

**Pharmacognostic and Phytochemical Evaluation of *Barleria Prionitis* Linn.****Reema Dheer<sup>1\*</sup>, Alok Khunteta***Professor, L.B.S. College of Pharmacy, Jaipur, Rajasthan, India***Received: 12-06-2018 / Revised: 25-07-2018 / Accepted: 20-08-2018****ABSTRACT**

Abstract: The *Barleria prionitis* Lin. (Acanthaceae), known as Vajradanti & Porcupine flower, is widely used in Indian traditional medicine. It is widely distributed throughout Africa, India, Sri Lanka and tropical Asia. The aim of the study was to investigate the pharmacognostical and phytochemical parameters of leaves and roots of *B. prionitis* L. (Acanthaceae). The leaf has antiseptic properties, its decoction is used for febrile catarrh, mouth wash to relieve toothache and as a paste it is applied over boils & glandular swellings. The extract of root has been observed to have 100% antifertility activity and the extract of plant, due to its antiseptic properties is incorporated into herbal cosmetics and hair products to promote skin and scalp health. The present study included macroscopical, microscopical, physiochemical, phytochemical, fluorescence analysis and HPTLC methods for standardization as recommended by WHO. Fresh leaves and shade-dried roots were taken for morphological studies. Free hand sections from freshly collected leaves and preserved root materials were prepared for microscopical studies. Total ash value, acid insoluble ash value, water-soluble ash value, water soluble, ethanol soluble and ether soluble extractive values were determined. Loss on drying, study of foreign organic matter, limit tests of Arsenic and Heavy Metals was carried out. Preliminary phytochemical analysis revealed the presence of flavonoides, alkaloids, saponins, steroids, glycosides, tannins and phenols. The present study and its findings would serve as an important source of information helpful in establishing the identity, purity and efficacy of the plant and provide an efficient and inexpensive medicine to the society for future applications.

**Key words:** Purity, Pharmacognostic, Herbal medicines.**Introduction**

Herbal remedies gained popularity due to their effectiveness, easy availability, low cost, and comparative freedom from serious toxic effects. Because herbal medicines are thought to be “natural”, many people think that they are safe. However, herbal products can be as toxic or even more toxic than prescription medicine. They can also have unwanted side effects, and cause drug interactions. This implies that for reproducible efficacy and maximum benefit of the herbs and the herbal products quality control is very essential, which can be achieved by standardization[1].

In course of time, it came into light that on account of various ecological factors, the same plant has varying properties depending upon the region and climatic conditions of its growth. Even in Ayurveda, the practice of preparing medicines by the physicians for the use of patients has largely been supplanted by the pharmaceutical industry. Due to increasing urbanization, the tendency is towards more and more dependence on readymade preparations. The increasing need of the population and the chronic shortage of the authentic plant raw material have made it incumbent that a proper control on the quality of the herbal medicine be maintained. The WHO has emphasized the need to ensure the quality control of medicinal plant products by using modern techniques and by applying suitable standards. Simple tests like microscopy, microcopy, and ash content, extractive values in various solvents and thin layer chromatography can reveal lot of information. It not only helps in

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establishing the correct botanical identity but also helps in regulating the chemical sanctity of the herbs.

The whole plant, leaves, barks and roots of *Barleria prionitis* are all used. The whole plant extract of *Barleria prionitis* contains iridoid glycosides, barlerin, and verbascoside, which have shown potent activity against respiratory syncytial virus in vitro and may account for the plants use in treating fever and several other respiratory diseases in herbal medicine [2]. The leaves are used to promote healing of wounds and to relieve joints pains and toothache [3]. The roots are used as a febrifuge and as a decoction they are employed as a mouth wash to relieve toothache, and as a paste they are applied over boils and glandular swellings was observed [4].

## Material and Methods

### Procurement and authentication of crude drug

Fresh botanically identified leaves and roots of healthy, mature plants of *Barleria prionitis* Linn. belonging to the family Acanthaceae were collected personally on 27<sup>th</sup> November, 2004 from the botanical Garden in Lal Bahadur Shastri College of Pharmacy, campus, where it grows naturally. The sample collected were authenticated by comparing with standard Herbarium sheets of the Herbarium of the Department of Botany, University of Rajasthan and also referring various national and regional floras of India. Few pieces of root samples were preserved in a glass bottle containing the solution of F.A.A. (Formalin- Aceto-Alcohol-95ml of 50% alcohol + 3 ml of formalin + 2ml of acetic acid ) for microscopical study. A few sample of whole roots and fresh leaves were dried under shade and kept for morphological study.

### Evaluation Parameters

**Macroscopic studies:** the morphological characters of the leaves and roots of *Barleria prionitis* linn. were studied by using a magnifying lens (10x) and dissection microscope (10x & 20x). morphological observations, which include shape, size texture, external surface, fracture, colour, odour, taste were noted down. photographs were taken as records for illustration of morphological study.

**Microscopic studies:** Free hand sections from freshly collected leaves and preserved root materials were prepared for microscopical studies. Glycerine (1:2) was used as medium for mounting. Chloral hydrate (4:10 solution B.P. was used as clearing agent. Phloroglucinol and HCL were used as staining agents to detect lignified elements. Saffranin was used for staining the sections.

Photomicrographs were taken for showing the distribution of tissues in transverse sections.

## Physical evaluation

### Determination of ash values

The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earthy matter. It is especially more applicable to powdered drugs. The adulteration of soil, sand, silica, limestone etc. can be detected by the ash values[5,6]The various ash values were determined as per the method prescribed in Indian Pharmacopoeia 1996, Vol-II.

### Determination of Total Ash Value

Two gms of accurately weighed air dried powder formulation was taken in a previously weighed clean and dry platinum crucible incinerated at 450 C until the ash became free from carbon which was confirmed by the white colour of the ash. The crucible containing the ash was kept in a desiccator and allowed to cool till a constant weight was obtained. The percentage total ash with reference to air-dried sample was calculated [7]. The results are given in Table 2.1

### From this ash the water soluble ash and acid insoluble ash were determined

#### Determination of Water Soluble Ash Value

The ash was boiled with 25 ml of water for 5 minutes and filtered through ashless filter paper. The residue collected on the filter paper was washed with hot distilled water. The filter paper was allowed to dry and ignited for 15 minutes at 450<sup>0</sup> C. The weight of insoluble ash was determined and subtracted from the total ash taken to obtain the water soluble ash. The percentage of water soluble ash was calculated with reference to air dried sample (I.P.1996 Vol-II). The results are given in Table 2.1[8-12]

#### Determination of Acid Insoluble Ash Value

The ash was boiled with 20 ml of hydrochloric acid (2M) for 5 minutes and filtered through ashless filter paper was washed with hot distilled water. The filter paper was allowed to dry and ignited to dull redness for 15 minutes and cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried sample (I.P.1996 Vol-II). The results are given in Table 1

### Solvent extractive values

The extraction of any drug material with a solvent yields a solution of different compounds. The composition of this solution will depend upon the drug and the solvent used. The use of a solvent can be the means of providing preliminary information on the quality of a particular drug sample[13-17]

**Determination of Water Soluble Extractive**

Five gms sample of leaf and root of *Barleria prionitis* was accurately weighed and macerated with 100 ml of chloroform water in a closed flask for 24 hours. The flask was shaken frequently during the first 6 hours and allowed to stand for 18 hours. The mixture was filtered and the filtrate evaporated to dryness in an evaporating dish and finally dried in an oven at 105<sup>0</sup> C and weighed. The percentage of water-soluble extractive was calculated with reference to air dried sample (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in Table 2.

**Determination of Ethanol Soluble Extractive**

Five gms sample of leaf and root of *Barleria prionitis* was accurately weighed and macerated with 100 ml of 90% ethanol in a closed flask for 24 hours. The flask was shaken frequently during the first 6 hours and allowed to stand for 18 hours. The mixture was filtered and the filtrate evaporated to dryness in a tared flat-bottomed shallow dish and dried further at 105<sup>0</sup> C and weighed [18-21]. The percentage of ethanol soluble extractive was calculated with reference to air-dried sample (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in Table 2

**Determination of Ether Soluble Extractive**

Accurately weighed, twenty five grams of sample of leaf and root of *Barleria prionitis* was packed in an extraction thimble of Soxhlet apparatus and 100 ml. Petroleum ether (40 to 60) was slowly added to the thimble chamber. The distillation flask was heated at 40<sup>0</sup> C and extraction continued for 3 hours. The extract was collected and evaporated to complete removal of petroleum ether. The semisolid mass so obtained was weighed. The percentage of ether soluble with reference to air dried sample was determined (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in Table 2

**Determination of loss on drying**

The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical changes or microbial contamination. Loss on drying or heating to constant weight can be determined for material which do not contain compounds, which are volatile at the temperature of drying.

Two gm of sample of leaf and root of *Barleria prionitis* was accurately weighed and transferred in a previously weighed weighing bottle. The bottle was stoppered loosely and placed in an oven at 105<sup>0</sup> C for 30 minutes. After drying the bottle was cooled to room temperature in a desiccator and weighed till a constant weight was obtained. The loss on drying was calculated with

reference to air-dried sample (Indian Pharmacopoeia 1996 Vol-II). The results are given in Table 2.3

**Determination of foreign organic matter**

Approximately 100gm of the sample of leaf and root of *Barleria prionitis* was taken and spread out in a thin layer on a glass plate. The samples were inspected thoroughly through a magnifying glass and the foreign organic matters present in the sample were removed (Indian Pharmacopoeia 1996 Vol-II). The results are recorded in the Table 3

**Determination of arsenic and heavy metals**

The medicinal plant materials can be contaminated with arsenic and heavy metals which can be attributed to many causes including environmental pollution and traces of pesticides. As these components even in trace amounts are dangerous, they have to be removed from the herbal drugs [22-25]. Limit tests for these materials have been prescribed in almost all the Pharmacopoeia throughout the world. As prescribed by WHO the following procedures have been recommended for their respective limit tests:

**Limit Test for Arsenic**

The amount of arsenic in the medicinal plant material is estimated by matching the depth of colour with that of a standard stain. The limit test was accomplished by using the following procedures (Indian Pharmacopoeia, 1996 Vol-II)

**Preparation of sample:** Accurately weighed, 35-70 gm of coarsely ground material, of leaf and root of *Barleria prionitis* was placed in a Kjeldahl flask, of capacity 800-1000 ml. 10-25 ml of water and 25-50 ml of nitric acid (~1000 g/l) was added. Then carefully 20 ml of sulfuric acid (~1760g/l), was added drop by drop, until all the organic matter was destroyed. This was confirmed when no further darkening of the solution was observed with continued heating, and a clear solution with copious vapors of sulfur trioxide was obtained. It was then cooled, and 75 ml. of water and 25 ml. of ammonium oxalate (25g/l) was added. It was heated again until sulfur trioxide vapors developed. It was then cooled, and transferred to a 250 ml volumetric flask, and diluted to the mark, with water [26-28].

An aliquot (25-50 ml) of the sample solution, which was being tested, (prepared as described above) was placed in the suitable apparatus. The yellow stain of the test sample was compared with that of the standard (a known quantity of dilute arsenic As TS. Prepared as per IP 1996) without delay, in daylight as the stains fade with time. Results are recorded in Table 4

**Limit Test for Heavy metals**

The procedure for the determination of lead and heavy metals was as the same as recommended by the WHO.

**Preparation of the sample:** For the wet digestion method in an open system, 200-250 mg of air-dried plant material of leaf and root of *Barleria prionitis* was accurately weighed, finally cut and homogeneously mixed, and was placed into a cleaned silica crucible. One ml. of the digestion mixture, was added and the crucible was covered without exerting pressure and placed in an oven with a controlled temperature and time regulator. It was heated slowly to 100°C and this temperature was maintained for upto 3 hours, then heated to 120°C and this temperature was maintained for 2 hours. The temperature was raised very slowly to 240°C, avoiding losses due to possible violent reactions especially in the temperature range of 160-200°C, and

maintained at this temperature for 4 hours. The remaining dry inorganic residue was dissolved in 2.5 ml. of nitric acid (~1000g/l) and used for the determination of heavy metals[27-30]

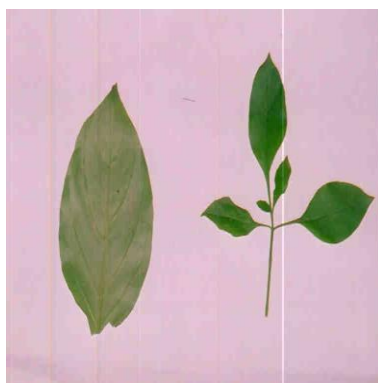
The colour produced, by the sample was compared with the colour produced by the standard (Indian Pharmacopoeia monograph 1996).

**Preliminary phytochemical screening :** The ethanolic extracts (method as described above) obtained were subjected to various qualitative tests in order to reveal the presence or absence of common phytopharmaceuticals by using standard tests. Results recorded in table 5.

### Results and discussion



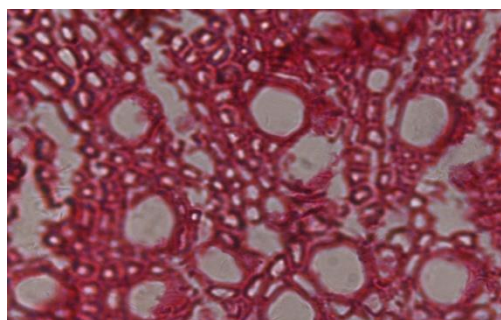
A



B



C



D



E

A (Fig: 1 Plant of *Barleria prionitis*)

B (Fig: 2 Leaves of *Barleria prionitis*)

C (Fig: 3 Dried root of *Barleria prionitis*)

D (Fig.4 Root Section)

E (Fig.5 Petiole)

**Table 1: Ash values of leaf and root samples of *Barleria prionitis***

		Total ash value %w/w	Water soluble ash %w/w	Acid insoluble ash %w/w
1	Leaf	17.64	8.24	0.988
2	Root	39.3	15.43	2.99

**Table 2 : Extractive values of leaf and root of *Barleria prionitis***

		Water soluble extractive %w/w	Ethanol sol. Extractive %w/w	Ether soluble extractive %w/w
1.	Leaf	35.85	16.31	7.65
2.	Root	14.039	2.07	0.93

**Table 3: Loss on drying and Foreign organic matter of leaf and root *Barleria prionitis***

	Loss on Drying %w/w	Foreign organic Matter
Leaf	18.43	Nil
Root	10.43	Nil

**Table 4: Limit test of Arsenic and heavy metals in the leaf and root sample of *Barleria prionitis***

	Limit test for Arsenic	Limit test for Heavy metals
Leaf	Less than 5ppm	Less than 10 ppm
Root	Less than 5ppm	Less than 10 ppm

**Table 5: Qualitative test of phytochemicals in the leaf and root sample of *Barleria prionitis***

1	Alkaloids	Mayer's reagent, Wagner's reagent, Dragendorff's reagent	Absent
2	Flavonoids	Ammonia test, Alkaline reagent test	Present
3	Tannins	Acetate test, Bromine water test	Present
4	Steroids	Salkowski test	Present
5	Protein	Biuret test, Ninhydrin test	Present
6	Gums and Resins	Ferric chloride test and HCl test	Absent
7	Terpenoids	With Chloroform and conc. Sulphuric acid	Present
8	Glycosides	Bontrager's test	Present
9	Saponins	Frothing test	Present

The morphological study revealed that the fresh leaves are dorsiventral dark green above, light green beneath; bitter in taste; ovate-elliptic lineolate in shape with thin texture. Dried roots were cylindrical, tortuous with numerous horizontally spreading lateral roots and rootlets. Light brown in colour; splintery and fibrous is fracture. Microscopical study revealed that the leaf contained caryophyllaceous (diacytic) stomata only in lower epidermis and absence of stomata in upper

epidermis. Presence of numerous cystoliths, sessile glandular hairs as well as non-glandular hairs in both the epidermis, acicular crystals of calcium carbonate, crescent shaped biocollateral vascular bundles were present.

Microscopical study of *Barleria prionitis* Linn revealed that the root is having presence of multilayered discontinuous with, narrow cortex which is devoid of starch grain, prismatic crystals of silica in some of the

cork cells, mostly uniseriate medullary rays reaching upto the centre core of xylems were seen. The major portion of the root is made up of xylem consisting of vessels, trachoids, fibres.

There was no foreign organic matter found in both the samples of the drug in the present study but the moisture contents were 18.43% w/w and 10.43% w/w in leaf and root respectively. On studying the limit of arsenic and heavy metals in the leaf and root sample, it was observed that the two of them complied with the standards i.e less than 5ppm for arsenic and less than 10 ppm for heavy metals.

The ash value of root was found to be more than the leaf. It could be due to soil contamination of the root sample. However the results obtained for the root and leaf sample were complying with that of the monograph (Ayurvedic Pharmacopoeia of India, Vol-III). The acid insoluble and the water insoluble ash also showed compliance with the monograph (Ayurvedic Pharmacopoeia of India, Vol-III). The various solvent extractive values were determined as per procedures of Indian Pharmacopoeia 1996. The ethanol soluble extractive value of root was found 2.07% w/w which is less than as prescribed by the monograph of *Barleria prionitis* in the Ayurvedic Pharmacopoeia of India Vol-III. This indicates that less organic constituents, soluble in ethanol are present in the root sample. However the water-soluble extractive value 14.04% w/w was in compliance with the monograph of *Barleria prionitis* (Table 2.2). The ether soluble extractive values of both root and leaf were as per the values indicated in monograph. The water-soluble and ethanol soluble extractive values of leaf were 35.85% w/w and 16.31% w/w respectively which also shows compliance with the monograph of *Barleria prionitis* (Ayurvedic Pharmacopoeia of India, Vol-III).

A standard procedure prescribed in the Indian Pharmacopoeia 1996 Vol-II specifies the limit of presence of arsenic, lead and other heavy metals. The results of the arsenic, lead and other heavy metals in the present study indicates that they are in compliance with the standard specified in Indian Pharmacopoeia 1996 Vol-II i.e less than 5 PPM of arsenic in both leaf and root sample. The limit of lead was also within limits i.e less than 10 PPM. Contamination by heavy metals of medicinal plants can induce toxic effects not only in humans but in plants themselves. It can also lead to qualitative and quantitative changes in the active [8].

### Conclusion

Authenticity of the plant is one of the most important parameter of the standardization of the plant. The

authentication of the selected herb was done by comparing and verifying herb's macroscopic and microscopic characters with the characters as prescribed in the Ayurvedic Pharmacopoeia of India. (Vol-III). Authenticity of the *Barleria prionitis*. was further conformed with the help of herbarium collection as available in the Department of Botany University of Rajasthan, Jaipur.

The macroscopic and microscopic characters were as those prescribed in the monograph of The Ayurvedic Pharmacopoeia of India (Vol-III). From the above studies carried out it can be concluded that the various parameters such as pharmacognostical, phytochemical and qualitative and semi-quantitative (limit tests) may be utilized for its identification and differentiation from other species. Further it is helpful in establishing the authenticity and purity of the herb as per guidelines of WHO. Phytochemical screening showed the presence of saponins, flavanoids, alkaloids, glycosides, tannins in the ethanolic extract of leaves and roots which indicates its usefulness in fever, whooping, cough, toothache, glandular swelling, anascara, rheumatic pain, stomach disorders, and catarrh in children. Flavonoids present in *B. prionitis* shows anti microbial activity. Due to presence of these compounds in the roots and leaves of *B. Prionitis*, this plant may have good anti-inflammatory, anti oxidant, hepatoprotective, antidiabetic, diuretic, as a febrifuge etc. Therefore *Barleria prionitis* linn can be considered as an important addition to the therapeutic armamentarium for the several ailments and futuristic research.

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