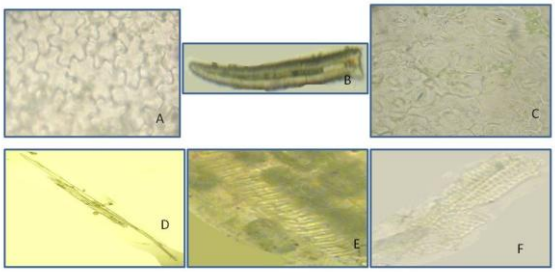

Fig. 1. Light microscopic photographs of *Barleria lupulina* Lindl.
A. Wavy epidermis. B. Multicellular covering trichome. C. Anisocytic stomata.
D. Stem fiber. E. Xylem vessel with helical thickening.
F. Xylem vessel with pitted thickening

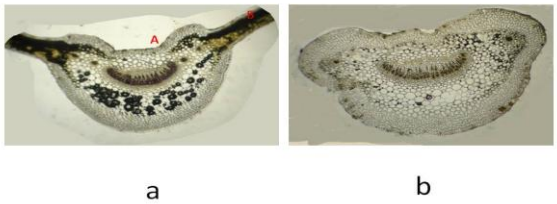

Fig. 2. Transverse section of *Barleria lupulina* Lindl leaf (a) and petiole (b)

A. Midrib
B. Lamina

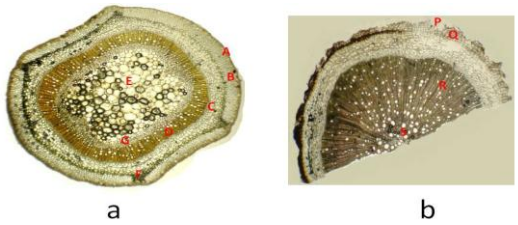

Fig. 3. Transverse section of *Barleria lupulina* Lindl stem (a) and root (b) showing secondary growth

A.Epidermis, B. Hypodermis, C. Secondary phloem, D. Secondary xylem, E. Pith, F. Collenchyma G. Primary xylem, P. Lenticel, Q. Cork cells, R. Secondary xylem, S. Primary xylem

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