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Phytochemical investigation and Evaluation of the Anti-arthritic activity of root extracts of *Berberis aristata*

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ABSTRACT: Background: *Berberis aristata* (*Berberidaceae*) is an important medicinal plant used in the traditional system of medicine for the treatment of inflammatory disorders. **Aim:** The root extracts of *B. aristata* were investigated for its anti-arthritic activity in albino rats. **Method:** The root was extracted by Soxhlation by using the ethanol, petroleum ether, diethyl ether and ethyl acetate as solvents. The evaluation of anti-arthritic activity of root extracts of *B. aristata* was carried out at dose of 200 mg/kg of body weight of rat, using formaldehyde and Complete Freund's adjuvant induced arthritis models. Aspirin (100 mg/kg) was used as a standard drug. **Results:** The ethanolic root extract of *B. aristata* exhibited significant anti-arthritic activity as compared to other extracts. The dose of 200 mg/kg of the ethanolic root extract of *B. aristata*, in formaldehyde induced arthritis model in rats produced 71.82 % and Complete Freund's adjuvant induced arthritis model produced 70.63 % inhibition of paw diameter respectively with that of the standard drug Aspirin (100 mg/kg) which produced 73.84 and 72.52 % inhibition respectively. Additionally, the plant exhibited remarkable anti-oxidant activity and phytochemical analysis revealed that the plant contains Polyphenols and Flavonoids. **Conclusion:** Taken together, these results support traditional use of *B. aristata* as a potent anti-arthritic agent that may be proposed for rheumatoid arthritis treatment.

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INTRODUCTION:

Rheumatoid arthritis is a systemic autoimmune disorder characterized by polyarticular symmetrical arthritis. Various inflammatory mediators produce joint inflammation with pain function loss, joint destruction and permanent deformity after a certain time if left untreated [1]. The prevalence of rheumatoid arthritis is consistent worldwide affecting about 0.5 to 1.0 % of the population. It usually occurs in people between 25 and 55 years of age. Women are affected more often than men at a ratio of 3 to 1. It is characterized by synovial hyperplasia, angiogenesis and mononuclear infiltration.

It progresses in 3 stages [2]. The drugs commonly in use for the treatment of inflammation and arthritis include glucocorticoids like cortisone and prednisone, NSAIDS like Ibuprofen and naproxen, disease-modifying anti-inflammatory and anti-rheumatic drugs like Methotrexate (MTX) and leflunomide. In recent days, researchers are directed towards the traditional system of medicine for the discovery of drugs that are long acting anti-inflammatory agents displaying minimum side effects [3,4].

In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions. Although the application of these medications has a sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms [5].

Berberis aristata DC is (Fig 1) one of the herbs mentioned in all ancient scriptures of Ayurveda, Charaka and Susruta have mentioned its different properties along with various uses for the treatment of numerous illnesses. *B. aristata* DC is an erect spinous shrub, often found in small patches on the bill slopes. This shrub is found growing wild in the sub-Himalayan tract at altitude ranging from 850 to 2,500 m. It also grows in the Nilgiris and in Ceylon. The plant possess the active constituents contain barberine, oxyberberine, berbamine, aromoline, karachine, palmatine, oxyacanthine and taxilamin [6,7]. The plant is useful in the treatment of jaundice and enlargement of spleen. The drug is also regarded as laxative, diaphoretic, antipyretic and antiseptic [8-11]. *B. aristata* have revealed its use in antimicrobial [12], hepatoprotective [13-14], immunomodulatory [15], and anti-depressant [16].

Fig 1. The flowering plant of *Berberis aristata.*

All parts of these plant extracts have been reported including diabetes mellitus [17], cytoprotective, antioxidant [18], anti-inflammatory [19], antiemetic, anti-pyretic, anti-pruritic [20-21].

Therefore, in light its use in traditional medicine, the present study was undertaken to investigate anti-arthritic activity of *B. aristata* root extract parts in experimental animal models.

MATERIALS AND METHODS:

Chemicals and drugs:

The chemicals used were Complete Freund's adjuvant (Sigma-Aldrich, USA), Formaldehyde (VWR, International Ltd), Aspirin (UNI-CHEM, India). All the other chemicals used were of analytical grade and purchase from HiMedia Laboratory, Mumbai.

Collection of plant material (Root):

The roots of *B. aristata* were collected from the state of Madhya Pradesh District Bhopal during the month of June. The plant has been identified and authenticated by Dr. Saba Naaz, Head of the Department Botany, Safia College of Science, Bhopal (M.P.). The plant part specimen was submitted as herbarium with Voucher specimen no 169/Bot./SaifSc./Bpl/2020. The fresh root parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water. The washed roots are shade dried and pulverized in a mechanical grinder to obtain coarse powder and stored in air tight containers for further use in the extraction process.

Preparation of extracts:

Exactly 2.5 kg dried and coarsely powdered root parts of the plant were used for the extraction procedure. The root parts were extracted with 90 % ethanol using Soxhlet apparatus (Borosil, India) with the solvents in increasing order of polarity starting with ethanol, petroleum ether, diethyl ether and ethyl acetate. By using a rotary evaporator (RE-201D, Kori Instruments, China), the extracts were concentrated under reduced pressure and then dried in open air, which gave a brownish black colored sticky residue. A portion of dried ethanolic extract (EE) was suspended in water and fractionated successively with petroleum ether (PEE - 40 to 60 °C), diethyl ether (DEE) and ethyl acetate (EAE). All the fractions were dried by distillation under reduced pressure.

Phytochemical screening:

The presence of phytoconstituents like Flavonoids, Tannins, Phenols, Alkaloids and Glycosides, Fats and Carbohydrates were investigated by using preliminary

phytochemical screening and the maximum contents were observed in ethanolic extracts of *B. aristata* [22].

Evaluation of anti-arthritic activity of *B. aristata:*

***Experimental animals*:**

All the experiments were carried out using male Albino rats weighing between 150 to 200 g. All the experimental procedures and protocols used in this study were reviewed by the Institutional Ethical Committee of Ravishankar college of pharmacy Bhopal, (M.P.) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal, Govt. of India (Approval no. 1733/PO/Ere/ S/13/CPCSEA). All animals were housed in polypropylene cages and maintained under standard laboratory conditions. Animals were housed at a temperature of 24±2 °C and relative humidity of 60 to 70 %. They were fed with a standard diet and water was given *ad libitum* and they were left for a week for acclimatization to animal house conditions. All experiments were conducted after overnight fasting but there was free access to water.

***By Formaldehyde induced arthritis in rats*:**

The rats were distributed into six groups (n = 5). Group I animals were administered with arthritic control (3 ml/kg distilled water). Group II animals were administered with standard drug Aspirin at a dose of 100 mg/kg b.w. The animals of Group III to VI were treated with ethyl acetate, diethyl ether, petroleum ether and ethanol root extract of *B. aristata* at a dose of 200 mg/kg b.w. (p.o). On day 1, 30 min subsequent to drug administration, arthritis was induced by sub plantar injection of 2 % formaldehyde solution (0.1 ml) and recurrent induction on day 3. Drug treatment was sustained for 10 days. Arthritis was evaluated by checking the mean increase in paw diameter for 10 days via digital Vernier Calliper. Percentage inhibition of knee joint edema by plant extract and aspirin was computed by correlating with untreated arthritic control rats, using the following formula [22,23].

Inhibition (%) = [(VC – VT)/ VT] × 100 ….(1)

Where, VC and VT are Joint edema of control and test groups.

***By Complete Freund Adjuvant (CFA) induced arthritis in rats*:**

The rats were allocated into seven groups (n = 5). Group I: Arthritic control rats (Rats were treated with 3 ml/kg b.w. of distilled water). Group II: Normal control rats (Rats were treated with 3 ml/kg b.w. of distilled water). Group III: Standard control rats (Rats were treated with Aspirin at a dose of 100 mg/kg b.w., p.o.). The animals of Group IV to VII were treated with ethyl acetate, diethyl ether, petroleum ether and ethanol root extract of *B. aristata* at a dose of 200 mg/kg b.w. (p.o). Arthritis was induced by injecting 0.05 ml of CFA subcutaneously into the left foot pad of each rat. The treatments were administered to rats, 1 day ahead of CFA injection and daily treatment continued for 11 days. The injected paw edema was appraised at 1, 4, 8 and 12 days after CFA injection through digital vernier caliper (Yuri YURI-05 Digital Vernier Caliper, Yuri, India). Percentage inhibition of edema was calculated as described earlier.

Statistical Data analysis:

Data are expressed as a mean ± SEM (Standard Error of Mean). Statistical analysis was performed by one-way ANOVA followed by Dunnet's test. P values <0.05 were considered as significant.

RESULTS AND DISCUSSION:

Phytochemical investigation:

The phytochemical study revealed that the root extract of *B. aristata* possess phytochemicals that are Alkaloids, Carbohydrates, Flavonoids, Glycosides, Proteins and Saponins. Results of extractive values such as ethanolic, petroleum ether, diethyl ether and ethyl acetate extract were calculated as 25.1, 14.2, 9.1, and 4.2 % respectively [24]. The extractive values and phytochemical screening of extract of *B. aristata* shown in Table 1 and 2. The phytochemicals, Alkaloids, Glycosides, Tannins, Phenols and Flavonoids are present in all the plant extracts. All solvent plant extracts does not contain Proteins and Saponins.

Table 1. Extractive values and appearance of various root extracts of *B. aristata.*

Extracts	Yield (%)	Visual Results
Ethanol	25.1	Semi Solid
Pet. ether	14.2	Semi Solid
Diethyl ether	9.1	Semi Solid
Ethyl acetate	4.2	Semi Solid

The LD_{50} values of all the extracts were found to be more than 1000 mg/kg b.w. of rat. After preliminary phytochemical screening it was observed that root extracts contains maximum amount of phytoconstituents which are responsible for inflammation and arthritic activities.

Table 2. Presence of various Phytoconstituents in root of *B. aristata*.

Phyto-constituents		ET	PE	DEE	EA
Alka-loids	Hager's test	+++	+++	++	+
	Mayers	+++	+++	++	+
Glycosides	Borntrager's test	++	+	+	-
	Keller killiani test	++	+	+	+
	Legal's test	++	+	+	-
Carbohydrates	Benedict's test	+	+	-	-
	Fehling's test	+	+	+	-
	Molish test	++	+	+	+
Phenols, Tannins	Bromine water Test	+	+	+	+
	Lead acetate Test	-	-	+	-
	$FeCl_3$ Test	+	-	+	+
Flavonoids	Shinoda Test	++	++	+	+
Proteins	Biuret Test	-	-	-	-
Saponins	Solubility Test	-	-	-	-

(+) – Present, (-) – Absent, (+++) – Abundant, (++) – Moderate, (+) – Fair, EE – Ethanol, PE – Petroleum ether, DEE – Diethyl ether and EA – Ethyl acetate.

Table 3. The effect of *B. aristata* against formaldehyde induced arthritis.

Group/ Treatment	Decrease in Paw Diameter (mm)			
	Day 2	Day 4	Day 7	Day 10
I/Control	7.99 ± 0.029	11.96 ± 0.021	16.02 ± 0.019	20.92 ± 0.016
II/Standard Aspirin (100 mg/kg)	4.01 ± 0.022*** (63.80)	4.37 ± 0.018*** (88.68)	3.92 ± 0.028*** (72.22)	3.53 ± 0.023*** (73.84)
III/ Ethyl acetate extract	5.46 ± 0.020*** (48.58)	5.71 ± 0.024*** (54.53)	5.32 ± 0.030*** (57.64)	4.95 ± 0.017*** (60.18)
IV/ diethyl Ether extract	5.50 ± 0.021*** (48.21)	4.95 ± 0.023*** (53.38)	4.98 ± 0.017*** (60.06)	4.41 ± 0.021*** (64.88)
V/ petroleum Ether extract	4.98 ± 0.023*** (41.34)	4.51 ± 0.015*** (46.87)	4.47 ± 0.024*** (64.41)	4.25 ± 0.021*** (69.81)
VI/ ethanol extract	4.38 ± 0.026*** (48.40)	5.33 ± 0.030*** (57.54)	4.85 ± 0.028*** (65.55)	4.65 ± 0.027*** (71.82)

Values in the parenthesis represent percentage inhibition of paw edema. The statistical analysis was carried out using two way ANOVA followed by Bonferroni posttest. *** = P < 0.001 when compared to control. Group I: Arthritic control (3 ml/kg distilled water). The dose was 200 mg/kg b.w. (p.o).

Table 4. The effect of *B. aristata* on CFA induced arthritis in rats.

Treatment/ Group	Decrease in paw diameter (mm)			
	Day 1	Day 4	Day 8	Day 12
I/ Arthritic Control (3 ml/kg distilled water)	4.21 ± 0.235	12.82 ± 0.029	16.50 ± 0.026	20.72 ± 0.023
II/ Normal Control (3 ml/kg distilled water)	2.09 ± 0.170***	2.14 ± 0.167***	2.22 ± 0.078***	2.25 ± 0.089***
III/ Standard Aspirin (100 mg/kg)	3.80 ± 0.028*** (12.06)	5.39 ± 0.020*** (45.05)	4.24 ± 0.019*** (60.27)	3.73 ± 0.039*** (72.52)
IV/ Ethyl acetate extract (200 mg/kg)	3.13 ± 0.029*** (24.72)	4.53 ± 0.029*** (49.22)	3.68 ± 0.037*** (52.24)	2.61 ± 0.023*** (57.98)
V/ Diethyl ether extract (200 mg/kg)	3.39 ± 0.020*** (34.01)	4.86 ± 0.032*** (45.06)	4.75 ± 0.019*** (52.95)	4.06 ± 0.022*** (62.34)
VI/ Petroleum ether extract (200 mg/kg)	3.40 ± 0.020*** (23.57)	5.88 ± 0.044*** (42.65)	5.47 ± 0.033*** (52.43)	4.45 ± 0.029*** (66.60)
V/ Ethanol extract (200 mg/kg)	3.72 ± 0.026*** (13.27)	6.32 ± 0.034*** (48.32)	5.95 ± 0.035*** (66.09)	5.01 ± 0.042*** (70.63)

Values in the parenthesis represent percentage inhibition of paw edema. The statistical analysis was carried out using two way ANOVA followed by Bonferroni posttest. *** = p < 0.001 compared to arthritic control.

Effect of *B. aristata* against formaldehyde induced arthritis:

The results displayed in Table 3, depicted that 200 mg/kg of ethanolic extract on 10th day showed more superior repression of paw edema 71.82 % ($p < 0.001$) as compared to 73.84 % reduction in paw edema by 100 mg/kg aspirin on 10th day.

Effect of *B. aristata* against CFA induced arthritis:

The Table 4 shows a significant ($p < 0.001$) decrease in paw diameter of treatment groups as compared to CFA control groups. Ethanolic, petroleum ether and diethyl ether extractat at a dose of 200 mg/kg exhibited 70.63, 66.60 and 62.34 % inhibition of paw edema respectively at the end of study period. These results were more pronounced than 100 mg/kg aspirin i.e., 72.52 %. The redness and swelling of affected joints were significantly less in treated as compared to arthritic control animals. A significant ($p < 0.001$) weight gain was observed in treated groups at the end of study.

All the extracts of *Berberis aristata* showed potent anti-arthritic activity and the potency of the extracts follows the order standard > EE > PEE > DEE > EAE (Fig 2). The results of formaldehyde induced arthritis model as well as complete Freund adjuvant (CFA) induced arthritis model indicate that among all the extracts, the ethanolic extract shows more potent activity.

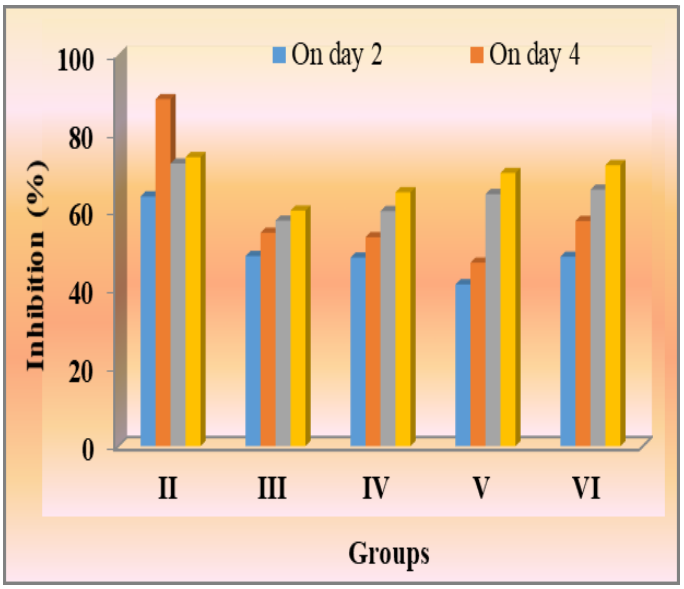

Fig 2. The anti-arthtritic activity of root extracts of *B. aristata.*

In the present investigation as the active fraction was ethanol, its analysis revealed the presence of Phenols, Alkanes, Carboxylic acids, Methyl, Carbonyl and carbon fluorine functional groups. The presence of Phenolics was further confirmed and active principles isolated from ethanol fraction were found to be quercetin, gallic acid, caffeic acid, p-coumaric acid, M-coumaric acid, ferulic acid, trans-4-hydroxy-3- methoxy cinnamic acid and sinapic acid. It has formerly been reported that phenolics and flavonoids possess anti-inflammatory and anti-oxidant activities. Previous works have shown that flavonoid quercetin exerts anti-inflammatory, anti-proliferative and anti-oxidative effects. Similarly, gallic acid and phenolic acids i.e., p-coumaric, caffeic, and ferulic acids have been reported as anti-inflammatory and free radical scavengers.

CONCLUSION:

In the view of above discussion, it is conceivable that roots parts of *Berberis aristata* has been observed to exert significant anti-arthritic effect in experimental studies. From the present experimental findings of pharmacological parameters observed from the current investigation. The present experimental study concluded that at the doses of 200 mg/kg ethanolic extract of *B. aristata* possesses potentially useful anti-arthritic activity since it gives a positive result in controlling inflammation in formaldehyde induced arthritis and adjuvant induced arthritic model in rats. The drug is a promising anti-arthritic agent of plant origin in the treatment of inflammatory disorders. In summary, this contemporary research lends pharmacological support to reported folkloric usage of roots parts of *B. aristata*in the treatment and management of painful arthritic inflammatory conditions that could conclusively establish roots of *B. aristata* as a potentially safer disease modifying agent in the treatment of RA.

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REFERENCES:

1. Tripathy S, Sahoo SP, Pradhan D, Sahoo S, Satapathy DK. Evaluation of antiarthritic potential of *Hybanthus enneaspermus*. Afr J Pharmacy Pharmacol, 2009; 3: 611-614.
2. Meera S, Kumar NS, Guptatyam VS. Screening of antiarthritic, anti-inflammatory and analgesic activity of a polyherbal formulation. Int J Pharmacol, 2008; 4: 398-402.

3. Patil RB, Nanjwade BK, Manvi FV. Effect of *Sesbania grandiflora* and *Sesbania sesban* Bark on carrageenan induced acute inflammation and adjuvant-induced arthritis in rats. Pharma Sci Monitor: An Int J Pharmaceut Sci, 2010; 1: 75-89.
4. Ekambaram S, Perumal SS, Subramanian V. Evaluation of anti-arthritic activity of *Strychnos potatorum* Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complement Altern Med, 2010; 10: 1-9.
5. Shah BN, Nayak BS, Seth AK, Jalalpure SS, Patel KN, Patel MA. Search for medicinal plants as a source of anti-inflammatory and anti-arthritic agents - A review. Pharmacog Mag, 2006; 2: 77-86.
6. Rahman AU, Ansari AA. Alkaloids of *Berberis aristata* - Isolation of Aromoline and Oxyberberine. J Chem Soc Pak, 1983; 5(4): 283-286.
7. Watt G. A dictionary of the economic products of India. Published under the authority of His Majesty's Secretary of State for India in Council, Kolkatta. London: Yohn Murry; 1889.
8. Chander V, Aswal JS, Dobhal R, Uniyal DP. A review on Pharmacological potential of Berberine; an active component of Himalayan *Berberis aristata*. J Phytopharmacol, 2017; 6(1): 53-58
9. Chandra AH, Singh GK, Rawal SR, Muniyari RMS, Dutt BI. Habitat-dependent variations in Berberine content of *Berberis asiatica* Roxb. ex. in Kumaon, Western Himalaya. Chem Biodiversity, 2010; 7(2): 415-420.
10. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. Vol. 1. New Delhi: Central Council for Research in Ayurveda & Siddha; 2000. pp. 121-127.
11. India, Ministry of Health and Family Welfare. The Ayurvedic Pharmacopoeia of India. Part I. Vol. II. New Delhi: Department of Indian Systems of Medicine & Homeopathy; 2001. pp. 34-48.
12. Musumeci R, Speciale A, Costanzo R, Annino A, Ragusa S, Rapisarda A. *Berberis aetnensis* extracts: Antimicrobial properties and interaction with ciprofloxacin. Int J Antimicrob Agents, 2003; 22: 48-53.
13. Singhal GD, Sharma KR. Ophthalmic and otorhinolaryngological considerations in ancient Indian surgery. Allahabad: Singhal Publications; 1976.
14. Acharya JT. Sushrutasamhita. Varanasi: Chaukhamba Orientalia; 1980.
15. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. Vol. 1. New Delhi: Central Council for Research in Ayurveda & Siddha; 2000. pp. 120-123.
16. Mukund S. Chemistry and Pharmacology of Ayurvedic medicinal plants. Varanasi: Chaukhambha Surabharati Prakashana; 2006.
17. Ni Yanxia. Therapeutic effect of berberine on 60 patients with non-insulin dependent diabetes mellitus and experimental research. Chin J Integr Tradi West Med, 1995; 1(2); 91-95.
18. Tomosaka H, Chin Y, Salim AA, Keller WJ, Chai H, Kinghorn AD. Antioxidant and cytoprotective compounds from *Berberis vulgaris* (Barberry), Phytother Res, 2008; 22: 979-981.
19. Akbulut M, Calisir S, Marakoglu T, Coklar H. Some physicomechanical and nutritional properties of Berberry (*Berberis Vulgaris* L.) fruits. J Food Process Eng, 2009; 32: 497-511.
20. Kafi M, Balandary A. *Berberis* production and processing Mashhad: Language and Literature; 2002.
21. Rounsaville TJ, Ranney TG. Ploidy levels and genome sizes of *Berberis* L. and *Mahonia* nutt. species, hybrids, and cultivars. Hortscience, 2010; 45: 1029-1033.
22. Manasi Khadanga, Durga Madhav Dash, Smruti Swarup Pradhan, Prabin Kumar Jani. Evaluation of Sedative and Hypnotic activities of Ethanolic extract of *Biophytum sensitivum* Linn. DC. J Pharm Adv Res, 2020; 3(3): 818-822.
23. Nayak BS, Ellaiah P, Dinda SC, Khadanga M, Mishra H, Nayak S. Anti-inflammatory activity of flavonoid compound isolated from *Gmelina arborea* fruits extract. Int J Appl Pharm Biol Res, 2017; 2(1): 1-10.
24. Kundan Singh Bora, Pant Nitish. Pharmacognostic evaluation and physico-chemical analysis of Ficus benjamina Linn. J Pharm Adv Res, 2020; 3(3): 823-828.

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