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Pharmacognostic Evaluation of the Roots of *Berberis chitria* Lindl.

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Abstract – *Berberis chitria* (family Berberidaceae) has a close affinity with *B. aristata,* used in traditional systems of medicine as a drug '*Daruharidra*' for skin disease, jaundice, affection of eyes, and rheumatism. Keeping this in view, in the present study attempts have been made to identify marker characters of *B. chitria* in order to differentiate the two species. Some of the diagnostic features of the root are patches of pericyclic fibre, pitted sclerieds and berberine containing cells and heterocyclic medullary rays. Besides, the physicochemical characters such as total ash; acid insoluble ash; alcohol and water soluble extractive; tannins; sugar and starch percentages has shown variations. The percentage of berberine as berberine hydrochloride was also calculated through HPTLC densitometric method and it was found little higher than *B. aristata* and B. *asiatica* i.e. 3.16%. Thus, this species can be utilized as a possible substitute to *Daruharidra.*
Keywords – *Berberis chitria*, HPTLC, Daruharidra, substitute

Introduction

Berberis chitria Lindl. is an important medicinal plant belonging to family Berberidaceae. It is very common in different markets of India as an adulterant/substitute to 'Daruharidra' i.e. *B. aristata.* The roots are used for treating a variety of ailments such as eye and ear diseases, rheumatism, jaundice, diabetes, fever, stomach disorders, skin disease, malarial fever and as tonic etc. (Watt, 1883; Kirtikar and Basu, 1933; Chopra *et al.* 1958; Anonymous, 1988). Its use in the management of infected wounds has also been described in Ayurvedic classical texts (Sushrut Samhita, 1963).

The major alkaloid of the plant is berberine, which is known for its activity against cholera (Dutta and Panse, 1962), acute diarrhoea (Lahiri and Dutta, 1967), amoebiasis, latent malaria and for the treatment of oriental sore caused by *Leishmania tropica* (Anonymous, 1988).

Over exploitation of *B. aristata* by different pharmaceutical industries created scarcity of the material that opened new vistas to identify a possible substitute for this species. Although a detailed pharmacognostic study of *B. aristata* and *B. asiatica* is reported by Srivastava *et al.* (2001 and 2003), but till date no detailed pharmacognostic details are available on *B. chitria.* Hence, the present study has been undertaken, which may be useful to pharmaceutical industries for the authentication of the commercial samples and to explore the possibilities of using this species as a substitute of *B. aristata.*

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Experimental

The plant material was collected from the Dhanaulti (Uttaranchal) region [Sharad, LWG 221241, 1998] and the roots were preserved in 70% ethyl alcohol for histological studies. Microtome sections were cut and stained with safranin and fast green and photographed with Nikon F70X camera (Johansen, 1940). Physicochemical and phytochemical studies like, total ash, acid insoluble ash, tannins, and total alkaloid contents were carried out on the shade dried powdered material according to the recommended procedures (Peach and Tracy, 1955; Anonymous, 1965; Siwon *et al.* 1980; Anonymous, 1984). The behavior of the powdered drug with different chemical reagents were also studied as per methods described by Chase and Pratt (1949) and Kokoski *et al.* (1958).

Results and Discussion

A brief taxonomic description of the plant (Plate 1) – A shrub 1 - 2.5 m high; stem rough pale brown, terete; spines 1 to 1.9 cm long; leaves 4 in each whorl, obovate lanceolate, 4 - 8 × 2 - 3 cm entire or 3 - 9 spinose, reticulate on both the surfaces, dull green above, pale yellowish green beneath; inflorescence 10 - 20 flowered in lax corymbose

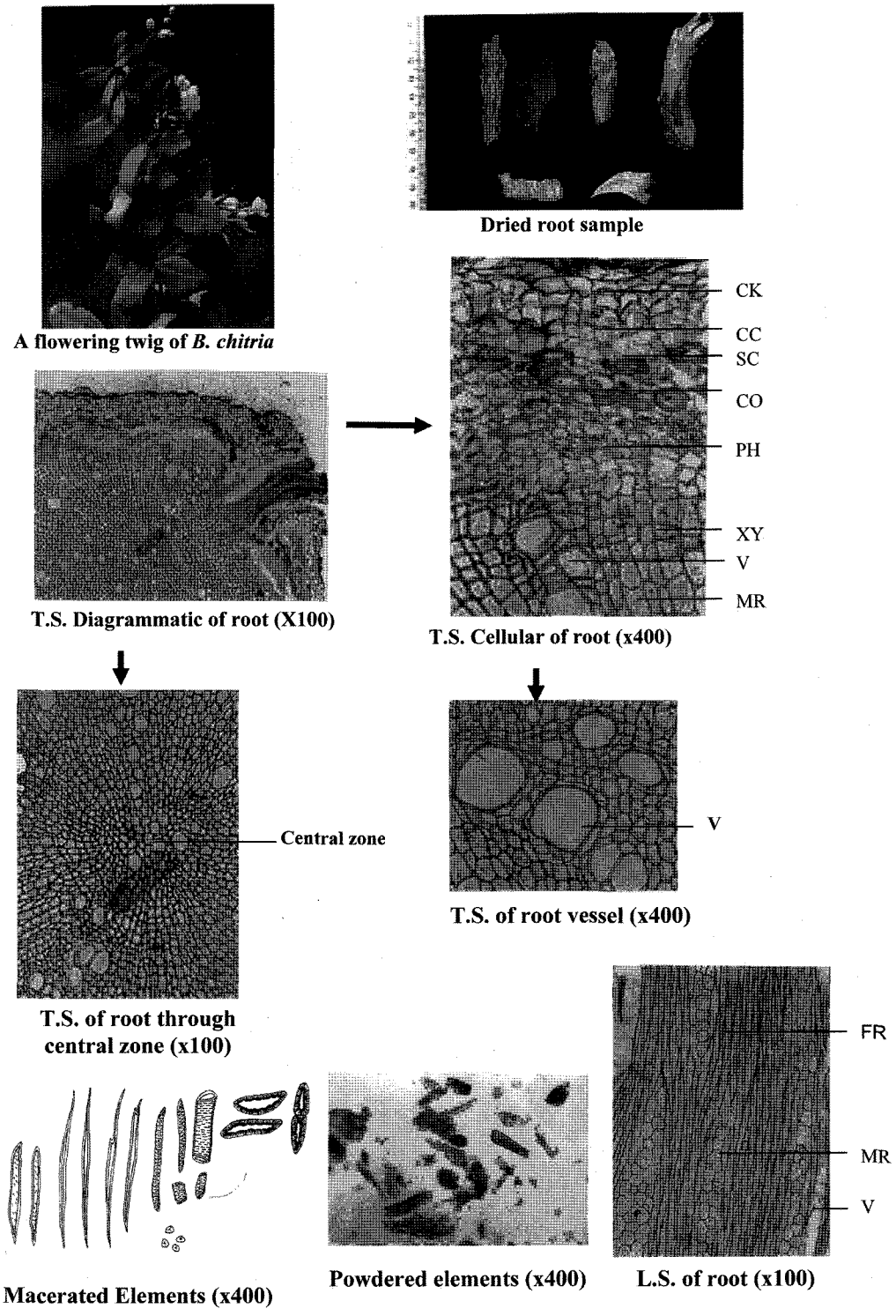

Plate 1. Microscopic characteristics of the roots of *B. chitria*. CC, cork cambium; CK, cork cells; CO, cortex; FR, fibre; MR, medullary ray; PH, phloem; SC, sclereids; V, vessels; XY, xylem.

racemes; flowers in groups of 3 - 5, glabrous rachis; bract 2 - 4 mm long; outer sepals unequal, oblong (lateral ones) or ovate (medium one); inner sepals larger; petals clawed, stamens 6; connectives capitate; anther linear; berries dark reddish brown, narrowly ovoid or ellipsoid, 12 × 4 - 6 mm.

Macroscopic charateristics of the root – Root cylindrical, hard, branched and gradually tapering, 3 to 8 cm in diameter often split longitudinally. Outer surface

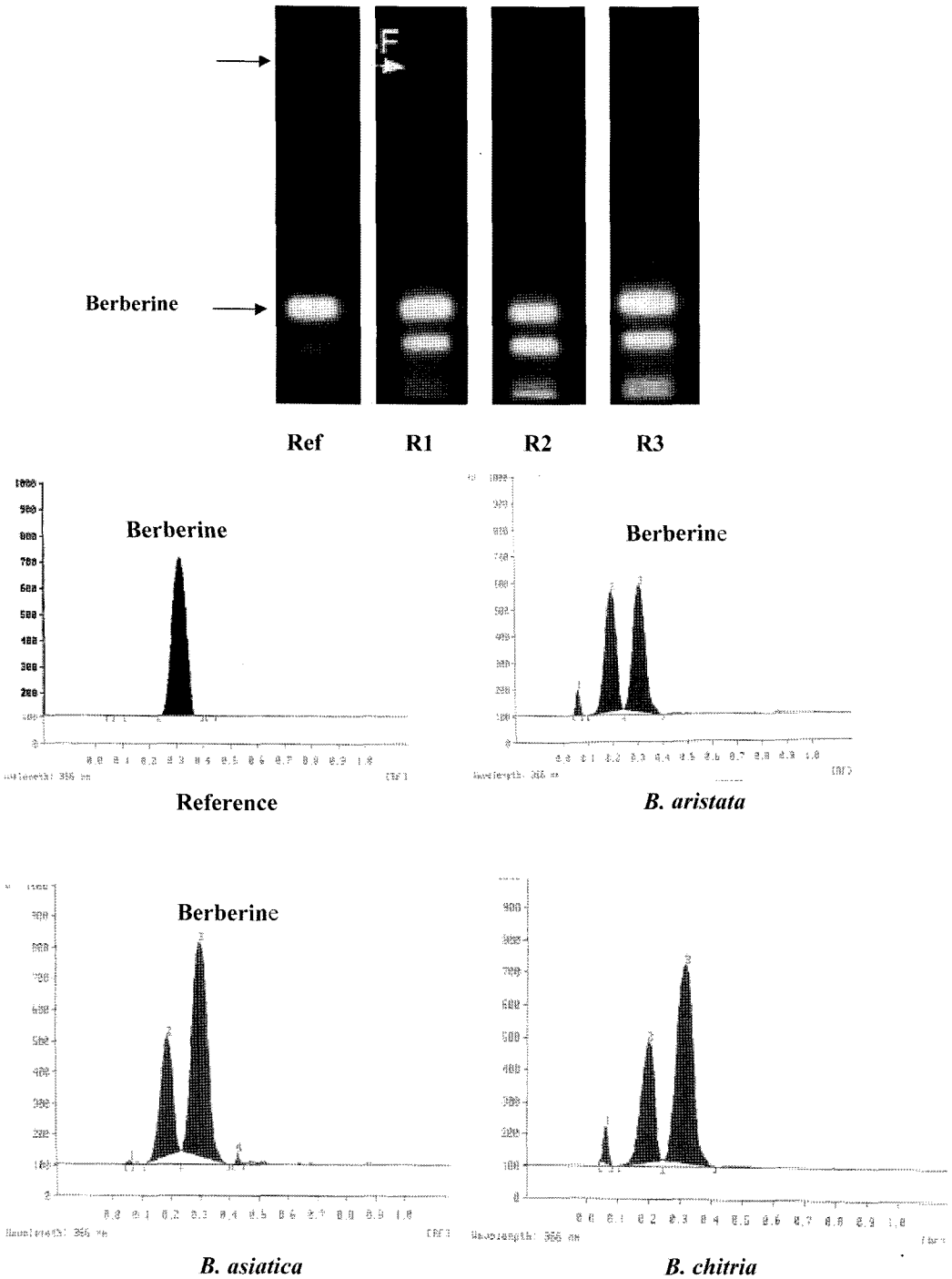

Plate 2. HPTLC profile of *Berberis chitria*, allied species and reference sample (Under UV- 366). ref, reference; R1, *B. aristata*; R2, *B. asiatica*; R3, *B. chitria*.

light brown in colour, smooth or longitudinally fissured in the case of thicker roots. Inner surface of the root is bright yellow in colour, distinctly radiated with narrow medullary rays. The bark is upto 5 mm thick, externally fissured and internally smooth; closely striated, having shining light brown colour (Plate 1). Fracture hard; odour faintly phenolic; taste very bitter.

Microscopic characteristics of the root – The transverse section is circular and regular in outline. The cork layers which forms the outer most zone are thin walled, dark brown in colour and composed of 8 to 10 layers of rectangular cork cells, followed by 1 or 2 layers of cork

cambium. Cortical zone composed of 18 to 20 layered rectangular parenchymatous cells. Sclerieds in 2 to 4 groups are frequently present in cortical zone. Pericycle sclerenchymatous and discontinuous and are comparatively lesser in number than other species of *Berberis*. Some cells are filled with starch and tannins. Presence of berberine containing cells is clearly visible in cork and cortical region. Phloem 3 to 5 cells deep and consists of sieve tubes, companion calls and phloem parenchyma. In between xylem and phloem, single layered cambium is present. Medullary rays thin walled, parenchymatous, heterogenous, 1 to 3 celled wide and 2 to 4 cells broad, with pitted cells and slightly radially elongated and are filled with starch and alkaloids. Vessels solitary sometimes in-groups of 2 or 3, associated with tracheids and fibres (Plate 1).

In transverse longitudinal section, rays are heterogenous, pitted filled with starch grains and alkaloidal contents (Plate 1).

On maceration, the vessels (160 × 19 μm) with annular, reticulate, spiral, scalariform thickening some tailed vessels are also observed. The tracheids with bordered pits measuring 348 × 12 μm and tracheidal fibres 477 × 10 μm and simple fibres 761 × 11 μm (Plate 1).

Study of powdered root – On microscopic examination, the powder shows the fragments of rectangular cork cells, cortical parenchymatous cells, pericyclic fibres, stone cells, spiral, pitted and reticulate vessels, tracheids with bordered pits, single or compound starch grains and pitted parenchymatous cells with yellow content (berberine alkaloid) (Plate 1).

Powder when treated with 1N-HCl and nitrocellulose in amyl acetate emits fluorescence yellow colour under UV 254.

Physico-chemical studies – Air-dried material was used for quantitative determination of different physico-chemical values and other phytochemical work. The values obtained are presented in Fig. 1.

Two gram of dried root powder was extracted in Soxhlet with hexane, chloroform, acetone, alcohol and water successively and percentage was calculated (Fig. 2) and tested for different constituents namely steroids and triterpenoids (LB test), flavonoids (Shinodas test), alkaloids (Mayer's reagent), tannins (ferric chloride test) and sugar (Fehling solution test). The study revealed that the triterpenoids are present in hexane and chloroform soluble parts, tannin is only in water soluble part while the resin present in acetone and alkaloids in chloroform, acetone, alcohol and water soluble parts.

HPTLC studies (Plate 2) – A densitometric HPTLC

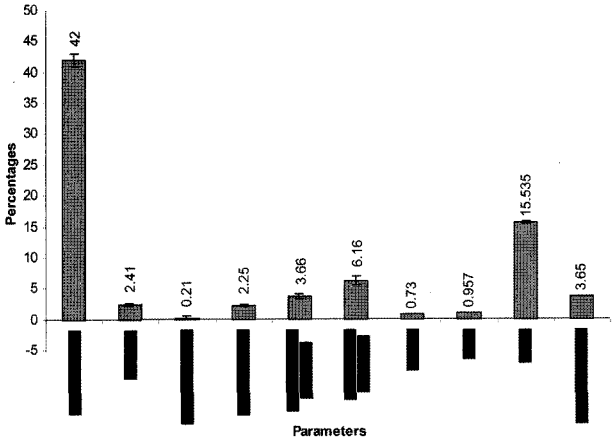

Fig. 1. Physico-chemical values of *B. chitria* root.

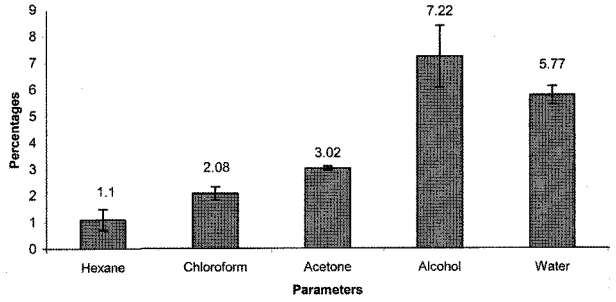

Fig. 2. Successive soxhlet extractive values of *B. chitria* root.

analysis was also performed for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug. In addition the study also explores the possibilities for using this species as a substitute of *B. aristata*. 1 gm powdered root was refluxed for 5 minutes on water bath with 5 ml methanol consecutively three times, filtered and filtrate taken as test solution along with reference berberine (7 μl of each) and was applied on HPTLC precoated silicagel G60 F_{254} Merck glass plates of 20 × 10 cm with the help of Camag Linomat-IV applicator and eluted the plate to a distance of 6.20 cm at room temperature (19 °C) in solvent system *n*-propanol : water : formic acid (90:8.0:0.4). The bands in the sample are obtained at R_fs 0.06, 0.20 and 0.32, which can be used as identifying markers. Berberine was identified at R_f 0.32 (Plate 2).

From the ongoing descriptions it is revealed that *B. chitria* could be identified from the presence of patches of pericyclic fibre, pitted sclerieds, berberine containing cells and heterocyclic medullary rays. On the contrary,

the chemical profile of all the three species i.e. *B. aristata*, *B. asiatica* and *B. chitria* are almost identical. Therefore *B. chitria* can be used as a substitute of *B. aristata*.

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