



Screening of solvent extracts of *Berberis aristata* for isolation of anti-inflammatory compound

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ABSTRACT

Berberis aristata (Berberidaceae) a well-known liver tonic is widely distributed throughout India. It has been traditionally used as a blood purifier. It is widely used in Chinese folk medicine as anti-arrhythmics, anti-hypertensive and anti-ophthalmic. Roots have been used in European folk medicine for inflammation. In the present study, the anti-inflammatory potential of solvent extracts of roots of *Berberis aristata* and isolation of anti-inflammatory compound from potent extracts was determined. Different solvent extracts of roots of *Berberis aristata* were prepared on the basis of increasing polarity viz. hexane, chloroform, ethanol and water by cold percolation method. These extracts were screened for in vitro anti-inflammatory activity via conventional procedures viz. HRBC membrane stabilization, inhibition of albumen denaturation and inhibition of heat induced hemolysis. Further these extracts were evaluated for anti-inflammatory potential against carrageenan induced albino rats. The potent extracts were screened for isolation of anti-inflammatory compound via chromatographic and spectroscopic techniques. Significant differences between the experimental groups were assessed by analysis of variance. It was found that the polar extracts showed potent inhibition of paw edema in comparison to non polar extracts in dose dependent manner. The non polar extracts showed anti-inflammatory activity but much minimum in comparison to that of polar extracts. The results revealed that ethanol extracts causes 80 % inhibition of paw edema in comparison to that aqueous extracts showing 72 % inhibition of paw edema at doses 50 mg/kg ($P < 0.05$). The extracts showed higher anti-inflammatory potential as the dose varies thus the extracts showed significant anti-inflammatory activity in dose-dependent manner. The ethanolic extracts exhibited significant membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane followed by aqueous extracts. The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization implies that the extract may also stabilize lysosomal membrane. High-performance liquid chromatography (HPLC) and FT-IR spectra results confirmed the presence of Berberine compound in potent ethanolic extract. The compound was further screened in particular dosage for anti-inflammatory activity in carrageenan induced albino rats against standard drug, Diclofenac sodium. The results confirmed the potent anti-inflammatory activity of Berberine. It was found that the compound showed potent reduction (80 %) in paw edema in comparison to standard drug (70 %) ($P < 0.05$). The results thus confirmed the potent anti-inflammatory activity of Berberine isolated from *Berberis aristata*.

Key words: *Berberis aristata*, solvent Extracts, Berberine, anti-inflammatory activity.

INTRODUCTION

Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto medicine for the treatment of disease [1]. It is estimated that plant materials are present in, or have provided the models for 50% Western drugs [2]. The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments [3]. Many plants have proved to successfully aid in

various ailments leading to mass screening for their therapeutic components. Today, the search for natural compounds rich in antimicrobial, antioxidant and anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic disorders (cancer, diabetes, arthritis, hypertension etc). Anti-inflammatory activities and active principles from plants of North West Himalaya Garhwal region were determined [4-9]. Natural products derived from plants offer a new source of biological that may have a great impact overall human health [10]. Typical inflammatory diseases such as rheumatoid arthritis, asthma, colitis and hepatitis are among the causes of death and disability in the world [11-13]. Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti inflammatory action [14]. Inflammatory response is a series of well coordinated dynamic mechanism consisting of specific vascular, humoral and cellular events that is characterized by the movement of fluids, plasma and inflammatory leukocytes (neutrophils, eosinophils, basophils and macrophages) to the site of inflammation [15, 16]. A variety of chemical mediators or signaling molecules such as histamine, serotonin, leukotrienes, prostaglandins and oxygen derived free radicals (O_2 , OH, ONOO⁻) are produced by inflammatory and phagocytic cells predominantly in the sequences which participates in onset of inflammation [17, 18]. *Berberis aristata* belongs to genus *Berberis* of family Berberidaceae. This genus is commonly known in English as berberry and in the vernacular as *Kashmol* or *Kinjosa*. Most of the species belonging to this genus are very popular as indigenous drugs in India [19]. It is an erect, glabrous, spinescent shrub that grows 3–6 m in height [20]. *Charaka Samhita* prescribed that the extract of the plant be taken internally for treatment of hemorrhage, piles, pruritus, and alopeci [21]. *Sushruta Samhita* described it as being useful internally in indigestion, deficiency of breast milk, and in uterine and vaginal disorders [22]. Among the over-the-counter preparations of this herb, *Daarvaadi Kashaaya* is prescribed for leucorrhoea and metrorrhagia, *Daarvaadi Churnam* for piles and internal abscesses, *Daarvibalaadi Ghritam* for bleeding piles, and *Daarvaadi Tailam* for massage in obesity. In the present investigation we have screened polar and non polar solvent extracts of whole plant of *Berberis aristata* for anti-inflammatory activities. The potent extract was utilized to isolate anti-inflammatory compound.

EXPERIMENTAL SECTION

The chemicals and reagents used were of Analytical Grade and were procured from Ranbaxy and CDH. The animal house used was ethical committee approved in Bundelkhand University, Jhansi (U.P), India.

Collection of Plant material

The roots of *Berberis aristata* belonging to Berberidaceae family was selected for the study. The herbariums of plant material were prepared and were further identified by Dr. Ajai Swami, Chinmaya Degree College, Haridwar (U.K), India. Roots of the plant were dried under shade and ground to form the fine powder.

Preparation of Solvent Extracts

The powdered plant material was soaked in approximately 400 ml of ethanol, water, hexane and chloroform separately on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatmann filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50°C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4°C. The extracts doses were optimized by determination of LD₅₀ of the dose. The dose at which death of the 50 % of the animal population in the group occurs is known as LD₅₀.

In vitro studies for determination of anti-inflammatory potential

(A) The human red blood cell (HRBC) membrane stabilization method

The method as prescribed [23] was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared (25 and 50 mg/ml) using distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were

considered. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

$$\text{Percent Protection (\%)} = (100 - \text{OD of drug treated sample} / \text{OD of Control}) \times 100$$

(B) Inhibition of Albumen Denaturation

Method as prescribed [24] was followed with minor modifications. The reaction mixture was consisting of test extracts (at concentrations 500 and 600 mg/ml) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percent inhibition (\%)} = (\text{OD of Control} - \text{OD of Sample} / \text{OD of Control}) \times 100$$

(C) Heat induced hemolysis

The reaction mixture (2 ml) consisted of 1 ml of test sample solution (at concentrations 500 and 600 mg/ml) and 1 ml of 10 % RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 minutes. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent hemolysis was calculated by the formula mentioned in the above procedure.

***In vivo* studies for determination of anti-inflammatory potential**

Animals

Extracts of whole plant of *Berberis aristata* were evaluated. Male albino rats (180–200 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals [25] The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1°C with a 12 h light/dark cycle. The *in vivo* anti-inflammatory activity was performed in Bundelkhand University, Jhansi (U.P), India. The institutional animal ethical committee has approved the protocol of the study.

Carrageenan-induced edema in rats

For screening *in vivo* anti-inflammatory activity for each of the extracts, 7 Groups of five animals each were used.

Group I: Treatment with Vehicle/Control (Distilled water); 10 ml/Kg

Group II: Treatment with Vehicle/Control (Ethanol); 10 ml/Kg

Group III: Treatment with Ethanolic extract of roots of *B. aristata* (Test); 25 & 50 mg/Kg

Group IV: Treatment with Aqueous extract of roots of *B. aristata* (Test); 25 & 50 mg/Kg

Group V: Treatment with Hexane extract of roots of *B. aristata* (Test); 25 & 50 mg/Kg

Group VI: Treatment with Chloroformic extract of roots of *B. aristata* (Test); 25 & 50 mg/Kg

Group VII: Treatment with Standard drug, Diclofenac Sodium (10 mg/Kg)

Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The solvent extracts of plant at dose of 25 and 50 mg/kg were administered orally 60 minutes before carrageenan injection. Diclofenac Sodium (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 1, 2, 3, and 4 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Phytochemical screening of the extract

The portions of the dry extracts were subjected to the Phytochemical screening using the method as adopted [26-28] Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars, cardiac glycosides and anthraquinones.

Test for Alkaloids

The 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Wagner's reagent Reddish orange colored turbidity or precipitation was taken as indicator for the presence of alkaloids.

Test for Tannins

About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% FeCl₃ was added to the filtrate. Deep green color appeared confirmed the presence of Tannins.

Test for Flavanoids

About 0.2 gm of the extract was dissolved in methanol and heated for some time. A chip of mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was Indicator of the flavanoids.

Test for Saponin

About 0.5 g of the plant extract was stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

Test for Steroids

Salkowski's method was adopted for the detection of steroids. About 0.5 g of extract was dissolved in 3 ml of chloroform and filtered. To the filtrate, conc. H₂SO₄ was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring [29].

Test for Cardiac glycosides

About 0.5 g of the extract was dissolved in 2ml of glacial acetic acid containing 1 drop of 1% FeCl₃. This was under laid with conc. H₂SO₄. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

Test for reducing Sugars

1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

Test for Anthraquinones

5ml of the extract solution was hydrolyzed with dil/conc. H₂SO₄. 1 ml of dilute ammonia was added to it. Rose pink colour confirmed the presence of anthraquinones.

Isolation and characterization of anti-inflammatory compound from the potent extract via chromatographic and spectroscopic techniques**Thin Layer Chromatography**

Preparative TLC was used for the isolation of compound (s) from the mixture, only small amounts can be obtained from each fractionation procedure. Fractions of the potent plant extracts chromatographed within the column were applied in the form of band on TLC plate. The plates used in this method were 0.5-1 mm thick (analytically TLC uses plates of 0.25 mm thickness). The plates were developed in the solvent, Chloroform: Methanol (CM) in a ratio of 9:1 to separate the compounds. R_f value of the compound isolated was calculated. The developed chromatogram was visualized in UV light, to detect the compound. The pure compound as viewed in the form of a single spot was pooled out from TLC silica gel for further analysis.

High Performance Liquid Chromatography

HPLC analysis was performed in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a Shimadzu LC- 2010 HPLC system (Kyoto, Japan), equipped with a Shimadzu LC 2010 UV-VIS detector with a thermostated flow cell and a selectable wavelength of 305 nm. The detector signal was recorded on a Shimadzu LC2010 integrator. The column used was a C-18 block heating-type Shim-pack VP-ODS (4.6 mm interior diameter × 150 mm long) with a particle size of 5 µm. Mobile phase was designed as per the nature of the compound, containing 50 % acetonitrile along with 50 % Phosphate buffer was used at a flow rate of 3.0 ml/min,

column temperature 25°C. Injection volume was 40 µl and detection was carried out at 346 nm having maximum absorbance.

Fourier Transform Infrared (FTIR) studies

The IR spectrum of isolated compound was recorded in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a computerized FT-IR spectrometer (Perkin Co., Germany) in the range of 4000-500 cm⁻¹ by the KBr pellet technique.

RESULTS AND DISCUSSION

Ethanollic and aqueous solvent extracts of roots of *Berberis aristata* were found to have significant anti-inflammatory activity at doses 25 and 50 mg/Kg during *in vitro* anti-inflammatory assay. During *in vivo* anti-inflammatory activity, the paw edema was reduced significantly in carrageenan induced albino rats through introduction of ethanolic extracts at a dosage 50 mg/Kg. Ethanolic extracts showed potent anti-inflammatory activity in comparison to aqueous extracts. The polar extracts showed potent anti-inflammatory activity in comparison to non polar extracts. The results revealed that ethanol extracts causes 80 % inhibition of paw edema in comparison to that aqueous extracts showing 72 % inhibition of paw edema at doses 50 mg/kg (P<0.05). The extracts showed higher anti-inflammatory potential as the dose varies thus the extracts showed significant anti-inflammatory activity in dose-dependent manner. The non polar solvent extracts viz. hexane and chloroformic extracts showed significant minimal anti-inflammatory activity in comparison to polar solvent extracts. The extracts showed higher anti-inflammatory potential as the dose varies.

In vitro studies for determination of anti-inflammatory potential

(A) The human red blood cell (HRBC) membrane stabilization method:

Amongst aqueous and ethanolic extracts, the ethanolic extracts at a concentration of 50 mg/ml showed 86.0 ±0.06 % protection of HRBC in hypotonic solution in comparison to aqueous extracts (82.2 ±0.06 %). The results were compared with standard Diclofenac Sodium which showed 83.54 ±0.06 % protections at 50 mg/ml. The results are shown in **Table 1** and **Figure 1**. *Berberis aristata* root extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane^[25] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.^[27] Some of the NSAIDs are known to posses membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [28].

Table 1: Percent activity of HRBC membrane stabilization

S.No.	Groups	Concentration of Extracts of <i>Berberis aristata</i>	Percent Protection± SD
I	Control	25 mg/ml	-
		50 mg/ml	-
II	Ethanolic extract	25 mg/ml	70.12 ±0.06
		*50 mg/ml	86.0±0.06
III	Aqueous extract	25 mg/ml	78.5 ±0.06
		*50 mg/ml	82.2 ±0.06
IV	Standard (Diclofenac Sodium)	25 mg/ml	72.34 ±0.06
		*50 mg/ml	83.54 ±0.06

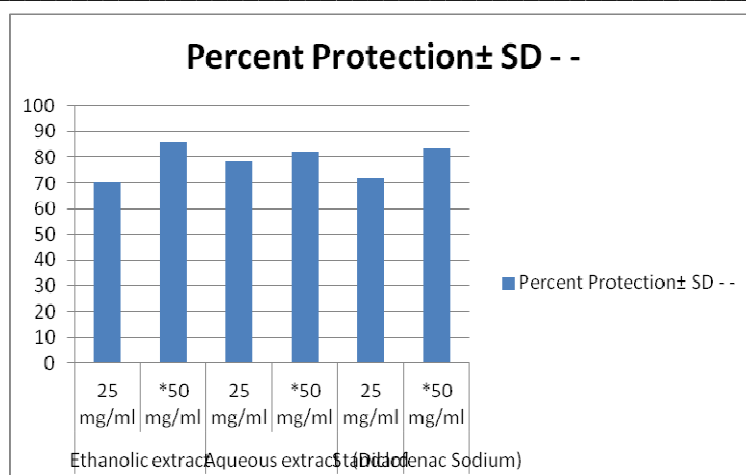


Figure 1: Percent activity of HRBC membrane stabilization

(B) Inhibition of Albumen Denaturation:

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. The ethanolic extracts were found to be effective in inhibiting heat induced albumin denaturation in comparison to aqueous extracts at a dose of 50 mg/ml. Maximum inhibition 88.05±0.06 was observed from ethanolic extracts followed by aqueous extracts (78.01±0.06). The results were compared with standard Diclofenac Sodium which showed 93.54 ±0.06 % inhibition in albumen denaturation at 50 mg/ml. The results are reported in **Table 2** and **Figure 2**. Since during inflammation condition, protein of the cell gets denatured, thus here albumen protein is used as a model whose protection in denaturation by plant extracts was studied.

Table 2: Percent inhibition of albumen denaturation

*Potent Extracts/Drug

S.No.	Groups	Concentration of Extracts of <i>Berberis aristata</i>	Percent Protection ± SD
I	Control	25 mg/ml	-
		50 mg/ml	-
II	Ethanolic extract	25 mg/ml	76.2 ±0.06
		*50 mg/ml	88.05±0.06
III	Aqueous extract	25 mg/ml	70.0 ±0.06
		*50 mg/ml	78.01±0.06
IV	Standard (Diclofenac Sodium)	*50 mg/ml	93.54 ±0.06

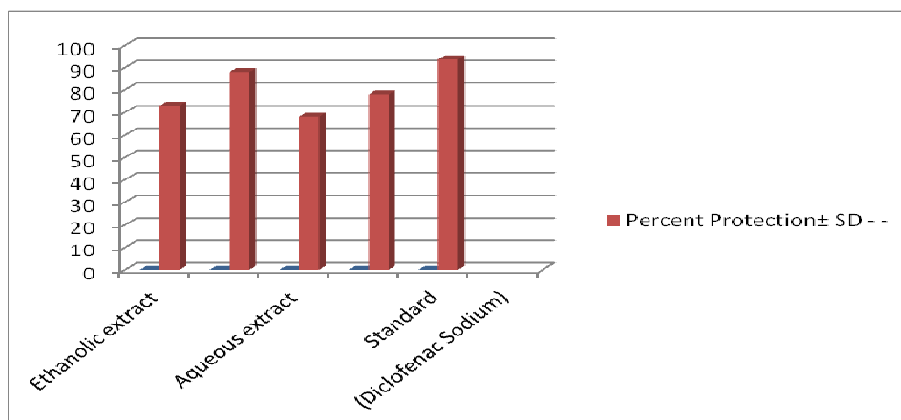


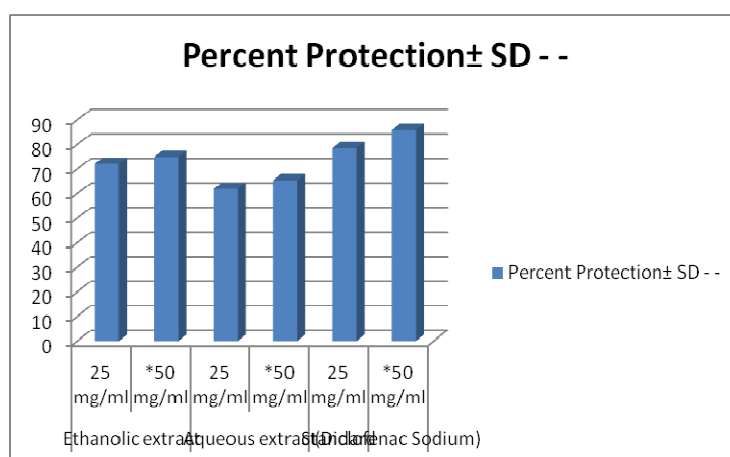
Figure 2: Percent inhibition of albumen denaturation

Heat induced hemolysis:

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different extracts of *Berberis aristata*. Both the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree. The maximum inhibition was recorded 75.02 ± 0.06 % from ethanolic extract followed by aqueous extracts (65.53 ± 0.05 %). The results were compared with standard Diclofenac Sodium which showed the maximum inhibition 85.92 ± 0.05 % at 50 mg/ml. The results are reported in **Table 3** and **Figure 3**.

Table 3: Percent Inhibition of Heat Induced Hemolysis

S.No.	Groups	Concentration of Extracts of <i>Berberis aristata</i>	Percent Protection \pm SD
I	Control	25 mg/ml	-
		50 mg/ml	-
II	Ethanolic extract	25 mg/ml	72.2 ± 0.06
		*50 mg/ml	75.02 ± 0.06
III	Aqueous extract	25 mg/ml	62.0 ± 0.06
		*50 mg/ml	65.53 ± 0.05
IV	Standard (Diclofenac Sodium)	25 mg/ml	78.54 ± 0.06
		*50 mg/ml	85.92 ± 0.05

**Figure 3: Percent Inhibition of Heat Induced Hemolysis*****In vivo* studies for determination of anti-inflammatory potential****Carrageenan-induced edema in rats**

The anti-inflammatory effects of the solvent extracts of *Berberis aristata* on carrageenan-induced edema in rat's hind paws are presented in **Table 4**. The anti-inflammatory activities of extracts were found to have effect in dose-dependent manner. There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, ethanolic extract and aqueous extract (50 mg/kg) showed a significant reduction in the edema paw volume. There was no reduction in inflammation found in case of rats treated with ethanol and distilled water. The results revealed that ethanol extracts causes 80 % inhibition of paw edema in comparison to that aqueous extracts showing 72 % inhibition of paw edema at doses 50 mg/kg ($P < 0.05$) in comparison to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.08 ± 0.05 , 0.10 ± 0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration. The non polar solvent extracts viz. hexane and chloroform extract showed much minimal anti-inflammatory potential in comparison to polar ethanol and aqueous extracts.

Table 4: Anti-inflammatory activities of different extracts of *Berberis aristata*
Paw volume (ml) ± SD

Experiment	Control	Diclofenac Sodium (10 mg/kg orally)	Ethanol extract (50 mg/kg)	Aqueous extract (50 mg/kg)	Ethanol (50 ml/kg)	Distilled water (50 ml/kg)
1h after treatment	0.25±0.05	0.21±0.05	0.23± 0.05	0.28±0.003	0.20±0.05	0.34±0.05
2h after treatment	0.25±0.05	0.18±0.05	0.20± 0.05	0.24±0.05	0.15±0.05	0.34±0.05
4h after treatment	0.25±0.05	0.16±0.05	0.08± 0.05	0.10±0.05	0.30±0.05	0.34±0.05

±, S.D, Standard Deviation ($P < 0.05$).**Phytochemical screening:**

Different conventional methods were followed to determine qualitatively the presence of phytochemical constituents present in the potent extracts. The ethanol and aqueous extracts of the plant possessed alkaloids, steroids, saponin, reducing sugars, tannins, cardiac glycosides and anthraquinones while only flavanoids were found to be absent. The results are indicated in **Table 5**.

Table 5: Phytochemical Screening of the active constituents

Solvent extracts	Phytoconstituents							
	Alkaloids	Tannins	Flavonoids	Saponin	Steroids	Cardiac glycosides	Reducing sugars	Anthraquinones
Ethanolic extract	+	+	-	+	+	+	+	+
Aqueous extract	+	+	-	+	+	+	+	+

*+, present; -, absent

Isolation and characterization of anti-inflammatory compound from the potent extract via chromatographic and spectroscopic techniques

The purity and character of the compound isolated, Berberine was confirmed by a combination of HPLC and Infra-red (IR) spectra as described below. The compound was screened further for in vivo anti-inflammatory activity on carrageenan induced albino rats. It was found that the compound showed potent reduction (80 %) in paw edema in comparison to standard drug (70 %) ($P < 0.05$).

Thin Layer Chromatography

Thin Layer Chromatography of pure compound, Berberine ($R_f = 0.56$) and standard ($R_f = 0.58$) was viewed under a UV light following chromatography through a silica gel column. A mobile phase containing chloroform and methanol (9:1, v/v) was used to separate the reaction product from starting material. The presence of a single spot confirms the purity of the reaction mixture. The results are shown in **Figure 4**.

**Figure 4: TLC chromatogram of Berberine isolated from *Berberis aristata* was viewed under UV light**

High Performance Liquid Chromatography

The retention time of the pure compound purified was found to be 13.2 minutes as compared to the Standard reference compound, which showed almost similar retention time while eluting out through the column at 346 nm. This analysis thus confirmed about the separation and identification of the purified active compound. The results are shown in **Figure 5**.

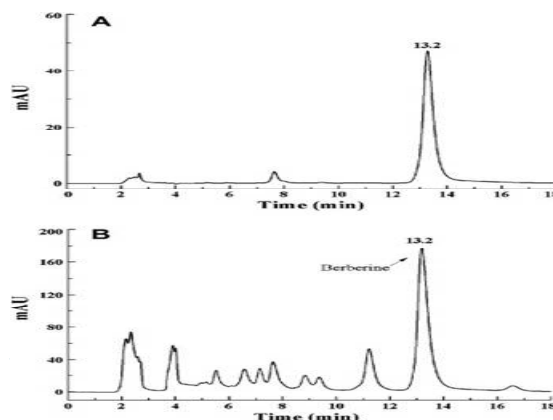


Figure 5: HPLC chromatogram of purified and isolated Berberine

Fourier Transform Infrared (FTIR) studies

The infra-red spectrum of a compound is essentially the superposition of absorption bands of specific functional groups, and IR studies may thus be qualitatively used for the confirmation of specific functional groups in a molecule. The IR spectrum of isolated compound was recorded in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a computerized FTIR spectrometer (Perkin Co., Germany) in the range of 4000–500 cm^{-1} by the KBr pellet technique. The FT-IR spectra peak of the compound merge with that of the spectra of reference/standard compound Berberine which partially confirmed that the compound purified is having the similar nature to that of the standard. The C-H stretching was found at 2820 cm^{-1} while C=C and C=N stretching was observed at 1597 cm^{-1} . The deformation in C-H was found from 1354-1383 cm^{-1} and C-O stretching was found at 1060 cm^{-1} . The results are indicated in **Figure 6**.

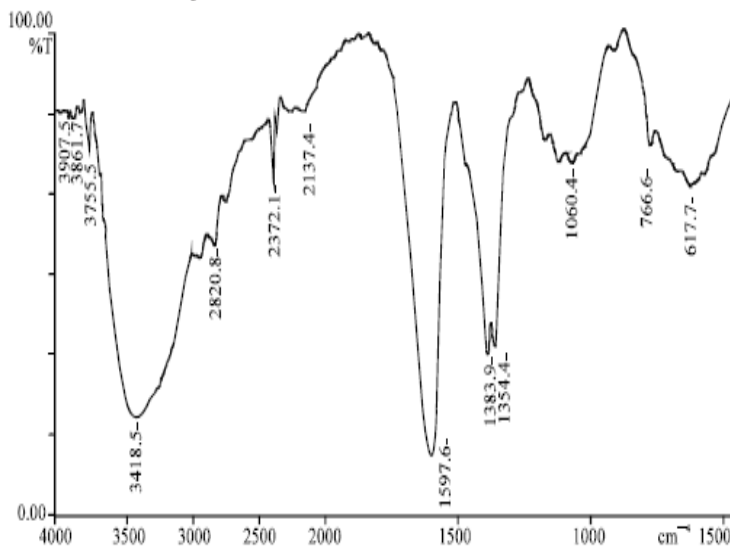


Figure 6: FT-IR spectra of Berberine

In the present investigation carried out to evaluate the anti-inflammatory potential of solvent extracts of *Berberis aristata* (Family: Berberidaceae) through *in vitro* and *in vivo* procedures, the results were found to be very surprising

and promising. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [21] and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage [22]. From the above study it was concluded that the ethanolic extract of *Berberis aristata* has significant membrane stabilization property compared to the aqueous extract of the same plant and it was comparable to the standard drug Diclofenac Sodium. Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation. From the above study it was concluded that the ethanolic extract of *Berberis aristata* had maximum albumen denaturation protection property as compared to the aqueous extract and other non polar solvent extracts of the plant. The results were found in reference to the standard drug Diclofenac Sodium. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The precise mechanism of this membrane stabilization is yet to be elucidated; it is possible that the *Berberis aristata* produced this effect by reducing the surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins. The above findings also confirmed that ethanolic extracts of *Berberis aristata* possessed maximum protection activity of RBC membrane by heat induction in comparison to aqueous extracts. This can be due to the reduction in heat induced by inflammation (associated with any disease) by the effect of plant extracts. When the extracts were evaluated for in vivo anti-inflammatory activity on carrageenan induced albino rats, the ethanolic and aqueous extracts also showed the similar pattern as that of in vitro studies. In the test groups, ethanolic extract and aqueous extract (50 mg/kg) showed a significant reduction in the edema paw volume. There was no reduction in inflammation found in case of rats treated with ethanol and distilled water. The results revealed that ethanol extracts causes 80 % inhibition of paw edema in comparison to that aqueous extracts showing 72 % inhibition of paw edema at doses 50 mg/kg ($P < 0.05$) in comparison to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.08 ± 0.05 , 0.10 ± 0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration. The non polar solvent extracts viz. hexane and chloroform extract showed much minimal anti-inflammatory potential in comparison to polar ethanol and aqueous extracts. The pure anti-inflammatory compound isolated from the potent ethanolic extract of the plant, *Berberis aristata* was Berberine as revealed from HPLC and FT-IR spectra. It was found that the compound showed potent reduction (80 %) in paw edema in comparison to standard drug (70 %) ($P < 0.05$).

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