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Review

Article.....!!!

**EVALUATION METHODS OF ETHANOLIC EXTRACT OF BARLERIA  
PRIONITIS LINN.**

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**KEYWORDS:**

*Barleria prionitis* Linn,  
*Acanthaceae*, Secondary  
Metabolites, Ayurvedic Herb,

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**ABSTRACT**

In recent time global demand for herbal medicines is increasing rapidly because of their higher safety margin and low cost. *Barleria prionitis* Linn. (Family *Acanthaceae*) is a well-known perennial, Ayurvedic herb distributed in tropical Asia, Africa. Several studies reveal that the whole plant or its specific parts (leaf, stem, root, bark, and flower) has been utilized for the treatment of a toothache, catarrhal affections, whooping cough, inflammations, glandular swellings, urinary infection, jaundice, fever, gastrointestinal disorders and as diuretic and tonic. A wide range of phytochemical constituents including balarenone, pipataline, barlerinoside, verbascoside, methyl ester, prionisides, acetylbarlerin, lupulinoside, shanzhiside, scutellarein have been isolated from the different parts of this plant. Extracts and isolated phytochemicals from this plant have been found to possess wide range of pharmacological include antimicrobial, anthelmintic, antifertility, antioxidant, antidiabetic, anti inflammatory, Antiarthritic, hepatoprotective, diuretic, anti diarrhoeal, enzyme inhibitory, anti viral, anti fungal, anti cancer and anti nociceptive activities without any toxic effects. However, significant presences of several new secondary metabolites strengthen the demand for further research based on its phytotherapeutical importance. Besides its numerous folk use, the present review also illustrates its phytochemical profile as well as pharmacological augmentation which will be helpful for future researchers.

**INTRODUCTION:**

*Barleria prionitis* generally known as Vajardanti it is utilized for different illnesses, for example, asthma, whooping hack, ailment, fever, heaps, ulcers, bothering, control wound healing, bleeding diseases liver diseases, bubbles, aggravation, solidness of appendages expanding force, gout, oedema, jungle fever, leukoderma scabies, toothache, joints torment, urinary contamination, jaundice, gastrointestinal clutters, hepatoprotective, snakebites, liver diseases and neuralgia. The word Herb has been shaped from the Latin word, herb and an old French word herb. These herbs that have restorative quality give discerning intends to the treatment of numerous interior ailments, which are generally viewed as hard to cure. *Barleria prionitis*, a perennial, acanthaceous, barbed, bushy medicinal plant, including in *Barleria* genus containing 300 species is famous for its medicinal value from ancient time.

**Common Names**

Common yellow nail dye, Porcupine flower, Hop headed *Barleria*, Thorny nail dye, Gorantachettu, Mullu goranta, Pachhammullu goranta, Vajradanti, Sahachara, Kurantaka, Espinosa Amarilla, Chemmulli, Koranda, shairiya, pita-saireyaka, piyaabaasaa, baana, jhinti, katsaraiyaa.

**Distribution**

Extensively found in India, it is distributed widely in throughout Asia including Malaysia, Pakistan, Philippines, Sri Lanka, Bangladesh, Yemen and tropical Africa. In India it is normally found in Andaman and Nicobar, Andhra-Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Goa, Gujarat, Jharkhand, Karnataka, Kerala, Lakshadweep, Maldives, Madhya Pradesh, Maharashtra, Orissa, Pondicherry, Rajasthan, Tamil Nadu, Uttarakhand, Uttar Pradesh and West Bengal. *Barleria prionitis* is a branched annual shrub of about 1–3 feet height with flowering and

Spiny invader. The Shrub is armed with 5-20mm long spines in leaf axils. Most often found on road sides. Due to presence of spines it is ignored by cattles and it is considered as weed. The *Barleria prionitis* is native to tropical areas of east Africa and Asia, but may be found throughout tropical Asia (India and Sri Lanka) and in South Africa also. The month of August to November is favorable for the flowering and fruiting within the plant. Flowers are yellow or whitish in color, tubular, 3-4 cm long and broad, sessile in leaf axils or in terminal branched spikes. *Barleria prionitis* considered yearly or perpetual plant amid the dry season. Its stems, leaves and blooms pass on yet roots alive. The vegetation develops stormy season.

**Stem**

Erect 1.8mm thick, terete, hard, glabrous, hubs swollen, spreading at hubs, youthful stem dark, somewhat four calculated with 3-4 divariate spines at hub of leaf. Its light tan or gray colored stems are stiff, round, cylindrical and glabrous. The stem part is generally single, but may have multiple stems or

branches near ground. Stem tube shaped with longitudinally orchestrated remotely greyish to light dark coloured. A couple of develop stems marginally empty.



### Flowers

Flowers are yellow or whitish in color, tubular, 3-4 cm long and broad, sessile in leaf axils or in terminal branched spikes. Sessile, regularly single in the lower axils, getting to be noticeably spicate above; bracts foliaceous, 16 by 4.5 mm, elongated, intense, abound tipped, about glabrous; bracteoles 1.3 cm long, barely straight, subulate, swarm tipped; calyx, isolated nearly to the base, one of the external sepals preferably more than 1.3 cm long. The white flower variety of *Barleria prionitis* is bitter in taste. The inverse sepal under 1.3 cm long, 3.4 mm wide both oval lanceolate, mucronate; the two internal sepals 1.5 mm wide and long as a shorter of the ones, direct lanceolate, mucronate crown, 3.2-4.5 cm long, yellow marginally pubescent outside, glabrous inside fairly two lipped.



### Leaf

Oval-ellipsoid moulded, variable in scrutinize to 10 cm long and 4 cm wide, elliptic whole, intense reticulate, unicostate, labours above, underneath petiole short. The base of leaves remain tapering into the petiole. Its elliptic leaf containing 5-20 mm long spines is about 3-10 cm long and 1.5-4 cm broad.



### Seeds

Oval-shaped seeds, 2cm long, containing two expansive 8mm long, 5mm wide level seeds with plush hairs. The seeds are flattened and remain covered with matted hairs. The weight of air dried seed is about 0.03g/seed. The fruits are ovoid and capsule shaped.



### PLANT PROFILE

Name: *Barleria prionitis*

Family: Acanthaceae

#### Scientific Classification

Kingdom	:	Plantae
Sub kingdom	:	Tracheobionta
Super Division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Sub Class	:	Asteridae
Order	:	Lamiales
Family	:	Acanthaceae
Genus	:	<i>Barleria</i> Linn
Species	:	<i>Barleria prionitis</i>



**Whole plant of Barleria prionitis**



**Spines part of Barleria prionitis**

### **PLAN OF WORK**

The following steps were performed on the ethanolic extract of whole plant of Barleria prionitis.

Collection of whole plant of Barleria prionitis.

1. Authentication of plant material.
2. Preparation of whole plant of Barleria prionitis using isolation apparatuses.
3. Preliminary phytochemical studies of extract to identify phyto constituents.

### **MATERIALS AND METHODS**

#### **Plant material**

The whole plant of Barleria prionitis was identified as Barleria prionitis whole plant by the scientist `F Botanical survey india.

#### **Preparation of Extraction**

The collected whole plant were, shade dried and then grind into coarse powder. The powder was then subjected to exhaustive extraction by the maceration process using ethanol solvent at room temperature for 7 days. After 7 days the mixture was strained, the marc (damp solid material) was pressed and the liquid was clarified by decantation after standing and later by filtration. The obtained filtrate was then concentrated using Rotovac at 45<sup>0</sup> C. the concentrate obtained was evaporated to dryness. The extract was weighed and used for further pharmacological studies.

#### **Chemical constituents**

Preliminary phytochemical analysis of hydroethanolic extract of *B. prionitis* whole plant indicated the presence of glycosides, saponins, flavonoids, steroids and tannins. Large amount of secondary metabolites such as glycosides, saponins, flavonoids, phenolic compounds, tannins, alkaloids, phytosterols, polyphenol and steroids are present in whole plant detected by different phytochemical tests. Its arial parts contains a lage quantities of glycosides (6-o-trans-p-coumaroyl-8-

oacetylshanzhiside methyl ester, barlerinoside, shanzhiside methyl ester, 6-o-trans-p-coumaroyl- 8-o-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside, and lupulinoside). The leaves and flowering tops were reported to rich in potassium salts. Several phytochemicals via, balarenone, pipataline, lupeol, prioniside A, prioniside B, and prioniside C, has been isolated from the ethanolic extract of *B. prionitis*. The air dried aerial parts of *Barleria prionitis* were extracted with 95% ethanol at room temperature and fractionated to yield phenylethanoid glycoside, barlerinoside along with other iridoid glycosides, namely: 7- methoxydiderroside and lupulinoside.

### PHYTOCHEMICAL COMPOSITION OF *BARLERIA PRIONITIS*

#### Plant

Part	Phytochemical/Nutrient	Test (extract details)
<b>Leaf</b>	Alkaloid	TLC (ME)
	Flavonoids	TLC (ME)
	Saponins	TLC (ME)
	Tannin	TLC (ME)
	Phytosteroids	TLC (ME)
	Phenolic compound	TLC (ME)
	Terpenoids	Not Specified
	Sterol (stigmasterol)	HPLC
	Essential oil	Not specified
<b>Aerial Part</b>	Glycosides	NMR
	Terpenoid (lupeol)	NMR (EE)
	Pipataline, Balarenone, 13,14-Seco-stigmasta-5,14- diene-3-ol	NMR (EE)
<b>Whole Plant</b>	Glycosides	Borntrager's test Legal's test
	Saponins	Frothing test
	Flavonoids	Ammonia test (HE), Alkaline reagent test, Shinoda test (CE),

	Phenolic compounds and Tannins	FeCl <sub>3</sub> test (HE, AqE), Lead acetate test, Bromine water test
	Steroids	Salkowski test (HE)
	Alkaloids	Mayer's reagent (PeE, ME), Hager's reagent (PeE), Wagner's reagent (EE), Dragendorff's reagent,
	Carbohydrate	Molisch test (ME, EE), Fehling's solution (ME), Fehling's solution B (EE),
	Phytosterols	Liebermann's test (ME), Liebermann Burchard test,
	Proteins and amino acid	Biuret test (ME, EE), Ninhydrin test (ME, EE),
	Polyphenol	Folin-ciocalteu test (AqE),
	7-rhamnosylglucoside	Not specified
	Anthraquinones	Chemical tests
<b>Flowers</b>	Flavonoid	Not specified
	Glycoside	Not specified
	Neohesperidoside	Not specified

## PHARMACOLOGICAL EVALUATION

Isolated phytochemicals extracts from *Barleria prionitis* Linn. is found to have a wide range of pharmacological activities like anthelmintic, antifertility, antimicrobial, antioxidant, antidiabetic, anti-inflammatory, anti-arthritic, hepatoprotective, diuretic, cytoprotective, antidiarrhoeal, anti-nociceptive and enzyme inhibitory activities without any toxic effects. Different activities are shown in different parts:-

1. Antibacterial activity - leaf and root
2. Antifungal activity – Bark
3. Antiviral activity – root
4. Antiarthritic activity – leaf
5. Antioxidant activity - leaf, flower
6. Antidiabetic activity - flower and leaf

7. Hepatoprotective activity – leaf
8. Anti inflammatory activity - aerial part
9. Cytoprotective activity – whole plant
10. Antidiarrhoeal activity - whole plant
11. Diuretic activity - aerial part
12. Anti nociceptive activity - whole plant
13. Enzyme inhibitory activity - aerial part

**Anti-fertility Activity:**

The significantly reduction in weight of testes, epididymides and ventral prostate along with reduction in total number of spermatids on oral administration of *Barleria prionitis* root extract have been found in male rats. It was observed that the root extract decreased production of round spermatids, sperm motility, spermatogonia, preleptotene spermatocytes population and mature leydig cells. Biochemical investigation revealed that the root extract was also reduced the total protein, glycogen, sialic acid contents of the testes, testicular glycogen contents, epididymides, ventral prostate and seminal vesicle. The Antifertility effect of root extract may be due to the presence of iridoid glycosides barlerin and acetyl barlerin via affecting the functions of testicular somatic cells.

**Antioxidant Activity:**

The whole plant extract of *B. prionitis* was reported to show potent antioxidant activity. In vitro study that the ethanol and aqueous extracts of whole plant possess significant antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2<sup>1</sup>-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), nitric oxide and hydroxyl radical scavenging assay and Fe<sup>3+</sup> reduction assay. Thabrew (2001), investigated effect of marketed preparation containing *Barleria prionitis* for antioxidant potential on rheumatoid arthritis patients. Study demonstrated that three months treatment of preparation has high antioxidant potential which was shown as initial activities of plasma antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase enhanced by 44.6%, 39.8% and 25.2%, respectively. The MeOH extract of root leaves and stems showed potent antioxidant activity. EtOH extract of whole plant of *B. prionitis* showed significant antioxidant activities. It was reported that the antioxidant activity of MeOH extract of leaf and stem were showed IC<sub>50</sub> values 63.41±0.32, 81.69±0, 40, respectively. Antioxidant potential of medicinal plant *Barleria prionitis* was studied by using DPPH assay. The ethylacetate soluble fractions have showed the maximum activity. Aerial parts of *Barleria prionitis* contain chemical constituent's phenylethanoid glycoside, barlerinoside; iridoid glycosides, shanzhiside methyl ester, 6-o-trans-p-coumaroyl-8-o-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydideroside and lupulinoside exhibited different free radical Scavenging

activities. It was observed that the leaves showed higher degree antioxidant potential and high phenolic content in comparison to flower and stem. The total phenolic contents were determined by Folin-ciocalteu method. The antioxidant potential and reducing power of all the prepared extracts were measured against DPPH as compared to standard ascorbic acid and BHA respectively. The result indicated that the phenolic contents were higher in methanolic extracts of leaf and stem.

### **Anti inflammatory Activity**

Several reports demonstrated the usage of *B. prionitis* in the treatment of inflammations. The anti inflammatory activity of *B. prionitis* was evaluated through in vitro enzyme based cyclooxygenase (COX-1 and COX-2) assays. It was found that the dichloromethane, petroleum ether and ethanol extracts of leaves, stems and roots exhibited significant inhibition of COX-1 and COX-2 with subsequent inhibition of prostaglandin synthesis that are involved in pain sensation. The Anti-inflammatory activity of *Barleria prionitis* whole plant extract have also been investigated and documented against carrageenan-induced paw edema in rats. Aqueous extract of root showed significant percentage inhibition of rat paw edema (52.56% & 55.76%) at a dose of 200 & 400 mg/kg respectively after 4 hr treatment. *Barleria prionitis* L. roots paste is used traditionally in the treatment of swelling and boils. The aqueous extract showed significant inhibition of edema as compared with reference drug indomethacin. The methanol extract of *Barleria prionitis* Linn. Showed significant anti-inflammatory activity comparable to control and standard drug indomethacin. Extract at 50, 100 and 200 mg/kg decreased granuloma weight from 15.32 to 36.4% gradually where phenylbutazone exposed 36.1% inhibition in cotton pellet granuloma rat model.

### **Antidiabetic Activity**

The antidiabetic activity of alcoholic extract of leaf and root of *B. prionitis* was evaluated by using alloxan monohydrate. *B. prionitis* leaves showed significant decrease in blood glucose level decrease in the body weight was also observed glycosylated haemoglobin and significant increase in serum insulin and liver glycogen level. *B. prionitis* root showed moderate but non-significant anti-diabetic activity in experimental animals. Different solvent extracts from leaves and stem parts of *B. prionitis* L. exhibited antibacterial activity against all Gram-positive bacteria studied (*Bacillus pumilus*, *Bacillus subtilis*, *Streptococcus pyogenes*, and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, *Serratia marcescens*, *Comamonas acidovorans*, and *Pseudomonas aeruginosa*). Maximum inhibition was delivered by methanol leaf extract against *B. cereus* which was followed by pet ether leaf extract against *E. coli*. Minimum inhibition was Shown by pet ether leaf extract against *Alcaligenes faecalis*, followed by methanol bark extract against *A. faecalis*. Antibacterial activity of the various extracts of *B. prionitis* was compared to the standard

antibacterial agent ampicillin tetracycline, and streptomycin, and it appeared to be almost the same. In another study, the petroleum ether extract of *B. prionitis* was most dynamic against *Pseudomonas putida* and *B. subtilis*.

### **Antifungal activity**

The acetone, methanol and ethanol extracts of *B. prionitis* bark showed antifungal activity against oral pathogenic fungus *Saccharomyces cerevisiae* and two strains of *Candida albicans* involved in oral diseases of human. Methanolic extract of *B. prionitis* bark showed much more potent activity against all the tested oral fungi than the standard drug amphotericin-B thus having a great potential to control candidiasis and other oral fungal infections. Petroleum ether, dichloromethane, ethanol and methanol extract of different parts of *Barleria prionitis*, *Barleria greenii* and *Barleria albostellata* studied for its antifungal activity. In another investigation, the leaf exudates and leaf tissue sap of *B. prionitis* L. have been assessed for antifungal activities against some fungi such as *Curvularia lunata*, *Curvularia clavata*, *Alternaria alternata*, *Nigrospora oryzae*, and *Cladosporium oxysporum*. The percentage inhibition of spore germination was calculated, and result revealed 40-85% inhibition of all of the species.

### **Hepato-protective Activity**

Iridoid enriched fraction from the ethanol-water extract of leaves and stem of *B. prionitis* was found to be significant hepatoprotection against carbon tetrachloride, galactosamine and paracetamol induced hepatotoxicity in mice and rats. The oral administration of iridoid fraction significantly reduced the hepatotoxin induced elevated levels of serum alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin and triglycerides in a dose dependent manner. The fraction was also increased the hepatic glutathione content and reduced the hepatic lipid peroxidation in response to the hepatotoxicity in mice and rats. A synergistic pharmaceutical composition having bioactive fraction of iridoid glucosides obtained from the *Barleria prionitis* was prepared and evaluated against carbon tetrachloride and acetaminophen induced liver toxicity.

### **Anti bacterial activity**

It has been reported that different solvent (ether, ethanol and chloroform) extracts of *B. prionitis* leaves and callus showed antibacterial activity against numbers of gram positive bacterial isolates while no or slight inhibitions were observed against the aqueous extracts. Ethanol, chloroform and petroleum ether extract of *B. Prionitis* showed comparative antibacterial activity on various bacterial species. The different extracts of the plant were first prepared on basis of various concentration levels and then extensively applied on selected bacterial culture media for determination of minimum inhibitory concentration and these extracts exhibited significant antibacterial activity. The antimicrobial activity of

leaf, stem and root of *B. prionitis* by using solvent chloroform, acetonitrile and ethanol was studied. Antimicrobial activity was done by gradient plate technique. Leaf, stem and root extracts of *B. prionitis* showed the significant antimicrobial activities.

#### **Anhelmintic activity**

The whole plant extract of *B. prionitis* was reported to show anthelmintic activity against *Pheretima posthuma*. Various concentrations aqueous and ethanolic extracts was evaluated in the bioassay involving determination of time of paralysis and time of death of the worms. Albendazole was used as standard anthelmintic drug. The results showed that the ethanolic and aqueous extracts significantly exhibited paralysis in worms in lower doses and also caused death of worms especially at higher concentration, as compared to standard drug.

#### **Anti viral activity**

Two new iridoid glycosides 6-o-trans-p-coumaroyl-8-o-acetylshanzhiside methyl ester and 6-o-cis-p-coumaroyl-8-o-acetylshanzhiside methyl ester showed potent *in vitro* activity against respiratory syncytial virus were isolated from *Barleria prionitis*. These bioactive phytochemicals revealed the potent antiviral activity against respiratory Syncytial virus (RSV) with EC<sub>50</sub> and IC<sub>50</sub> values of 2.46 and 42.2 µg mL<sup>-1</sup>, respectively<sup>12</sup>.

#### **Anti diarrheal activity**

Butanol fraction of *B. prionitis* leaves showed significant anti-diarrheal activity. *In vivo* study showed that the butanol fraction dose dependently inhibited the castor oil induced diarrhea and PGE<sub>2</sub> induced enteropooling in sprague-dawley rats. The butanol fraction also reduced the gastrointestinal motility in response to charcoal-induced gut transit changes.

#### **Anti-nociceptive Activity**

Extract of flower (200 mg/kg) increased analgesia-meter-induced force and exhibited resistance against pain. It also inhibited acetic acid induced pain as 30.6% where phenylbutazone (100 mg/kg) presented 34.6%. *In vivo* study showed that the flower extract dose dependently provided a significant increase in the analgesio meter induced force and exhibited significant resistance against pain in mice. The flower extract also provided dose dependent significant reduction in writhing characterizes by the reduction in acetic acid induced abdominal cramping.

#### **Diuretic Activity**

Diuretic activity of *B. prionitis* flower extract was investigated using by administration of normal saline solution. Administration of aqueous flower extract was significantly increased the urination and sodium elimination but not potassium in rats. The diuretic effect of flower extract was comparable and significant with the reference drug furosemide. Aqueous root extract (100 mg/kg) produced significant

diuresis ( $12.58 \pm 0.80$  urine volume in 24 hr) compared with furosemide at 20 mg/kg ( $12.58 \pm 0.80$  urine volume in 24 hr) and increased sodium elimination.

### Enzyme Inhibitory Activity

The methanolic extracts of *B. prionitis* leaf, stem and root exhibited AChE inhibitory activities and the leaf and stem extracts exhibited higher potency of inhibition in compare the root extract. Several glycosides include barlerinoside, shanzhiside methyl ester, 6-*O*-trans-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetyl barlerin, pataline, lupeol, 7-methoxydiderroside, 13, 14-seco-stigmasta-5, 14-diene-3-*a*-ol and lupulinoside have been isolated from the aerial parts of *B. prionitis* and these compounds showed different levels of AChE inhibitory activity.

### CONCLUSION

Herbal medicine plays a major role in the development of modern civilization. *B. prionitis* is important plant in ayurvedic medicine in India. This review article briefly explains the traditional uses and pharmacological actions of *B. prionitis*. From this review it is conspicuous that several portions of *Barleria prionitis* individually or jointly administered successfully by traditional practitioners specifically against fever, severe pain, asthma, ulcer etc. The plant has found to be broad spectrum of activities due to presence of active constituents like glycosides, flavonoid, saponin, steroid and tannins. The plant have reported to have variety of pharmacological actions like antioxidant, antibacterial, anti-inflammatory, anti-arthritic, hepatoprotective, antifungal, antiviral, mast cell stabilizing, antifertility and gastoprotective activity of the extract and isolated molecule of the plant. In future study, the conversion of these pharmacological activities in to the modern drugs, proper scientific evaluation includes isolation of responsible phytochemicals, their mechanism of actions, toxicity and proper standardization need to be explored. Along with its several folk use, this review also defines its phytochemical profile as well as pharmacological augmentation which will be helpful for future researchers.

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