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Pharmacognostic Evaluation of the Roots of *Berberis tinctoria* Lesch.

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Abstract – *Berberis tinctoria* (Berberidaceae), commonly known as Nilgiri Barberry is a common allied species to *B. aristata*, used in India Traditional Systems of Medicine by the name of '*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. tinctoria*. Some of the diagnostic features of the root are patches of pericyclic fibre, pitted sclerieds, crystals, 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 also shown some variations. The percentage of berberine as berberine hydrochloride was also calculated through HPTLC densitometric method and it was found almost similar to *B. aristata, B. asiatica* and *B. chitria* i.e. 3.36%. Thus it can be explored as a possible source of substitute to *B. aristata.*

Keywords – *Berberis tinctoria*, HPTLC, Daruharidra, Substitute

Introduction

Berberis tinctoria Lesch., common Nilgiri Barberry is an important medicinal plant belonging to family Berberidaceae. It is very common in Nilgiri hills of Tamilnadu and sold in market as an adulterant/substitute to '*Daruharidra*' i.e. *B. aristata*. The root 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 Berberis species is berberine (Bhakuni *et al.*, 1968), which is known for its activity against cholera (Dutta & Panse, 1962), acute diarrhoea (Lahiri & Dutta, 1967), amoebiasis, latent malaria and for the treatment of oriental sore caused by *Leishmania tropica* (Anonymous, 1988). However, antiviral activity (Vijayan *et al.* 2004), Hepato Protective and Antioxidant Role of *Berberis tinctoria* Lesch (Murugesh *et al.*, 2005) has also been established.

Threat to *B. aristata* by different pharmaceutical industries created scarcity of raw material. This opens new vistas to exploit other *Berberis* species as a possible substitute for *B. aristata*. Although a detailed pharmacognostic study of *B. aristata, B. asiatica* and *B. chitria* is reported by Srivastava *et al.* (2001, 2004 & 2006), but till date no detailed pharmacognostic details are available on *B. tinctoria*. 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*.

Experimental

The plant material was collected from the Pykara of Nilgiri Hills, Ootacamund (Tamilnadu) [Sharad LWG 221324, 2000] 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). Physico-chemical and phytochemical studies like, total ash, acid insoluble ash, tannins and total alkaloids were calculated from 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 was also studied as per methods described by Chase and Pratt (1949) and Kokoski *et al.* (1958).

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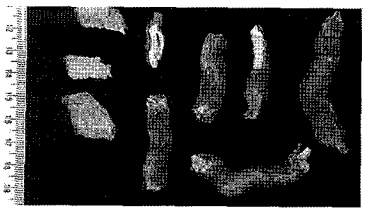
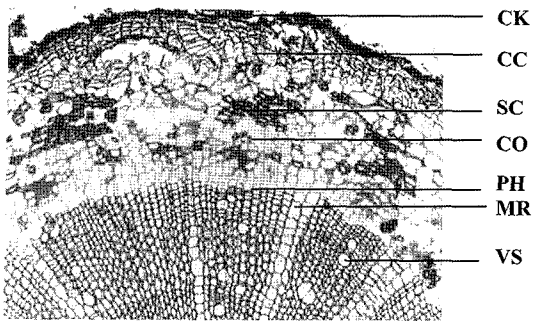
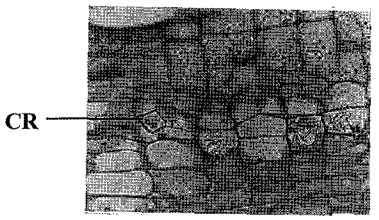
1. A Fruiting Twig



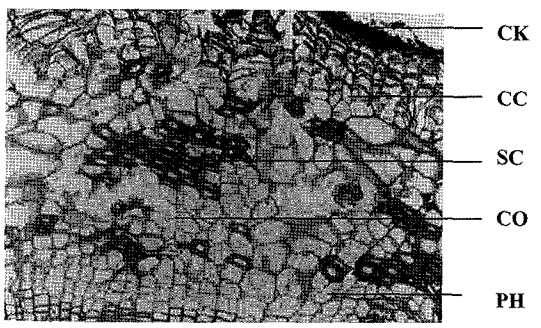
2. Dried Roots



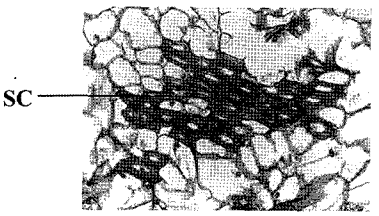
3. T.S. Cellular of Root (x25)



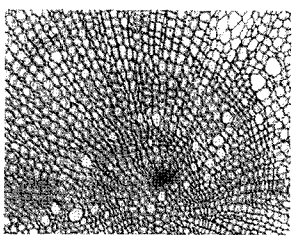
6. T.S. through cortical zone showing crystals (x25)



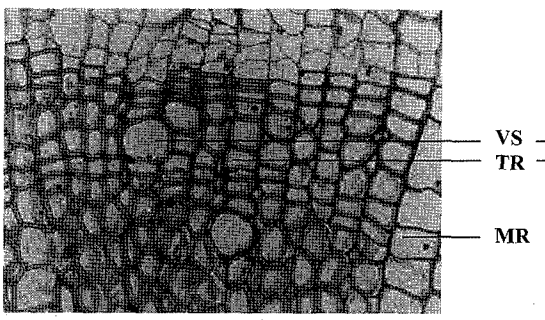
4. T.S. Cellular of Root showing cortical zone (x40)



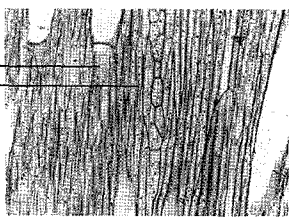
7. T.S. through cortical zone showing sclerenchymatous patches(x25)



8. T.S. Root through central region (x25)



5. T.S. Cellular of Root showing vascular zone(x40)



9. L.S. Root showing xylem elements (x40)

Plate. 1. Macroscopic & Microscopic characters of *Berberis tinctoria* root.
BC, Berberine containing cell; CC, Cork cambium; CK, Cork cells; CO, Cortex; CR, Crystals; MR, Medullary ray; PH, Phloem; SC, Sclereids; V, Vessels; XY, Xylem. Ref, Reference; BT, *Berberis tinctoria.*

Results and Discussion

A Brief Taxonomic Description of the plant – A shrub, but very variable in size and form; in the open often only 2 or 3 feet high, but in a shola sometimes reaching 15 feet with stem as thick as one's arm and long

scandent branches bearing numerous slender leafy twigs; wood very tough, bright yellow in color. Leafy twigs green or purple, grooved and angular, studded with triple spines in the axils of which are tufts of leaves. Leaves when young purplish, obovate, entire or with a few spiny teeth, glabrous, 1 to 2 1/2 inches. Racemes of flowers drooping, sometimes branched; pedicels slender, red 1/2-inch; petals notched. Berry sausage-shaped when young, eventually top-shaped, 1/3 by 1/6 inch, purplish red, turning to a dark-blue with glaucous bloom, with the dry style and large round stigma still attached.

Distinguished from *B. aristata* DC by the slender drooping pedicels and the shape of the fruit.

Macroscopic characters of the root – Roots are woody, grayish white, cylindrical, with smooth surface and thin brittle bark. Cut surface deep yellow. The main features distinguishing it from other species are a smooth surface of grayish white colour and a deep yellow wood. Fracture hard; odour phenolic; taste bitter (Plate-1).

Microscopic characters of the root – The transverse section is circular and regular in outline. The layers of cork, which forms the outer most zones is thin walled, suberized, dark brown in colour and composed of 8 to11 layers rectangular cork cells. Following the cork layers, 2 or 3 layers of cork cambium is present. Cortical zone is secondary in nature and composed of 17 to 22 layers of rectangular parenchymatous cells. Sclerieds are frequently present in cortical zone and are of 2 to 4 in groups. Frequent patches of sclerenchymatous pericyclic fibres have also been observed in this region. Cells filled with tannins and starch grains are also seen. Pericyclic fibre patches are comparatively lesser in number than other species. Presences of berberine containing cells are clearly visible following the cortical zone is secondary phloem region, which is 4 to 6, cells deep. Secondary xylem contains vessels very distinct distributed among xylem fibres, solitary or sometimes in-groups of 2 or 3; tracheids and fibres are present. Vessels and tracheids occupy central zone (Plate-1).

In transverse longitudinal section, rays are heterogenous, 2 to 5 cells broad, pitted and filled with starch grains and alkaloidal content (Plate-1).

Measurement of macerated elements – On maceration, the vessels (132.118 × 22.328 μm) with annular, reticulate, spiral, scalariform and some with simple pits are observed and tailed vessels are also observed. The trachieds with bordered pits measuring 254.836 × 12.206 μm and tracheidal fibres 308.924 × 12.518μm and simple fibres 506.809× 10.284 μm are clearly discernible.

Study of powder – On microscopic examination the powder revealed the presence of fragments of rectangular cork cells, cortical parenchymatous cells, pericyclic fibres, stone cells, spiral, pitted and reticulate vessels, tracheids with bordered pits and starch in single or compound grains.

The behavior of the powdered drug with different chemical reagents was also studied as per standard methods. Powder when treated with 1N-HCl and nitrocellulose in amyl acetate emits fluorescence yellow colour under UV 254 (Table-1).

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

Table 1. Florescence powder study of *B. tinctoria* root

S. No.	treatment	day light	UV-254 nm	UV-366 nm.
1.	Powder (P) as such	yellowish brown	yellowish brown	brownish yellow
2.	P + Nitro-cellulose in amyl acetate	yellowish dark brown	yellowish brown	brownish yellow
3.	P + N. NaOH in water	brown	fl. greenish brown	black
4.	P + 1N NaOH + Nitro-cellulose in acetate	brown	brown with greenish tinge	reddish yellow
5.	P + 1N HCl + Nitro-cellulose in amyl acetate	brown	fl. yellowish green	reddish yellow
6.	P + 1N NaOH in Methanol	brown	brown with greenish tinge	black
7.	P + 50% KOH	brown	brown with greenish tinge	black
8.	P 1N HCl	greenish yellow	brown with greenish tinge	black
9.	P + 50% H_2SO_4	greenish yellow	yellowish green	black
10.	P + 50% HNO_3	red	dark brown with greenish tinge	black
11.	P + Conc. HNO_3	yellowish red	fl. green	black
12.	P + Acetic acid	yellowish brown	fl. green	black
13.	P + Conc. H_2SO_4	black	black	black
14.	P + Iodine water	dark brown	brownish with greenish tinge	reddish brown

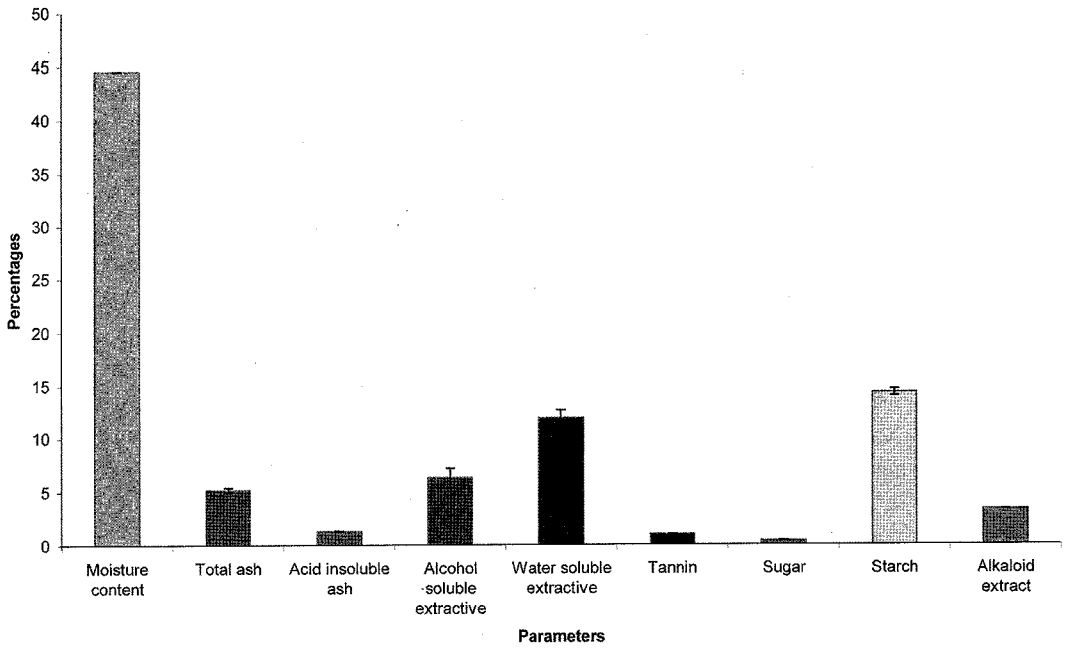


Fig. 1. Quantitative physico-chemical analysis of *B. tinctoria* root.

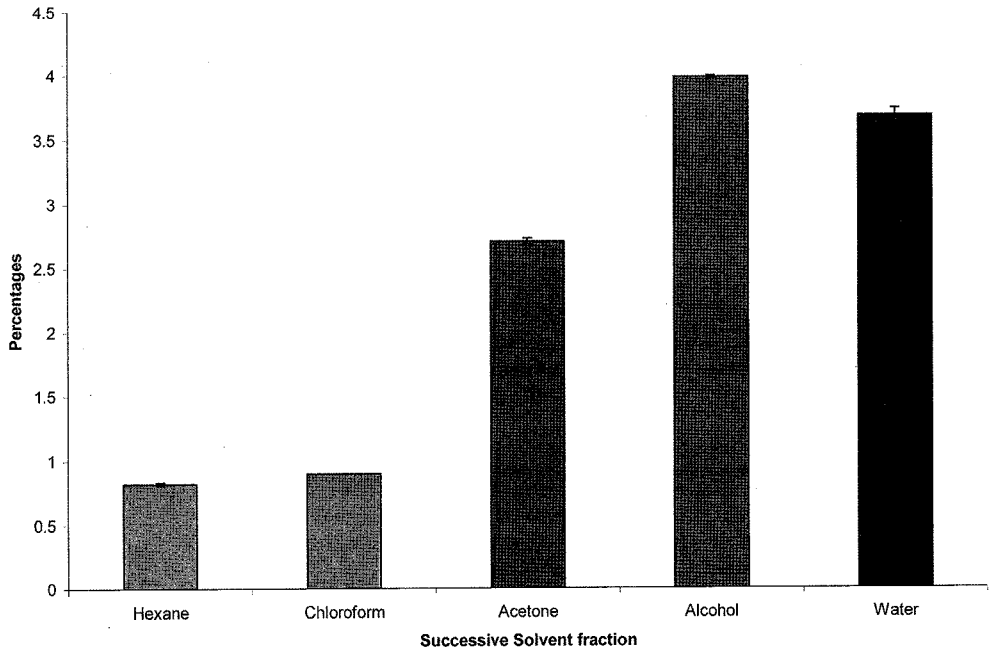


Fig. 2. Successive soxhlet extractive values of *B. tinctoria* root.

2 gm 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. (Table-2)

HPTLC studies (Plate-2) – A densitometric HPTLC 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 this study also explores the possibilities

Table 2. Phytochemical screening of the successive solvent extractives of *B. tinctoria* root

extractive	triterpenoids & steroids	saponins	flavanoids	tannins	reducing sugars	resins	glycosides	alkaloids
hexane	+	–	–	–	–	–	–	–
chloroform	+	–	–	–	–	–	–	+
acetone	+	–	–	–	–	+	–	+
alcohol	–	–	–	–	–	–	–	+
water	–	–	–	+	–	–	–	+

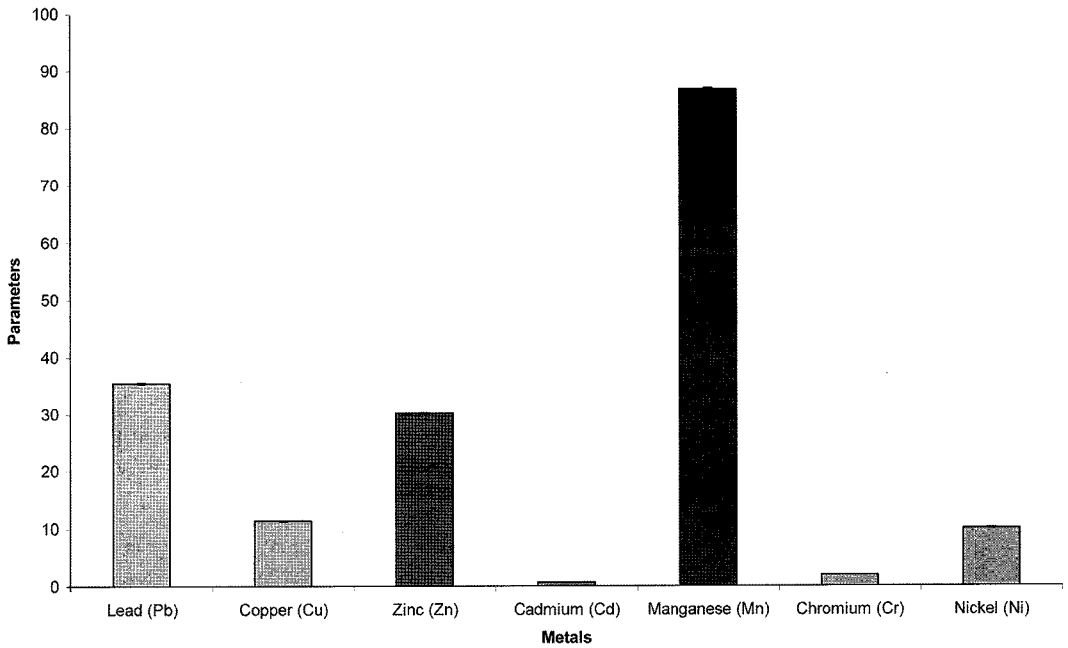


Fig. 3. Heavy metal analysis of *B. tinctoria* root.

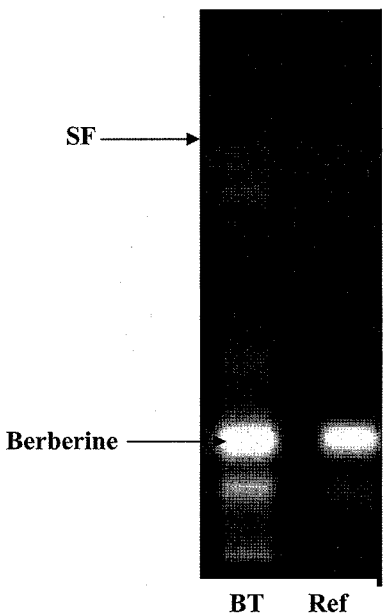


Fig. 4 HPTLC profile of *B. tinctoria* root and Berberine reference. Ref, Reference; BT, *Berberis tinctoria.*

for using this species as a substitute of *B. aristata* on the basis of its active secondary metabolite *i.e.*, berberine. 1 gm powdered root was refluxed for 5 minutes on water bath with 5 ml methanol consequently 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 0.17 and 0.30, which can be used as identifying markers. Berberine was identified at R_f 0.30 (Fig. 4 & 5).

Heavy metal studies – A heavy metal analysis was also done to check the quality of toxic metals in the root (Fig. 3).

Conclusion

From the ongoing descriptions it is revealed that *B.*

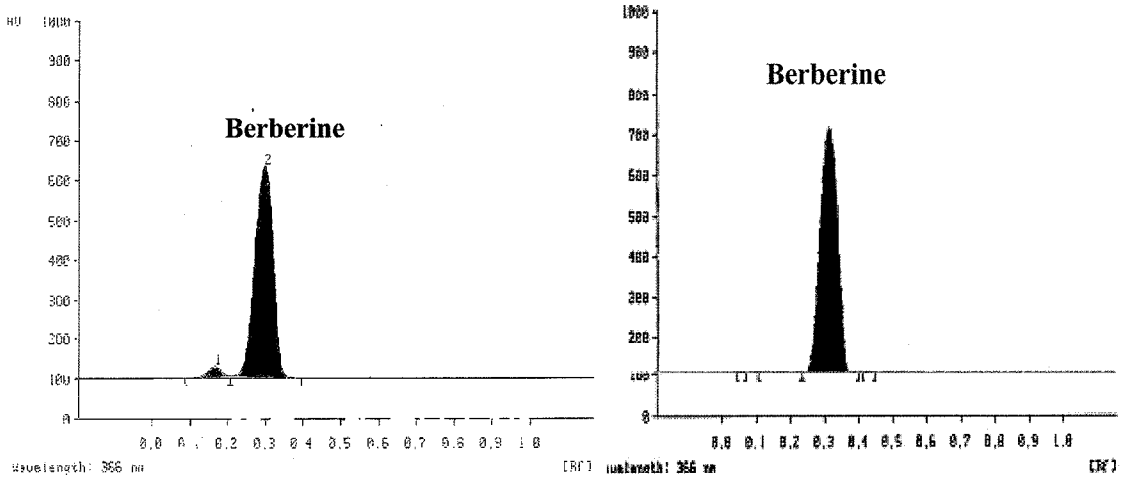


Fig. 5. Densitometric Chromatogram of *B. tinctoria* root and Berberine reference.

tinctoria could be identified on the basis 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 with *B. tinctoria* (Srivastava *et al.* 2001; 2004 & 2006) Therefore *B. tinctoria* can be explored as a substitute of *B. aristata.*

Acknowledgements

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