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Comparative pharmacognostical studies of three *Mahonia* species: Exploring the possibilities as a substitute for the Ayurvedic drug "Daruharidra"

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Mahonia species (Family: Berberidaceae) is a well-known plant used in traditional systems of medicine for the treatment of fever, cold, jaundice, diarrhoea, dysentery, dermatitis and eczema and for postnatal treatment. The present communication deals with comparative pharmacognostical studies and HPTLC quantification of a benzylisoquinoline alkaloid, berberine, of three *Mahonia* species, viz., *Mahonia leschenaultii* (Wallich *ex* Wight & Arn.) Takeda (ML), *Mahonia napaulensis* DC. (MN) and *Mahonia borealis* Takeda (MB). The macroscopic examination showed characteristic differences in leaf, inflorescence and fruit of the three species. The pharmacognostical parameters, viz., moisture content, ash and extractive values of samples were found to be within the limits of standard. The phytochemical evaluation of metabolites through spectroscopy reveals the presence of flavonoids, phenolics, starch, sugar and tannin and the former was found to be the highest (0.45%) among all. HPTLC quantification showed that the maximum content of berberine was found in *Mahonia leschenaultia* i.e., 0.197%±0.01 dry wt. basis. The study explores the possibilities of *Mahonia* species as a substitute of the Ayurvedic drug "Daruharidra" i.e., *Berberis aristata* and will also aid in quality control of products containing "Daruharidra".

Keywords: Berberine, HPTLC, *Mahonia borealis*, *Mahonia leschenaultii*, *Mahonia napaulensis*
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Mahonia genus (Family: Berberidaceae) includes about 109 species of evergreen shrubs[1]native to Eastern Asia. In India, it is found in Kerala, Tamil Nadu, Karnataka (Nilgiri and Pulney hills), Uttarakhand, Himachal Pradesh, Jammu & Kashmir (Western Himalayas) at an altitude of 6000 feet[2,3]. About 13 species of *Mahonia* are reported from India[4] and is closely related to genera *Berberis*, often included within it by some botanists. However, *Mahonia* species are differentiated from *Berberis* by their large, pinnate leaves (10-15 cm long) with 5-15 leaflets and long inflorescence (9-28 cm). *Mahonia* species are well-known medicinal plants and are widely used in folk medicine for the treatment of a variety of ailments[5]. *Mahonia leschenaultia* is a shrub locally called "Thovari" by the Todas tribes of the Nilgiris, "Todas plant" and holy-leaved berry. A paste of stem bark is used for the treatment of fever, cold and jaundice and for postnatal treatment[6]. *Mahonia borealis* commonly known as Gurang, has edible fruits and the roots are used for the treatment of diarrhoea and dysentery. Root and stem are also used for yielding a natural dye[3,5]. *Mahonia napaulensis*, commonly known as "Jamanemandro" in Nepali and "Michiki swan" in Newari, is traditionally used for the treatment of skin diseases such as psoriasis, dermatitis and eczema[7]. Besides this, a wide range of pharmacological activities have been reported for *Mahonia* species, i.e., anticancer, antibacterial, antifungal, antioxidant, antiproliferative and anti-inflammatory[8-11].

The roots of *Mahonia* species are rich in alkaloids like berberine, neprotine, barbamine, oxyacanthine, tetrahydro berberine, columbamine, coptisine, palmatine and jatrorrhizine[12-16]. The major alkaloid berberine exhibits a wide range of biological activities viz., anti-proliferative, anti-migratory[17], antimicrobial[18], antiprotozoal, antiplatelet[19] and anti-diabetic[20]. The present study deals with comparative morpho-anatomical studies, pharmacognosy and HPTLC analysis of three *Mahonia* species to explore the possibilities for using these *Mahonia* species as potential substitute for *Berberis aristata*. This study will also aid in authentication of raw materials for maintaining the batch-to-batch consistency in raw materials and/or formulations containing *Mahonia* species.

Materials and Methods

Chemicals and reagents

HPTLC pre-coated silica gel 60 GF_{254} (10×10 cm, 0.2 mm thick) plates were purchased from Merck

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(Germany). Berberine (> 98%) was procured from Sigma Aldrich (USA). All the other chemicals and reagents were of analytical grade and purchased from Thermo Fisher.

Plant materials

The roots of three *Mahonia* species, viz., *Mahonia leschenaultia* (ML), *Mahonia napaulensis* (MN) and *Mahonia borealis* (MB) were collected from the Nilgiri hills of Ootacamund, Tamil Nadu (India). The GPS coordinates are 11˚26'00" N, 76˚35'26" E at an elevation of 2076 m. The collected samples were authenticated by Dr. Sharad Srivastava (Sr. Principal Scientist, Pharmacognosy Division, CSIR-NBRI; Lucknow, Uttar Pradesh, India). The herbarium specimen was prepared and deposited in the institute's herbarium repository with voucher numbers (305481, ML; 305482, MN and 305483, MB). The collected roots were washed, chopped, shade dried (25˚C±3) and pulverised to a coarse powder (40 mesh) for further study.

Morphological and anatomical analysis

Morphological aspects such as colour, texture, fracture, taste and odour were examined. For the anatomical characterisation, root samples were fixed in FAA 70[21] solution and preserved in 70% ethyl alcohol[22]. The transverse section of the root was done by rotary microtome and, stained using astra blue and/or fuchsine dye[23]. Then the section was mounted on a glass slide with Canada balsam as a fixative. The slides were analysed and photomicrograph was taken by a digital microscope, Nikon, Japan (Model-Eclipse Ci).

Physicochemical and phytochemical evaluation

The various physicochemical parameters, viz., moisture content, ash values (total ash, acid insoluble and water soluble ash) and extractive values (hexane, alcohol and water soluble) were estimated as per the standard protocol of the Ayurvedic Pharmacopoeia of India[24]. The phytochemicals like sugar and starch[25], phenolic[26], flavonoid[27] and tannin[28] were estimated through spectrophotometric method.

HPTLC analysis

Preparation of plant extract

The crude powder (2 g) of each sample was subjected to cold extraction with HPLC grade methanol (4×50 mL).Continuous shaking was done with rotary shaker for 6-8 h and then allowed to stand at room temperature (25˚C±2) for 18 h, the process was repeated for three days. The extract was filtered and the pooled filtrate was concentrated in arotatory evaporator (Buchi, Switzerland) with constant pressure (40 mbar) and temperature (20˚C±2). Finally, the concentrated extract was lyophilized (Labconco, USA) toa solid residue and stored at 4˚C for analytical studies.

Preparation of stock and working solutions

The stock solution of berberine was freshly prepared by dissolving 1 mg in HPLC grade methanol (1 mL) and was stored at 4˚C until analysis. Prior to HPTLC analysis, aliquots of the stock solution (standard) were diluted to get a working solution of 0.1 mg ml^{-1}. Samples were prepared by dissolving 1 mg of lyophilized methanolic extract in methanol to obtain a working concentration of 1 mg mL^{-1}. Working dilutions of standards and samples were filtered through a 0.45 mm Millipore membrane filter (Pall, USA).

HPTLC instrumentation and chromatographic condition

A CAMAG Linomat V automated thin layer chromatography (TLC) sample applicator (CAMAG ATS 4) was used to dispense the aliquot of working solution of standards and plant samples. The chromatogram was developed in the CAMAG twin through glass chamber with humidity control (CAMAG ADC 2). The slit dimension was 5×0.35 mm, scanning speed was 100 mm/s and scanning of bands was performed using CAMAG TLC Scanner III in ultraviolet (UV) absorbance mode by CAMAG vision CATS IV software (version 3.2.1) under absorbance-reflectance mode.

Separation and development of the HPTLC chromatogram for quantification of berberine was performed on a Merck TLC aluminium pre-coated silica gel 60 GF_{254} (10×10 cm, 0.2 mm thick) plate. Aliquots of each extract (standard and samples) were applied to the plate as 8 mm wide bands, positioned 10 mm above the bottom and 15 mm from the side of the plate with the help of nitrogen flow. Following sample applications, the TLC plate was developed in a CAMAG developing chamber, preconditioned and optimized for 20 min at room temperature (25°C±2) and relative humidity 55%±2 for better resolution. The plate was allowed to develop up to 80 mm from the point of application (total length run by the mobile phase). After development, plate was dried within the developing chamber and scanning was performed at λ_{max} of 350 nm in absorbance-reflectance mode.

Berberine was quantified based on area vs. concentration of a standard marker, and results are expressed on dry weight basis of crude plant material.

Statistical analyses

The data were recorded as mean ± standard deviations of three replicates and one-way analysis of variance (ANOVA) was used to calculate the level of significance (XLSTAT, 2010, Microsoft Corporation, USA).

Results

Morphology and anatomy

Mahonia leschenaultii leaves are imparipinnate, alternate, opposite, petiolate, leaflets are 7 to 13 in pairs, lateral leaflets are usually sessile and terminal one is sessile or petiolulate, oblong or ovate, margin and apex is prominently spiny dentate. Inflorescence is terminal raceme (approx. 26 cm long) with numerous small yellow flowers (Fig. 1a). Fruits are berry, 7- 8 mm long, 4 -5 mm broad, ellipsoid-globose and purple coloured. Roots are long, woody, cylindrical, outer brownish thin brittle bark and internally yellowish (Fig. 1b).

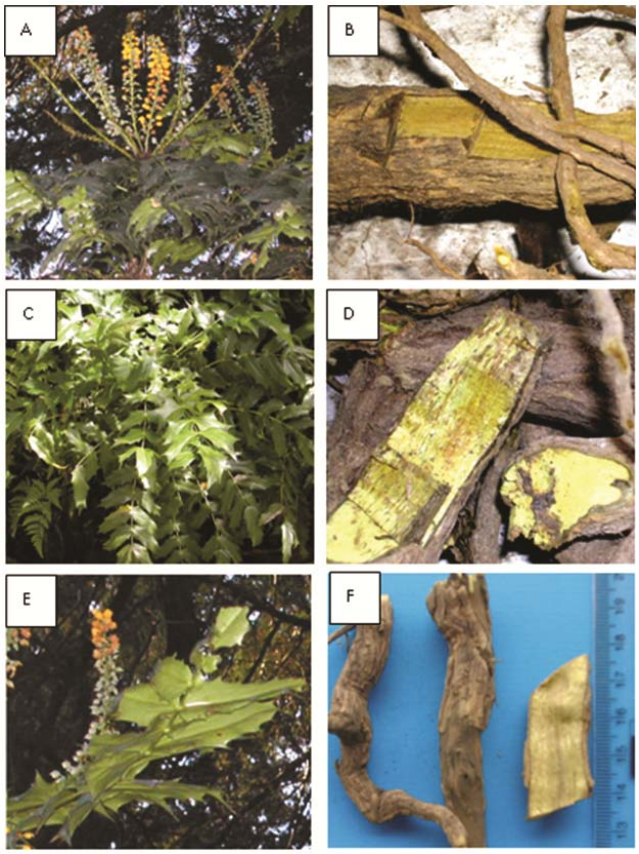

Fig. 1 — Morphological characteristics of *Mahonia* species. Morphological appearance of *Mahonia leschenaultia* plant (a) and root (b); *Mahonia napaulensis* plant (c) and root (d); *Mahonia borealis* flowering twig (e) and root (f).

Leaves of *Mahonia borealis* are large, imparipinnate, opposite, 5 to 9 pairs leaflet with terminal one. Lower leaflets are small in comparison to upper, sessile or petiolulate, margin and apex are spiny dentate and prominent veins (netted) on both sides (Fig. 1c). Fruit are berry, 7 - 8 mm long, 4 - 5 mm broad and globose or ovoid. Inflorescence and roots characteristics are almost similar to ML (Fig. 1d).

Mahonia napaulensis leaves are also large, dark green, imparipinnate, opposite, leaflets are elliptic to ovate, sessile or petiolulate, 4 to 11 pairs leaflet with terminal one. Margin and apex of leaflets are prominently spiny dentate, terminal leaflets are larger in comparison of lateral. Inflorescence is fascicled racemes approximately 20 cm long, with many tiny yellow flowers (Fig. 1e). Fruits are berries, obovoid to subglobose and purplish black. Morphology of root is almost similar to ML (Fig. 1f). Odour of roots is characteristic and bitter in taste.

The anatomical descriptions of the transverse section (mature roots) of three *Mahonia* species along with *Berberis aristata* are mentioned in Table 1. However, differences are observed in the arrangement of sclereids in cortical region. The cortical region is compressed due to secondary growth and is made up of parenchymatous cells, containing sclereids (Fig. 2a, f, k). Sclereids are present mainly in groups or sometime solitary. In ML sclereids are present in group of 6-9 (Fig. 2b); MN contains mainly solitary sclereids, but sometime in groups of 2 or 3 (Fig. 2g). On the other hand, sclereids in MB mainly found in groups of 6-11 and rarely solitary (Fig. 2 l). In longitudinal section, medullary rays are heterogeneous and arrangements are horizontal, along with vessels and fibers; tracheids are found along with the xylem vessels (Fig. 2e, j, n).

Pharmacognostical parameters

The root samples of different *Mahonia* species were collected as per good collection practice guidelines (GCP), therefore the foreign organic matter was nil. The moisture content among all 3 species varied from 6.32 - 6.51%. The total ash value was found to be higher than the water-soluble and acid-insoluble ash. The samples were extracted with different solvents from low to high polarity and hexane-soluble extractives were found lowest in all

Table 1 —Comparative morpho-anatomical features of root of *Mahonia* species.

Characters	*Mahonia leschenaultii* (Wallich *ex* Wight & Arn.) Takeda	*Mahonia napaulensis* DC.	*Mahonia borealis* Takeda	*Berberis aristata* DC. (Srivastava *et al*., 2001)
Macroscopic characters	Woody, cylindrical, with smooth surface and thin brittle bark. Outer brownish and internal bright yellow wood	Woody, cylindrical, brittle bark, smooth surface of greyish brown and internal yellowish	Woody, cylindrical, rough friable bark, internal smooth and bright yellow	Woody, yellowish brown, cylindrical, rough covered with thin brittle bark
Cork	Cork was formed by multiple rows of compactly arranged thick-walled, suberized rectangular cells	Outermost layer is formed of 16-20 layers of suberized cork cells	Cork is made up of 14-20 layered compactly arranged suberized cells	Cork is composed of 13-15 layered rectangular cells
Secondary cortex	Broad, made up of parenchymatous rounded or oval cells, multilayered. Thick-walled, sclereids were present in isolation or in groups of 6-9 throughout the cortex	Broad, many layers of parenchymatous rounded or oval cells. Thick-walled sclereids were mainly present in isolated form or in groups of 2-3	Broad, multilayered parenchymatous cells. Sclereids were present in groups of 5-12 and rarely isolated	Broad, multilayered rectangular parenchymatous cells. Sclereids are mostly solitary or in group of 2–5. Some cells are filled by yellow colored alkaloid (Berberine).
Secondary Tissue	Secondary phloem is lying underneath the wider cortex; secondary xylem is transverse with uniseriate or biseriate medullary rays in continuation with xylem, alternating with sieve tubes, parenchyma and xylem fibers, xylem vessels are isolated or in groups of 3–7 (Fig. 2c)	Secondary phloem is lying underneath the cortex; multiseriate medullary rays containing starch grains are present in continuation with xylem vessels. Xylem vessels are isolated or in groups of 2–5 (Fig. 2h)	Secondary phloem is present below the cortex; secondary xylem is transverse by mainly uniseriate medullary rays and sometime multiseriate containing starch grains in continuation with xylem vessels. Xylem vessels are mainly isolated or in groups of 2-4	3-4 layered secondary phloem is present just below the cortex; heterogeneous medullary rays are present in continuation with xylem vessels. Secondary xylem is 8-10 cells wide and consists of vessels, fibers, tracheids and parenchyma
Pith	Parenchymatous, narrow (Fig. 2d)	Parenchymatous, narrow (Fig. 2i)	Parenchymatous, narrow (Fig. 2m)	Parenchymatous, narrow

Table 2 —Physicochemical parameters of *Mahonia* species.

Plant name	Moisture content	Total ash	Acid-insoluble ash	Water-soluble ash	Hexane-soluble extractive	Alcohol-soluble extractive	Water-soluble extractive
Mahonia leschenaultii	6.32±0.03	5±0.06	0.6±0.01	3±0.05	0.52±0.03	5.04±0.04	8.23±0.05
Mahonia napaulensis	6.51±0.04	3.81±0.05	0.75±0.03	2.01±0.03	0.41±0.06	3.43±0.03	7.85±0.06
Mahonia borealis	6.45±0.04	3.45±0.03	0.8±0.03	2.2±0.04	1±0.03	4.17±0.06	7.46±0.4

Values are mean (n=3) ± Standard deviation, Values are in percentage (%)

the three samples, followed by alcohol-soluble and water-soluble extractives (Table 2). However, it is observed that there is no significant variation ($p>0.05$) in the moisture content, acid-insoluble ash, water-soluble ash, hexane-soluble and alcohol-soluble extractive values (Table 2).

The different phytochemical parameters, viz., total phenolic content, flavonoid, sugar, starch and tannin of all the samples are depicted in Fig. 3. A comparative phytochemical study of all three species, showed no significant variation in quantified metabolites. Total phenolic content was found to be highest (0.45%), followed by flavonoid, starch, sugar and tannin respectively.

Quantification of berberine

A densitometric HPTLC analysis was performed for the development of a characteristic TLC fingerprint profile, which may be used as a biomarker for quality evaluation and standardisation of herbal drugs. In addition, this study also explores the possibilities for using these *Mahonia* species as a substitute for *B. aristata* on the basis of its secondary metabolite, i.e., berberine (Fig. 4). The chromatographic separation of berberine was done in

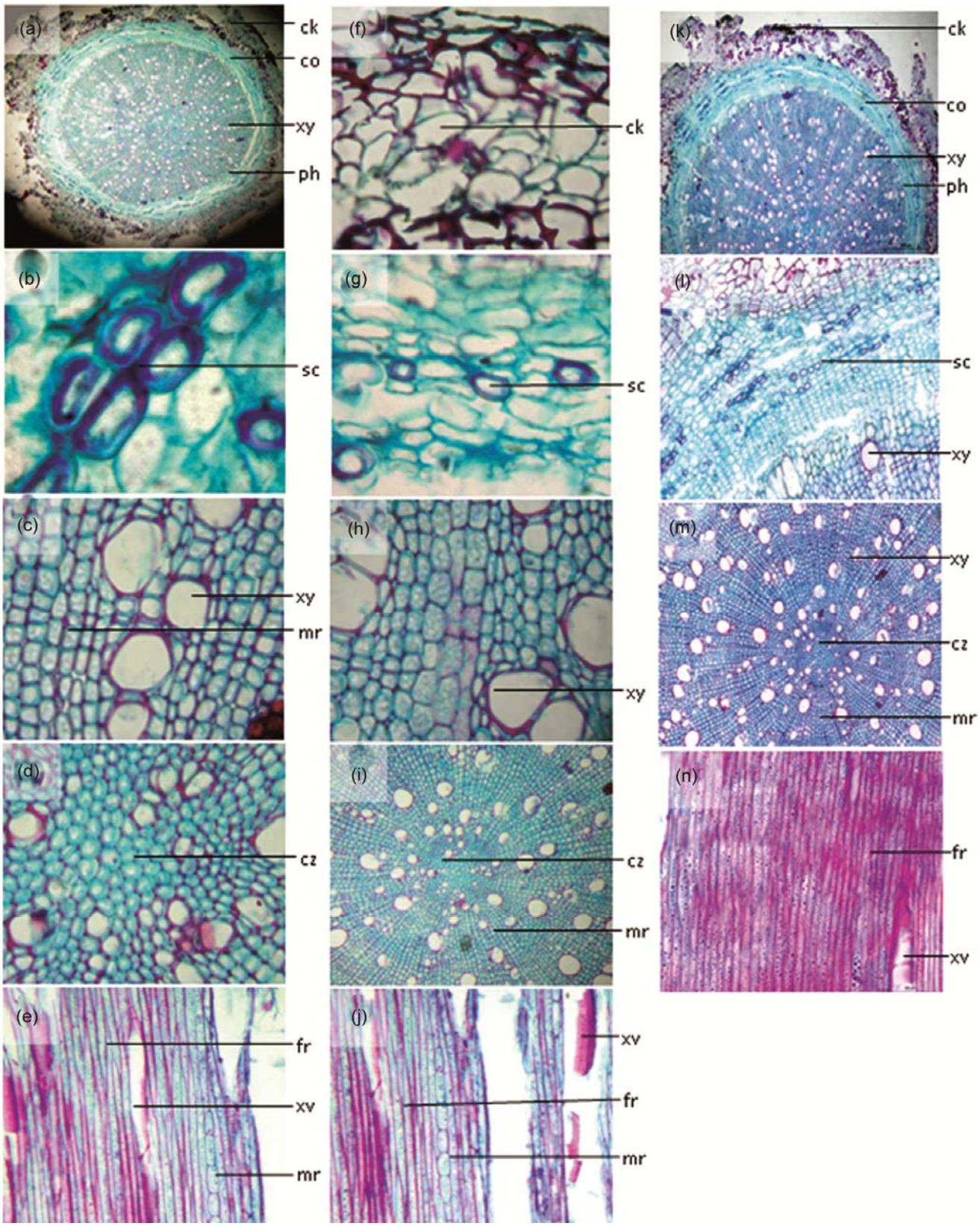

Fig. 2 — Anatomical characters of roots of *Mahonia leschenaultii* (a, b, c, d & e), *Mahonia napaulensis* (f, g, h, i & j) and *Mahonia borealis* (k, l, m & n). Abbreviations: ck - cork cells; co - cortex; cz – central zone; fr – Fibres; mr - medullary rays; ph - phloem; sc - sclereids; xv – xylem vessels; xy – xylem.

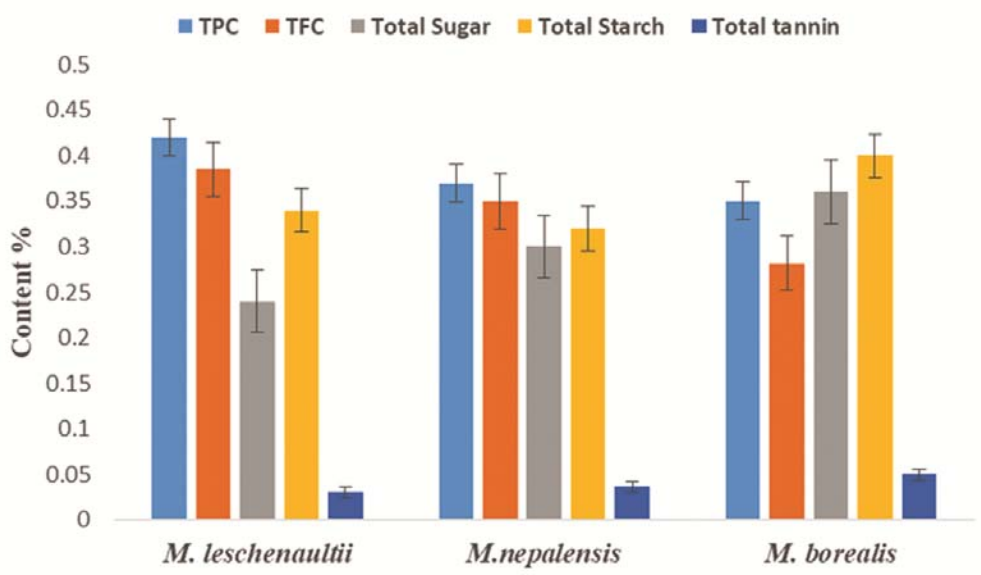

Fig. 3 —Phytochemical estimation of *Mahonia* species. (TPC: total phenolic content, TFC: total flavonoid content, error bars represent standard error).

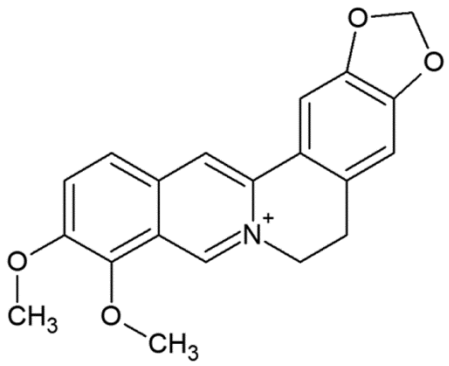

Fig. 4 —Chemical structure of berberine

the tertiary solvent system of n-propanol: water: formic acid (9:1:0.1 v/v/v) and after development, the image of TLC plate was taken at 366 nm. Identification of berberine was done by comparing the UV absorption spectra and R_f of standard and sample on TLC plate (Fig. 5). The berberine was identified at R_f0.32±0.03 in samples and scanning was carried out at λ_{max} of 350 nm in absorbance-reflectance mode (Fig. 6). The berberine was found to be 0.197%±0.01, 0173%±0.03 and 0.151%±0.01 in ML, MN and MB respectively on dry weight basis in crude samples.

Discussion

Morphological investigation of aerial plants of all three *Mahonia* species showed imparipinnate, alternate, opposite venation of leaves with sessile or petiolulate leaflets. Leaves, inflorescence and fruits are key features to identify these *Mahonia* species. The roots of all three species have similar morphological features and cannot be differentiated by external examination. However anatomical investigation of roots showed several differentiating features, i.e., there is a difference in the distribution pattern of sclereids in the cortical region. Most sclereids in *Mahonia napaulensis* are found solitary, whereas in *Mahonia leschenaultii* and *Mahonia borealis* they are found in groups and rarely solitary. Similar anatomical characters were found in the root of *Berberis aristata*[29] and are described in Table 1 for comparison.

The physicochemical standards, viz., total ash, acid insoluble ash, alcohol and water soluble extractive of *Mahonia leschenaultii* roots observed in our study are on par with the published literature[30]. However, physicochemical data on the roots of *Mahonia borealis* and *Mahonia napaulensis* is missing, thus the evaluation of these species became quintessential for establishing the quality standards. The presence of various phytochemicals was qualitatively screened in *Mahonia leschenaultii* and *Mahonia napaulensis*[7,31], but the data on *Mahonia borealis* is lacking. Thus, the comparative study of these three *Mahonia* species is relevant for quality regulation and to check for adulteration. Further, it is found that the physicochemical and phytochemical profiles of targeted *Mahonia* species are on par with *B. aristata*[29]. Additionally, the major isoquinoline alkaloid of "Daruharida", i.e., Berberine is also found

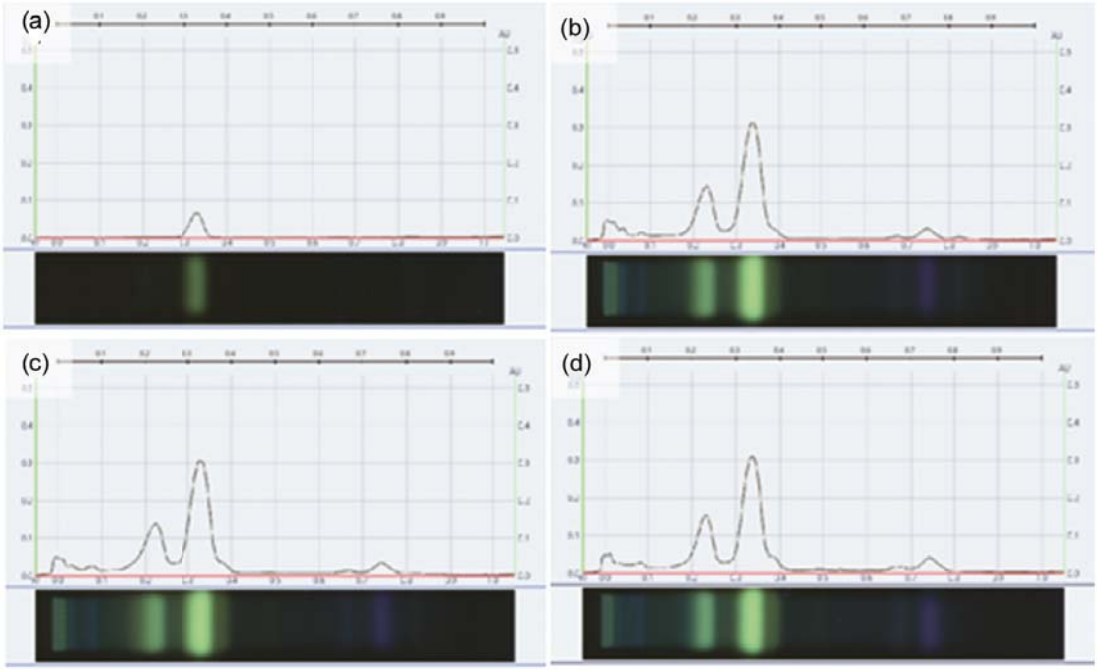

Fig. 5 —HPTLC profile and densitometric chromatogram of berberine (a), *Mahonia leschenaultia* (b), *Mahonia napaulensis* (c) and *Mahonia borealis* (d) scanned at 350 nm.

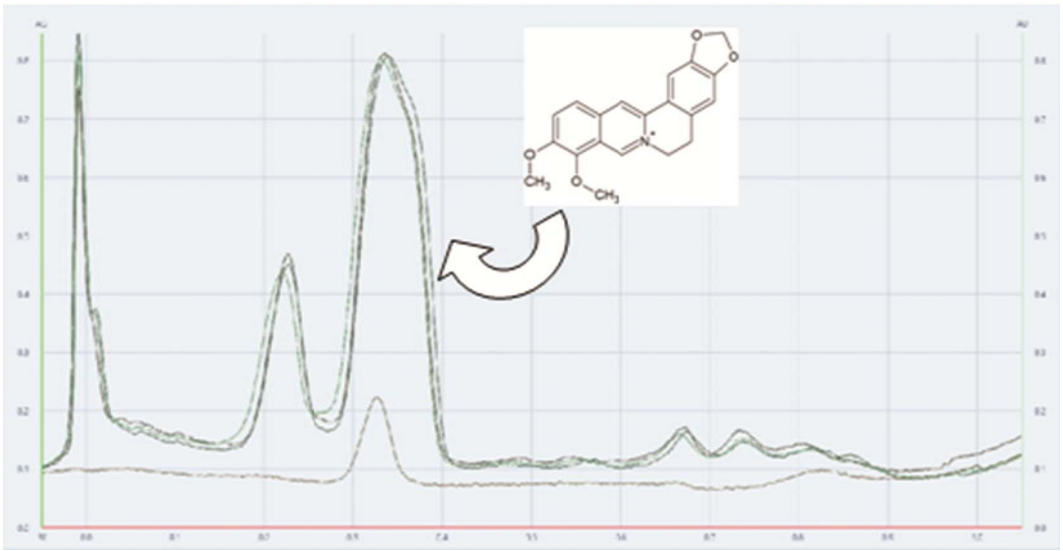

Fig. 6 —Purity spectrum of berberine and *Mahonia* species, scanned at 350 nm.

in *Mahonia* species and thus they can be used as an alternative to the former.

Conclusion

The pharmacognostic parameters of *Mahonia leschenaultii*, *Mahonia napaulensis* and *Mahonia borealis* were standardised for quality regulation of these species in herbal drug industry. HPTLC profiling of targeted species reveals that the berberine content is available in the range from 0.151 to 0.197% and thus it may also be used as a potential substitute of *B. aristata* (Daruharidra).

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Conflict of Interest

The authors declare no potential conflict of interest.

Author's Contributions

M K C carried out most of experimental work and drafted the manuscript. A M has done HPTLC and corrected the manuscript. S S designed the study, supervised the whole experimental process and edited the manuscript.

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