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Analysis of Berberine Content using HPTLC Fingerprinting of Root and Bark of Three Himalayan *Berberis* Species

[1,2]Harish C. Andola, [1]R.S. Rawal, [3]M.S.M. Rawat,
[1]I.D. Bhatt and [4]Vijay Kant Purohit
[1]Biodiversity Conservation and Management Thematic Group,
G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal,
Almora, 263 643-Uttarakhand, India
[2]Herbal Research and Development Institute,
Centre for Aromatic Plants, Selaqui, Dehradun, Uttarakhand, India
[3]Department of Chemistry, H.N.B. Garhwal Central University,
Srinagar Garhwal, 246 174-Uttarakhand, India
[4]High Altitude Plant Physiology Research Centre,
H.N.B. Garhwal Central University, Srinagar Garhwal, 246 174-Uttarakhand, India

Abstract: The Indian Himalayan Region (IHR) is well known for richness and diversity of valuable but highly sensitive species of medicinal and aromatic plants. Among medicinal plants of the region, genus *Berberis* has gained an important place in different systems of medicine. Every parts of the species used in medicinal purpose. This genus represents richness of species across Indian Himalayan Region (IHR) with wide ranging distribution from low lying sub-tropical to High alpine areas. The roots stem bark and fruits of various *Berberis* species in the region are well recognized for their alkaloid contents. In recent years, the global demand for these alkaloids and their derivatives has grown substantially. Its medicinal uses are reported Indian pharmaceutical codex and in different traditional medicines various Analytical tools such as High performance liquid chromatography, Gas chromatography, Gas chromatography and mass spectroscopy infrared spectroscopy progress in natural product chemistry have always liked with innovation in analytical technology. Among the chromatographic techniques High performance thin layer liquids chromatography more popular in quality control and standardization of traditional herbs like *Berberis* keeping in view of this goal of the present study to develop chromatography fingerprinting of three high value Himalyan *Berberis* species plant parts. The berberine content in root and stem bark of three *Berberis* (i.e., *B. asiatica*, *B. aristata, B. lycium*) was estimated using a simple High Performance Thin Layer Liquid Chromatography (HPTLC). The comparative methods assessment revealed that the berberine content varied both in root and stem bark samples. More berberine content observed in root samples as compared to bark of all the investigated species. Among the species *Berberis asiatica* contain more berberine significantly ($p<0.05$) as compared to *B. lycicum* and *B. aristata*

Key words: Comparative methods assessment, *Berberis* species, root, stem bark, HPTLC, fingerprinting

Corresponding Author: Harish C. Andola, Biodiversity Conservation and Management Thematic Group, G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora, 263 643-Uttarakhand, India

INTRODUCTION

Daruharidra (*Berberis*), an important ingredient of Ayurvedic system of medicine, is in continuous use for health care in India and other parts of globe. It is used as a single plant remedy or in polyherbal formulation in organized systems of medicine such as Ayurveda, Siddha and Unani. The species of the genus *Berberis* is known for berberine alkaloid and used as a remedy for eye disease, diarrhea, etc. The root are extensively used in various indigenous system of medicine for treating eye, ear diseases, rheumatism, jaundice, diabetes, fever, stomach disorder, skin disease, malarial fever, etc. (Watt, 1883; Kirtikar and Basu, 1933; Annonymous, 1988). While considering the quality of drugs from plant origin, several analytical techniques have been developed. One such method is to select a known active constituent or a marker compound as qualitative and quantitative target to assess authenticity and inherent quality. However, the chemical components and their contents of crude drug often vary depending on the geographical locations of the habitats, seasons; plant parts harvesting and post harvesting, etc., all these factors influence the production of secondary metabolites in plants (Cüneyt *et al*., 2007). In addition, the alkaloids in plant are to some extent under genetic control but they are also greatly affected by varying growth conditions arising from climate and soil differences (Nautiyal, 2007; Andola *et al*., 2010). In recent year advancement in of chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines (Gong *et al*., 2005). Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and its products and therefore be used for authentication and identification of the herbal products. Based on the concept of phytoequivalence, the chromatographic fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines (Liang *et al*., 2004). Keeping the above in view a sample HPTLC method has been standardized for analysis for analysis of berberine content in different *Berberis* species. However, HPTLC methods have been standardized for *Berberis asiatica* root (Srivastava *et al*., 2004, 2006). Hence, the present study has been taken which may useful to pharmaceutical industry for the authentication of the commercial sample and explorations the possibility of the using this species substitute of each others. In view of the above the comparative study of root and bark sample in three different species is yet not be carried out.

MATERIALS AND METHODS

Plant material of (*Berberis asiatica*, *B. aristata* and *B. lycium*) was collected from Garasin Uttarakhand in West Himalaya, in 2006. The material was brought to the laboratory and dried at room temperature (25±2°C). The dried samples were powdered using Wiley grinder mill and stored in desiccators.

Extraction of Plant Material

Air dried (25±2°C) sample of root and bark of three different *Berberis* species (1 g powdered) was refluxed for 5 min on the water bath with 5 mL methanol consequently three times and concentrated under vacuum and finally made upto 20 mL with HPLC grade methanol and ready for HPTLC analysis.

Chromatographic Condition

Chromatography was performed on glass bucked silica gel 60 G F_{254} Merck glass plates of 20×10 cm with the help of Camang Linomat-IV applicator and eluted the plate to a distance

of 6.20 cm at room temperature (25°) in solvent system n-propanol: water: formic acid (90:8.0:0.4). sample solution along with reference berberine (7 μL of each) and was applied on 7 mm wide bands positioned 15 mm from the bottom and 20 mm from the side of the plate, using a Camag Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nL sec^{-1} from the syringe. These parameters were kept constant throughout the analysis of samples (Srivastava *et al*., 2006).

Detection and quantification: After sample application plates were developed in a Camag twin through glass tank pre-saturated with the mobile phase n-propanol: water: formic acid (90:8.0:0.4) for 1 h and poured in twin through glass solvent development chamber well in advance to allow completed saturation. This was further enhanced by keeping one filter paper along one well of the twin through chamber. The plate was kept in a chamber and solvent front was allowed to develop on the plate. The HPTLC runs were made under laboratory conditions of 25±2°C and 50% relative humidity. After drying, the spot were visualized under Camag UV cabinet (254 and 366 nm). Quantitative analyses of the compounds were done by scanning the plates using Camang TLC Scanner model 3 equipped with Wincats software (Camag). The following conditions were applied silt width condition 6x0.45 mm, wavelength (254 and 366 nm). The identification of berberine was confirmed by superimposing the UV spectra of the samples and standards within the same R_f0.29 window.

A densitometry HPTLC analysis was also preformed for the development of characteristic fingerprint profile, which may be used as marker for quality evaluation and standardization of the drugs.

Calibration

The content of berberine compound determined by using a calibration curve established with seven dilution of each standard, at concentration range from 2 to 8 ng. Each concentration peak area was plotted against the concentration of berberine injected. The linear regression of stands curve was determined 0.996. The liner regression is y = 5018x+14019.

Accuracy

Accuracy was measured by analysis of standard solution spotted at different concentration on the same days. The RSD was found 3.0% which was considered acceptable. Repeatability: different samples were chromatographed in triplicate using the same equipments on different days. The RSD was <2.7 % which was considered acceptable.

RESULTS AND DISCUSSION

The berberine content in root samples was invariably more (Table 1) *B. asiatica* contain maximum amount of berberine significantly ($p<0.05$) (root 4.3%, stem bark 3.0%), followed *B. lycium* root contain (root 4.0%, stem bark 2.9%) and *B. aristata* contain (root 3.8%, stem bark 2.6%). It is well reported fact that berberine occurs in more amount in organs of the plants which grow in absence of light, i.e., roots and rhizomes (Cromwell, 1933). This is in agreement with the earlier reports from 8 *Berberis* species, which indicated a significant decline in berberine content in all parts of *Berberis* species at high altitude areas as compared to those at lower altitudes in west Himalayan region (Chandra and Purohit, 1980; Nautiyal, 2007; Andola *et al*., 2010). The present study showed the greater variation in berberine content among plant parts (Table 1). This is in agreement also with the previous reports on *Berberis* species where higher berberine content was observed in root sample than the stem

Table 1: Berberine % in root and stem bark of investigated species

	Berberine (%)	
Species name	Root	Stem bark
Berberis asiatica	4.30	3.00
Berberis aristata	3.80	2.60
Berberis lycium	4.00	2.80
LSD at (p<0.05)	0.28	0.26

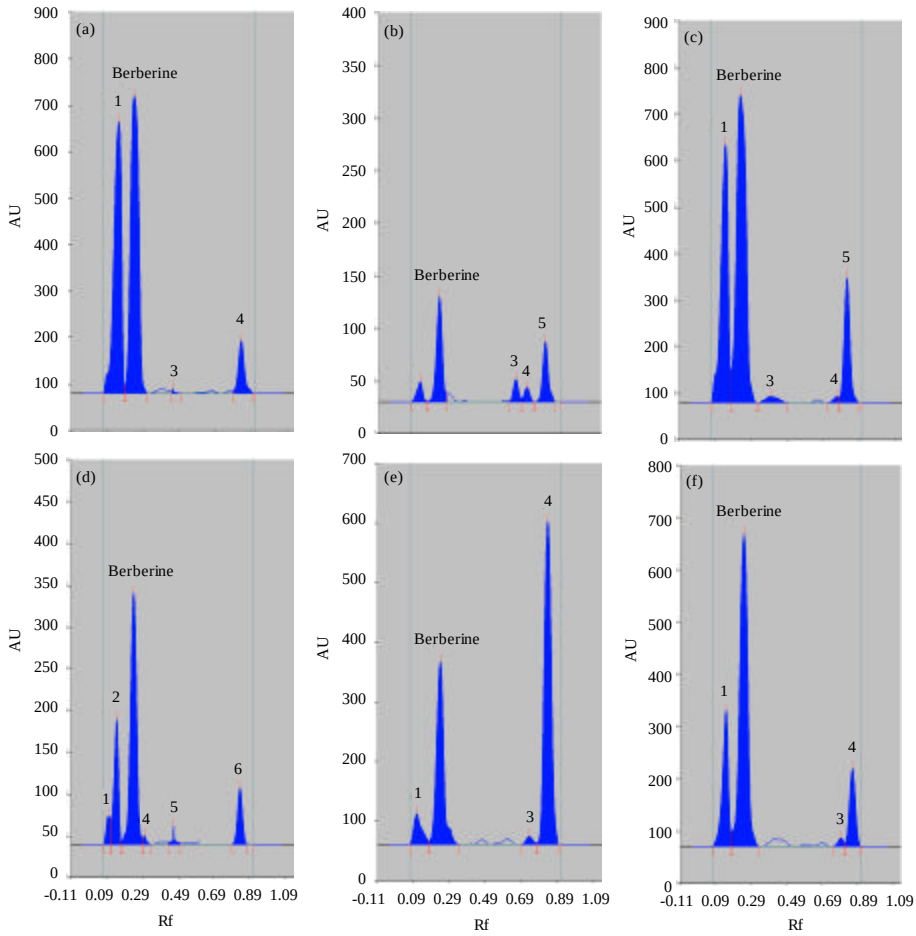

Fig. 1: HPTLC fingerprinting of root and stem bark sample of selected *Berberis* species; (a) Track 2, ID: *Berberis asistica* (root), (b) Track 3, ID: *Berberis aristata* (root), (c) Track 4, ID: *Berberis lycium* (root), (d) Track 8, ID: *Berberis asistica* (bark), (e) Track 9, ID: *Berberis aristata* (bark), (f) Track 10, ID: *Berberis lycium* (bark)

bark (Nautiyal, 2007; Mikage and Mouri, 1999). The high berberine content in roots of other species *Mahonia* sp. of family Berberidaceae has also been reported by Greathouse and Watkins (1938). Screening of root and stem bark of thee *Berberis* species i.e., *Berberis asiatica*, *Berberis aristata*, *Berberis lycium* using HPTLC fingerprinting showed that root and bark of (Fig. 1-3). The HPTLC Chromatograph showed that *B. asistica* root possess total four compounds, however stem bark contained 6 compounds. Similarly, root of *B. aristata*

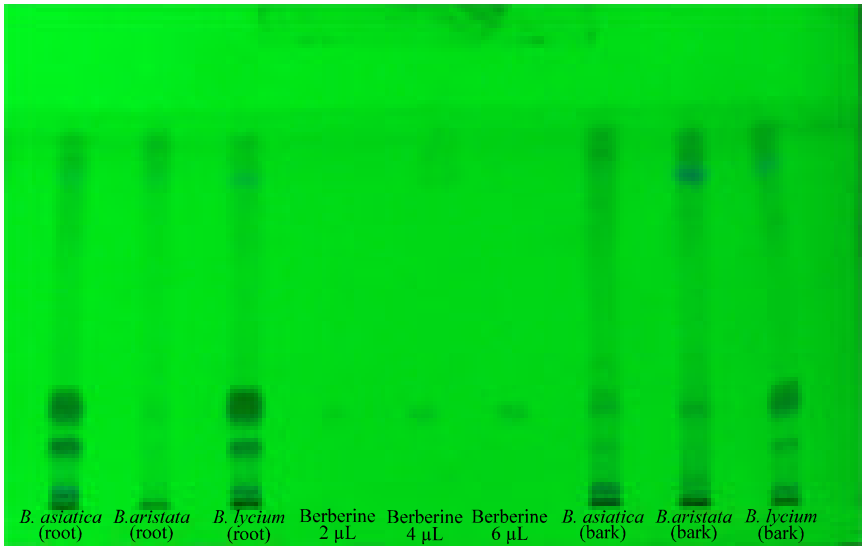

Fig. 2: Image at 254 nm root and stem bark sample of selected *Berberis* species

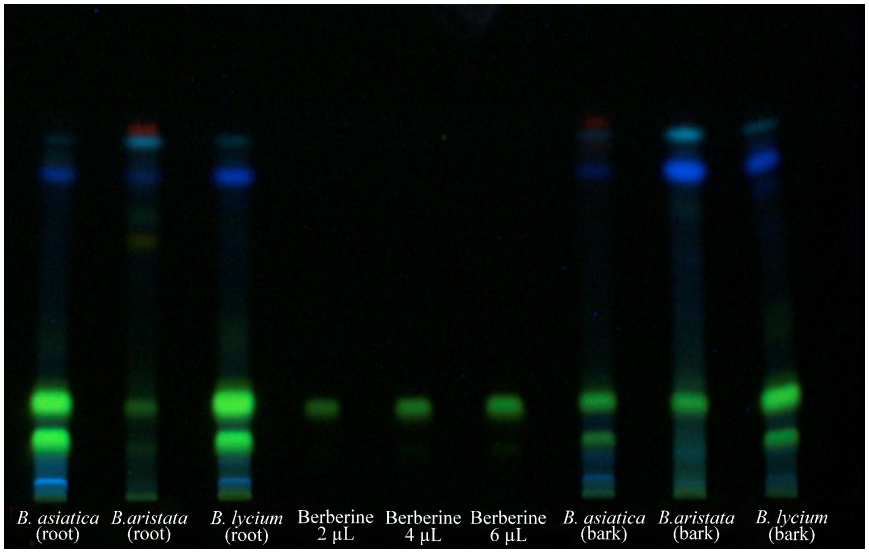

Fig. 3: Image at 366 nm root and stem bark sample of three *Berberis* species

and *B. lycium* contained 5 compounds; however stem bark of both the species contained 4. This indicates the variation in quantitative character of stem bark and root samples of different *Berberis* species. Plant secondary metabolites are also affected by different size classes. Reports indicated that taxol of *Taxus baccata*, known to be influence qualitatively and quantitatively by plant age, variety, season, time of day and weather. Seasonal variation in taxoid and highest level of paclitaxel were found in needles *Taxus baccata* between month of February and April (Hook *et al*., 1999). This variation may be related to flower developments and emergence of new shoot growth and would appear to be dependence on

other factors e.g., variety or cultivar, location, weather during the year, soil types and nutrients etc. (Hook *et al*., 1999) resent study concluded that all the species contain more or less similar berberine content and can be used as a substitute of each others. Also, the result reveled that present study can be used to explore the qualitative composition of root and bark all the species. Root of *B. asiatica* possessed total four compounds but stem bark of contained six compounds. Root of *Berberis aristata* contains five compounds but *B. lycium* contains also contains five compound. The HPTLC analysis in species indicates the variations in quantitative and quantitative character. Study to emphasize the compositional patterns of the species. In addition, the study explore the possibilities for these species as a used for as a substation on the basis of chief active constituents berberine. In summary, the HPTLC methods for the simultaneous analysis of berberine form root stem bark reported here is very simple, sensitive, economic and suitable for rapid screening of large number of sample analysis.

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