

## EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF METHANOLIC EXTRACT OF *BERLERIA PRIONITIS* LINN. AERIAL PARTS

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### ABSTRACT

**Objective:** The aim of the present study is to evaluate the aerial parts of a plant of *Berberia prionitis* Linn., for its antiasthmatic activity with the separation of active moieties.

**Methods:** Adult wistar albino rats were used for the anti-inflammatory study. Histamine-induced bronchospasm was conducted on isolated goat trachea.

**Results:** The dried and powdered aerial parts of *Berberia prionitis* was extracted with continuous soxhlet extraction with Petroleum ether (40-60 ° C), Chloroform, Ethyl acetate, Acetone, Methanol, and Hydroalcoholic solvents. Preliminary phytochemical screening of all extracts was done. Preliminary animal studies by *In vitro* isolated goat trachea chain preparation of all extracts were done to find out the potent extract. In this study, the methanolic extract of aerial parts of *Berberia prionitis* was found to be potent comparative to another extract. The results of carrageenan induced rat paw oedema model indicated the dose-dependant anti-inflammatory activity. As compared to standard drug (Indomethacin), methanolic extract showed similar activity which was found to be statistically significant ( $P < 0.0001$ ). The extent of DPPH radical scavenging was determined by calculated the  $IC_{50}$  value of methanolic extract *Berberia prionitis* (133.5) compared with the Ascorbic acid (114.7) taken as standard. In the present study, the histamine-induced dose-dependent contraction of goat tracheal chain was significantly inhibited ( $p < 0.001$ ) by methanolic extract of aerial parts of *Berberia prionitis* (200  $\mu\text{g/ml}$ ). Thus the present study revealed that the methanolic extract of *Berberia prionitis* (MEBP) has significant antihistaminic (H1 receptor antagonist) activity.

**Conclusion:** In view the fact that tribal have well experienced the antiasthmatic effects of the roots of *Berberia prionitis* Linn. The results of our study, for the first time, show that the methanolic extract of aerial parts of *Berberia prionitis* Linn. possesses antioxidant, anti-inflammatory, Bronchodilator properties and therefore can be used for the antiasthmatic treatment.

**Keywords:** Anti-asthmatic, Antihistaminic, Anti-inflammatory, *Berberia prionitis*

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### INTRODUCTION

Asthma was the most frequent primary diagnosis followed by AR, COPD and rhinosinusitis. Given the ongoing rapid urbanization of India, the frequency of respiratory diseases and their economic burden will continue to rise [1]. *Berberia prionitis* Linn. is a widely dispersed indigenous plant throughout the Indian subcontinent. In the Ayurvedic medicine of the India, it has a significant place due to its biological and pharmacological activities. The various parts of *Berberia prionitis* widely used to cure an array of ailments by different ethnic communities [2]. The whole plant or its parts like leaf, root, stem, bark and flower has been widely utilized for the cure of catarrhal affections, swellings, whooping cough, inflammations, toothache, glandular swellings, urinary infection, fever, gastrointestinal infections, diuretic and also in the treatment of dental infections [3].

### MATERIALS AND METHODS

#### Plant materials

The aerial parts of plant *Berberia prionitis* were collected from the outfield of Amravati city, Maharashtra, India in the month of November-December. The plant materials (Ref. No-GVISH/BOT/REPORT/10/2015) were identified and authenticated by Dr. S. N. Malode, Head, P. G. Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati (M. S.), India.

#### Preparation of extract of *Berberia prionitis* linn

The dried and powdered aerial parts of *Berberia prionitis* (500 gm) were extracted with Continuous soxhlet extraction with Petroleum ether (40-60 ° C), Chloroform, Ethyl acetate, off the solvents and evaporated to dryness using a water bath to get the crude extract. All extracts were dried.

#### Experimental animals

For anti-inflammatory activity, Wistar albino rats weighing 140–160 g of either sex were used. Six animals were group housed in polypropylene cages (640 x 410 x 250 mm high) and kept in well cross ventilated room at the same experimental condition explained above. They were provided with standard rodent pellet diet and tap water *ad libitum* except the food was withdrawn 18–24 h before the experiment [4]. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Government College of Pharmacy, and Amravati, India (approved number-1370/ac/10/CPCSEA).

#### Acute toxicity test (determination of LD50)

The crude methanolic extracts and isolated compound were used for the test. Wistar rat (200–250 g) of either sex were used. This method involved an initial dose-finding the procedure, in which the animals were divided into five groups of three animals per group. Doses of 200, 600, 1000, 1500 and 2000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for 24 h for mortality and general behavior [5].

#### Phytochemical screening of extracts of *Berberia prionitis* linn

All extracts of *Berberia prionitis* were subjected to phytochemical screening for tannins, glycosides, steroids, terpenoids, flavonoids, and alkaloids according to the methods of Trease and Evans.

#### Screening for antiasthmatic activity

##### A preliminary animal study by *in vitro* isolated goat trachea chain preparation of all extracts of *Berberia prionitis* Linn

Isolated adult goat tracheal tissue was obtained immediately after the slaughter of the animals. Trachea was cut into individual rings

and tied together in series to form a chain. Trachea was suspended in bath of Krebs's solution and was continuously aerated at  $37 \pm 0.5$  °C. Dose-response curve (DRC) of histamine in plain Krebs's solution and in different doses of *B. prionitis* different extracts viz., Pet. Ether, Chloroform, Ethyl acetate, Acetone, Methanol and Hydroalcoholic in Krebs's solution were taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record DRC of histamine, in the absence and in the presence of drug extracts.

#### Isolation of phytoconstituent from the selected extract

On the preliminary pharmacological evaluation of Antihistaminic activity, methanolic extract (ME) was found most active extract, and it was then subject to column chromatography. The concentrated methanolic extract (20 g) was chromatographed through a column of silica gel 60-120 mesh L. R. (diam. 4 cms X length 45 cms). The column being successively eluted with increasing polarities of chloroform, chloroform: methanol and methanol. The elution was carried out chloroform: methanol in graded mixture i.e. 100, 95:05, 90:10, 85:15, 80:20 up to 100 methanol. From the above elution, two different fractions i.e. fraction A and B were collected. The concentration of other elutes gave only brown resinous masses which were not processed further.

#### Antioxidant activity

##### *In vitro* DPPH radical scavenging activity

The free radical scavenging activity of various extract can be measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Prepare 0.1 mmol solution of DPPH in Methanol and add 1.0 ml of this solution to 3.0 ml of methanolic *Berberia prionitis* extract and isolated fraction (F1BP) in

different concentrations (25-800 µg/ml). Thirty minutes later, measure the absorbance at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

#### Preparation of extract dilution

50 mg of *B. prionitis* methanolic extract was 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 (50, 100, 150, 200, 250 µg/ml) were prepared by diluting serially with methanol.

#### Anti-inflammatory activity

##### Carageenan induced paw edema in rat

Carrageenan-induced hind paw edema model can be used for determination of anti-inflammatory activity. 60 min after the oral administration of methanolic *B. prionitis* extracts (200 mg and 400 mg/kg) and fraction 1 BP (F1BP), allow each rat to inject with the freshly prepared suspension of carrageenan (0.5 mg/25 mcl) in physiological saline into subplantar tissue of the right hind paw. As the control, Inject 25 micro liter saline solutions into that of the left hind paw. Measure the Paw edema in the intervals of 0, 1, 2,3,4,5 and 6 hr. after induction of inflammation. Measure the difference in footpad thickness. Compare the mean values of treated groups with those of a control group and analyzed by using statistical methods. Use Indomethacin (10 mg/kg) as the reference drug.

Table 1: Experimental design: carageenan induced paw edema in rat

Group	Treatment	Dose
I	Control (Normal saline)	2 ml/Kg b. wt
II	Standard Indomethacin	10 mg/Kg b. wt
III	Methanolic extract of <i>Berberia prionitis</i> (Lower)	200 mg/Kg b. wt
IV	Methanolic extract of <i>Berberia prionitis</i> (Higher)	400 mg/Kg b. wt
VII	Fraction 1 <i>Berberia prionitis</i> (Lower) (F1BP)	200 µg/ml
VIII	Fraction 1 <i>Berberia prionitis</i> (Higher)	400 µg/ml

#### Bronchodilator activity

##### Inhibition of histamine-induced contraction in isolated goat tracheal chain preparation. (*In vitro* model)

Isolated adult goat tracheal tissue was obtained immediately after slaughter of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs's solution and was continuously aerated at  $37 \pm 0.5$  °C. Dose-response curve (DRC) of histamine in plain Krebs's solution and in different doses of methanolic extract of *B. prionitis* and isolated fraction (F1BP) in Krebs's solution were taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record DRC of histamine, in absence and in the presence of drug extracts.

#### Statistical analysis

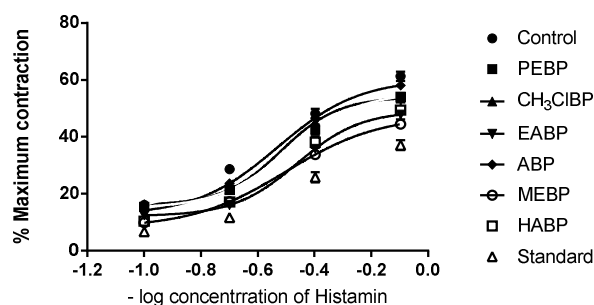
The statistical analysis was performed by using one-way analysis of variance (ANOVA) followed by Dunnett's test for individual comparison of groups with the control. The p values less than 0.001 were considered as significance.

## RESULTS AND DISCUSSION

### A preliminary animal study by *In vitro* isolated goat trachea chain preparation of all extracts of *Berberia prionitis*

It was observed that methanolic extract of *Berberia prionitis* (MEBP) inhibits contraction produced by histamine in these tissue preparations as compared to the pet ether, chloroform, ethyl acetate, acetone and hydroalcoholic extract of BP. Histamine (30µg/ml) was taken in different dose level and DRC was plotted. Study revealed

that Methanolic extract exhibits significant ( $p < 0.001$ ) percentage decreased contraction at concentration 200 µg/ml in goat tracheal chain preparation.



Graph 1: Effect of all extracts of aerial parts of *Berberia prionitis* on histamine induced bronchospasm, values are expressed as mean  $\pm$  SEM \*\*\* $p < 0.0001$ ; ns = nonsignificant, compared with standard Group (one-way ANOVA followed by Dunnett's Multiple Comparisons test)

### Acute toxicity study of aerial parts methanolic extract of *Berberia prionitis* linn

The methanolic extract of *B. prionitis* (MEBP) and isolated fraction F1BP were administered to a group of experimental animals at

doses that is 100 200 and 400 mg/kg was selected for this study. The acute oral toxicity study was carried out as per the OECD guidelines found that both methanolic extract and isolated fraction were safe at limit dose of 2000 mg/kg with no mortality in studied animals. 1/10<sup>th</sup> of these doses i.e. 200 mg/kg and doubling of that dose that is 400 mg/kg and half of 1/10 dose that is 100 mg/kg were used in the subsequent study respectively.

#### Phytochemical screening of extracts of *Berleria prionitis* linn

In phytochemical screening methanolic BP extract contain Alkaloids, Phenolic and Tannins, Saponin, Glycoside, Flavonoid, Phytosterol, Protein, Carbohydrate.

#### Isolated compound from aerial parts of *Berleria Prionitis* Linn

#### TLC profiling of isolated compound (F1BP) from aerial parts of *Berleria prionitis* linn

Fraction A was eluted from Chloroform: methanol (80:20), resulted as a single compound, which was confirmed by TLC

(CHCl<sub>3</sub>: MeOH; 7:3). The product was designated as F1BP. Visualization was carried out by spraying Vanillin-Sulphuric acid reagent (VS) and detection was carried out visually in visible light and under UV light.

Dipping the plate in iodine chamber also shows color spot. Rf value of F1BP was found to be 0.34.

#### Antioxidant activity

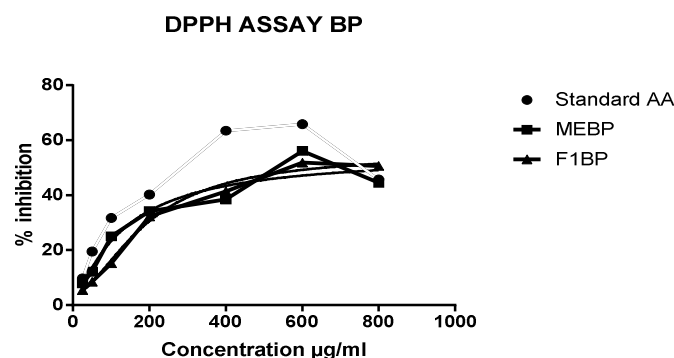
##### *In vitro* DPPH radical scavenging activity

The result of DPPH scavenging activity assay in this study indicates the methanolic extract was potentially active. The scavenging activity of methanolic extract compared with the standard drug ascorbic acid suggest that the plant is also a potent scavenger of free radicals. The antioxidant activity on the basis of their IC<sub>50</sub> values was methanolic BP extract (IC<sub>50</sub> 133.5µg/ml) and isolated compound F1BP (191.6 µg/ml). Results were compared with ascorbic acid (IC<sub>50</sub> 114.7µg/ml).

Table 2: Percent inhibition of DPPH absorbance at different concentrations of standard (ascorbic acid)

Concentration (µg/ml)	% Inhibition		
	MEBP	F1BP	STANDARD
25	07.92	5.48	9.75
50	12.19	8.53	19.51
100	25	15.24	31.70
200	34.14	32.31	40.24
400	38.41	41.46	63.41
600	56.09	51.82	65.85
800	44.51	50.60	45.73

Absorbance of control: 0.164, values are expressed as mean±SEM, \*\*p<0.01, \*\*\*p<0.0001; ns =non-significant, compared with Standard (one-way ANOVA followed by Dunnett's Multiple Comparisons test).



Graph 2: Effect of *B. Prionitis* methanolic extract and an isolated fraction on DPPH antioxidant assay, values are expressed as mean±SEM, \*\*p<0.01, \*\*\*p<0.0001; ns =non-significant, compared with Standard (one-way ANOVA followed by Dunnett's Multiple Comparisons test)

Table 3: Comparison of IC<sub>50</sub> of extracts and isolated fraction from *B. prionitis*

<i>B. prionitis</i> extract	IC <sub>50</sub> µg/ml
Standard Ascorbic acid	114.7
MEBP	133.5
F1BP	191.6

Mean values (P<0.001) according to Duncan's Multiple Range Test.

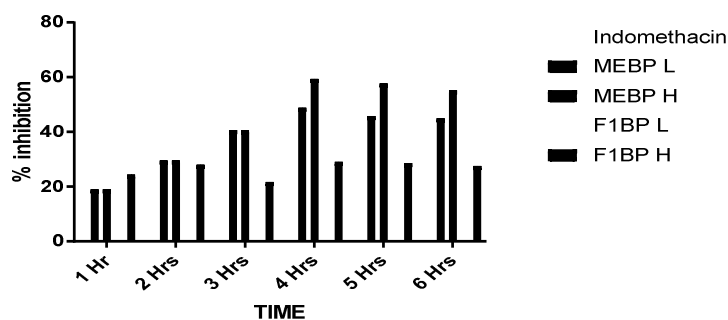
Table 4: Anti-inflammatory activity of methanolic extract of *B. prionitis* L. by carrageen induced rat paw edema

Group	Dose mg/Kg	% Inhibition					
		1 h	2 h	3 h	4 h	5 h	6 h
Indomethacin	10	48.64	57.35	63.51	61.84	58.57	56.52
MEBP	200	18.91	29.41	40.54	48.68	45.71	44.92
	400	43.24	26.47	51.35	59.21	57.63	55.07
F1BP	200	18.91	20.58	10.81	17.10	10	10.14
	400	24.32	27.94	21.62	28.94	28.57	27.53

**Anti-inflammatory activity****Carageenan induced paw edema**

In the carageenan induced rat paw edema test (table-I, fig-I andII), methanolic extract of BP (MEBP) and isolated compound (F1BP) (200 mg/kg) showed significant (\*\*P<0.0001), with 48.68 % and 17.10 % inhibition of edema respectively at the end of 4 h. At a dose of 400 mg/kg methanolic extract of BP (MEBP) and isolated compound

(F1BP) showed significant (\*\*P<0.0001) with % and 59.21 and 28.94% inhibition of edema respectively at the end of 4 h as compared with reference drug indomethacin (\*\*P<0.0001) with 61.84 % inhibition of edema. The carageenan induced rat paw edema is a biphasic process [19, 20]. From the result methanolic extract of BP (MEBP) and isolated compound (F1BP) 400 mg/kg showed more percentage of inhibition against carageenan induced paw edema which is comparable with reference standard Indomethacin.



**Graph 3: Effect of *B. Prionitis* methanolic extract and isolated fraction on carrageenan-induced rat paw oedema, values are expressed as mean±SEM (n = 6), \*\*p<0.01, \*\*\*p<0.0001; ns = non significant, compared with disease control group (one-way ANOVA followed by Dunnett's multiple comparisons test)**

**Bronchodilator activity****Inhibition of histamine-induced contraction in isolated goat tracheal chain preparation. (In vitro model)**

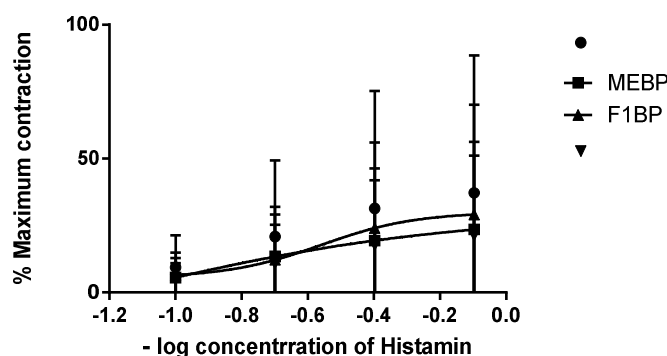
It was observed that methanolic extract of *Berleria prionitis* Linn. Inhibits dose-dependent contraction produced by histamine (30

µg/ml) as indicated in the graph of maximum percentage of contractile response v/s negative log molar concentration of histamine. Study revealed that methanolic extract of *Berleria prionitis* exhibits significant (p<0.001) percentage decreased contraction at concentration 200 µg/ml in goat tracheal chain preparation

**Table 5: Effect of *Berleria prionitis* methanolic extract on histamine-induced contraction on isolated goat tracheal chain preparation**

Dose of histamine (30 µg/ml)	% maximum contraction (mean±SEM)			
	Control group	MEBP	F1BP	Standard
0.1	17.8±0.90	10.78±1.31	12.46±0.61	9.21±0.52
0.2	41.03±1.69	26.63±1.30	24.16±2.19	21.19±0.74
0.4	62.51±1.28	38.49±1.24	46.65±1.41	34.87±0.54
0.8	73.45±0.89	46.65±1.40	58.09±1.04	42.45±1.14

Each value represents the mean±SEM; ns Not significant, \*P<0.05, significant; \*\*\*P<0.0001 vs. control (histamine), one-way ANOVA followed by Dunnett's t-test.



**Graph 4: Bronchodilator activity Of *B. prionitis* extract and isolated fraction (Inhibition of tracheal tissue contraction, values are expressed as mean±SEM\*\*P>0.001 significant compared to control group by one way ANOVA followed by Dunnett's multiple comparison test)**

**CONCLUSION**

The results of the investigation revealed that methanolic extracts of the *B. prionitis* showed significant DPPH radical activity which was calculated in terms of IC50. There was no major difference in the

DPPH radical scavenging activity of methanolic extract and isolated compound.

From the present study, it is concluded that methanolic extract of *B. prionitis* showed maximum percentage inhibition (59.21%) of rat

paw edema at a dose of 400 mg/kg. Anti-inflammatory activity was found to be dose-dependent.

Histamine contracts the tracheobronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than a guinea pig tracheal chain. The goat tracheal muscle has H1, M3, and B2 receptors. The stimulation of H1 receptors caused contraction of the bronchial smooth muscle. In the present study, there is a right side shift of Dose Response Curve (DRC) of histamine in the presence of a methanolic extract of *Berberia prionitis* indicating antiasthmatic action.

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#### AUTHORS CONTRIBUTIONS

All the author have contributed equally

#### CONFLICT OF INTERESTS

Declared none

#### REFERENCES

1. Alope Gopal Ghoshal, GD Ravindran, Paras Gangwal, Girish Rajadhyaksha, Sang-Heon Cho. The burden of segregated respiratory disease in India and the quality of care in these patients: results from the Asia-Pacific burden of respiratory diseases study. *Lung India* 2016;33:611-9.
2. Ajeet Singh, Navneet. A review: traditional, ethnomedicinal utilization, pharmacological properties, and phytochemistry of *Berberia prionitis* Linn. *Int J Pharm Sci Rev Res* 2017;44:19-26.
3. Kamini Singh, Deepika Sharma, Gupta Rs. A comprehensive review on *Berberia Prionitis* (L.). *Asian J Pharm Clin Res* 2017;10:22-9.
4. Sachin Parmar, Amit Gangwal, Navin Sheth. Evaluation of the antiasthmatic activity of a polyherbal formulation containing four plant extracts. *J Curr Pharma Res* 2010;2:40-4.
5. J Shetty Akhila, Shyamjith, Deepa, MC Alwar. Acute toxicity studies and determination of median lethal dose. *Curr Sci* 2007;93:7.
6. Alam MM, Anis M. The medicinal plants of gunung leuser national park, Indonesia. *J Ethnopharmacol* 1987;19:85-8.
7. Alzweiri M, Sarhan AA, Mansi K, Hudaib M, Aburjai T. Ethnopharmacological survey of medicinal herbs in Jordan, the Northern Badia region. *J Ethnopharmacol* 2011;137:27-35.
8. Aneja KR, R Joshi, C Sharma. The potency of berberia prionitis L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin. *New York Sci J* 2010;3:5-12.
9. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish J Biol* 2006;30:177-83.
10. Banerjee D, Maji A, Banerji P. *Berberia prionitis* linn: a review of its traditional uses, phytochemistry, pharmacology and toxicity. *Res J Phytochem* 2012;6:31-41.
11. Chen JL, Blanc P, Stoddart CA, Bogan M, Rozhon EJ, Parkinson N, et al. New iridoids from the medicinal plant *Berberia prionitis* with potent activity against the respiratory syncytial virus. *J Nat Prod* 1998;61:1295-7.
12. Kapoor A, Shukla S, Kaur R, Kumar R, Lehra KS, Kapoor S. Preliminary phytochemical screening and antioxidant activity of whole plant of *Berberia prionitis* Linn. *Int J Adv Pharm Biol Chem* 2014;3:410-9.
13. Khadse CD, Kakde RB. Anti-inflammatory activity of aqueous extract fractions of *Berberia prionitis* L, roots. *Asian J Plant Sci Res* 2011;1:63-8.
14. Maji AK, Bhadra S, Mahapatra S, Banerji P, Banerjee D. Mast cell stabilization and membrane protection activity of *Berberia prionitis* L. *Pharmacogn J* 2011;3 Suppl 24:67-71.
15. Sharma P, Sharma GN, Shrivastava B, Jadhav HR. Evaluation of antioxidant potential of *Berberia prionitis* Leaf and stem. *Am J Phytomed Clin Ther* 2014;2:1177-86.
16. Singh B, Bani S, Gupta DK, Chandan BK, Kaul A. Anti-inflammatory activity of TAF an active fraction from the plant *Berberia prionitis* Linn. *J Ethnopharmacol* 2003;85:187-93.
17. Trease, William C Evans. *Textbook of Pharmacognosy*. 16 edition. Elsevier publication; 2009.