INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Preliminary Phytochemical Screening and antioxidant activity of Whole plant of *Barleria prionitis* linn.

Amit Kapoor, Shiwani Shukla, Rajinder Kaur, Rajesh Kumar, Kuldeep Singh Lehra , Shilpa Kapoor.

Lala Lajpat Rai College of Pharmacy, Moga, Punjab, India.

ABSTRACT

Traditional plants contains various secondary metabolites such as phenolic compounds, cartenoids, ascorbic acid, thiols and tocopherols which have shown antioxidant activity that include scavenging free radical species, inhibiting the production of reactive species, inhibiting the production of reactive species resulting from normal cell metabolism.The present study was undertaken to analyse the antioxidant activity of various fractions of 90% methanolic extract of medicinal plant *Barleria prionitis* Linn Family- Acanthaceae by using DPPH assay. The extent of DPPH radical scavenging was determined by calculated the IC_{50} value of hexane, chloroform, ethylacetate and butanol soluble fractions of methanolic extract and compared with the Ascorbic acid taken as standard. The ethylacetate soluble fractions have shown the maximum activity among all. The order of the antioxidant potency of the different fractions of methanolic extract were in the order of ethyl acetate> butanol >chloroform> methanolic > hexane.

Key words: *Barleria prionitis* Linn, DPPH assay, Secondary metabolites, Antioxidant, IC_{50}.

INTRODUCTION

Oxygen is an essential element for life to perform biological functions such as catabolism of fats, proteins and carbohydrates in order to generate energy for growth and other activities. Although Oxygen is not dangerous by itself, but is involved in the generation of various kinds of "reactive oxygen species" (ROS). ROS can interact with bio-molecules and ultimately lead to free radical chain reactions. Free radical chain reactions are produced in mitochondrial respiratory chain, liver mixed function oxidase, xanthine oxidase activity, atmospheric pollutants and fro transition metal catalysts, drugs and xenobiotics[1]. ROS attacks the unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane protein leading to cell inactivation[2]. ROS attacks DNA and causes mutation leading to cancers[3]. ROS also leads to pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenration, Parkinson's daseases, mongolism, ageing process and dementia. For the treatment of diseases caused by ROS antioxidant are used. Antioxidants are composed of a group of compounds and enzymes potent enough to remove free radicals before they cause tissue damage.

Types of Antioxidants:

There are two types of antioxidants: **Enzymatic antioxidants** and **Non-Enzymatic antioxidants.** The most efficient enzymatic antioxidants involve glutathione peroxidase, Catalase and superoxide dismutase. Nonenzymatic antioxidants include Vitamin E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), melatonin, carotenoids, natural flavonoids, and other compounds[4].

Some antioxidants are produced in the body while others must be sequestered from the diet or through supplementation. Most citrus and dried fruits, cruciferous vegetables, garlic, onions, carrots, tomatoes, sweet potatoes, sesame and olive

oil are rich sources of antioxidants. There are thousands of naturally occurring and synthetic antioxidants known; these antioxidants belong to different classes of compounds, such as carotenoids, polyphenolics, polyamines, gallic acid derivatives, tannins and catechins. Examples include phytic acid, lipoic acid, bilirubin, melatonin, quercetin, carnosol, camosic acid, hydroxytyrosol, rutin, butylatedhydroxyanisole, and butylatedhydroxy toluene. Vitamins E and C are among the most effective antioxidants with preventive effects against heart diseases and cancer[5], also regarded as natural antioxidants obtained from diet.

Table 1: Types of Free Radicals

ROS	Types	Symbol
Radical	Superoxide	$O_2^{\cdot-}$
	Hydroxyl	$^{\cdot}OH$
	Alkoxyl	$LO^{\cdot}/RO^{\cdot}$
	Peroxyl	$LOO^{\cdot}/ROO^{\cdot}$
	Nitric Oxide	$NO^{\cdot}$
	Thiyl radical	$R\text{-}S^{\cdot}$
Non-Radical	Hydrogen Peroxide	H_2O_2
	Hypochlorus Acid	HOCl
	Ozone	O_3
	Singlet oxygen	$^1O_2$
	Peroxy Nitrite	ONOO-
	Lipidperoxide	LOOH

Plant secondary metabolites such as phenolic compounds, cartenoids, ascorbic acid, thiols and tocopherols have shown antioxidant activity that include scavenging free radical species, inhibiting the production of reactive species, inhibiting the production of reactive species resulting from normal cell metabolism. Thereby prevent the damage to lipids, proteins, nucleic acids and subsequent cellular damage and death[6]. Flavonoids are polyphenolic compounds that are abundant in fruits, vegetables, red wine, tea and chocolate

Barleria prionitis L. commonly called 'Porcupine flower' **is** especially well known for treating bleeding gums and toothache. Because of its anti dontalgic property it is known as 'Vajradanti'[7]. *B. prionitis* has been the centre of interest to pharmacologists as it exhibits a variety of pharmacological activities *viz.* antibacterial activity[8,9,10], antifungal activity[10,11], anthelmintic activity[12], antifertility activity[13,14], antidiabetic activity[15], anti-diarrheal activity[16], enzyme inhibitory effects[9,11,17,18], anti-inflammatory activity[19,20,21], anti-arthritic activity[22], cytoprotective activity[22,23], hepatoprotective activity[24], diuretic effect[25], anti-nociceptive activity[26] etc. Keeping in mind the traditional/ alternative and complementary medicinal uses and diverse activity potential, *B. prionitis* seems to hold a great potential for in depth investigation for various biological activities, especially antioxidant activity. Several phytochemicals has been isolated from *B. prionitis* viz. balarenone, pipataline, lupeol, prioniside a, prioniside b, prioniside c, barlerinoside, verbascoside, shanzhiside methyl ester etc.

Preliminary studies carried out by previous workers revealed the presence of iridoids and steroidal compounds in plant. Different extracts of the plant had also shown significant anti-oxidant activity. These observations indicated that there is merit to carry out detailed phytochemical and pharmacological studies on this plant. It was decided to done the work on different solvents fractions of 90% methanolic extract of the *Barleria prionitis* for the antioxidant activity of *B. prionitis which* may be due to the presence of barlerinoside, shanzhiside methyl ester, 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside[9,10].

Therefore, in lieu of above deliberations it was thought worthwhile to investigate anti-bacterial studies and implement the following plan of work.

1. Phytochemical screening.
2. Subjecting *B. prionitis* leaves for antioxidant activity.

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The whole plant of *B. prionitis* was collected from HARI OM HERBS of Santinagar Chhutmalpur in july 2012. The plant was authenticated by Dr. K. MADHAVA CHETTY, Sri Venkateswara university, TIRUPATI and the plant specimen is kept at the Herbarium of Lala Lajpat Rai College of Pharmacy, Moga. The cleaned whole plant of *Barleria prionitis* were shade dried at room temperature, coarsely powdered and stored in air tight container till further use.

Extract Preparation

The ground powdered material(500g) were exhaustively defatted with pet. ether by maceration for 2 days at room temperature with occasional shaking. This process of extraction was repeated for four times, filtered and concentrated on rotary evaporator. The marc left behind was divided into three parts and extracted with 90% methanol, 50%

methanol and 100% aqueous solvent respectively by maceration process for 2 days at room temperature with occasional shaking. This process was repeated four times for each extract. The ME was suspended in water and partitioned with chloroform, ethyl acetate and n-butanol to yield chloroform, ethyl acetate, n-butanol and aqueous soluble fractions.

Phytochemical Screening

Weighed quantity of the various extracts, were subjected to preliminary phytochemical screening using standard methods[27]. All the extracts of *B. prionitis* whole plant were screened for different classes of phytoconstituents viz. alkaloids, steroids, terpenoids, anthraquinone, glycosides, flavonoids, tannins and phenolic compounds, saponins, carbohydrates, proteins and amino acids using specific standard reagents[28].

ANTI-OXIDANT STUDIES

1, 1-DIPHENYL-2-PICRYLHYDRAZYL DPPH ASSAY[29]:

Principle

DPPH is nitrogen centered free radical that show strong absorbance at 517 nm. DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. The free stable radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors; this ability was absorbance decrease at 517 nm produced by the addition of the antioxidant to a DPPH solution in ethanol or methanol. This method is widely used to check the free radical scavenging antioxidants. To evaluate the antioxidant activity of, specific compounds or extracts (antioxidant) were allowed to react with a stable radical $DPPH^{\bullet}$ in a methanol solution. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values.

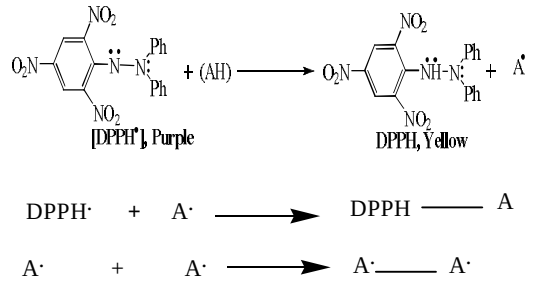

Fig. 1: Free radical scaveenging reaction of DPPH by antioxidants.

Preparation of dilution

50 mg of each of *B. prionitis* extracts (90%, 50% methanolic & Aq.) were weighed separately and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions. Lower concentrations (25, 50, 100, 150, 200, 250 µg/ml) were prepared by diluting serially with methanol

Preparation of standard dilution

Ascorbic acid weighed (50 mg) separately and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions. Lower concentrations (5, 10, 15, 20, 25, 30 µg/ml) were prepared by diluting serially with methanol.

Method

The stable (DPPH was used for determination of free radical-scavenging activity of the extracts. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extract were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula

$$\text{Inhibition (\%)} = \left[\frac{AB-AA}{AB}\right] \times 100$$

Where AB is the absorbance of control; AA is the absorbance of samples.

IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of *B. prionitis* extracts (90%, 50% methanolic & Aq.) which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50 %.

The measurements were carried out three times and their scavenging effect was calculated based on the percentage of DPPH scavenged.

Preparation of fraction from 90% ME of *B. Prionitis:*

On the basis of antioxidant potential by DPPH study, 90% ME has shown maximum antioxidant property. So this extract was further used for fraction with different organic solvent. 90% ME was first suspended in distilled water. Then fractionated gradiently (non-polar to polar) with different solvents. Solvents used for fractionation was chloroform, ethyl acetate and n-butanol. Furthermore, fractions obtained from these solvents were estimated for total phenolic content. Fractions having more phenolic content were charged into column for isolation of pure compound(s).

Table 2: Results of phytochemical screening of various extracts of *B. prionitis*

S. no	Phytochemical screening	Pet ether extract	Chloroform extract	Methanol extract	Water extract
1.	Alkaloids 1. Mayer's reagent 2. Hager's reagent 3. Wagner's reagent 4. Dragendorff's reagent	 + + + +	 - - - -	 + - + +	 - - - -
2.	Phenolic compounds and Tanins 1. $Fecl_3$ 2. Lead acetate test 3. Bromine water test	 - - -	 - - -	 + + +	 + + +
3.	Saponin 1. Frothing test	 -	 -	 +	 +
4.	Carbohydrates 1. Molisch test 2. Fehling's solution A 2. Fehling's solution B 3. Benedict's test	 - - - -	 - - - -	 + + + +	 - - - -
5.	Protein and Amino acids 1. Millon's test 2. Biuret test 3. Ninhydrin test	 - - -	 - - -	 - + +	 - - -
6.	Glycosides test 1. Borntrager's test 2. Legal's test	 - -	 - -	 + +	 - -
7.	Flavonoids test 1. Alkaline reagent test 2. Shinoda test	 - -	 + +	 + +	 + +
8.	Phytosterols test 1. Liebermann's test 2. Libermann Burchard test	 - -	 - -	 + +	 + +

Note:'+' signify presence of the compound; '-' signify absence of compound

Table 3: Percent inhibition of DPPH absorbance at different concentrations of standard (ascorbic acid)

Concentraction of ascorbic acid (µg/ml)	Absorbance (Mean ± S.D)	% Inhibition
2	0.060±0.002	29.41
10	0.052±0.003	39.22
25	0.040±0.002	52.66
50	0.021±0.006	76.92
75	0.007±0.0005	91.76

Average absorbance of control: 0.085

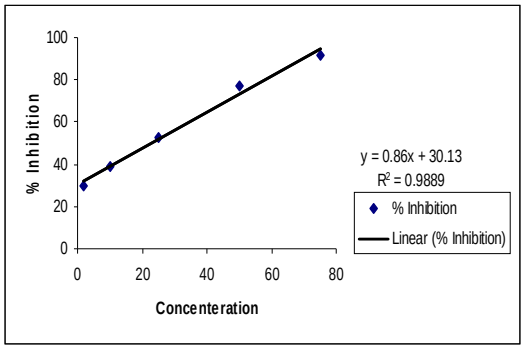

Fig. 2: Free radical scavenging effect of ascorbic acid (standard) in DPPH assay.

Table 4: Percent inhibition of DPPH absorbance at different concentrations of methanolic extract of *B. prionitis*.

Concentration (µg/ml)	Absorbance (Mean ± S.D)	% Inhibition
5	0.197 ± 0.006	2.90
10	0.185 ± 0.004	8.86
25	0.178 ± 0.002	12.31
50	0.164 ± 0.002	19.20
75	0.128 ± 0.001	36.90
100	0.104 ± 0.003	48.76
125	0.089 ± 0.002	56.15
150	0.062 ± 0.004	69.44
200	0.030 ± 0.001	85.22

Average absorbance of the control: 0.203

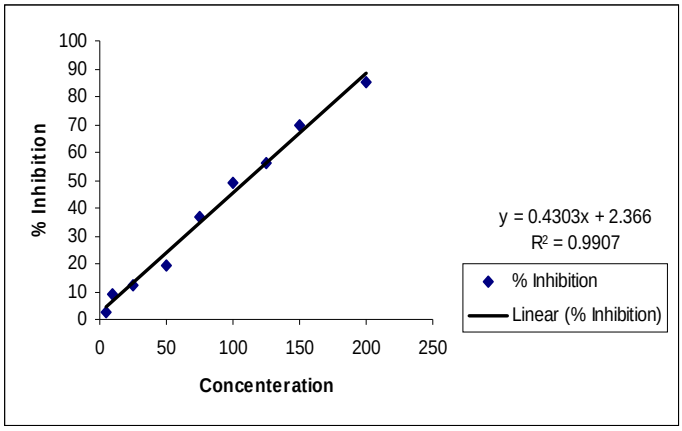

Fig. 3: Free radical scavenging effect of methanolic extract of *B. prionitis* in DPPH assay.

Table 5: Percent inhibition of DPPH absorbance at different concentrations of hexane soluble fraction of *B. prionitis.*

Concentration (µg/ml)	Absorbance (Mean ± S.D)	% Inhibition
5	0.166 ± 0.003	8.28
10	0.159 ± 0.004	12.51
25	0.152 ± 0.002	16.02
50	0.139 ± 0.005	23.20
75	0.128 ± 0.005	35.19
100	0.116 ± 0.003	29.28
125	0.106 ± 0.004	41.43
150	0.098 ± 0.004	45.85
200	0.084 ± 0.003	53.59

Average absorbance of the control: 0.181

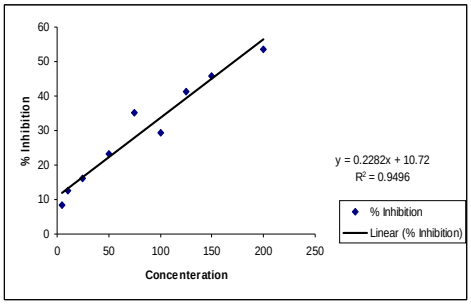

Fig. 4: Free radical scavenging effect of hexane soluble fraction of *B. prionitis* in DPPH assay.

Table 6: Percent inhibition of DPPH absorbance at diffetent concentrations of chlorofonn soluble fraction of *B. prionitis.*

Concentration (µg/ml)	Absorbance (Mean ± S.D)	% Inhibition
5	0.118 ± 0.003	22.36
10	0.105 ± 0.001	30.92
25	0.092 ± 0.003	39.47
50	0.078 ± 0.003	48.68
75	0.062 ± 0.002	59.21
100	0.040± 0.001	71.60
125	0.024 ± 0.004	84.21
150	0.011 ± 0.001	92.76

Average absorbance of the control: 0.152

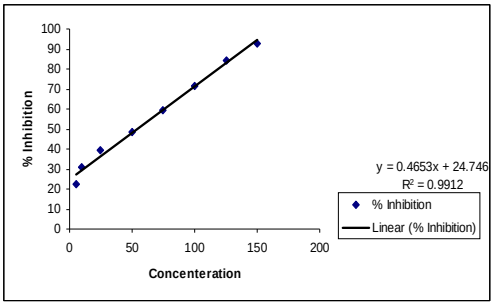

Fig. 5: Free radical scavenging effect of chloroform soluble fraction of *B. prionitis* in DPPH assay.

Table 7: Percent inhibition of DPPH absorbance at different concentrations of ethyl acetate soluble fraction of *B. prionitis*.

Concentration (µg/ml)	Absorbance (Mean ± S.D)	% Inhibition
2.5	0.138 ± 0.004	22.90
5	0.128 ± 0.004	29.49
10	0.110 ± 0.003	38.54
25	0.086 ± 0.004	51.95
50	0.041 ± 0.003	77.09
75	0.005 ± 0.001	97.20

Average absorbance of the control: 0.179

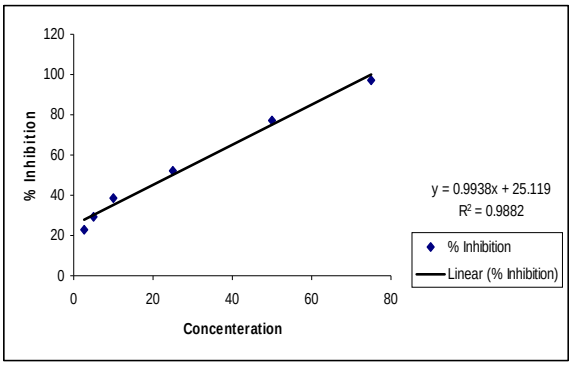

Fig. 6: Free radical scavenging effect of ethyl acetate soluble fraction of *B. prionitis* in DPPH assay.

Table 8: Percent inhibition of DPPH absorbance at different concentrations of butanol soluble fraction of *B. prionitis*

Concentration (µg/ml)	Absorbance (Mean ± S.D)	% Inhibition
2.5	0.125 ± 0.004	29.77
5	0.117 ± 0.005	34.26
10	0.108 ± 0.003	39.32
25	0.085 ± 0.003	52.24
50	0.056 ± 0.002	68.53
75	0.026 ± 0.002	85.39
100	0.005 ± 0.005	97.19

Average absorbance of the control: 0.178

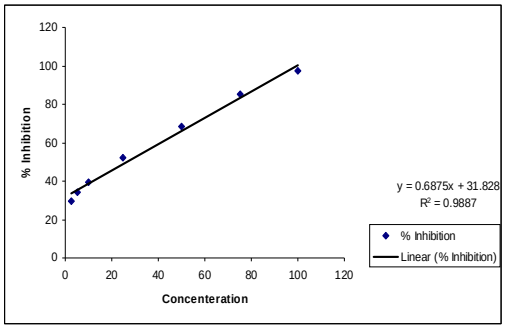

Fig. 7: Free radical scavenging effect of butanol soluble fraction of *B. prionitis* in DPPH assay.

RESULTS & DISCUSSION

The methanolic extract of *Barleria prionitis* linn shows the presence of phenols, flavonoids, glycosides, proanthocyanidins, alkaloids and tannins. Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activity[30]. Phenolics are the mostly wide spread secondary metabolite in plant kingdom. These diverse group of compounds have potential of natural antioxidant and have ability to act as both efficient radical scavengers. The antioxidant activity of phenols is due to their redox properties, hydrogen donors and singlet oxygen quenchers[31].

Preliminary studies carried out by previous workers revealed the presence of flavonoids, iridoids and steroidal compounds in *Barleria prionitis* linn. Different extracts of the plant had also shown significant anti-oxidant activity. Antioxidant activity of *B. prionitis* which may be due to the presence of barlerinoside, shanzhiside methyl ester, 6-*O*-trans-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside.

The result of DPPH scavenging activity assay in this study indicates the ethyl acetate soluble fractions of methanolic extract was potentially active. The scavenging activity of ethyl acetate soluble fractions compared with the standard drug ascorbic acid suggest that the plant is also a potent scavenger of free radicals. However further study aimed at characterization of active constituents responsible for antioxidant activity. Overall, the ethyl acetate soluble fractions of methanolic extract of *Barleria prionitis* linn have most potent antioxidant activity.

CONCLUSION

Antioxidant activity of the hexane soluble, chloroform soluble, ethyl acetate soluble and butanol soluble fractions of methanolic extract were determined *in vitro* by using 1, 1-diphenyl-2-picrylhydrazine assay. The results of the investigation revealed that some of the extracts of the *B. prionitis* showed significant DPPH radical activity which was calculated in terms of IC_{50}.

The highest DPPH radical scavenging activity was found in the ethyl acetate soluble fraction (Table 9) while hexane soluble fraction did not show any activity. The potency of the extracts were in the order of ethyl acetate> butanol >chloroform> methanolic > hexane.

Sample	*B. prionitis* IC_{50}(µg/ml)
Ascorbic acid	23.10
Methanolic extract	110.79
Hexane soluble fraction	170.77
Chloroform soluble fraction	53.88
Ethyl acetate soluble fraction	25.22
Butanol soluble fraction	26.46

ACKNOWLEDGEMENT

The authors are grateful to Dr B. S Bajwa, principal of Lala Lajpat Rai college of pharmacy, moga to provide us all facilities required during preparation of this article.

REFERENCES

1. Ames BN, Shigenaga MK and Hagen TM. Oxidants, antioxidants and the generative disease of aging, Proc Natl Acad Sci USA. 1993; 90: 7915-7922.
2. Dean RT and Davies MJ. Rective species and their accumulation on radical damaged proteins, Trends Biochem Sci. 1993; 18: 437-441.
3. Ceruti P. Oxy-radicals and cancer. Lancet. 1994; 344: 862-863
4. Sies H. Oxidative stress: oxidants and antioxidants. Experimental Physiology. 1997; 82: 291-295
5. Lee JK and Min DB. Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals, Comprehensive Review of Food Science and Food Safety. 2004; 3: 21-33.
6. Chanwithees A, Teerawutgulrag A, Rakariyatham Nuansri. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand, Food Chemistry. 2005; 92: 491–497.
7. Banerjee AK, Maji S, Mahapatra S and Banjeri P. Barleria prionitis linn.: A review of its traditional uses, phytochemistry, pharmacology and toxicity, Research journal of phytochemistry. 2012; 6: 31-41.
8. Shukla P, Singh A, Gawri S, Alexande A and Sonwane S. In vitro propagation of Barleria prionitis Linn and its antibacterial activity. Int. J. Pharma Prof. Res. 2011; 2: 198-200.
9. Kosmulalage KS, Zahid S, Udenigwe CC, Akhtar S, Ata A and Samarasekera R. Glutathione S-transferase,

acetylcholinesterase inhibitory and antibacterial activities of chemical constituents of Barleria prionitis. Z. Naturforsch. 2007; 62b: 580-586.
10. Aneja KR, Joshi R and Sharma C. Potency of Barleria prionitis L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin, New York Sci. J. 2010; 3(11): 5-12.
11. Amoo SO, Ndhlala AR, Finnie JF and Staden JV. Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three Barleria species. S. Afr. J. Bot. 2011; 77: 435-445.
12. Chavan CB, Hogade MG, Bhinge SD, Kumbhar M and Tamboli A. In vitro anthelmintic activity of fruit extract of Barleria prionitis Linn. Against Pheretima posthuma. Int. J. Pharm. Pharm. Sci. 2010; 2: 49-50.
13. Gupta R, Thakur B, Singh B, Singh HB, Sharm VD, Katoch, VM and Chauhan SVS. Anti-tuberculosis activity of selected medicinal plants against multi- drug resistant Mycobacterium tuberculosis isolates, Indian J Med Res. 2010; 131(6): 809-813.
14. Verma PK, Sharma A, Joshi SC, Gupta RS and Dixit VP. Effect of isolated fractions of Barleria prionitis root methanolic extract on reproductive function of male rats: Preliminary study, Fitoterapia. 2005; 76: 428-432
15. Dheer, R. and Bhatnagar, P. A study of the antidiabetic activity of Barleria prionitis Linn, Ind. J. Pharm. 2010; 42(2): 70-73.
16. Jaiswal SK, Dubey MK, Verma AK, Das S, Vijaykumar M and Rao CV. Evaluation of iridoid glycosides from leave of Barleria prionitis as an anti-diarrhoeal activity: An Ethnopharmacological study, Int. J. Pharm. Sci. 2010; 2: 680-686.
17. Ata A, van den Bosch SA, Harwanik DJ and Pidwinski, GE. Glutathione S-transferase and acetylcholinesterase-inhibiting natural products from medicinally important plants, Pure Appl. Chem. 2007; 79: 2269-2276.
18. Ata A, Kalhari KS and Samarasekera R. Chemical constituents of Barleria prionitis and their enzyme inhibitory and free radical scavenging activities, Phyto chem. Lett. 2009; 2: 37-40.
19. Amoo SO, Finnie JF and Staden JV. In vitro pharmacological evaluation of three Barleria species, Journal of Ethanopharmacology. 2009; 121(2): 274-277.
20. Singh B, Bani S, Gupta DK, Chandan BK, and Kaul A. Anti-inflammatory activity of TAF an active fraction from the plant Barleria prionitis Linn, J. Ethanopharmacol. 2003; 85: 187-193.
21. Khadse CD and Kakde RB. Anti-inflammatory activity of aqueous extract fractions of Barleria prionitis L. roots, Asian J. Plant Sci. Res. 2011; 1: 63-68.
22. Manek RA, Sheth NR, Vaghasiya JD, Malaviya SV, Jivani NP and Chavda JR. Study on herb-herb interaction potential of Glycyrrhiza glabra with Solanum xanthocarpum and Adhatoda vasica on mast cell stabilizing activity, Int. J. Pharmacol. 2011; 7: 589-598.
23. Maji AK, Bhadra S, Mahapatra S, Banerji P and Banerjee D. Mast cell stabilization and membrane protection activity of Barleria prionitis Linn, Pharmacog. J. 2011; 3: 67-71.
24. Singh B, Chandan BK, Prabhakar A, Taneja SC, Singh J, Qazi GN. Chemistry and hepatoprotective activity of an active fraction from Barleria prionitis Linn. In experimental animals, Phytother Res. 2005; 19(5): 391-404.
25. Musale SB, Jagtap VA, Patil MS, Chittam KP and Wagh KP. Diuretic activity of Barleria prionitis Linn Flower extract, Int. J. Drug Discovery Herbal Res. 2011; 1: 20-21.
26. Jaiswal SK, Dubey MK, Das S, Verma AR, Vijayakumar M and Rao CV. Evaluation of flower of Barleria prionitis for anti-inflammatory and anti-nociceptive activity, Int. J. Pharm. Biol. Sci. 2010; 1: 1-10.
27. Ambi AA, Abdurrahman EM, Sule MI, Pateh UU, Abdurrahman YR and Ibrahim NDG. Phytochemical Screening and Histopathological Studies on the Seeds of Colocynthis citrullus in Albino Rats, Nigerian Journal of Pharmaceutical Sciences. 2007; 6(2): 7-13.
28. Khandelwal KR. Preliminary Phytochemical Screening in Practical Pharmacognosy, Niralli Parkashan, Pune. 2010; 149-56.
29. Choi CW et al. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison, Plant Science. 2002; 163: 1161-1168.

30. Van A et al. Structural aspects of antioxidant activity of flavonoids, Free Radical Biol Med. 1996; 20: 331-342.
31. Riceevans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant derived polyphenolic flavonoids, Free Rad Res. 1995; 22: 375-383.