

Full Length Research Paper

Ethnobotanical studies on *Berberis aristata* DC. root extracts

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The aqueous and alcoholic extract of fresh *Berberis aristata* DC roots, as well as aqueous extract of dried roots were compared for their antibacterial and antifungal activities by the disc diffusion method. All three extracts showed wide antibacterial activity against Gram-positive bacteria. Among the Gram-negative bacteria tested, the antibacterial activity was limited to *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* type 1 and *Vibrio cholerae*; with the best activity against *V. cholerae*. MICs of the alcoholic extracts against Gram-positive bacteria ranged between 3.8×10^{-3} to 6.1×10^{-3} mg/ml and for Gram-negative bacteria from 6.1×10^{-3} to 7.6×10^{-3} mg/ml. The MICs for *Candida* species ranged between 0.02 to 3.8×10^{-3} mg/ml and for *Aspergillus* species, it was 3×10^{-3} mg/ml. All three extracts also had antifungal activity against the fungal species tested, except *Candida krusei*. The extracts of *B. aristata* also demonstrated anti-inflammatory, analgesic, and antipyretic activities. Chemical analysis revealed the presence of alkaloids, amino acids, tannins, terpenes, resins, phenols and reducing sugars as major compounds. FTIR-spectral analysis of all the extracts revealed the presence of berberine, as a major constituent, along with other chemical constituents.

Key words: Antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, berberine.

INTRODUCTION

Berberis aristata DC. (Berberidaceae), known locally as Chitra and Dar-Hald in Hindi, and Dar-E-Hald in Urdu, is a spinous shrub native to mountainous parts of North India and Nepal. These shrubs are distributed throughout the Himalayas, from Bhutan to Kunawar (altitude 6-10,000 ft), Nilgiri hills (altitude 6-7,000 ft) and Sri Lanka (altitude 6-7,000 ft) (Khory and Kartak, 1985; Kirtikar and Basu, 1995). The plant is an erect glabrous spinescent shrub, 3-6 m in height with obovate to elliptic, subacute to obtuse, entire or toothed leaf ($3.8-10 \times 1.5-3.3$ cm long), flowers are yellow in corymbose racemes, petiole distinct up to 4 mm, inflorescence a single drooping raceme (2.5-7.5 cm long), and dense-flowered. Pedicles

are stout, 4-6 mm long, fruits are 7-10 mm long, ovoid, bluish black or bright red in color and covered with a thick pale or bluish white bloom and are born in pendulous clusters (Khory and Kartak, 1985; Kirtikar and Basu, 1995). Ethnobotanical studies indicate that the decoction of *B. aristata* leaves, commonly known as Rasaut, is an alterative and deobstruent, and is commonly used to treat skin diseases, menorrhagia, diarrhea, cholera, jaundice, eye and ear infections, as well as urinary tract infections. The decoction of the root is used as a wash for infected wounds and ulcers, and is said to help healing and promote cicatrisation (Khory and Kartak, 1985; Kirtikar and Basu, 1995). *B. aristata* extracts have also been reported to cure hepatotoxicity (Gilani and Janbaz, 1995). Despite the medicinal importance of this plant species, especially root extracts, reports on its antibacterial and antifungal activities are still limited (Dutta and Panse, 1962). During our search of literature we found studies on other *Berberis* spp., namely *B. asiatica* (Hashmi and

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Hafiz, 1986; Singh et al., 2007), *Berberis thunbergii* (Li et al., 2007), *Berberis aetnensis* (Musumeci et al., 2003; lauk et al., 2007), *Berberis heterophylla* (Freile et al., 2003), *Berberis chitria* (Singh et al., 2007) and *Berberis lycium* (Singh et al., 2007).

However, we could get very few studies exploring the antimicrobial and other medicinal properties of *B. aristata*. Of these existing studies, a more recent report of Singh et al. (2007) described the antimicrobial activities of hydroalcoholic (50%) extracts of four *Berberis* species, including that of *B. aristata*. In this present study, we adopted different approach to test aqueous and alcoholic extracts separately, instead of using hydroalcoholic extracts, and we used different and exhaustive range of bacterial and fungal isolates, including both standard as well as clinical strains. Furthermore, anti-inflammatory, analgesic, and antipyretic properties of the extracts were also investigated since the infectious diseases, which are traditionally treated with *Berberis* species usually, also have these symptoms.

MATERIALS AND METHODS

The roots of *B. aristata* were procured from Kashmir, identified by Dr. Badruzzaman Siddiqui, Department of Botany, Aligarh Muslim University, Aligarh. Voucher specimens are deposited in the herbarium of Medicinal Plant Unit of Central Council for Research in Unani Medicine at Aligarh, Uttar Pradesh, India.

Physicochemical and qualitative-chemical analysis

Ash values, extraction values, and moisture content were measured in *B. aristata* roots according to Afaq et al. (1994). Qualitative chemical analysis was performed as described by Bhattacharjee and Das (1969).

Plants extracts

Both aqueous and alcoholic extracts were prepared as described by Singh and Singh (2000) with minor modifications as adopted in our previous studies (Shahid et al., 2007).

Aqueous extracts

Fresh roots of *B. aristata* (15 g) were thoroughly washed in sterile double distilled water (DDW), surface sterilized in 70% ethanol (v/v) for 30 s, and then washed three times in sterile DDW. The sterilized materials were ground with a sterile pestle and mortar in sterile distilled water (150 ml). The homogenized tissue was centrifuged at 7,000 rpm for 15 min, supernatant was filter-sterilized and used as the aqueous extract.

Alcoholic extracts

To prepare alcoholic extracts, fresh roots (15 g) were homogenized in 95% ethanol (30 ml) and centrifuged as outlined above. The supernatant was put in a hot water bath at 60°C to evaporate the organic solution. The extract was re-dissolved in 95% ethanol to achieve the desired concentrations (100 mg/ml). The extracts were filter-sterilized before use.

Aqueous extract of dried root

The roots (15 g) were thoroughly washed and surface-sterilized as described above, and subsequently dried completely before being ground into a powder. The powdered root material was dissolved in sterile distilled water (150 ml) and kept overnight before using for antimicrobial studies.

Microorganisms

Clinical isolates of Gram-positive and Gram-negative bacteria, and the fungi were obtained from the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh. The clinical bacterial species used were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella typhimurium*, *Shigella dysenteriae* type 1, and *Vibrio cholerae*. Bacterial control strains were originally provided by Dr. Sunil Gupta, Joint Director of National Institute for Communicable Diseases (NICD), New Delhi, and the fungal control strains were provided by Dr. Ashok Rattan, Director, New Drug Discovery Research, Ranbaxy, Gurgaon, India. The control bacterial species used were *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853). The clinical fungal strains were *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida keyfr*, *Candida parapsilosis*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger*, and the control strains were *C. parapsilosis* (ATCC 22019), *C. keyfr* (Nyl/1), and *A. fumigatus* (ATCC 204305). Bacterial strains were grown on blood agar or MacConkey agar plates at 37°C and maintained on nutrient agar slants, while the fungi were grown at 25°C and maintained on Sabouraud's Dextrose agar (SDA) slants.

Antimicrobial susceptibility testing

Antibacterial tests was performed as per National Committee for Clinical Laboratory Standards (NCCLS; now CLSI) (2000) and antifungal testing was performed according to NCCLS (1997) (document M27-A for yeasts) and NCCLS (2002) (document M 38-A for filamentous fungi). Discs containing aqueous and alcoholic extracts at concentrations of 50, 25 and 12.5 µg/disc were used for susceptibility testing. An inoculum size of 10⁶ cfu/ml of bacteria or 2×10⁵ yeast cells or fungal spores was used for inoculating the susceptibility plates. Mueller Hinton agar (M 173; Hi Media, India) was used for antibacterial susceptibility testing, whereas SDA was used for the antifungal susceptibility testing. All of the plates were incubated either at 37°C overnight for bacterial strains, or at 25°C for 3-7 days for fungi. Ciprofloxacin was used as standard antibiotics, and nystatin, fluconazole, and amphotericin B discs were used as standard antifungal drugs. All experiments were performed in triplicate.

Determination of minimum inhibitory concentrations (MICs)

MIC was determined for the alcohol extracts. MICs were determined by the broth microdilution method according to the method defined by National Committee for Clinical Laboratory Standards (NCCLS, 2000), with minor modifications. Briefly, A doubling dilution of the extracts were prepared using RPMI-1640 broth (HiMedia, India) supplemented with 0.3 g/L L-glutamine (HiMedia, India), 0.165 M MOPS buffer (35.5 g/L; HiMedia, India) and 0.01% DMSO (Qualigens Fine Chemicals, India). Extracts were dissolved in

DMSO, and further diluted 1:50 in RPMI-1640 medium. Microtitre plates were prepared containing 100 µl of dilution of extracts with final concentrations ranging from 25 to 9.5×10^{-5} mg/mL. Standardized inoculum of each bacterial species was added. For each test there was a sterility control well containing RPMI only, two sets of growth control wells, one containing RPMI broth alone and the other containing RPMI broth with DMSO, and a drug control containing an aqueous or alcohol extract. The microtitre plates were incubated either at 37°C for bacteria for 24 h or at 30°C for fungi for 96 h. The lowest concentration that did not show any visible growth was considered the MIC. All the MIC experimentations were performed in duplicate.

Detection of anti-inflammatory activity by Carrageenin induced rat paw edema test

Carrageenin induced rat paw edema test was performed according to the method of Winter et al. (1962) with minor modifications. Albino rats, weighing 150 – 200 g, were divided into four groups containing 6 animals in each group. The volume of right hind paw was measured with the help of a plethysmometer by dipping the animal paw up to the indelible mark just below the knee joint. The liquid displaced by the paw denoted the paw volume (in ml). Animals in groups 1 and 2 were fed ethanolic and aqueous extracts, respectively, at a dose of 50 mg/100 g. Animals in group 3 served as controls and were administered with 02 ml distilled water or alcohol per 100 g feed, during testing of aqueous and alcoholic extracts, respectively, for anti-inflammatory activity. The anti-inflammatory drug Diclofenac sodium (Biochem. Pharma industries Mumbai) was orally administered to group 4 in the dose of 0.5/100 g. One hour after treatment, animals were injected with 0.1 ml of a suspension of lambda-type carrageenin in normal saline under the planter aponeurosis of the right hind paw. The thickness of the paw was measured at 1, 2, 3, 4 and 5 h after the injection of carrageenin. The percent inhibition in paw volume between the test and standard control was calculated in comparison with the control group according to the formula:

$$i = 100 \left(1 - \frac{a - x}{b - y} \right)$$

where, i = percent inhibition; a = right hind paw volume (mean) of test/standard animals after carrageenin injection; b = right hind paw volume (mean) of control animals after carrageenin injection; x = right hind paw volume (mean) of test/standard animals before carrageenin injection; y = right hind paw volume (mean) of control animals before carrageenin injection. The mean paw thickness of test/standard group was analyzed statistically using test of variance (ANOVA) in comparison with control group.

Analgesic activity

Analgesic activity was determined according to the method of Eddy and Leimbach (1953) with minor modifications. Albino rats, weighing 100-150 g, were divided into three groups having 6 animals in each group. The initial reaction time of all the animals was recorded by putting them on the hot plate maintained at 55.5°C before administering the drugs or distilled water. Licking of paw or jumping was taken as the index for reaction to heat. One test group received 50/100 g of the alcoholic extract orally and the other test group received 50/100 g of aqueous extract orally. The control group was either given 1 ml/ 100 g of distilled water or 1 ml/ 100 g of alcohol, orally, for respective experimentations. The reaction time of each animal was again recorded after administration of the drug, at 20 min interval, for the next 2 h. The mean reaction time of the test

group of each drug, at each interval of the testing, was compared statistically with the mean reaction time of the control group at the corresponding intervals by using test of variance (ANOVA).

Test for antipyretic activity

The antipyretic activity test was carried out by using the Diphtheria-Pertussis-Tetanus (D.P.T.) vaccine as the pyrexia-inducing agent. Rabbits, weighing 1.0 – 1.5 kg, were divided into three groups containing five animals in each group. Two groups served as test groups and one group as the control group. The initial temperature of each animal was recorded using a clinical thermometer, inserted half inch deep into the rectum. Alcoholic and aqueous extracts were administered at a dose of 200 mg/kg orally to the respective test groups, and the control group was administered water in the usual manner. The D.P.T. vaccine (Central Research Institute, Kasauli, Himachal Pradesh, India) was injected intravenously in the ear vein at a dose of 0.5 ml/kg. Rectal temperature of the test and control groups was recorded after vaccine injections at 30-min intervals for a period of three hours. The mean rectal temperature of the test group was compared with that of the mean rectal temperature of the control group at corresponding intervals and analyzed statistically by using test of variance (ANOVA).

FTIR-spectral analysis

FTIR-spectral analysis of the extracts was performed in the section of Analytical Chemistry, Department of Chemistry, Aligarh Muslim University, Aligarh, India. The method described by Socrates (1980) was used and the FTIR-spectrum of l-13α-estrone methyl ether was used as a control.

Statistical analysis

Data were statistically analyzed by using either one- or two-way analysis of variance (ANOVA). Mean comparisons were made by least significant difference at the 5% probability level. P values ≤ 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Alcoholic and aqueous extracts had superior extraction values compared to petroleum ether (60-80°C), diethyl ether, chloroform, benzene, and ethyl alcohol (95%). In addition to aqueous and alcohol extracts, the root powder dissolved in distilled water was also used for analyzing the antimicrobial potential since the powdered root is commercially available and is being prescribed in alternative medicine in India. The chemical analyses of the roots of *B. aristata* showed the presence of alkaloids, amino acids, flavonoids, phenol, proteins, sterols/terpenes, reducing sugars, non-reducing sugars, resins, saponins and tannins.

Antibacterial activity

All three extracts showed wide antibacterial activity against Gram-positive bacteria; best being given by extracts at a concentration of 50 µg/disc (Table 1). Among

Table 1. Inhibition zones (mm) of aqueous and alcoholic extract of *Berberis aristata* against Gram-positive and Gram-negative bacterial species.

Bacteria tested	Alcoholic extract ($\mu\text{g}/\text{disc}$)			Aqueous extract ($\mu\text{g}/\text{disc}$)			Powdered root in DW ($\mu\text{g}/\text{disc}$)			Ciprofloxacin
	50	25	12.5	50	25	12.5	50	25	12.5	
<i>Staphylococcus aureus</i>	27.9±2.7 ^C	22.0±2.6 ^b	16.0±2.2 ^c	9.0±1.5 ^d	-	-	9.0±1.6 ^d	-	-	24.0±2.2 ^b
<i>Staphylococcus epidermidis</i>	29.9±2.7 ^b	22.0±2.3 ^b	18.0±1.8 ^b	9.0±1.6 ^d	-	-	-	-	-	14.0±0.0 ^f
<i>Streptococcus pyogenes</i>	29.9±3.0 ^b	26.0±2.7 ^a	20.0±3.0 ^a	12.0±2.1 ^b	9.0±2.6 ^b	8.1±1.3	10.0±2.7 ^c	8.0±0.0 ^c	-	22.0±4.9 ^c
<i>Streptococcus viridans</i>	17.8±0.1 ^f	12.0±1.5 ^d	8.0±2.0 ^g	-	-	-	-	-	-	14.0±2.2 ^f
<i>Enterococcus faecalis</i>	15.9±2.2 ^g	11.0±1.3 ^e	-	-	-	-	-	-	-	14.0±0.0 ^f
<i>Bacillus subtilis</i>	28.0±0.2 ^c	16.0±2.5 ^c	10.0±0.1 ^e	10.0±0.0 ^c	-	-	8.0±1.6 ^e	-	-	22.0±0.0 ^c
<i>Bacillus cereus</i>	24.0±1.6 ^d	12.0±1.5 ^d	9.0±1.3 ^f	9.0±1.6 ^d	-	-	9.0±1.6 ^b	-	-	22.0±0.0 ^c
<i>Escherichia coli</i>	14.0±1.3 ^h	10.0±0.0 ^f	-	10.0±1.5 ^c	-	-	9.0±1.5 ^d	-	-	18.0±2.7 ^d
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	-	22.0±0.0 ^c
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	12.0±0.0 ^g
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	NT
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	NT
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	-	-	18.0±1.3 ^d
<i>Salmonella paratyphi A</i>	-	-	-	-	-	-	-	-	-	22.0±2.2 ^c
<i>Salmonella typhimurium</i>	10.0±2.7 ¹	-	-	-	-	-	-	-	-	NT
<i>Shigella dysenteriae</i> type 1	22.0±1.5 ^e	16.0±2.2 ^c	12.0±2.5 ^d	12.0±2.0 ^b	9.0±2.5 ^b	-	12.0±0.0 ^b	9.1±1.3 ^a	-	16.0±1.6 ^e
<i>Vibrio cholerae</i>	32.0±1.9 ^a	26.0±1.5 ^a	20.0±2.3 ^a	14.0±1.7 ^a	10.0±0.0 ^a	9.0±1.7	14.0±0.0 ^a	9.0±0.0 ^b	-	16.0±0.0 ^e
<i>S. aureus</i> ATCC 25923	30.0±1.3 ^b	22.0±2.1 ^b	16.0±2.5 ^c	12.0±1.5 ^b	9.0±0.0 ^c	-	9.0±1.5 ^d	-	-	28.0±2.6 ^a
<i>E. coli</i> ATCC 25922	18.0±2.9 ^f	10.0±1.3 ^f	-	10.0±1.3 ^c	-	-	10.0±2.7 ^c	-	-	24.0±0.0 ^b
<i>P. aeruginosa</i> ATCC 27853	-	-	-	-	-	-	-	-	-	22.0±0.0 ^c

Diameter of zone of inhibition is a mean of triplicates \pm SE (mm). Differences were assessed statistically using one way ANOVA followed by Tukey's test $P \leq 0.05$. The mean represented by same letter is not significantly different within the column.

the Gram-negative bacteria tested, the antibacterial activity was limited against *E. coli*, *S. typhimurium*, *S. dysenteriae* type 1 and *V. cholerae*, the best activity being against *V. cholerae* (Table 1). MICs of the alcoholic extracts against Gram-positive bacteria ranged between 6.1×10^{-3} to 3.8×10^{-3} mg/ml and for Gram-negative bacteria from 6.1×10^{-3} to 7.6×10^{-3} mg/ml. The antibacterial activity of the extracts against clinical isolates was comparable to those of standard strains (see Table 1). It is interesting to note that

the Gram-negative bacteria reported here as susceptible to the extracts of *B. aristata* are important human pathogens responsible for causing diarrhea and dysentery.

Antifungal activities

All the three extracts showed antifungal activity against the *Candida* and *Aspergillus* species tested, except *C. krusei* (Table 2). Best activity

was given at a concentration of 50 $\mu\text{g}/\text{disc}$ of the three concentrations used (50, 25 and 12.5 $\mu\text{g}/\text{disc}$). Of the three types of extracts, the best results were obtained by using the alcoholic extracts (Table 2). The MICs for *Candida* species ranged between 0.02 to 3.8×10^{-3} mg/ml and for *Aspergillus* species, it was 3×10^{-3} mg/ml.

Although antimicrobial studies on other *Berberis* species exist in the literature, such studies on *B. aristata* are limited. Comparing our results with a more recent short report of Singh et al. (2007), we

Table 2. Inhibition zones (mm) of aqueous and alcoholic extracts of *Berberis aristata* against fungal species.

Fungi tested	Alcoholic extract (µg/disc)			Aqueous extract (µg/disc)			Powdered root in DW (µg/disc)			Fluco nazole	Nystatin	Ampho tericin B
	50	25	12.5	50	25	12.5	50	25	12.5			
<i>Candida albicans</i>	38.0±1.0 ^b	28.0±2.9 ^b	18.0±3.0 ^b	26.0±2.5 ^c	18.0±1.8 ^a	12.0±0.0 ^a	31.0±3.6 ^b	22.0±2.0 ^b	14.0±0.0 ^a	-	26.1±1.7 ^b	24.0±1.5 ^c
<i>Candida krusei</i>	-	-	-	-	-	-	-	-	-	32.0±2.6 ^b	28.0±0.0 ^a	26.0±1.5 ^b
<i>Candida tropicalis</i>	38.1±0.2 ^b	26.0±2.8 ^c	16.1±2.9 ^c	27.0±3.3 ^b	15.0±3.0 ^b	10.0±1.8 ^b	29.0±2.0 ^c	24.0±2.3 ^a	16.0±1.6 ^a	32.0±3.0 ^b	26.0±3.3 ^b	20.8±0.1 ^e
<i>Candida keyfr</i>	40.3±0.1 ^a	32.0±2.3 ^a	21.9±6.6 ^a	26.0±2.2 ^c	12.0±0.0 ^d	10.0±0.0 ^b	29.0±2.0 ^d	15.9±2.0 ^d	14.0±2.8 ^b	38.0±2.1 ^a	26.0±1.6 ^b	24.0±0.0 ^c
<i>Candida parapsilosis</i>	30.0±2.6 ^d	22.5±0.9 ^d	14.0±1.0 ^d	20.0±0.0 ^d	12.0±2.5 ^d	8.0±0.0 ^c	22.0±4.0 ^f	14.0±0.0 ^e	12.0±1.6 ^c	22.0±4.0 ^c	22.0±4.2 ^d	24.0±1.5 ^c
<i>Aspergillus fumigatus</i>	36.1±5.1 ^c	26.0±2.3 ^c	14.0±1.0 ^d	-	13.9±1.8 ^c	10.1±5.1 ^b	19.0±0.0 ^h	14.1±1.7 ^e	8.0±0.0 ^f	-	24.0±1.6 ^c	18.1±3.4 ^f
<i>Aspergillus flavus</i>	18.1±2.2 ^f	12.0±2.3 ^f	7.0±2.2 ^f	-	-	-	18.0±0.0 ^f	11.0±0.0 ^g	7.0±0.0 ^g	-	20.0±1.6 ^e	16.1±2.7 ^g
<i>Aspergillus niger</i>	21.0±1.3 ^e	14.5±2.2 ^e	8.8±0.0 ^e	-	-	-	20.0±2.0 ^g	12.0±1.8 ^f	7.0±1.8 ^g	-	21.1±1.6 ^d	16.0±0.0 ^g
<i>C. parapsilosis</i> ATCC 22019	36.1±2.6 ^c	24.1±2.6 ^d	14.0±0.0 ^d	26.0±0.0 ^c	14.0±1.6 ^c	8.0±1.5 ^c	29.0±3.1 ^d	14.1±2.7 ^e	9.0±1.5 ^e	22.0±0.0 ^c	28.0±2.1 ^a	32.0±1.6 ^a
C. keyfr (Ny/l)	38.1±3.4 ^b	26.0±1.5 ^c	16.0±0.0 ^c	30.1±9.9 ^a	18.0±0.0 ^a	12.0±2.4 ^a	32.0±2.0 ^a	18.0±2.3 ^c	11.0±1.6 ^d	32.1±3.2 ^b	28.0±2.1 ^a	26.0±1.5 ^b
<i>A. fumigatus</i> ATCC 204305	36.1±2.9 ^c	28.0±1.5 ^b	16.0±2.2 ^c	-	-	-	26.0±2.1 ^e	18.0±0.0 ^c	12.0±1.5 ^c	18.1±4.8 ^d	26.1±1.6 ^b	22.0±1.6 ^d

Diameter of zone of inhibition is a mean of triplicates ±SE (mm). Differences were assessed statistically using one way ANOVA followed by Tukey's test $P \leq 0.05$. The mean represented by same letter is not significantly different within the column.

emphasize that we adopted different experimental approach testing both alcoholic and aqueous extracts separately rather than using hydro-alcoholic (50%) extract, and the activities were performed on different list of bacteria and fungi. We tested a total of 20 bacterial and 11 fungal species (including clinical and standard strains) whereas Singh et al. (2007) tested 11 bacterial and 8 fungal species (majority being standard strains). The spectrum of activity was different in our results as compared to those of Singh et al. (2007) especially the activity against *V. cholerae*. Native berberine has already been reported to possess bactericidal action against *V. cholerae*. Furthermore, we noticed better antimicrobial activity by alcoholic extract in comparison to aqueous extract and probably this could be the reason Singh et al. (2007) found lower spectrum of antimicrobial activity since they used the hydro-alcoholic mixture and thereby decreasing the

concentration of alcoholic extract. The differences in our study and that of Singh et al. (2007) are also expected since variable concentrations of berberine had already been reported in species of *Berberis* collected from different altitudes and are suggested probably as a result of adoptive response to altitudinal gradient in addition to genetic characters (Chandra and Purohit, 1980).

Anti-inflammatory activity

Alcoholic and aqueous extracts showed good activity against acute inflammation and significant activity was achieved at two hours after Carrageenin injection (Figure 1). The alcoholic extract (50/100 g) showed non-significant results after one hour. However, the results of aqueous extract (50/100 g) were significant after one hour. After 3 h of carrageenin injection, the results were found

to be significant in both test groups indicating the late onset of effect of alcoholic extract. Aqueous extract was effective in the early phase of acute inflammation and alcoholic extract in the later phase of acute inflammation. This suggest that the alcoholic extract may be acting by blocking the mediators released in the later phase (i.e. prostaglandin), while the aqueous extract may be acting by blocking the mediators released in the early phase (i.e. bradykinin, histamine, and serotonin), as well as by blocking the mediators released in the later phase (i.e. prostaglandin).

Test for analgesic activity

A significant increase in the reaction time was observed with 50/100 g of the alcoholic and aqueous extracts, as compared with the reaction time of the control group (Figure 2). The reaction

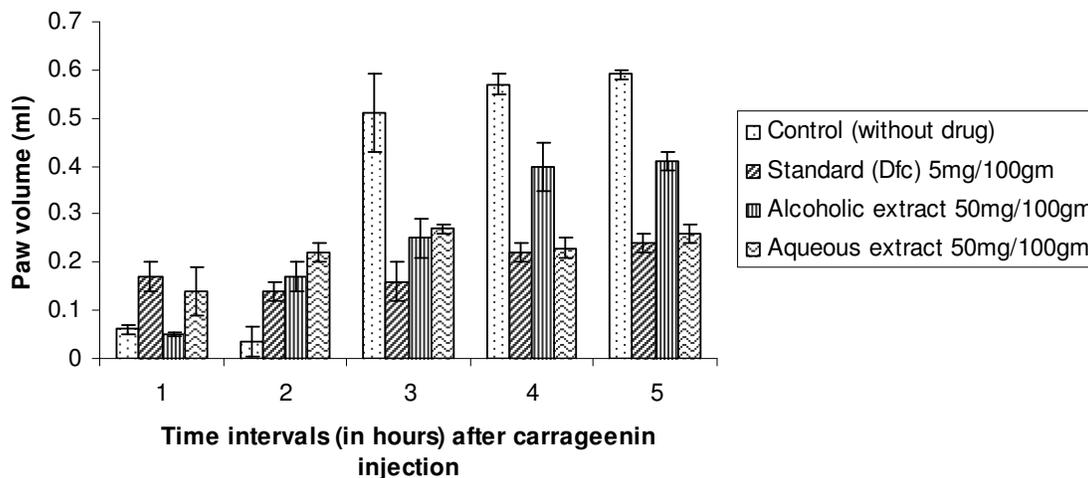


Figure 1. Anti inflammatory effect of *Berberis aristata* in carrageenin-induced rat paw edema test with diclofenac (Dfc) as a standard drug.

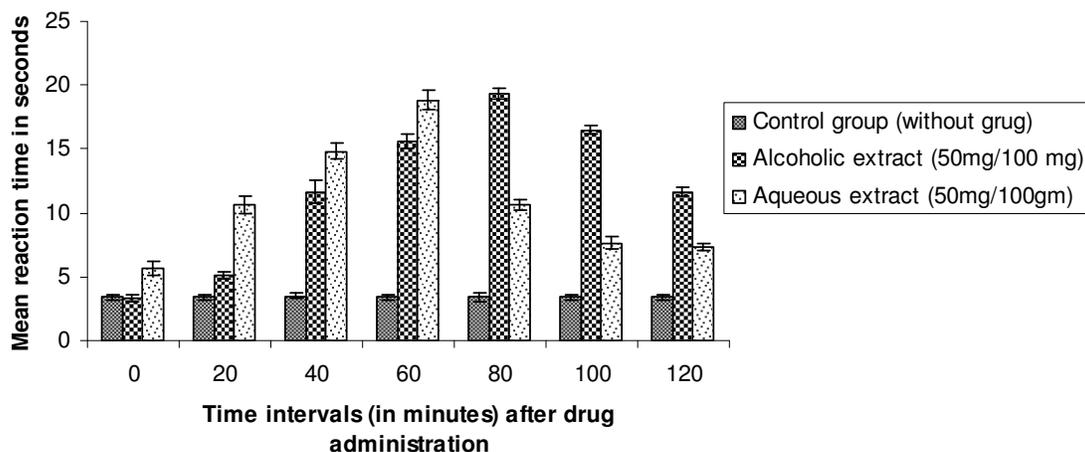


Figure 2. Analgesic effect of *Berberis aristata* by Eddy's Hot-plate test.

time of the animals receiving alcoholic extracts was significant throughout the whole experiment, whereas the group receiving aqueous extract, the reaction time started to decrease after an interval of 100 min (Figure 2). Based on this study, it was concluded that both alcoholic and aqueous extracts showed good analgesic activity. However, there was a shorter duration of effectiveness for the aqueous extract.

Test for antipyretic activity

The alcoholic extracts in the dose of 200 mg/kg produced a significant antipyretic effect (rectal temperature) at the intervals of 30 min ($37.87 \pm 0.09^{\circ}\text{C}$; $P < 0.05$), 60 min ($38.37 \pm 0.02^{\circ}\text{C}$; $P < 0.05$), 90 min (38.82 ± 0.03 ; $P < 0.01$), and 120 min (38.93 ± 0.22 ; $P < 0.01$). However, the results were non-significant at an interval of 180 min

(39.60 ± 0.27 , NS). The aqueous extracts in the dose of 200 mg/kg produced a significant antipyretic effect at the intervals of 30 min (38.22 ± 0.08 ; $P < 0.05$), 60 min (38.37 ± 0.08 ; $P < 0.05$), 90 min (38.78 ± 0.28 ; $P < 0.01$), 120 min (39.22 ± 0.20 ; $P < 0.05$), 150 min (39.52 ± 0.20 ; $P < 0.05$) and 180 min (39.22 ± 0.15 ; $P < 0.05$) (Figure 3). The aqueous extracts demonstrated significant antipyretic activity, which was present throughout the duration of the study, while the results for alcoholic extracts were non-significant in the later period.

FTIR spectral analysis

IR spectral data revealed presence of peak (1558 cm^{-1}) which denotes the presence of isoquinoline and, thus, suggests the presence of berberine in the extracts. Analysis of all the three extracts revealed presence of

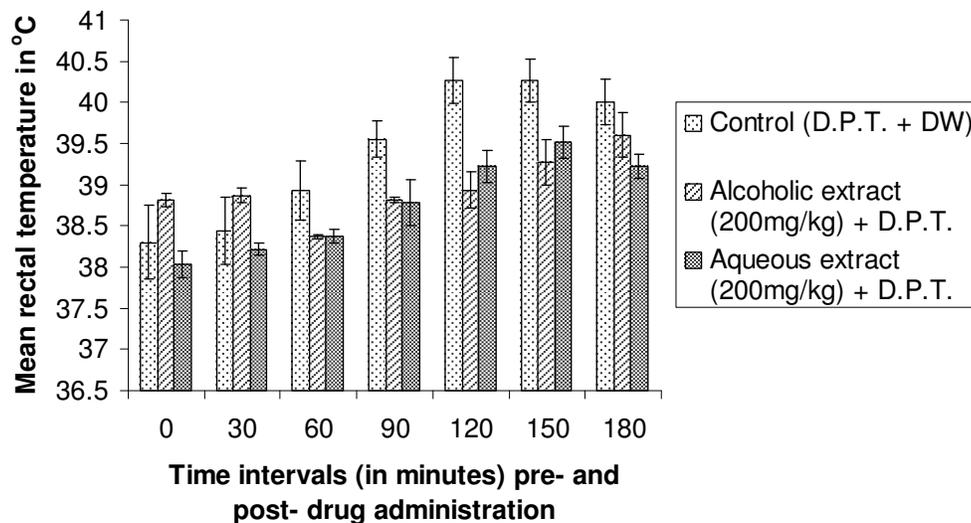


Figure 3. Antipyretic effects of *Berberis aristata*.

berberine along with other chemical constituents.

In the existing literature, berberine has been reported to be produced by numerous *Berberis* spp. belonging to family *Berberidaceae*, including, *Berberis nepalensis*, *B. asiatica*, *Berberis vulgaris*, *Berberis lycium* (Chandra and Purohit 1980), *Berberis aetnensis* (lauk et al., 2007), *Berberis stolonifera* (Stadler et al., 1988), *Berberis chitria* (Hussaini and Shoeb, 1985) and also by other plant species like *Thalictrum* spp., including *Thalictrum flavum* (Samanani et al., 2002) and *Thalictrum minus* (Terasaka et al., 2003), *Mahonia* spp. (Cernáková and Kostálová, 2002), and *Coptis* spp. (Lee et al., 2005). Native berberine, a benzyloquinoline alkaloid, has already been reported to possess antimicrobial activities against a wide variety of microorganisms including Gram-positive and Gram-negative bacteria, fungi, and protozoa (Amin et al., 1969; Birdsall and Kelly, 1997; Park et al., 1999; Park et al., 2001; lauk et al., 2007). Berberine has also been reported to possess activity against methicillin-resistant *Staph. aureus* (Yu et al., 2005). However, the berberine is being reported to be readily extruded by multidrug resistance pumps (MDRs) present in numerous bacterial cells and thereby bacteria escaping cell death (Stermitz et al., 2000). Interestingly, several *Berberis* spp., including *Berberis fremontii* which is used in Native American traditional medicine, are also found to synthesize an inhibitor of the NorA MDR pump of a human pathogen *S. aureus*. The inhibitor was identified as 5'-methoxyhydrocarpin (5'-MHC) which had no antimicrobial activity alone but strongly potentiated the action of berberine by inhibiting the MDR-dependant efflux of berberine (Stermitz et al., 2000). Similarly, in few other reports, crude extract from *Berberis* spp. was found to have better antimicrobial activity in comparison to alkaloid fraction and native berberine (lauk et al., 2007). It is plausible that possibly either other active compounds or inhibitors of the

efflux pumps are present in crude extracts and thus it is suggested that more extensive studies are needed to explore the presence of inhibitors or other active constituents from unexplored *Berberis* spp., including *B. aristata*. Apart from antimicrobial activities, berberine has also been reported to possess anti-inflammatory, analgesic, and antipyretic potentials (Kupeli et al., 2002; Yesilada and Kupeli, 2007). Very recently, we also demonstrated antifilarial potential of the extracts derived from *B. aristata* roots against *Setaria cervi* microfilariae (Rizvi et al., 2008).

Conclusion

In nutshell, extracts of *B. aristata* have remarkable antibacterial and antifungal potentials against clinical and standard strains and thus could be used to derive antimicrobial agents especially against *V. cholerae*, *Staphylococcus* species, *Candida* species and *Aspergillus* species. As the extracts of *B. aristata* also have other activities, including anti-inflammatory, analgesic, and antipyretic properties; and it could be used to derive active components possessing these properties. Drug prepared from extracts of *B. aristata* could be an excellent drug for cholera.

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