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PHARMACOGNOSTICAL AND PHYSICOCHEMICAL STUDIES ON THE LEAVES OF *BARLERIA PRIONITIS* (L.)

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ABSTRACT

The *Barleria prionitis Lin*. (Acanthaceae), known as Vajradanti & Porcupine flower, is widely used in traditional medicine. According to survey, any report was not available on microscopic and physicochemical properties of *Barleria prionitis Lin*. The present study deals with Pharmacognostical examination and physicochemical constant of *Barleria prionitis Lin.* leaf**.** The leaf has antiseptic properties, its decoction is used for febrile catarrh, mouth wash to relief toothache and as a paste it is applied over boils & glandular swellings. Pharmacognostical evaluation includes macroscopy, microscopy, surface preparation and physicochemical parameter. The macroscopic features of the leaves are opposite, acute apex, elliptic-ovate in shape, entire margin, long petiole. Microscopically it consists of single layer of epidermis. Lamina is uniformly flat with even surface & contains single layer of palisade cell. Collateral vascular bundles and cross celled stomata. Physicochemical studies like ash value, loss on drying and extractive value. These finding will be useful towards establishing pharmacognostic standards which help in identification, purity and classification of the plant.

INTRODUCTION: The *Barleria prionitis* Linn. (Acanthaceae) is widely distributed throughout Africa, India, Sri Lanka and tropical Asia. It is well adapted to the climate of northern Australia, which has distinct wet and dry seasons. Barleria is considered to be a perennial species because it lives for more than one year. Porcupine flower grows in a wide variety of well – drained soils derived from igneous, metamorphic and sedimentary rocks [1, 2].

During the dry season when virtually no rain falls (May – September), its stem, leaves and flowers die off although the roots remain alive. Flowering occurs at the end of the wet and start of the dry season (April – May). Fruiting occurs several months after flowering, at the end of the dry season and it will give yellow – orange flower. While seeds germinate early in the wet season, following the first significant storms and grow steadily during the remaining the wet season. *Barleria* is a large polymorphic wide spread genus of herbs, shrub, up to 3 meter in height, found growing throughout the hotter parts of India. It is a much branched, prickly rarely climbers, 300 species are present worldwide. It is the third largest genus in the family Acanthaceae. *Barleria* grows on a wide variety of soil type and seems to prefer well - drained soil. Plants are propagated by seeds or cuttings and require pruning to keep them trim .It is also commonly grown as a hedge plant in garden [2, 3].

It is widely used in traditional medicine to treat infection related ailments. It is mainly used for medicinal purpose. Plant pacifies vitiated *vata*, *pitta* and it has numerous medicinal properties including treating fever, respiratory diseases, toothache, joint pains, burns, dental caries, and nocturnal ejaculation

and a variety of other ailments and it has several cosmetic uses [4]. It is widely used in urinary and paralytic affection, reuthmetic pain, and itch. A mouthwash made from root tissue is used to relieve toothache and treat bleeding gums. To increase sexual vigor, seed extract is administered daily once for a night.

Seed paste is used for a toothache. Because of its antiseptic properties, extracts of the plant are incorporated into herbal cosmetic and hair products to promote skin and scalp health. Juice of leaf administered in a little honey or sugar in catarrhal affections of children, which is accompanied by fever and much phlegm [5, 6, 7].

MATERIALS AND METHODS: The proposed material for study of *Barleria prionitis Lin.* was procured from south Gujarat region with the help of local tribe and field botanist. Care was taken to select healthy plant for normal leaves. The species for the proposed study was identified as *Barleria prionitis Lin.* by Dr. Minoo. H. Parabia, Botanist, Bapalal Vaidya Botanical Research Centre HOD, Department of Bioscience, Veer Narmad South Gujarat University, Surat, Gujarat.

Prepared herbarium was submitted and plant was certified as *Barleria prionitis* Linn. specimen no. - UCV/02/20112009. The leaves were collected and stored properly. The leaves were washed with water and dried in sunlight for one hour and then it was dried in shade. The dried leaves were powdered with the help of grinder and were passed through the sieve no. 60. Coarse powder was used for physiochemical work.

Chemicals and Instruments: Magnus MLX microscope, lucida camera, stage and eye piece micrometer and other basic equipments, glass wares are used for the present study. Solvents like conc.HCL, Phlouroglucinol, Chloral hydrate, Ethanol, Chloroform, Sulphuric acid and dilute HCL are used.

Pharmacognostical Studies:

Macroscopic Studies: The morphological characters like condition, type, size, shape, apex, margin, venation, base, petiole, surface, phyllotaxy, color, odor and taste of *Barleria prionitis Lin*. leaves were studied.

Microscopic Studies:

Collection of Specimen: Utmost care was taken to select healthy plant. The required sample of *Barleria prionitis Lin.* Leaf was cut and removed from the plant and washed with water.

Sectioning: The fresh leaf collected from the plant was washed and put on the slide. The thin T.S. was taken with the help of sharp blade. Then, it was cleared by treating with the 90% chloral hydrate solution. For the staining of that T.S., it was treated with conc. HCL solution and then with Phlouroglucinol solution. Lignified cells were colored to pink. For study of the morphology of the stomata the surface of the leaf was prepared [8].

Photomicrographs: Photographs of the T.S. of *Barleria prionitis Lin.* leaf at a different magnification were taken with Magnus MLX microscopic unit. Resolutions 5X, 10X & 100X are used for magnification of the detail view of T.S as well as surface preparation.

Quantitative Microscopy:

Stomatal Number: It is the average number of stomata per square mm of the epidermis of the leaf. Middle part of the leaf was cleared by boiling with Chloral hydrate solution. Upper and lower epidermis were peeled out separately with help of forceps & kept it on slide and mounted in glycerin water. With the help of micrometer, 1mm square was drawn. Number of stomata and epidermal cell which were present in the area of 1 sq.mm were counted. Recorded the result and calculated the Stomatal Index [9].

Stomatal Index: It is calculated by using this formula:

$$I = S/E + S \times 100$$

I = Stomatal Index, S = No. of stomata per unit area, E = No. of epidermal cells in the same unit area.

Physicochemical Studies: The powder leaves of *Barleria prionitis* Linn. determined the Ash value, Extractive value, Loss on drying and Foaming Index [10, 11, 12].

Determination of Ash Values:

Total Ash: Accurately weighed about 3 gms of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to

500-600°C until it is white, indicating the absence of carbon. Cool and weigh. This process repeated till constant weight. Then the percentage of total ash was calculated with reference to the air dried drug**.**

Water Soluble Ash: The total ash was boiled with 25 ml. of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Acid Insoluble Ash: The total ash was boiled with 25 ml of 2 N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot Water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Sulphated Ash: About 3 gm. of accurately weighed air dried powdered drug was taken in a tarred silica crucible, which was previously ignited and weighed. Then ignite gently first until the drug was thoroughly charred. The crucible was cooled and residue was moistened with 1ml of concentrated sulphuric acid, heated gently until the white fumes were no longer evolved and then ignited at 800 ± 25°C until all the black particles has disappeared. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed for cooling and weighed to get a constant weight.

Determination of Solvent Extractive Values: 5 gm. of drug was macerated with 100 ml of different solvents (90% alcohol, 90% chloroform) in closed flask for 24 hrs. It was shaken frequently during first 6 hrs and allowed standing for 18 hrs. Thereafter it was filtered. 25ml out of filtrate was evaporated to dryness in tarred flat bottomed shallow dish at 105°C and weighed, calculated with reference to the air-dried drug.

Loss on drying: About 3.0 gm powdered drug was weighed accurately in a tarred porcelain dish which was earlier dried at 105°C using hot air ovens at constant weight. Using the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

Foaming Index: Weighed accurately about 1 gm. of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintained at moderate boiling at 80-90°C for about 30 minutes. Then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100 ml (V_1). Cleaned 10 stopper test tubes were taken and marked with 1 to 10. The successive portions of 1, 2 ml up to 10 ml drug was taken in separate tubes and adjusted remaining the volume with the liquid up to 10 ml in each.

After closing the tubes with stoppers, Shook them for 15 seconds and allowed to stand for 15 minutes then measured the height. If the height of the foam in each tube is less than 1cm, the foaming index is less than 100(not significant). Here, if the foam is more than 1cm height after the dilution of plant material in the **sixth** tube, then corresponding number of the test tube was the index sought.

If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the plant material needs to be measured and transferred to a 100ml volumetric flask (V_2) and volume is to be maintained up to 100ml and follow the same procedure.

Foaming Index was calculated by using this formula:

Foaming Index = 1000/a in case of V_1;

Foaming Index = 1000 × 10/a in case of V_2

Where, a = volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

RESULTS: The Macroscopical studies of leaves showed that very thin and delicate leaf with some other characteristics like type, base, margin, apex and organoleptic characters like color, odor, and test of leaves are shown in (**Table 1, Fig.1**)

TABLE 1: MACROSCPICAL CHARACTERS OF *BARLERIA PRIONITIS LIN*. LEAF

Parameter	Observation
Condition	Fresh
Type	Simple
Size	Length : 11-12 cm Width : 6-7 cm
Shape	Elliptic to elliptic –ovate
Margin	Entire
Apex	Acute
Base	Attenuate
Petiole	0- 2.5 cm
Venation	Reticulate
Phyllotaxy	Opposite
Surface	Upper surface: Glabrous Lower surface : Pubescent
Color	Upper surface : Dark green Lower surface : Light green
Odor	Slightly aromatic
Taste	Mucilaginous
Extra feature	Decussate leaf with narrow & pointed at both ends.

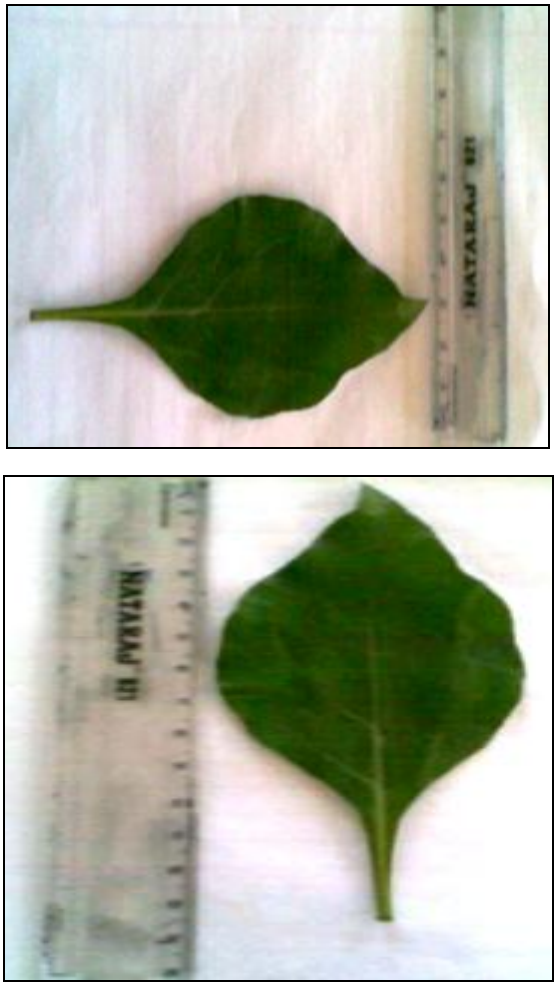

FIG. 1: PHOTOGRAPH OF *BARLERIA PRIONITIS* LINN. LEAF (LENGTH & WIDTH)

Microscopical studies showed the T.S. of leaf of *Barleria prionitis* Linn. and following characters are observed (**Fig. 2, Table 2**).

- Midrib is distinct from the lamina .Midrib consists of spongy parenchymatous cell having about 6-8 layers and cells are circular or angular with intracellular spaces & loosely arranged. Vascular bundle consists of thick vertical band of xylem & fairly wide band of phloem. It is a single & hemispherical in shape. Xylem elements are spherical, thick walled. It is systematically arranged and consists of upper & lower collenchymatous cells with thick walled.

- Lamina is flat and even surface. Mesophyll tissue is differentiated into single layer palisade cells & spongy parenchymatous cells. Trichome is absent.

TABLE 2: STAINING / DIAGNOSIS / MICRO – CHEMICAL TESTS

Reagents	Observations	Characterization
Phloroglucinol + Conc. HCl	Pink	Vascular Bundles
Dilute HCl	Soluble	Calcium Oxalate
Sulphuric acid (60 % w/w)	Soluble	Calcium Oxalate

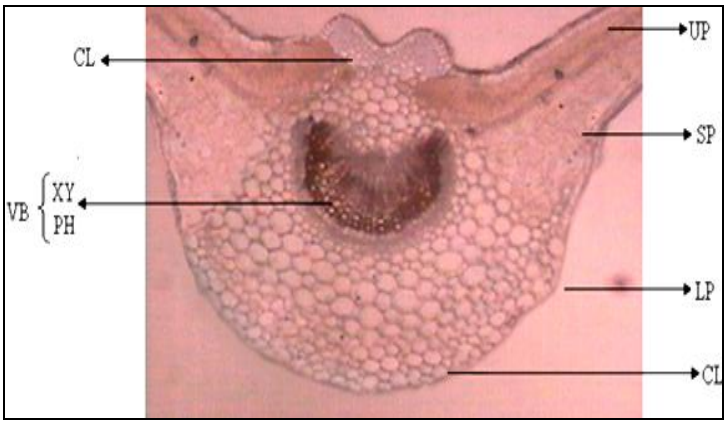

FIG. 2: PHOTOGRAPH OF T.S. OF *BARLERIA PRIONITIS* LINN. LEAF

Legends of figure: VB- Vascular Bundle, **XY-** Xylem; **PH-** Phloem; **SP-** spongy; parenchyama, **UP-** Upper Epidermis; **LP**-Lower Epidermis; **CL** -Cholenchayma.

- Each stomata is surrounded by varying number of subsidiary cells so the stomata are diacytic type (**Fig. 3**)

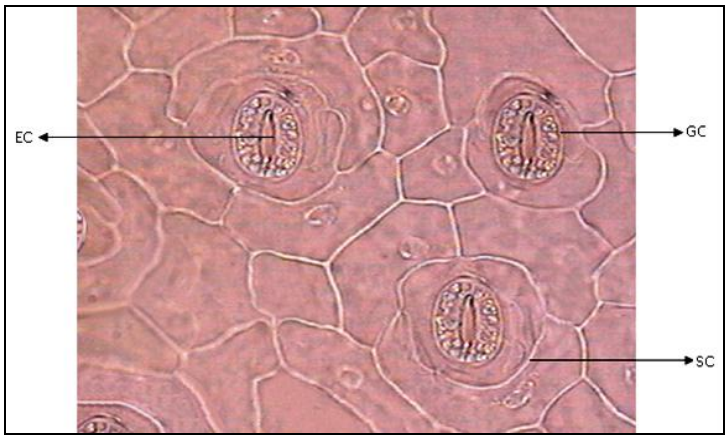

FIG. 3: SURFACE PREPARATION OF *BARLERIA PRIONITIS* LINN. LEAF

Legends of figure: GC- Guard cell, **EC**- Epidermal cell, **SC**- Subsidiary cell

Quantitative microscopy of leaves studies includes Stomatal Number and Stomatal Index is 63-68 and 42.28 – 43.58 respectively (**Table 3**). Physicochemical analysis of *Barleria prionitis Lin*. leaves powder is contained Ash values, Extractive Values, Loss on drying and foaming index (**Table 4**).

TABLE 3: LEAF CONSTANTS FOR *BARLERIA PRIONITIS* LINN.

Leaf Constants	Value
Stomatal number	63-68
Stomatal index	42.28 – 43.58

TABLE 4: PHYSICOCHEMICAL CONSTANT OF *BARLERIA PRIONITIS LIN.* LEAVES POWDER

Analytical Parameter	Value obtained on dry weight basis (%w/w)
Total ash	19.33%w/w
Acid insoluble ash	10.2% w/w
Water soluble ash	4.05% w/w
Sulphated ash	11.56% w/w
Loss on drying	3.36% w/w
Alcohol soluble extractive value	17.6% w/w
Aqueous extractive value	12.0% w/w
Foaming index	Not significant

CONCLUSION: The Pharmacognostical study is one of the major criteria for identification and authentication of plant drugs. The present study on Pharmacognostical characteristics of *Barleria prionitis* Linn. leaf will provide useful information for its standardization and classification on the basis of microscopy and physicochemical constants.

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