

Physico Chemical Characterization and Anti Inflammatory Activity of Stem Extracts of *Berberis aristata* DC and *Cosinium fenestratum* Linn in Carrageenan Induced Wistar Rats

S Tamilselvi¹, Padma Venkatasubramanian*² and N S Vasanthi¹

¹Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam-638452, TamilNadu, India

²Institute of Ayurveda and Integrative Medicine (IAIM), Bangalore -560106, Karnataka, India

ABSTRACT

An initial study on the physico-chemical properties of *Berberis aristata* and *Cosinium fenestratum*, both used as Daruharidra by the Ayurvedic drug industry, recorded all the tested parameters within the limits of Ayurvedic Pharmacopeia of India. Also, analysis of secondary metabolite of the plants was carried out focusing on the presence and quantification of berberine in the samples. Further, the potential difference in the anti-inflammatory activity of the aqueous and methanolic stem extracts of the two species was compared using carrageenan induced Wistar rats model. At a dose of 25 mg/kg, the aqueous and methanolic crude extracts of both the plant species showed significant inhibition of rat paw edema at various time intervals viz., 0, 30, 60, 120 and 240 minutes as against the control standard drug indomethacin ($p < 0.01$, $p < 0.001$).

Keywords: antiinflammation, Berberine, *Berberis aristata*, *Cosinium fenestratum*, Physico-chemical analysis.

INTRODUCTION

Herbal medicines are at great demand globally for primary healthcare due to their higher safety margins and cost effectiveness. Quality control of herbal medicines generates a lot of problems involving even the selection of the right kind of plant material for therapeutically efficacious compounds. Herbal medicines are being manufactured on large scale where manufacturers face many problems such as low-quality raw material, lack of authentication of raw material, non-availability of standards, lack of proper standardization methodologies of single drugs and formulations and lack of quality control parameters. Classical evaluation of herbal drugs is available based on Rasa, Guna, Virya, Vipaka and Karma etc.

In the global view although there is shift towards the use of herbal medicine, consumers prefer to choose products with established standards. Therefore, it is a prime need to standardize Ayurvedic preparations to guarantee their purity, safety, potency and efficacy.

Herbal products represent a number of unique problems related to quality which are further complicated by the use of combination of herbal ingredients being used in traditional practice. Therefore, in case of herbal drugs and products the standardization should encompass entire field of study from cultivation of medicinal plant to its clinical application. WHO involves in standardization and quality control of herbal crude drugs^[1] to monitor the physicochemical evaluation of crude drugs covering the aspects of selection and handling of crude material, safety, efficacy, stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.

Daruharidra, an important ingredient of traditional Indian ayurvedic system of medicine, is continuously used

*Corresponding author:

S. Tamilselvi

Bannari Amman Institute of Technology,

Sathyamangalam-638401,

Tamilnadu, India

E-mail - tmlsaran@gmail.com

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for health care in India and other parts of the globe^[2,3]. Ayurvedic Pharmacopeia of India correlates Daruharidra to *Berberis aristata* DC of family Berberidaceae, a spinous shrub native to mountains of North India and Nepal and commonly known as 'Daruhaldi'^[4,5]. In South India, *Cosinium fenestratum* is traded as substitute for *B. aristata* in the name of Daruharidra^[3-17] and native to Western Ghats as 'tree turmeric'. Daruharidra is useful in a vast range of disease conditions including inflammations, wounds, ulcers, jaundice, burns, skin diseases, abdominal disorders, diabetes, fever and general debility^[2,6,7]. Both *B. aristata* and *C. fenestratum* are reported to possess the alkaloid called berberine, which is known to possess anti-inflammatory property for various ailments^[8]. The ethnobotanical studies conducted on albino rats using alcoholic and aqueous root extracts of *B. aristata* showed significant activity on acute inflammation after two hours of carrageenan injection^[9]. Studies also indicated that the topical instillation of aqueous extracts of *Curcuma longa* and *B. aristata* showed potent anti-inflammatory activity against endotoxin induced uveitis in rabbits^[10]. Though the above studies report the anti-inflammatory property of *B. aristata* for various infections, no valid scientific evidence is available to legitimise the use of *C. fenestratum* as a substitute of *B. aristata*.

The present study was attempted to evaluate and compare the physico chemical properties of *B. aristata* and *C. fenestratum*, both traded as Daruharidra, and the anti-inflammatory activities of their aqueous and methanolic stem extracts in carrageenan induced Wistar rats for drug validation.

MATERIALS AND METHODS

Plant collection

B. aristata and *C. fenestratum* samples were collected and vouched by qualified taxonomists from the Institute of Ayurveda and Integrative Medicine (IAIM) Herbarium. Voucher specimens were deposited in the Repository of Medicinal Resources at IAIM, Bangalore, Karnataka, India.

Extraction of plant material

The extraction of stem samples was carried out using methanol^[11].

Physicochemical Analysis

The following physicochemical parameters were analysed based on the protocols of Ayurvedic Pharmacopeia of India (API)^[5]

Foreign Organic Matter: Foreign matter is a material consisting of parts of the medicinal plant material or materials other than those named, any organism, part of product of an organism, other than that named in the specification and description of the plant material concern. Foreign matter consists of mineral admixtures adhering to the medicinal plant materials, such as soil, stones, sand and dust. Known quantity of sample was weighed and spread in a thin layer and the foreign matter was sorted into groups either by visual inspection or with the help of magnifier. The sorted foreign matter was weighed and expressed as % foreign matter.

Moisture content: Moisture content of the samples was determined using infrared moisture balance model-M-3A Deluxe Voltage-230VAC (Advance Research Instrument Co).

Total Ash values: The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non physiological ash", which is residue of extraneous matter (e.g. sand and soil) adhering to the plant surface. When vegetable drugs are incinerated, they leave an inorganic ash. In some plants, the total ash is of importance and indicates the extent of care taken in the preparation of the drug. Carbon must be removed at as low a temperature (450°C) as possible because alkali chlorides, which may be volatile at high temperatures, would otherwise be lost. The total ash usually contains carbonates, phosphates, silicates and silica. 4g of the ground air dried samples are weighed and the content is spread into an even thin layer on a previously ignited, dried and tarred silica crucible. Silica crucibles were placed in the muffle furnace and the temperature was adjusted 450–500°C and allowed to ignite until it was white, indicating the absence of carbon. Crucibles were removed from muffle furnace, allowed to cool for 30 minutes in a dessicator and weighed without delay.

The content of total ash in mg/g of air dried material was calculated and expressed as % Ash by the following formula:

$$\text{Total Ash \%} = (B-C) \times 100/A$$

Where, A-Sample weight in (g); B-Weight of dish + contents after drying (g); C-Weight of empty dish (g)

Acid insoluble ash: Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter.

This measures the amount of silica present, especially as sand and siliceous earth. To the silica crucible containing the total ash obtained, 25 ml of hydrochloric acid was added, covered with a watch glass and boiled gently for 5 minutes on a burner. The insoluble matter was collected on an ash less filter paper by filtration and rinsed this filter paper repeatedly with hot water until the filtrate is neutral/free from acid. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450–500°C. The silica crucible was removed from the muffle furnace and allowed to cool in a dessicator for 30 minutes, weighed without delay. The content of acid insoluble ash was calculated using the formula

$$\text{Acid insoluble ash \%} = (B-C) \times 100/A$$

Where, A- Sample weight in g; B - Weight of dish + contents after drying (g); C - Weight of empty dish (g).

Comparison of HPTLC fingerprints and Quantification of Berberine

Sample preparation: 1 g of selected stems (Mesh no. 85) was extracted in water bath with methanol and filtered through Whatman No.1 filter paper. The bark was subjected to extraction until colorless with the same menstrum. The extracts were combined and evaporated to dryness. The residue obtained was redissolved in methanol (10 ml) from which 1ml was taken and made upto 10 ml and aliquots were taken for TLC analysis^[11].

Standard berberine hydrochloride preparation: Berberine hydrochloride was purchased from Natural Remedies Pvt. Ltd., Bangalore (90% pure, HPLC Grade). Standard stock solution of berberine (0.1 mg/ml) was prepared in methanol in a 10 ml volumetric flask.

Analysis was performed on 20 cm X 10 cm TLC silica gel 60 F254 plates. Standard and the *B. aristata* and *C. fenestratum* sample solutions were applied to the plate using Linomat 5 (Camag, Switzerland) automated spray- on band applicator equipped with a 100 microlitre Hamilton syringe and operated with the Band length - 8 mm, Dosage speed -150 nL/s, Distance between bands - 12.5 mm, Distance from the plate edge -15 mm and Distance from the bottom of the plate - 10 mm. Development of the plates was carried out allowing 10 min for solvent saturation of the twin – trough chamber (Camag, Switzerland) at ambient temperature. A solvent system consisting of n – butanol:glacial acetic acid:water (14:3:4, v/v/v) for a total of 21ml of volume of solvent mixture for the

migration distance was 80 mm. After development, the plate was dried using a hair dryer and evaluated visible light, 254 nm, 366 nm and after derivatisation with anisaldehyde-sulfuric acid reagent. The images were recorded using Reprostar 3 (Camag, Switzerland). Rf values of the markers and the compounds of interest were measured. Quantification of berberine was done at 343 nm using a calibration curve.

Method validation: Linearity was determined by using a standard solution of 100 mg/10 ml in methanol (n=3). For calibration curves, an appropriate volume of berberine stock solution was diluted in methanol, and using HPTLC concentration levels ranging between 20 and 100 ng / spot (n=3) were analyzed.

Experimental Animals

Wistar rats of either sex weighing 180–220g were procured from Central Animal House, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu, India. The temperature in the experimental room was at 22°C (± 4°C) with 60% ±2 RH with appropriate lighting (12h light and dark cycle). Animals were housed in polycarbonate cages with stainless steel metal grades in bottom and were accessed to unlimited water supply and food. Experimental protocol was approved from Institutional Animal Ethical Committee (IAEC) and carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) by the J.S.S. College of Pharmacy under JSSCP/IAEC/PH.D/PH.COLOGY/01/2012–13.

Wistar rats of 180–220g were divided into 6 groups with 6 animals in each group as follows. Carrageenan indicates 1% w/v solution is prepared by dissolving 100mg of carrageenan in 10ml of 0.9% w/v NaCl and Indomethacin suspension was prepared accurately suspending 100 mg of Indomethacin in 10ml of distilled water using 1%w/v CMC (carboxy methyl cellulose) as suspending agent. A stock solution was prepared containing 10mg/ml of the drug.

Group-I: Carrageenan (Normal control)

Group-II: Carrageenan + Indomethacin 10 mg/kg

Group-III: Carrageenan + *Berberisaristata* (Aqueous extract – 25mg/kg)

Group-IV: Carrageenan + *Cosiniumfenestratum* (Aqueous extract – 25 mg/kg)

Group-V: Carrageenan + *Berberis aristata* (Methanolic extract – 25 mg/kg)

Group-VI: Carrageenan + *Coscinium fenestratum* (Methanolic extract – 25 mg/kg)

Acute paw edema was produced by injecting carrageenan 1% w/w (0.1ml) into the sub plantar region of the left hind paw in the rats. The methanolic and aqueous extracts (200 mg/kg) and Indomethacin 10 mg/kg administered orally one hour before testing. The control group received vehicle 0.1 ml/100gm. The paw volume was measured by using digital plethysmometer (UGO Basile, Italy) at 0, 30, 60, 120 and 240 minutes after carrageenan challenge^[12].

Statistical analysis

Data obtained from this study was performed using one-way ANOVA followed by Bonferroni multiple comparison test at 95% significance and expressed as mean \pm SEM

RESULTS

Physicochemical characterization of B. aristata and C. fenestratum samples

Physicochemical analyses of both the plant species recorded that all the parameters comply with API limits. The details are given in Table 1.

Table 1: Physicochemical characterization of *Berberis aristata* and *Coscinium fenestratum*

Parameters	<i>Berberis aristata</i>	<i>Coscinium fenestratum</i>	API Limits
Foreign organic matter (% w/w)	NIL	NIL	NMT 2 %
Moisture content (% w/w)	7.60	8.20	NA
Total Ash (% w/w)	2.17	2.11	NMT 14%
Acid insoluble ash (% w/w)	0.39	0.62	NMT 5 %
Alcohol soluble extractive (% w/w)	6.67	6.54	NLT 6 %
Water soluble extractive (% w/w)	9.36	12.46	NLT 8 %

NA- Not available; NMT - Not more than; NLT – Not less than

Quantification of Berberine by HPTLC

The linearity curve of Berberine was calculated and graphically represented with standard berberine using which the berberine content in *B. aristata* and *C. fenestratum* was detected and represented in Table 2.

Table 2: Quantification of Berberine by High Performance Thin Layer Chromatography

Sample	Berberine content (%)
<i>Berberis aristata</i>	0.180
<i>Coscinium fenestratum</i>	0.380

HPTLC Fingerprinting of samples

The HPTLC fingerprinting of *B. aristata* and *C. fenestratum* was compared. Standard berberine showed Rf value of 0.47 which was noticed in both the species (Table 3). Hence, the method is valid and can be used to distinguish *B. aristata* and *C. fenestratum*. Although very large variations were not seen between the two species, it can be inferred that the geographical locations among these two plants do not affect the amount of berberine present.

Experimental mice study

The details of inflammation induced in the different animal groups treated with 1% w/v carrageenan, standard

Table 3: Rf values *Berberis aristata* and *Coscinium fenestratum* samples and standard Berberine

Sample	UV 254nm	UV 366nm
Standard Berberine	0.47 (Dark)	0.38, 0.47 (Yellow fluorescent)
<i>Berberis aristata</i>	0.22 (Blue) 0.38, 0.47 (all Dark)	0.22 (Blue), 0.38, 0.47 (Yellow fluorescent)
<i>Coscinium fenestratum</i>	0.02, 0.22 (Blue) 0.38, 0.47, 0.68 (all Dark)	0.02 (Blue fluorescent) 0.38, 0.47 (Yellow fluorescent), 0.58 (Green) 0.77 (Blue)

drug indomethacin 10 mg/kg, and aqueous and methanolic stem extracts of *B. aristata* and *C. fenestratum* 25 mg/kg are provided in Figure 1. The stem extracts of *B. aristata* and *C. fenestratum*, and the standard drug indomethacin had significant anti-inflammatory activity on paw edema as against the control from 60 minutes onwards which was maintained up to 240 minutes ($p < 0.01$, $p < 0.001$). Though

at 60 minute, indomethacin treated group showed significantly higher anti-inflammation than the rest of the groups, at subsequent time intervals of 120 and 240 minutes, no such significant difference was observed. There was no significant difference in the anti-inflammatory activity of *B. aristata* and *C. fenestratum*. The results were comparable with that of the standard drug indomethacin @10 mg/kg.

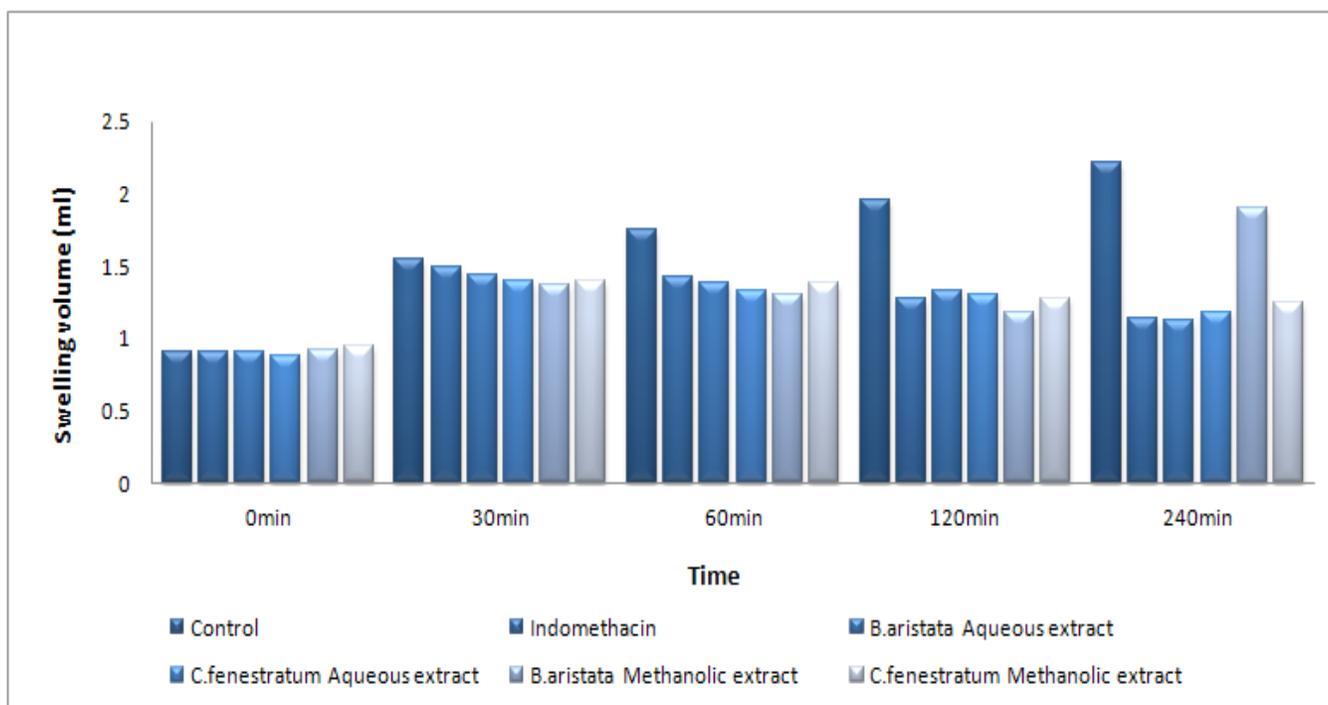


Figure 1: Inhibitory effect of Aqueous and Methanolic extracts of *Berberis aristata* and *Coscinium fenestratum* on carrageenan-induced edema of the hind paw in rats

DISCUSSION

Physicochemical analyses of plant extracts provide essential information regarding the chemical constituents for the pharmacological and pathological discovery of novel drugs^[16]. The results of the physicochemical

tests carried out in the present study confirmed that all the tested parameters of the samples were within the permitted levels of API and can be used for further phytochemical analysis. TLC and HPTLC profiling of the extracts confirmed the presence of phytochemicals. The Rf values of *B. aristata* and *C. fenestratum* were found to

be highly similar and were further addressed for animal model study to validate their respective anti inflammatory property.

The results of the animal study demonstrated the anti-inflammatory property of both *B. aristata* and *C. fenestratum* extracts that significantly inhibited paw oedema induced by carrageenan in rats. Carrageenan induced paw edema model is generally used to study anti-inflammatory activity of drugs^[15]. The inflammatory condition, a biphasic response, induced by carrageenan, could be attributed to the step-wise release of vasoactive substances such as histamine, bradykinin and serotonin in the early phase and prostaglandins in the acute phase^[13,14]. These chemical substances probably increase the vascular permeability, thereby promoting accumulation of fluid in tissues that accounts for oedema. Similar inference has been obtained in studies conducted on anti inflammatory activities of root and leaf extracts of *B.aristata*^[9]. The ability of the extracts to reduce the size of oedema produced by carrageenin suggests that they contained chemical components that may be active against inflammatory conditions.

CONCLUSION

The present study scientifically validates the anti-inflammatory property of *B. aristata* and *C. fenestratum*. Similarity observed in the anti-inflammatory activity of both the herbal drugs appears to fulfil the necessary criteria expected to use *C. fenestratum* as equivalent/substitute to *B. aristata*.

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