Int J Pharm Bio Sci 2014 Jan; 5(1): (P) 556 - 570

Review Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

A REVIEW ON THE PHARMACOGNOSY AND PHARMACOLOGY OF THE HERBALS TRADED AS 'DARUHARIDRA'

S.TAMILSELVI*[1], S.P.BALASUBRAMANI[2], PADMA VENKATASUBRAMANIAN[2] AND N.S. VASANTHI[1]

[1] Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam - 638452, TamilNadu, India
[2] Institute of Ayurveda and Integrative Medicine (IAIM), Bangalore -560106, Karnataka, India

ABSTRACT

Daruharidra (*Berberis aristata* DC) has been used in ayurveda and traditional Chinese medicine for more than 3000 years. It is a red-listed endemic medicinal plant species of conservation concern and has become very important in recent years due to its rarity and huge demand in the medicinal plant sector. However, many other species such as *Berberis asiatica* Roxb., *Berberis lycium* Royle., *Cosinium fenestratum* (Gaertn.) Coleb and *Morinda umbellate* L. are recommended as ayurvedic substitutes of Daruharidra and traded in the market. In ayurveda, it has been reported to be diaphoretic and diuretic; used as a tonic and also in the preparations of formulations for treating eye diseases, jaundice and skin diseases, diarrhoea, syphilis, chronic rheumatism, urinary disorders etc. From time to time, a number of reports on the various pharmacognosical and pharmacological properties of original Daruharidra (*B.aristata*) and its substitutes have been reported. This review analyses traditional medicinal usage, and pharmacognosical and pharmacological investigations done on the endangered medicinal herb Daruharidra and its substitutes.

KEY WORDS: Daruharidra*, Berberis aristata,* Substitutes, Traditional medicine, Pharmacognosy, Pharmacology

S.TAMILSELVI
Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam - 638452, TamilNadu, India

***Corresponding author**

INTRODUCTION

Daruharidra (in Sanskrit means 'the wood having yellow color') is one of the herbs mentioned in ancient scriptures of Ayurveda. Caraka and Susruta have mentioned its different properties along with various indications of its use. Caraka has categorized Daruharidra as stanyasodhana (lactode purant), lekhana (a reducing herb), arsoghna (antihaemorrhoidal), kandughna (antihaemorrhoidal), kandughna (antipruritic), svedala (promotes sweating), rasayana (rejuvenative). Susruta have mentioned it as ropana – a wound healer. Ayurvedic Pharmacopeia of India correlates Daruharidra to *Berberis aristata* of family Berberidaceae. The root and wood are rich in a yellow alkaloid berberine, a bitter substance, which dissolves in acids and forms salts of the alkaloid. Daruharidra has been observed to be diaphoretic, rejuvenating, antipyretic properties and as Raja Nighantu (bitter tonic). The plant is native to Himalayas at an elevation 2000 to 3500 metres and predominantly found in the Nilgiri mountain range in Southern India. The shrub grows upto 1.5 – 2.0 metres in height, with a thick woody root covered with a thin brittle bark. The leaves are cylindrical, straight, tapering, very sharp, hard, smooth spine with yellow, numerous, stalked, arranged in drooping racemic flowers and small berry, ovoid and smooth fruits. The flowering season of this plant is observed from April to May. Some of the major Daruharidra formulations are Darvyadi kavatha, Darvyadi leha, Darvyadi taila, Rasanjana and Dasanga lepa. Due to several clinical important formulations, Daruharidra is of trade importance (high volume/high value) and is of conservation concern being an endemic species. Market survey in India indicates that *Berberis asiatica*, *Berberis lycium*, *Cosinium fenestratum* and *Morinda umbellata* are traded as substitutes of *B. aristata*.[1] Literature survey of these plant species indicates that they possess a wide range of pharmacological activity, except for *M. umbellata*.

TRADITIONAL MEDICINAL USES

B. aristata is used to treat all types of ENT infections, wound healing, dysentery, indigestion, uterine and vaginal disorders.[2] It is also used as a tonic and used to cure ulcers and fevers and as an important ingredient of several polyherbal formulations for treating diarrhea,[3,4] cholera [5] and eye diseases[6] including opthalmia and other disorders which are cured by applying the dried extract of the root externally to the eyelids. Tender leaf buds are used to treat dental caries. "Rashut" decoction prepared from root is widely used in ayurveda. *B. asiatica* root has been reported to be efficient against a variety of ailments and diseases such as rheumatism, jaundice, diabetes, fever, stomach disorders, skin disease, and malarial fever.[7] Their roots have been used as antiperiodic, diaphoretic and antipyretic, and bark as tonic and antiperiodic.[8] *B. lycium* roots possess medicinal properties and used to treat eye complaints [9], menorrhagia, chronic diarrhea, febrifuge and piles.[10] Leaves are used to treat jaundice and stem used in the treatment of diabetes, wounds, broken bones, ulcers and sore eyes. Gilani and Janbaz in 1999 reported in some areas of India and Pakistan the fruits of this plant are used as a tonic against liver and heart diseases and also possess antihistaminic activity, stomachic, astringent, antipyretic and diaphoretic properties.[11] Stems of *C. fenestratum* exhibits medicinal properties and is widely used for the treatment of kapha, vata, skin diseases, diseases of the eye, inflammations, wounds, ulcers, jaundice, diabetes, dysentery, fever and general weakness and are commercially used as ropes in Sri Lanka. [12] The boiled roots and leaves of *M. umbellate* are used in traditional medicine with the powdered leaves used to treat dysentery, diarrhoea and also reported to possess antileukemic and antioxidant properties and the fruits are considered edible.

PHARMACOLOGICAL STUDIES

Pharmacology is the branch of medicine and biology concerned with the study of drug action

(Table 1 and 2). More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function. Following reports explain the possible effects of Daruharidra pharmacology.

Anti-inflammatory activity

The anti-inflammatory property of berberine sulphate from *B.aristata* was reported in the year 1977. Aqueous extracts of the roots of *B.aristata* (500-1000 mg/kg) showed significant anti-inflammatory effect in rats with carrageenan induced paw edema which is comparable with that induced by 10 mg diclofenac sodium. [13] Anti inflammatory effects of *Curcuma longa* and *B. aristata* in endotoxin induced uveitis in rabbits by grading the clinical signs and histopathological changes and estimating the inflammatory cell count, protein, and TNF-α level in the aqueous humor observed indicated the topical instillation of aqueous extracts of *C.longa* and *B.aristata* showed potent anti-inflammatory activity against endotoxin induced uveitis in rabbits. The ethnobotanical studies conducted on alcoholic and aqueous root extracts of *B.aristata* showed significant activity on acute inflammation after two hours of carrageenan injection indicating the effectiveness of aqueous extract in the early phase by blocking the mediators released (i.e. bardykinin, histamine and serotonin) and of the alcoholic extract in the later phase by blocking the mediators released (i.e. prostaglandins). [14] Methanolic and aqueous extracts of *B. aristata* and C. *fenestratum* have shown similar anti-inflammatory activity in carrageenan induced raw paw edema in rat model. [15]

Anti-microbial activity

The alkaloid berberine from *B.aristata* was reported to possess antibacterial effects against trachoma. [16] Efficacy of berberine at a concentration of 0.2%, when compared to 20% sulfactamide, was found to be superior in both clinical course of trachoma and in achieving a fall in the serum antibody titers against *Chlamydia trachomatis* in the treated patients. Differential antifungal activity of methanolic extracts of *B.aristata* roots against three common forest fungal pathogens has been reported. [17] The effect of two natural products namely berberine, an alkaloid from *B. aristata* and santonin from *Artemisia maritima* on spore germination of some saprophytic and obligate fungi, individually and also in combination were reported by observing the significant block in spore germination of most of the tested fungi and mixture of both alkaloids was found to be more effective than individual ones. [18] Studies conducted with six ear pathogens against the root and leaf extracts of *B.aristata* recorded antimicrobial activity against all the 6 pathogens by the root extract while leaf extract was effective against 5 pathogens indicating the possible use of acetonic root extracts of *B.aristata* against ear infection.[19] The methanolic extract of stem bark of *B.asiatica* was reported to possess effective antimicrobial activity against a number of microorganisms by comparing the different solvent extracts of stem bark for antimicrobial property suggesting the presence of other antimicrobial agents in addition to berberine in the plant.[20] The reports on the isolated flavanoids quercetin and kaempferol from the leaves of *B.lycium* revealed the antimicrobial activity against *Staphylococcus aureus* and *E.coli.* The antimicrobial activity of hydroalcoholic extracts of four *Berberis* species were tested against eleven bacterial and eight fungal strain.[21] The root extracts of *B. lycium, B. aristata* and *B asiatica* showed significant antifungal activity against *Aspergillus niger* and *A.flavus* and specifically the root extract *B. aristata* gave low minimum inhibitory concentration values against three bacterial strains and a fungal strain while stem extract demonstrated low activity against two bacterial strains. The pharmacological effects of berberine from *C. fenestratum* have been well investigated and reported to be active against a number of gram-positive as well as gram-negative bacteria and also against a number of fungi.[22] Water extract from *C. fenestratum* was shown to exhibit antibacterial activity against *Clostridium* species.[23] The stem of *C. fenestratum* extract has been reported to exert hypotensive, and hepatoprotective activities.[24,25] The methanol

extract of *C. fenestratum* was found to have strong anti-plasmodial effect inhibiting the growth of the chloroquine-resistant *P. falciparum* strain FCR-3. The ethanolic extract of *C. fenestratum* significantly suppressed *in vitro* anti-herpes simplex virus type 1 (HSV-1) plaque formation in Vero cells. [26] In clinical tests in Vietnam, the aqueous methanol extract of *C. fenestratum* showed distinct activity on *Staphylococcus aureus* and *Streptococcus hemolyticus*, which may cause inflammation and infection especially in women after childbirth.[22]

Cytotoxic and Antitumour Activity

Studies conducted on the methanolic extracts of *B.aristata* stem and rhizomes of *Hemidesmus indicus* on MCF7 breast cancer cell line proved the extracts to have *in vitro* cytotoxic activity.[27] The polar components of *C. fenestratum* are cytotoxic against laryngeal cancer cell lines.[22] The methanolic extract of *B. aristata* is confirmed to be a potential anticancer herb against colon cancer due to its COX-II inhibitory property on proliferation of human colon cancer cell line (HT29).[28] Evaluation on the antitumour activity of aqueous and alcoholic extracts of *B. aristata* in swiss albino mice on primary stages of cancer and found the possible anticancer activity by *in vitro* cytotoxic avtivity in the brine shrimp lethality bioassay method confirmed the *in-vivo* antitumour activity in the ehrlich ascites carcinoma model.[29] The anti-neoplastic activities of different root extracts of *B. lycium* along with berberine and palmatine were investigated in p53-deficient HL-60 cells and demonstrated that they regulated protein expression and protein activation in HL-60 cells and downregulated two potent onogenes Cdc-25A and cyclin D1. [30] Antiproliferative activity of the methanolic, methanol-water and water extracts of *C. fenestratum* when evaluated in human HT-1080 fibrosarcoma cells, exhibited antiproliferative activities. *C. fenestratum* also showed selective activity against lung carcinoma and lung metastatic cell lines, A549, LLC and B16-BL6. The leaf powder of *M. umbellata* is reported to possess excellent antileukemic and antioxidant anthraquinones.[31]

Hypoglycemic effect and Anti diabetic activity

The anti-diabetic activity of stem bark of *B.aristata* in alloxan induced diabetic rats was evaluated and reported the ethanolic extract to reduce blood glucose level in diabetic rats.[32] The analysis of serum urea, protein, blood cholesterol, total lipids, SGOT and SGPT, body weight and liver glycogen showed reduced levels than standard levels. *B.aristata* root extract was found to possess anti hyperglycemic and strong anti oxidative properties by reducing blood glucose level in alloxan induced diabetic rats, restoring antioxidant status, reducing oxidative stress and modulating enzymes for glucose metabolis.[33] The extract of the root of *B. aristata* was reported to have strong potential to regulate glucose homeostasis through decreased gluconeogenesis and oxidative stress.[33] The methanolic extract of *B.aristata* stem bark was reported to possess blood glucose lowering potential and *in vitro* antioxidant property.[34] Ethanolic, acidified-basified and chloroform: methanol fractions isolated from the root bark of *B.aristata* and treated with alloxan induced diabetic rabbits found a significant decrease in the blood glucose level at 2, 4, 8 and12 hrs of observation for both normal and alloxan induced rabbits, which was higher than gliclazide confirming the roots bark of *B.aristata* possess hypoglycemic activity for all the above mentioned fractions.[35]

Comparative study of the effects of the crude extract of *B.lycium* with pure berberine and as an attempt to validate its use as a therapeutic agent, demonstrated *Berberis* extract and berberine had similar effects on all parameters *viz.,* glucose tolerance, glycosylated haemoglobin, serum lipid profiles and body weight of experimental animals measured and the extract was comparable in efficacy to berberine.[36] Antihyperglycemic effect of the ethanolic extract of root samples of *B.lycium,* when studied using alloxon induced diabetic rats, indicated significant hypoglycemic activity. Similarly, antidiabetic claim of *B.lycium* suggested the possible use of water extract as an adjunct to insulin.[36, 37] Hypoglycemic activity was reported for the alcoholic stem

extract of *C. fenestratum* and aqueous stem extract of *C. fenestratum* for the treatment of diabetes mellitus, evaluated in streptozotocin-nicotinamide induced type 2 diabetic rats.[38, 39] Studies suggested the possible use of *C. fenestratum* ethanolic extract in controlling diabetes mellitus by markedly decreasing the plasma glucose level in diabetic rats and exhibited antihyperglycemic activity by stimulating insulin secretion and α-glucosidase inhibition.

***Antidiarrhoeal* activity**

Study with berberine from the roots and barks of *B.aristata* reported the inhibition of secretary response of heat labile enterotoxins of *Vibrio cholerae* and *Escherichia coli* in rabbit ligated intestinal loop model and infant mouse assay and possible clinical effectiveness in treating acute diarrheal disease.[40] The effectiveness of alcoholic extract of the stem of *B.aristata* against castor oil induced diarrhoea was analysed in rats indicating the antienteropooling activity (prevention of induced intestinal fluid accumulation) of the extract. The leaf powder of *M. umbellata* has been used in treating diarrhoea and dysentery.[41]

Other Pharmacological Activities

Effects of *B. aristata* on lipid profile and blood coagulation in hyperlipidemia induced rabbits, when evaluated, revealed the hypolipidemic effects of *B. aristata* and also indicated the probable influence on blood coagulation which is of importance in cardiovascular diseases.[42] Based on the observations on uterine weight, bone loss, ash content, biomechanical, biochemical and histopathological changes in ovariectomized rats, the aqueous - methanol extract of *B. aristata* was reported to possess potent anti-osteoporotic activity.[43] Studies conducted to test the anti-cirrhosis activity of ethanolic and aqueous extract of whole plant of *B.aristata* against dimethylnitrosamine induced liver cirrhosis in rat's model recorded significant increase in the survival time and decrease in cirrhotic nodules indicating the protective effect of the extracts.[44] Evaluations of the hepatoprotective and antioxidant effects of the methanol and aqueous extracts of *B .asiatica* against CCl_4- induced hepatic injury in rats reported the effective hepatoprotective and antioxidant activity of the extract which reduced the serum marker enzymes and restored the antioxidant levels.[45] Role of *B.lycium* in reducing serum cholesterol in broilers was carried out and also reported to possess anti-bacterial and anti-diabetic effect and is used in treatment of bleeding piles.[46, 47, 48]

Hypotensive action was recorded when ethanolic stem extract of *C.fenestatum* was tested in anaesthetised dogs, rats and guinea pigs in a dose dependent manner.[24] Antioxidant effect of methanol extract of *C. fenestratum* stem powder was examined using carbon tetrachloride-intoxicated rat liver as the experimental model and in streptozotocin-nicotinamide induced type 2 diabetic rats revealing the effectiveness of *C. fenestratum* in combating oxidative stress due to hepatic damage.[25, 38] Studies conducted to test the hypotensive and vasorelaxant effects of water extract of *C. fenestratum* demonstrated the effectiveness of the plant extract in reducing blood pressure in anesthetized normotensive rats.[49] The stem of *C. fenestratum* extract has been reported to exert hypotensive and hepatoprotective activities with antinociceptive effects.[24,25,50] Studies concluded that *C. fenestratum* possesses neurotoxicity and induced neurobehavioral changes in rats.[51] There are also reports on the alcoholic stem extract possessing good hypolipidemic activity (http://www.freshpatents.com). Evaluations with respect to the wound healing potential of ethanolic extract of *C. fenestratum* using albino rats excision wound model and incision wound model were based on the wound contracting ability, epithelisation period and tensile strength.

The question of authenticity

When these plants show diverse pharmacological actions with different dosage preparations, using of substitutes is not appropriate for which robust objective methods. Current pharmacognosy practices use morphology, microscopy, phytochemistry to distinguish the different plant species. It is

therefore required to develop standards with authentic plant accessions for each of the species (Table 3).

MORPHOLOGICAL DESCRIPTION OF THE PLANTS

Berberis aristata D.C. is a large shrub which grows up to 1.8 – 3.6 m high with 10 – 20 cm stem diameter. It has thick woody roots which are cylindrical, yellowish brown and covered with thin brittle bark with pale yellowish brown, cylindrical and strongly striate twigs. The leaves are obovate, spinous toothed and gradually narrowed with prominent reticulate nerves. Numerous flowers are arranged as stalked inflorescence with ovoid, blue black with persistent style and stigmatous fruits.[52, 53, 54, 55]

Berberis asiatica Roxb., is an evergreen shrub growing to 1.2-1.8 m high at a medium rate and with 10 cm wide stems. Its bark is rough, furrowed and corky and pale yellowish and glabrous twigs. Leaves are oblong, elliptic, or broadly obovate with large distant spinous teeth and the flowers are hermaphrodite. The pollination is observed to be by insects or self resulting in fruits are 7-10 mm long, ovoid, and blue black with glaucous bloom with a distinct style. The plant is capable to grow in heavy clay and nutritionally poor soils.[56]

Berberis lycium Royle., is a 2-4 m high semi deciduous shrub with pale yellow coloured roots that are rich in alkaloids (berberine, etc.) and other phytochemicals. Leaves are narrowly obovate-oblong, with a few large spinous teeth, arranged alternately on stem which is whitish grey in colour. Flowers are yellow in colour found in axillary clusters and are characteristically longer than the leaves with black coloued fruits. *Berberis lycium* is valued mainly for its fruits and roots.[36]

Coscinium fenestratum (Gaertn.) Colebr., is a large woody climber with cylindrical and yellowish stem of 10 m long and 10 cm diameter. Its leaves are simple, oblong, deltoid, alternate and minutely tomentose beneath, smooth above, with yellowish tint. Flowers are yellow in colour and unisexual in supra axillary inflorescence yielding smooth globose drupes containing one globose seed.[22]

Morinda umbellate Linn., is a large, climbing shrub, with long, hairy, slender branches. Leaves are elliptic, smooth on the upper surface, hairy beneath, and pointed at both ends. The flowers appear in clusters on long stalks and arranged like rays of a star. Fruit is compound, and irregularly lobed that mature from green to orange.[57]

PHARMACOGNOSTIC STUDIES

Pharmacognosy is the study of the physical, chemical, biochemical and biological properties of drugs, potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources. Daruharidra and various endangered plant species require highly sophisticated techniques for their identification. Various pharmacognostic parameters including macroscopy, microscopy, chemomicroscopy and behaviour of powdered drug on treatment with different chemical reagents were studied on the stems of *Berberis aristata* to supplement information in regard to its identification parameters.[27]

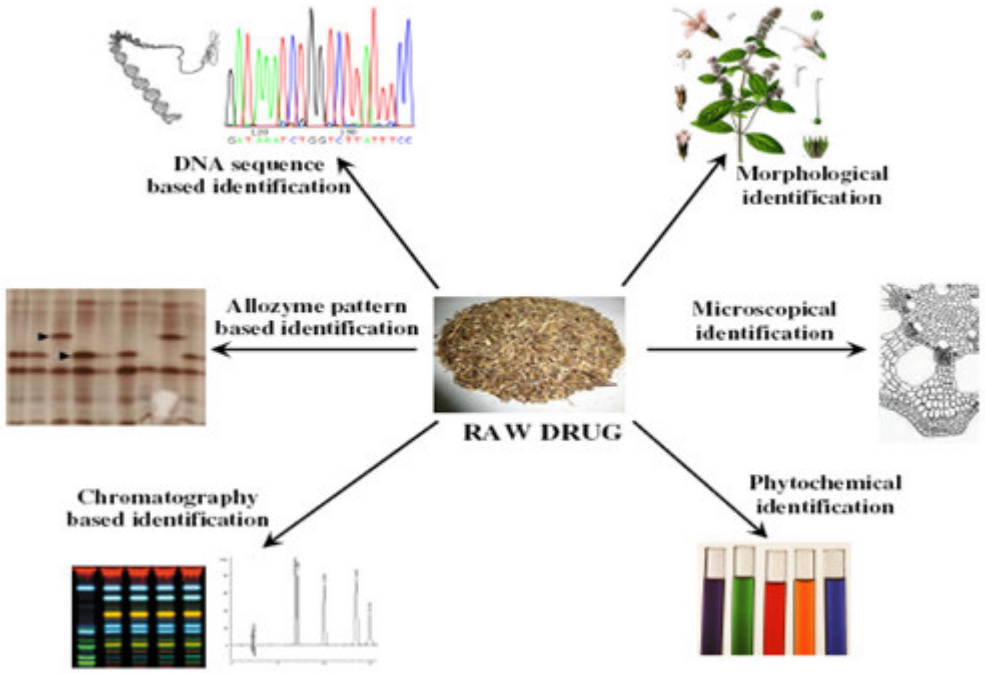

Figure 1
Choice of Pharmacognosy methods available

i. Macroscopical and Microscopical analysis

A detailed pharmacognistic study including the macroscopic character and systematic anatomical study of the root of *B.asiatica* was carried out.[56, 58] Similarly, the anatomical studies of the roots and rhizomes of *B. lycium* were reported.[59] A detailed account of the morphological and anatomical features of *C. fenestratum* has been described.[22, 38]

ii. Physicochemical analysis

Elaborate study carried out on the various physicochemical properties viz., ash content, water and alcohol soluble extract, tannins, sugars, starch and alkaloid content to identify the diagnostic features of the root of *B.asiatica.* [56, 58] Various studies concluded that *B.asiatica* can be a substitute for *B.aristata*. A detailed chemical analysis of the roots and rhizomes of *B. lycium was* reported along with various physical, chemical, nutritional, anti-nutritional and organoleptic qualities of wild fruit *Kasmal* (*B. lycium*). Evaluations indicated the presence of high moisture content with low protein, fat, ash and fibre contents. Among the nutritional content, vitamin C, β-carotene and mineral and antinutritional components (phytic acid and phytate phosphorous) were detected to be low and anthocyanins were in high percentage. The chemical and mineral constituents of possible pharmacological interest of the wild *B. lycium* indicated that the content of moisture, ash and protein increased in different parts in descending order i.e. root < shoot < leaves whereas fat and fiber contents decreased in ascending order i.e. root > shoot > leaves.[59, 60] A systematic pharmacognostic study (Table 4 and 5) including microscopic characters, fluorescence analysis, physicochemical phytochemical screening and TLC was performed on *M. umbellate* with results suggesting these parameters can be used as a diagnostic tool for the identification of this plant and differentiating it from its adulterants.[57]

iii. Phytochemical Analysis

Isolation of tertiary alkaloids such as berlamine, dihydroberlamine and noroxyhydrastine from the roots of *B.aristata* was reported. Similarly, studies on the stem of *B.aristata* identified the presence of berberine, ceryl alcohol, hentriacontane, sitosterol, palmitic acid, oleic acid and saponin. The phytochemical screening

of the methanolic extract of stems of *B. aristata* showed the presence of alkaloids as active chemical constituents.[28] Phytochemical screening of *B.aristata* stem with various solvents revealed the presence of phenolic compounds, tannins, flavonoids, phytosterols, saponins and glycosides in it.[27] Studies on the extraction of air dried materials of *B.asiatica* revealed the presence of triterpenoids in hexane and chloroform soluble parts, tannin in water soluble part, resin in acetone and alkaloids in chloroform, acetone, alcohol and water soluble parts.[56] Different pharmacognostic studies revealed the presence of triterpenoids resin in acetone and alkaloids in chloroform, acetone, alcohol and water soluble parts which can be used as diagnostic features of the root of *B. asiatica*.

iv. High performance thin layer chromatography (HPTLC) finger printing and High performance liquid chromatography (HPLC) Analysis

A precise, sensitive and reproducible method of detection, quantification and monitoring berberine in *B.aristata* was developed using HPLC. A spectrophotometric method was developed for the simultaneous estimation of curcumin and berberine using methanoloic extract, in pure and combined form of *C. longa* and *B. aristata* without any prior purification step. [61] The developed method has been employed to analyse the two markers viz., curcumin and berberine in polyherbal formulations. HPTLC analysis was carried out for development of characteristic fingerprint profile of different extracts of *B. asiatica* root can be used as markers for quality evaluation and standardization of the drug. Habitat dependent variation in berberine content of the roots and stems of *B.asiatica* was recorded using HPLC analysis.[62] Quantitative analysis of berberine in stem pieces *C.fenestatum* was done by HPLC.[63,64] HPTLC finger printing study was conducted to identify variation in berberine content among the ten Thailand market stem samples of *C. fenestratum* although recorded a significant difference in berberine content among the samples, TLC densitometric fingerprinting showed a similar pattern with berberine as a major component suggesting a possible guideline for identification of raw material and extracts of *C. fenestratum* for pharmaceutical preparations.[65]

v. Molecular Markers

Molecular markers to authenticate the species of *Berberis* viz., *B. aristata, B. asiatica* and *B. lycium* developed by using DNA ITS (internal transcribed spacer) sequence.[66] Their data from sequence alignment indicated the ITS1, 5.8S rRNA gene and ITS2 regions of each species were quite unique and different from each other. Maximum homology was observed in the ITS1 regions of *B. asiatica and B. lycium* while lowest was in the ITS1 regions of *B. asiatica and B. aristata.* The 18S rRNA gene and ITS gene sequences with PCR-RFLPs were proven to be powerful molecular markers for identifying *C. fenestratum* and distinguishing it from *Arcangelisia flava*; *Fibraurea tinctoria*;, the other two Menispermaceae plants.[67] Similarly, to strengthen the pharmacognosy parameters, molecular identification of *C. fenestratum* was performed based on the nuclear DNA ITS sequence.[68] Their study recorded a species-specific DNA marker for easy identification of *C. fenestratum* and as a molecular pharmacognosy tool in quality control of herbal raw drugs.

Table 1
Properties of various substitutes of Daruharidra

Activity	*B. aristata*	*B. asiatica*	*B. lycium*	*C.fenestratum*	*M. umbellate*
Anti-inflammatory	√				
Anti-microbial	√	√			
Analgesic	√				
Anti-pyretic	√				
Anti-hepatotoxic	√	√			
Anti-oxidant	√	√		√	
Anti-diabetic / Hypoglycemic	√		√	√	
Cytotoxic	√				
Anti-osteoporetic	√				
Inotropic	√				
Anti-diarrhoea	√				
Anti-tumor	√		√		
Wound healing				√	

Table 2
Phytochemical constitution of Daruharidra on comparison with its substituents

Compound	*B. aristata*	*B. asiatica*	*B. lyceum*	*C.fenestratum*	*M. umbellate*
Berberine	√	√	√	√	√
Berbamine	√	√	√		
Palmitine	√		√	√	
Oxyberberine	√			√	
Oxycanthine	√				
Aromoline	√				
Anti-diabetic / Hypoglycemic	√		√	√	
Cytotoxic	√				
Anti-osteoporetic	√				
Inotropic	√				
Anti-diarrhoea	√				
Anti-tumor	√		√		
Wound healing				√	

Table 3
Daruharidra and its permitted substitutes

S.No	Botanical Name	Classification	Common Names
1.	*Berberis aristata* DC	Kingdom: Plantae Phylum: Magnoliophyta Class: Magnoliopsida Order: Ranunculales Family: Berberidaceae Genus: Berberis Species: Berberis aristata / asiatica / lyceum	Barberry, Tree turmeric (English) Daruharidra, Darvi, Darurajani (Sanskrit) Maramanjal (Tamil & Malayalam)
2.	*Berberis asiatica* Roxb		Ucikkala (Tamil) Chutro (Local name in Nepal)
3.	*Berberis lycium* Royle		Sumbal (Urdu) Churku, Ishkeen (Local name in Pakistan)
4.	*Coscinium fenestratum* (Gaertn.) Colebr	Kingdom: Plantae Phylum:Tracheophyta Class: Magnoliopsida Order: Ranunculales Family:Menispermaceae Genus: Coscinium Species: Coscinium fenestratum	Tree turmeric (English) Canda, Daruharidra (Sanskrit) Atturam (Tamil) Maramanjal (Malayalam)
5.	*Morinda umbellate* Linn	Kingdom: Plantae Phylum: Magnoliophyta Class: Magnoliopsida Order: Rubiales Family: Rubiaceae Genus: Morinda Species: Morinda umbellate	Pitadaru (Sanskrit) Indian mulberry (English) Nuna (Tamil)

Table 4
Pharmacological studies of Daruharidra and its substitutes

Plant species	Activity	Preparation	Animal Model	Dosage	Effect
Berberis aristata	Anti-inflammatory activity	Aqueous Root extracts	Rats with carrageenan induced paw edema	500-1000 mg/kg	Significant reduction of paw size
		Alcoholic and aqueous root extracts	Rats with carrageenan induced paw edema	50 mg/100 g	Significant reduction of paw size
		Aqueous extracts of *C.longa* and *B.aristata*	Endotoxin induced uveitis in rabbits	150-200 µl	Improvement of chronic uveitis
	Antimicrobial activity	Methanolic root extract	Against forest fungal pathogens	0.1-0.5 mg/ml	Inhibitory zone
		Hydroalcoholic root extract	Eleven bacterial and eight fungal strains	0.31µg /ml	Inhibibatory zone
		Aqueous and alcoholic extracts of root	Bacteria and Fungi	50 µg/disc	Inhibibatory zone
		Aqueous & acetonic extract	Five different ear infecting pathogens	3.12 mg/ml	Inhibibatory zone
	Analgesic activity	Aqueous and alcoholic extracts of root	Albino rats	50/100 g	Significant increase in reaction time
	Antipyretic activity	Aqueous and alcoholic extracts of root	Rabbits	200 mg/kg	Rectal temperature
	Antihepatotoxic activity	Aqueous methanol fruit extract	Swiss male mice and Male albino wistar rats	500 mg/kg	Induced rise in serum transaminases and reduced paracetamol and CCl_4-induced hepatic damage
	Antihyperglycemic and antioxidant activity	Ethanolic root extract	Alloxan Induced male albino wister rats	250 mg/kg	Lowered blood glucose level, restored antioxidant status
	Antihyperglycemic activity	Ethanol extract	Alloxan Induced male wistar albino rats	71.42 and 100 mg/kg	Reduction of serum glucose level
	Antidiabetic activity	Methanolic extract of stem bark	Male wistar albino rats	250 mg/kg	Change in the body weight
	Hypoglycemic activity	Ethanolic fraction of root bark, Acidified- basified fraction Chloroform-methanol fraction	Alloxan induced diabetic female albino rabbits	0.5-1.5g/kg 100 and 125 mg/kg 4 and 5 mg/kg	Stimulates the release of insulin and possess insulin like action
		Methanol extract of stem	Male albino wistar rats	250 and 500 mg/kg	Reduced the serum lipid levels
	Cytotoxic activity	Methanolic extracts of stems	MCF7 cell lines	1 mg/ml	Cells viability decreased
	Anti-osteoporotic activity	Aqueous-methanol extract	Ovirectomized female sprague-dawley rats	500 mg9/kg	Significant increase in uterine weight, femer BMD, ash content and lumbar hardness
	Inotropic activity	Aqueous-methanol extract of fruit	Guinea pig	2 mg/ml	Modulatory effect on actin myosin cooperativity
	Antidiarrhoeal activity	Ethyl alcohol extract of stem	Wistar albino rats	250 mg/kg 500 mg/kg	Increased the reabsorption of water by decreasing intestinal motility
	Antitumour activity	Aqueous and alcoholic extract	Ehrlich Ascites Carcinoma induced albino mice	3289 and 66 mcg/ml	Significant increase in life span and decrease in cancer cell number
B.asiatica	Hepatoprotective and antioxidant effect	Aqueous & methanol extract	Wistar albino rats	200-300 mg/kg	Prevented the CCl_4 elevation of serum enzymes
	Antimicrobial	Aqueous &	20 different	156.25-	Inhibibatory zone

	activity	methanolic extract of stem bark	microorganisms	625.00 µg/ml	
		Petroleum ether, Chloroform, Ethyl acetate, Acetone, methanol and Water extracts of fruit	13 Bacteria and fungus strains	10 mg/ml and 50 mg/ml	Inhibibatory zone
Berberis lyceum	Anti-neoplastic activity	n- butanol extract Ethyl acetate extracg Water extract	HL-60 human promyelocytic cells	0.6 and 1.2 µg/ml	Inhibited the expression of the proto-oncogene cyclin D1.Induced the acetylation of α-tubulin which induced apoptosis
	Anti hyper glycemic effect	Aqueous and ethanol extracts	Wistar rats	50 and 100 mg/kg	Reduction of serum glucose levels
	Hypoglycemic activity	Various extracts of roots	Rabbits	250 and 500 mg/kg	Reduced the blood glucose levels
Coscinium fenestratum	Antihyperglycemic activity	Ethanol extract	Steptozotocin induced diabetic male sprague dawley rats	10 µg/ml	Stimulates insulin secretion and α-glucosidase inhibition
	Antioxidant activity	Alcoholic stem extract	Streptozotocin-nicotinamide induced diabetic rats	500mg/kg	Enhanced and protective effect on cellular antioxidants
	Wound Healing activity	Ethanol extract	Wistar strain albino rats	10% w/w ointment	Increased wound contracting ability, epithelisation period and tensile strength

Table 5
Parts of the plant used for targeting various compounds

Plant species	Part used	Compound isolated
Berberis aristata	Roots and stem bark	Berberine, Berbamine, Aromoline, Palmatine, Oxycanthine and Oxyberberine
	Roots and bark	Calumbamine, Umballiatine, Jatrorrhizine and Hydrastine
	Root	Karachine and Taxilamine
	Fruits	Citric acid and Malic acid
	Flowers	E-caffeic acid, Quercetin, Chlorogenic acid, Meratin, and Rutin
	Heartwood	n-docosane lanost-5-en-3β-ol
Berberis asiatica	Roots and stem	Berberine
Berberis lycium	Root and stem part of plant	Berberine, Berbamine, Chinabine, Karakoramine, Palmatine Balauchistanamine, Gilgitine, Jhelumine, Punjabine, Sindamine,Chinabine Acetic acid, Maleic acid, Ascorbic acid
Cosinium fenestratum	Root and Stem	Berberine, Protoberberine, Jatrorrhizine, Magnoflorine, Berberrubine, Thalifendine, Palmitine, Oxyberberine, Ceryl-alcohol, Saponin, Hentriacontane, Sitosterol, Palmitic acid, Oleic acid and Sitosterol glucoside,N,N-dimethyllindacarpine, Oxypalmitine, 8-Oxotetrahydrothalifendine, 8-oxoisocorypalmine, 8- oxothaicanine, 8-oxo-3-hydroxy 2,4,9,10 tetramethoxy berberine, 8-oxocanadine,12,13-dihydro-8-oxoberberine, 5,6,13,13a tetrahydro-9,10-dimethoxydibenzo(a,g) 1,3-benzodioxalo(5,6a) quinalizine -8-one, Stigmasterol, Berlabine, Dihydroberlabine, Noroxyhydrastinine.

CONCLUSION

Daruharidra has been correlated to *B. aristata* as per the Ayurvedic literature. Since it is an endemic species of conservation concern, it is in practice that *C.fenestatum* is used as its substitute in certain parts of India. Exploitation of *B.lycium*, *B.asiatica* and *M.umbellata* as substitutes to Daruharidra requires critical scientific understanding in terms of their chemical constituents and property. There is a lack of evidence based data detailing the pharmacological and pharmacognosical effects of *B.lycium*, *B.asiatica* and *M.umbellata* and hence future studies may be carried out to prove the potential of these plant species. Attempts are being made to extend the work upto clinical trial stages as most of the information available on the pharmacological activities is purely academically oriented. Identification of a valid substitute to *B. aristata* will reduce the pressure on this red listed Himalayan species and also suggest industries for an authentic, effective and economically viable substitute.

ACKNOWLEDGMENT

The authors wish to acknowledge the Management of Bannari Amman Institute of Technology, Sathyamangalam, Tamil Nadu, India and Institute of Ayurveda and Integrative Medicine, FRLHT (Foundation for Revitalization of Local Health Traditions), Bangalore, India.

Conflict of Interest
Conflict of interest declared none

REFERENCES

1. Ved DK, Goraya GS. Demand and supply of medicinal plants in India. Bishan Singh Mahendra Pal Singh, Dehradun and FRLHT, Banglore. (2008).
2. Gupta SK, Agerwal R, Srivastava S, Agarwal P, Agarwal SS, Saxena V, et al. The anti-inflammatory effects of Curcuma longa and Berberis aristata in enditoxin-induced uveitis in rabbits. Investigative Opthalmology and Visual Science. 49(9):4036-4040, (2008)
3. Kirtikar KR, Basu BD. Indian Medicinal Plants Latit Mohan Basu, Leader Road, Allahabad: India. 1664-1665, (1984).
4. Jain SP, Singh SC. Ethno-Medico-Botanical Survey of Ambikapur District MP, India. Fourth International Congress of Ethnobiology, NBRI, Lucknow, UP, India. (1994).
5. Dutta NK, Panse MV. Usefulness of berberine (an alkaloid from *Berberis aristata*) in the treatment of cholera (experimental). Indian J. Med. Res. 52:732-736, (1962).
6. Babbar OP, Chhatwal VK, Ray IB, Mehra MK. Effect of berberine chloride eye drops on clinically positive trachoma patients. Ind. J. Med. Res. 76:83-88, (1982).
7. Watt G. Economic Products of India V, The Superintendent of Government Printing. India, 652–653, (1883).
8. Chopra RN, Chopra TC, Handa KL, Kapoor LD. Chopra's Indigenous Drugs of India. Vol. 284. UN Dhar and Sons Pvt; (1958).
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, National Institute of Science Communication (CSIR), New Delhi, 170; (1999).
10. Ghosh AK, Rakshit MM, Ghosh DK. Effect of berberine chloride on *Leishmania donovani*. Ind. J. Med. Res. 78:407–416, (1983)
11. Shamsa F, Ahamadiani A, Khosrokhavar R. Antithistaminic and anticholinergic activity

of Berbery fruit (*Berberis vulgaris*) in the guinea pig ileum. Ethnopharmacol. 64:161-166, (1999).
12. Varier P S,*Cosinium fenestratum* In: Indian Medicinal Plants, Compendium of 500 species, Hyderabad.,India: Orient Longman Ltd., 2: 191-193, (1994)
13. Rajput N, Nigam JM, Srivastava DN, Sahni YP. Anti-inflammatory activity of *Adhatoda vasica* and *Berberis aristata* on carrageenin induced paw oedema in rats. Journal of Natural Remedies. 4(1): 97 – 102, (2004).
14. Shahid M, Rahim T, Shahzad A, Latif TA, Fatma T, Rashid M, et al. Ethnobotanical studies on Berberis aristata DC. root extracts. African J. Biotechnol. 8:556–563, (2009)
15. Sekhar S, Karmakar R, Ramachandra KK, Ramachandrappa SN, Prakash HS. Potential anti-inflammatory bioactives from medicinal plants of Western Ghats, India. Pharmacognosy Communications. 2(2): 2-12, (2012).
16. Khosla PK, Neeraj VI, Gupta SK, Satpathy G. Berberine, a potential drug for trachoma. Rev Int Trach Pathol Ocul Trop Subtrop Sante Publique. 69:147–165, (1992).
17. Rajasekaran RA, Pant J. The genus Berberis Linn.: A review. Phcog. Rev. 2:369-385, (2008).
18. Singh B, Srivastava JS, Khosa RL, Singh UP. Individual and combined effects of berberine and santonin on spore germination of some fungi. Folia Microbiol. (Praha). 46:137-142, (2001).
19. Sharma C, Aneja KR and Kasera R. Screening of *Berberis aristata* DC. for Antimicrobial potential against the pathogens causing ear infection. International Journal of Pharmacology. 7(4): 536-541, (2011).
20. Bhandari DK, Nath G, Ray AB, Tewari PV. Antimicrobial activity of crude extracts from *Berberis asiatica* stem bark. Pharmaceutical Biology. 38(4); 254–257, (2000).
21. Meenakshi S, Shrivastava S, Rawat AKS. Antimicrobial activities of Indian Berberis species. Fitoterapia, 78 (7-8):574-576, (2007).
22. Tushar KV, George S, Remashree AB, Balachandran I. *Coscinium fenestratum* (Gaertn.) Colebr. – A review on this rare, critically endangered species and highly traded medicinal species. Journal of Plant Sciences. 3(2):133-145, (2008).
23. Nair R, Kalariya T and Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol. 29: 41-47, (2005).
24. Singh GB, Singh S, Bani S, Malhotra S. Hypotensive action of a *Cosinium fenestratum* stem extract. J Ethanopharmacol. 38:151-155, (1990).
25. Venukumar MR, Latha MS. Antioxidant effect of *Coscinium fenestratum* in carbon tetrachloride treated rats. Indian Journal of Physiology and Pharmacology. 46(2):223-8, (2002).
26. Ekalaksananan T, Pientong C, Tattawasart U and Kongyingyoes B. *In vitro* anti-herpes simplex virus type 1 activity of *Coscinium fenestratum* (Gaertn.) Colebr. and *Stephania venosa* (Blume) Spreng. Proceeding of the 10th International Congress of Ethnobiology. Nov. 5-9, (ICE), Chiang Rai, Thailand. (2006).
27. Mazumder PM, Das S, Das S, Das MK. Cytotoxic activity of methanolic extracts of *Berberis aristata DC* and *Hemidesmus indicus* R.Br.in MCF7 Cell Line. Journal of Current Pharmaceutical Research. 01: 12-15, (2011).
28. Das S, Das MK, Mazumder PM, Das S, Basu SP. Cytotoxicity activity of methanolic extract of *Berberis aristata* DC on colon cancer. Global J. Pharmacol. 3: 137-140, (2009).
29. Porwal KP, Kumar K, Jain N, Pathak KA, Jain P. Evaluation of antitumour activity of *Berberis aristata DC.* Indian drugs. 47 (5):17-20, (2010).
30. Khan M, Giessrgl B, Vonach C, Madlener S, Prinz S, Herbaceck I, et al. Berberine and a *Berberis lycium* extract inactivate Cdc25A and induce a tubulin acetylation that correlate with HL-60 cell cycle inhibition

and apoptosis. Mut. Res. 683:123-130, (2010).
31. Rusia K, Srivastava SK. Antimicrobial activity of some Indian medicinal plants. Ind. J. Pharm. Sci. 50: 57-58, (1988).
32. Semwal BC, Gupta J, Singh S, Kumar Y, Giri M. Antihyperglycemic activity of root of *Berberis aristata* DC. in alloxan-induced diabetic rats. Int. J. Green Pharm. 3:259-262, (2009).
33. Singh J, Kakkar P. Anti hyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. J. Ethnopharmacol. 123(1): 22-26, (2009).
34. Gupta JK, Mishra P, Rani A, Mazumder PM. Blood glucose lowering potential of stem bark of *Berberis aristata* DC in alloxan-induced diabetic rats., Iranian Journal of Pharmacology & Therapeutics. 9:21-24, (2010).
35. Akhtar MS, Sajid SM, Akhtar MS. Hypoglycaemic effect of *Berberis aristata* root, its aqueous and methanolic extracts in normal and Alloxan induced diabetic rabbits. Pharmacology Online. 2:845-856, (2008).
36. Gulfraz M, Mehmood S, Ahmad A, Fatima N, Praveen Z, Williamson EM. Comparison of the antidiabetic activity of *Berberis lyceum* root extract and berberine in alloxan-induced diabetic rats. 22(9):1208-1212, (2008).
37. Ahmad A, Pandurangan A, Koul S, Sharma BM. Antidiabetic potential of *Berberis aristata* bark in alloxan induced diabetic rats. IJPSR. 3(11): 4425-4428, (2012).
38. Punitha ISR, Rajendran K, Shirwaikar A, Shiwaikar A. Alcoholic Stem Extract of *Coscinium fenestratum* Regulates Carbohydrate Metabolism and Improves Antioxidant Status in Streptozotocin-Nicotinamide Induced Diabetic Rats. *e*CAM.. 2(3): 375-381, (2005).
39. Shirwaikar A,Rajendran K, Punitha ISR. Antidiabetic activity of alcoholic stem extract of streptozotocin nicotinamide induced type 2 diabetic rats. J Ethanopharmacol. 97: 369-374, (2005).
40. Sack PB, Froehlich JL. Berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins. Infect Immun. 35(2):353-365, (1982).
41. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous Drugs of India. Academic Publishers: Calcutta. 2:508-674, (1958).
42. Razzaq FA, Khan RA, Feroz Z and Afroz S. Effect of *Berberis aristata* on lipid profile and coagulation parameters. African Journal of Pharmacy and Pharmacology. 5(7): 943 – 947, (2011).
43. Yogesh HS, Chandrashekhar VM, Katti HR, Ganapaty S, Raghavendra HL, Gowda GK, et al. Anti-osteoporotic activity of aqueous-methanol extract of *Berberis aristata* in ovariectomized rats. J Ethnopharmacol. 24(134(2)):334-8, (2011).
44. Ajmire PV. Effect of *Berberis aristata* DC. against dimethylnitrosamine induced liver cirrhosis in rat model. Journal of Pharmacy Research. 4(11), (2011).
45. Tiwari BK, Khosa RL. Evaluation of the hepatoprotective and antioxidant effect of *Berberis asiatica* against experimentally induced liver injury in rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2(1): 92-9, (2010).
46. Chand N, Durrani FR, Qureshi MS, Durrani Z. Role of *Berberis lycium* in reducing serum cholesterol in broilers. Asian-Australasian. J. Ani. Sci. 20(4):563-568, (2007).
47. Singh M, Srivastava S, Rawat AKS. Antimicrobial activities of Indian Berberis species. Fitoterapia. 78:574–576, (2007).
48. Sharifii A, Hassani B. Vacuum Drying of Barberry Fruit (*Berberis vulgaris*) and Selection of a Suitable Thin Layer Drying Model. Research Journal of Applied Sciences, Engineering and Technology. 5(5): 1668-1673, (2013).
49. Wongcome T, Panthon A, Jesadanont S, Kanjanapothi D, Taesotikul T, Lertprasertsuke N. Hypotensive effect and toxicology of the extract from *Coscinium fenestratum* (Gaertn.) Colebr. Journal of Ethnopharmacology. 111(3)3: 468–475, (2007).

50. Chitra K, Sujatha K, Dhanuskha SH, Mangathayaru K ,Vasantha J, Janani S, et al. Antinociceptive effects of *Coscinum fenestratum*(Gaertn.) on mouse formalin Test. Biomed.Res. 15:73-75, (2004).
51. Wattanathorn J, Uabundit N, Itarat W, Mucimapura S, Laopatarakasem P, Sripanidkulchai B. Neurotoxicity of *Coscinium fenestratum* stem, a medicinal plant used in traditional medicine. Food Chem Toxicol. 44(8):1327, (2006).
52. Parmar C, Kaushal MK. *Berberis aristata*.,Wild Fruits.,Kalyani Publishers New Delhi., India., 10-14, (1982).
53. Prajapati ND, Purohit SS, Sharma AK, Kumar TA. Handbook of medicinal plants. Agro bios, Jodhpur. 210, (2003).
54. Ali M, Sharma SK. Heterocyclic constituents from *Berberis lycium* roots. Ind. J. Hetero. Chemistry. 6**:**127-130, (1996).
55. Ali MN, Khan AA. Pharmacognostic studies of *Berberis lycium* Royle and its importance as a source of raw material for the manufacture of berberine in Pakistan. Pak J. Fore. 26, (1978)
56. Srivastava SK*,* Rawat AKS and Mehrotra S*.* Pharmacognostic Evaluation of the. Root of *Berberis asiatica*. Pharma. Biol. 42: 467-473, (2004).
57. Doymaz I, Ismail O. Drying characteristics of sweet cherry. J. Food Inst. Chem. E. 89(1): 31-38, (2010).
58. Srivastava SK, Khatoon S, Rawat AKS, Mehrotra S, Pushpangadan P. Pharmacognostic evaluation of the root of *Berberis aristata* DC. Natural Product Sciences*.* 7(4):102–106, (2001).
59. Mahmood A, Ahmad M, Jabeen A, Zafar M, Nadeem S. Pharmacognostic Studies of Some Indigenous Medicinal Plants of Pakistan. *Ethnobotanical Leaflets.* 9:1, (2005).
60. Sood P, Modgil R, Sood M. Physicochemical and nutritional evaluation of indigenous wild fruit *Kasmal, Berberis lycium* Royle. Indian Journal of Natural Products and Resources. 1(3) 362-366, (2010).
61. Pundarikakshudu, Dave HN. Simultaneous determination of Curcumin and berberine in their Pure Form and from the Combined Extracts of *Curcuma Longa* and *Berberis aristata.* International Journal of Applied Science and Engineering. 8(1):19-26, (2010).
62. Andola HC, Rawal RS, Rawat MSM, Bhatt ID, Purohit VK. Analysis of berberine content using HPTLC fingerprinting of root and bark of three Himalayan Berberis species. Asian J Biotech. 2:239–245, (2010).
63. Narasimhan S, Nair GM. Effect of auxins on berberine synthesis in cell suspension culture of *Coscinium fenestratum* (Gaertn.) Colebr.-A critically endangered medicinal liana of Western ghats. Indian J.Exp. Biol. 616-619, (2004a).
64. Narasimhan S, Nair GM. Release of berberine and its crystallization in liquid medium of cell suspension cultures of *Coscinium fenestratum* (Gaertn.) Colebr. Curr.Sci. 86:1369-1371, (2004b).
65. Rojsanga P and Gritsanapan W. Variation of Berberine content in *Cosinium fenestratum* Stem in Thailand market Mahidol University. Journal of Pharmaceutical Sciences. 32 (3-4): 66-70, (2005).
66. Balasubramani SP, Murugan R, Ravikumar K, Venkatasubramanian P. Development of ITS sequence based molecular marker to distinguish, *Tribulus terrestris* L. (Zygophyllaceae) from its adulterants. Fitoterapia. 81:503–508, (2010).
67. Wongbutdee J. Physiological effects of Berberine. Thai Pharm Health Sci J. 4:78–83, (2009).
68. Balasubramani SP, Venkatasubramanian P . Molecular Identification and Development of Nuclear DNA ITS Sequence Based Marker to Distinguish *Coscinium fenestratum* Gaertn. (Menispermaceae) from its Adulterants. Current Trends in Biotechnology and Pharmacy. 5(2):1163-1172, (2011).