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Quantification of Berberine in different Berberis Species and their Commercial Samples from Herbal Drug Markets of India through HPTLC

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Abstract

A simple, precise, and convenient HPTLC method has been established for the analysis of Berberine, the major marker compound extracted from the root and stem of different *Berberis* species and their commercial samples in the name of *Daruharidra*. Chromatography was performed on silica gel $60F_{254}$ plates with *n*-propanol:water:formic acid (90:8.0:0.4) as mobile phase. Detection and quantification were performed densitometrically at λ_{max} = 360 nm with berberine as external standard. The method is characterized by high sensitivity and linearity over wide range of concentrations. Berberine concentration in different species and their commercial counterpart were calculated. This will be utilized by pharmaceutical industries for the bioprospection of allied *Berberis* species for commercial exploitation and batch to batch consistency of raw materials.

Key Words: Berberine; Densitometry; HPTLC; *Berberis*

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1- Introduction

High-performance thin-layer chromatography (HPTLC) is commonly used for identification, assay, purity testing and for content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmaceuticals, nutraceuticals). It has also emerged as an important analytical tool for detection and characterization of the active ingredients in crude drug formulations. In HPTLC, densitometry has been used to test purity [1], for assay of pharmaceutical dosage forms [2], and to obtain a chromatogram for herbal fingerprinting [3,4].

Berberis aristata DC. known, as *'Daruharidra'* in Ayurveda is a versatile medicinal plant used singly or in combination with other medicinal plants for treating a variety of ailments like jaundice, enlargement of spleen, leprosy, rheumatism, fever, morning/evening sickness and snakebite [5-10]. In addition, the decoction of root or stem of *Berberis*-known as '*Rasaut'* is specifically used in eye disease, skin disorders and indolent ulcers [11-13]. Although the roots of *B. aristata* are considered as the genuine drug, the study revealed that different species of *Berberis viz*. *B. asiatica, B. chitria*, and *B. lycium* are also being used as *Daruharidra* in different parts of the country. The study also shown that most of the market materials sold as *Daruharidra* consist of mostly the mixture of root & stem parts different *Berberis* species [14-16].

In view of its great importance as major ingredient in different herbal formulations worldwide, a detailed HPTLC study of four *Berberis* species *viz*. *B. aristata* DC., *B. asiatica* Roxb. ex DC., *B. chitria* Lindl. and *B. lycium* Royle collected from wild and ten commercial samples which are implicated as *Daruharidra* procured from various important crude drug markets of India *viz.* Aligarh, Amritsar, Bangalore - I, Bangalore - II, Delhi, Hyderabad, Jammu, Lucknow, Trichur, Varanasi has been carried out with the aim to authenticate and check the adulteration/substitution in different commercial crude drug samples. Berberine was used as external standard.

2- Experimental

2-1 Plant Material

Roots and stems of the plant were collected from Dhanulti region of Uttarakhand, India and were authenticated by Dr A.K.S. Rawat, Scientist, NBRI, Lucknow, India and ten commercial samples procured from Aligarh, Amritsar, Bangalore-1, Bangalore-2, Delhi, Hyderabad, Jammu, Lucknow, Tirchur and Varanasi markets of India. The crude samples were submitted to drug depository of the Institute along with the voucher specimen numbers. (Table 1&2)

2-2 Chemicals and standard compounds

Reagents (analytical grade) used were from Merk (Germany) and standard *viz.* Berberine is from Sigma-Aldrich (Steinheim, Germany).

2-3 Analytical Procedures

2-3-1 Standard Solutions

Accurately weighed Berberine standard (1mg) was dissolved in acetone (1mL) to prepare a 1 mg mL^{-1} standard solution.

2-3-2 Sample Preparation

The powdered (100mesh) dried root (5g) were soaked in methanol (4 × 20 ml, each for 1 h). The extracts were combined, filtered, and evaporated to dryness by rotary evaporation. Accurately weighed methanol extract (10 mg) was dissolved in methanol (10 ml) to prepare a 1 mg ml^{-1} solution.

2-3-3 Chromatographic conditions

Chromatography was performed on 20 cm × 10 cm glass-backed HPTLC plates coated with 0.25-mm layers of silica gel Si $60F_{254}$ (E. Merck, Germany). Different volumes of standard solution and 5 µl of the extract solutions were applied to the plates as bands, 6 mm long and 8 mm apart, by using Camag Linomat V (Switzerland) sample applicator equipped with a 100µl micro syringe.

2-3-4 Detection, quantification and calibration of Berberine

Plates were developed to a distance of 80 mm, with *n*-propanol:water:formic acid (90:8.0:0.4), as mobile phase, in a 20 cm × 10 cm Camag glass twin-trough chamber previously saturated with mobile phase vapor; the temperature was 25°C and the relative humidity was 37%. After removal of the plates from the chamber completely dried in air at room temperature (25°C) and peak areas for the samples and standard were recorded by densitometry in absorbance/reflectance mode at λ max = 360 nm, by means of a Camag TLC Scanner 3 equipped with WINCATS version 3.2.1 software. Typical chromatograms are shown in Figure 1-21 and the calibration plot obtained by diluting standards in different concentrations (µL).

2-3-5 Validation

The precision of the scanner was checked by scanning the same spots five times and the coefficient of the variance was calculated. The repeatability of the method was also established by applying 5µg per spot of each standard solution five times and the coefficient of variance was calculated. The limit of detection and quantification were also determined. (Table 3&4)

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3- Results and Discussion

Different compositions of the mobile phase were checked and the desired resolution of Berberine with reproducibility of peaks was achieved by using *n*-propanol:water:formic acid (90:8.0:0.4) (v/v). Under these conditions the R_f of Berberine was 0.32 and the compound was well resolved from other components of the extract.

The calibration curve of Berberine was linear and in the range of 1µg to 13µg. Linear regression and R_f of Berberine are given in Table 3. The amount of Berberine in the roots of different *Berberis* species and it market samples were also presented in Fig. 22

4- Conclusions

Berberine, one of the major alkaloids was quantified through HPTLC densitometric method and it was found more in roots as compared to stem i.e. 2.25 – 5.20% and 1.02-2.01% respectively. Its concentration was also varied from species to species i.e. maximum in roots of *B. chitria* (5.20%) followed by *B. lycium* (3.99%), *B. aristata* (3.55%) and *B. asiatica* (2.25%).

An extensive market survey was done to check the quality of raw material (adulteration/substitution) available in different parts of the country. On morphological studies it was observed that most of these commercial samples are either mixture of two species or substituted with some other species than *B. aristata.* This was also confirmed with the analysis done through HPTLC (Table 2 & Fig. 22). Thus this study will be a useful tool for checking adulteration/substitution of *Berberis* in pharmaceutical preparations. It will also be useful for maintaining batch to batch consistency of crude samples/herbal formulations commercialized by pharmaceutical industries.

Acknowledgments

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Table 1: *Berberis* species collected

S. No.	Samples	Voucher Specimen No. (LWG)
1.	*Berberis aristata* DC.	221239
2.	*B. asiatica* Roxb. ex DC.	221240
3.	*B. chitria* Lindl.	221241
4.	*B. lycium* Royle	221238

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Table 2: Commercial samples of *Daruharidra* procured from Indian herbal drug markets

S. No.	Place of collection	Voucher Specimen No. (LWG)	Source of supply	Vernacular name	Part Identified (Morphologically)
1	Aligarh	221242	Dehra Dun	Daruharidra	Stems of *Berberis* spp.
2	Amritsar	221243	Dehra Dun	Daruharidra	Roots of *Berberis* spp.
3	Bangalore - 1	221244	Delhi market	Daruharidra/ Maramanjal	Stems of *Coscinium fenestratum*
4	Bangalore - 2	221245	Local supplier	Daruharidra/ Maramanjal	Roots of *C. fenestratum*
5	Delhi	221246	Dehra Dun	Daruharidra	Mixture of *Berberis* spp.
6	Hyderabad	221247	Delhi	Daruharidra	Stems of *Berberis* spp.
7	Jammu	221248	Local supplier	Daruharidra	Roots of *Berberis* spp.
8	Lucknow	221249	Dehra Dun	Daruharidra	Mixture of *Berberis* spp.
9	Trichur	221250	From southern hills	Daruharidra/ Maramanjal	Roots of *Berberis* spp.
10	Varanasi	221251	Dehra Dun	Daruharidra	Roots of *Berberis* spp.

Table 3: R_f values by HPTLC and linear regression equations for the determination of berberine

Compound	R_f value	Regression equation	r^2
Berberine	0.32	y = 20.72 + 11318.80X	0.999

Table 4: Validation data for the HPTLC method for the estimation of berberine

Property	Berberine
R_f	0.32
Instrumental precision (CV, n=5)	0.324
Repeatability (CV, n=5)	0.979
Limit of detection (LOD)	0.34 μg
Limit of quantification (LOQ)	1.04μg
Linear regression	0.999
Calibration range	1-13 μg
Specificity	Specific
Robustness	Robust

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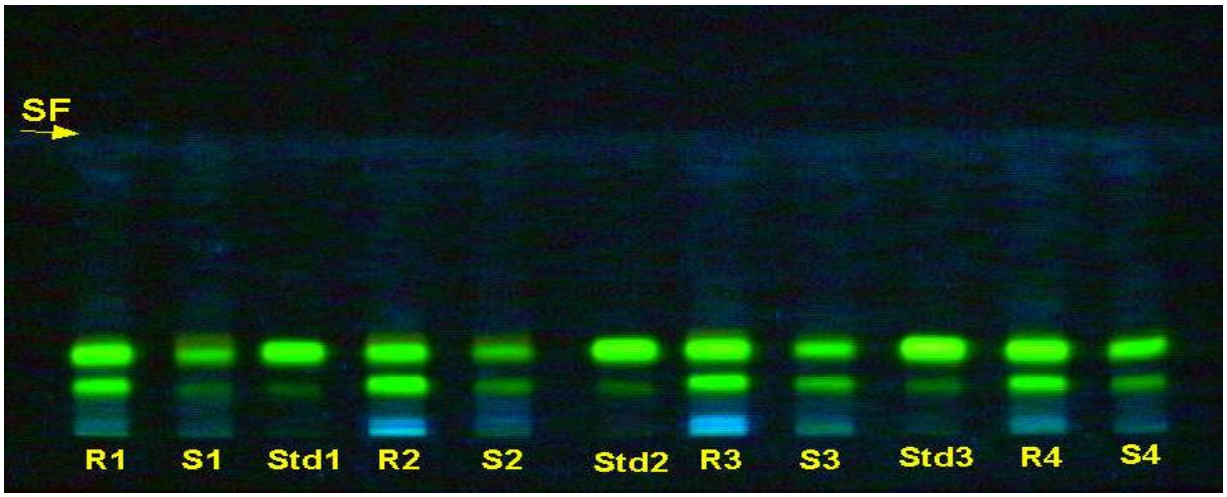

Fig. 1 HPTLC profile of roots and stem of different *Berberis* species (UV 366nm)

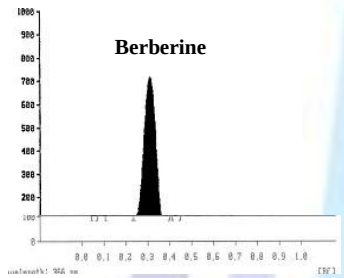

Fig. 1 Chromatogram of reference sample

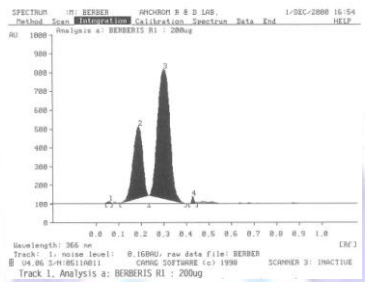

Fig. 2 Chromatogram of *B. aristata* Root

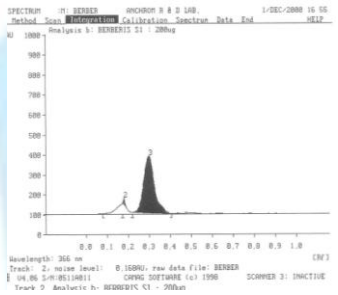

Fig. 3 Chromatogram of *B. aristata* stem

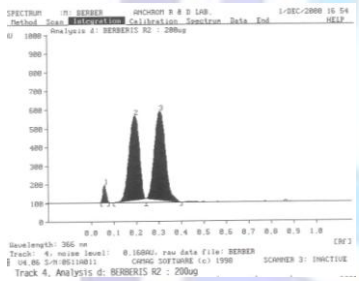

Fig. 4 Chromatogram of *B. asiatica* Root

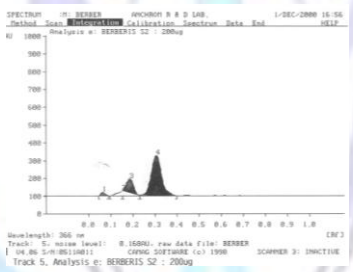

Fig. 5 Chromatogram of *B. asiatica* stem

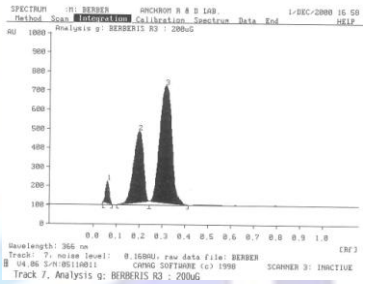

Fig. 6 Chromatogram of *B. chitria* Root

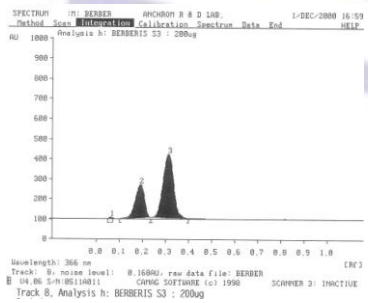

Fig. 7 Chromatogram of *B. chitria* stem

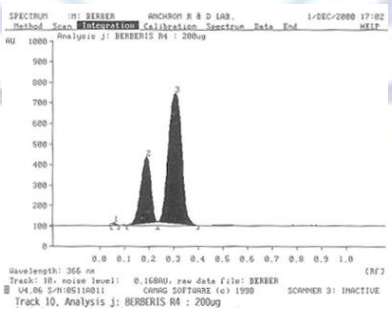

Fig. 8 Chromatogram of *B. lycium* Root

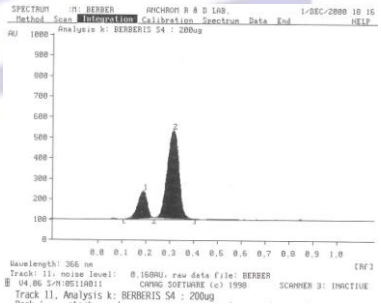

Fig. 9 Chromatogram of *B. lycium* stem

ABREVIATIONS (Fig.1):
R1: *Berberis aristata* root; S1: *B. aristata* stem; Std1: Berberine; R2: *B. asiatica* root; S2: *B. asiatica* stem; Std2: Berberine; R3: *B. chitria* root; S3: *B. chitria* stem; Std3: Berberine; R4: *B. lycium* root; S4: *B. lycium* stem

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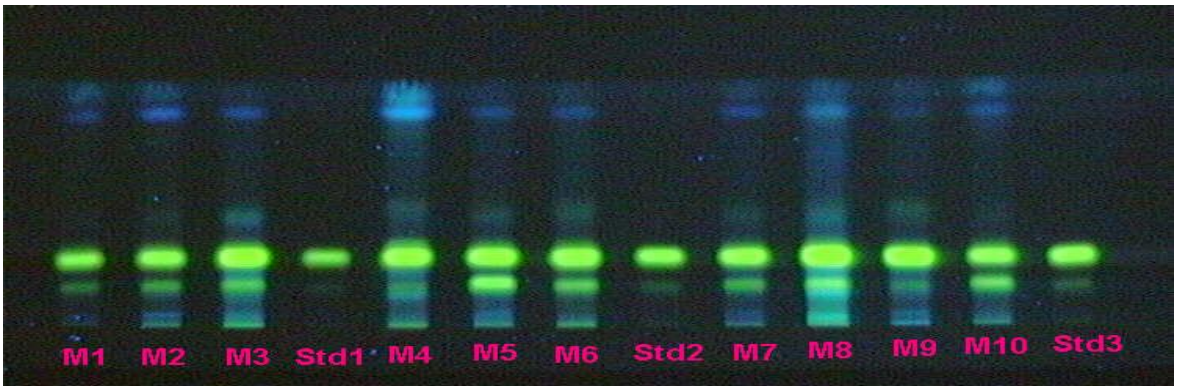

Fig. 10 HPTLC profile of different crude market samples sold by the name of *Daruharidra* (under UV 366)

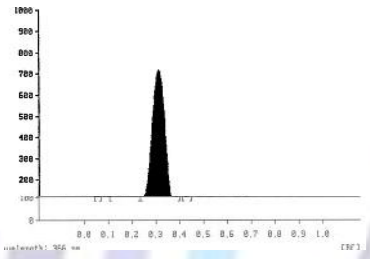

Fig. 11 Chromatogram of reference sample

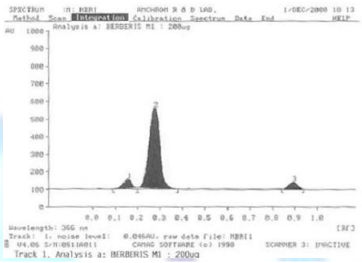

Fig. 12 Chromatogram of Aligarh sample

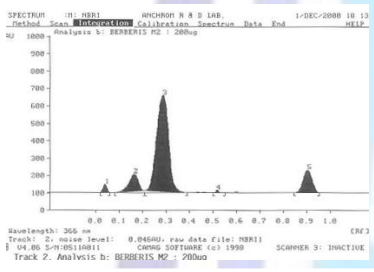

Fig. 13 Chromatogram of Amritsar sample

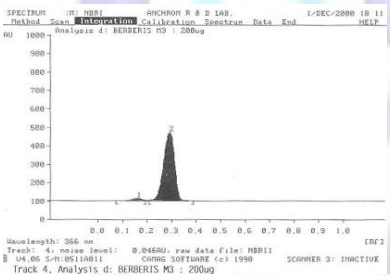

Fig. 14 Chromatogram of Bangalore-1 sample

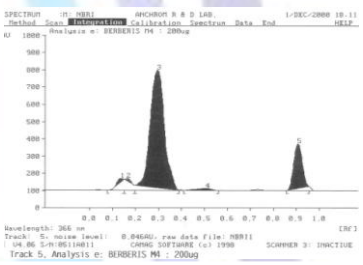

Fig. 15 Chromatogram of Bangalore-2 sample

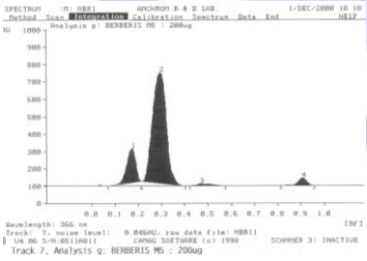

Fig. 16 Chromatogram of Delhi sample

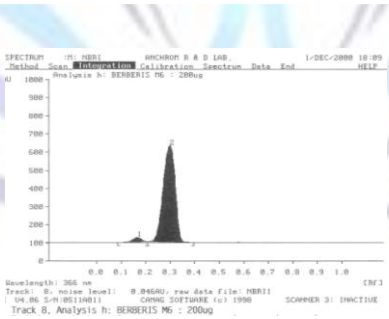

Fig. 17 Chromatogram of Hyderabad sample

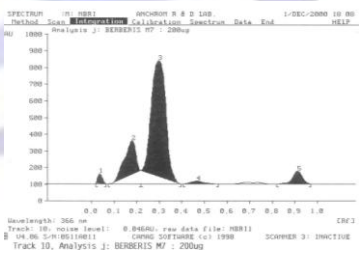

Fig. 18 Chromatogram of Jammu sample

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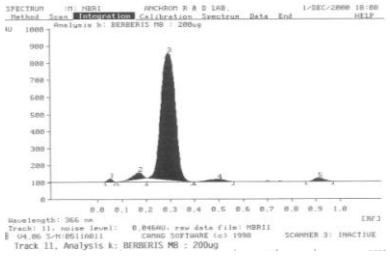

Fig. 19 Chromatogram of Lucknow sample

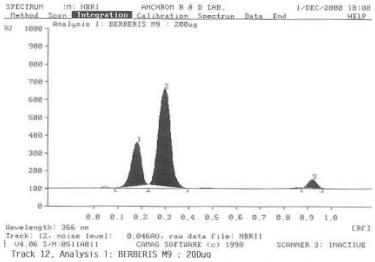

Fig. 20 Chromatogram of Tirchur sample

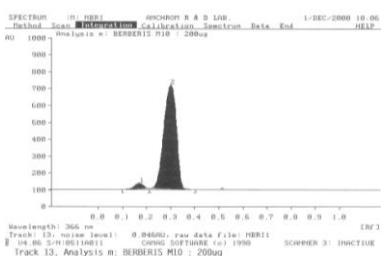

Fig. 21 Chromatogram of Varanasi sample

ABREVIATIONS (Fig.10):
M1: Aligarh; M2: Amritsar; M3: Bangalore-1; Std1: Berberine; M4: Bangalore-2; M5: Delhi; M6: Hyderabad; Std2: Berberine; M7: Jammu; M8: Lucknow; M9: Tirchur; M10: Varanasi; Std3: Berberine;

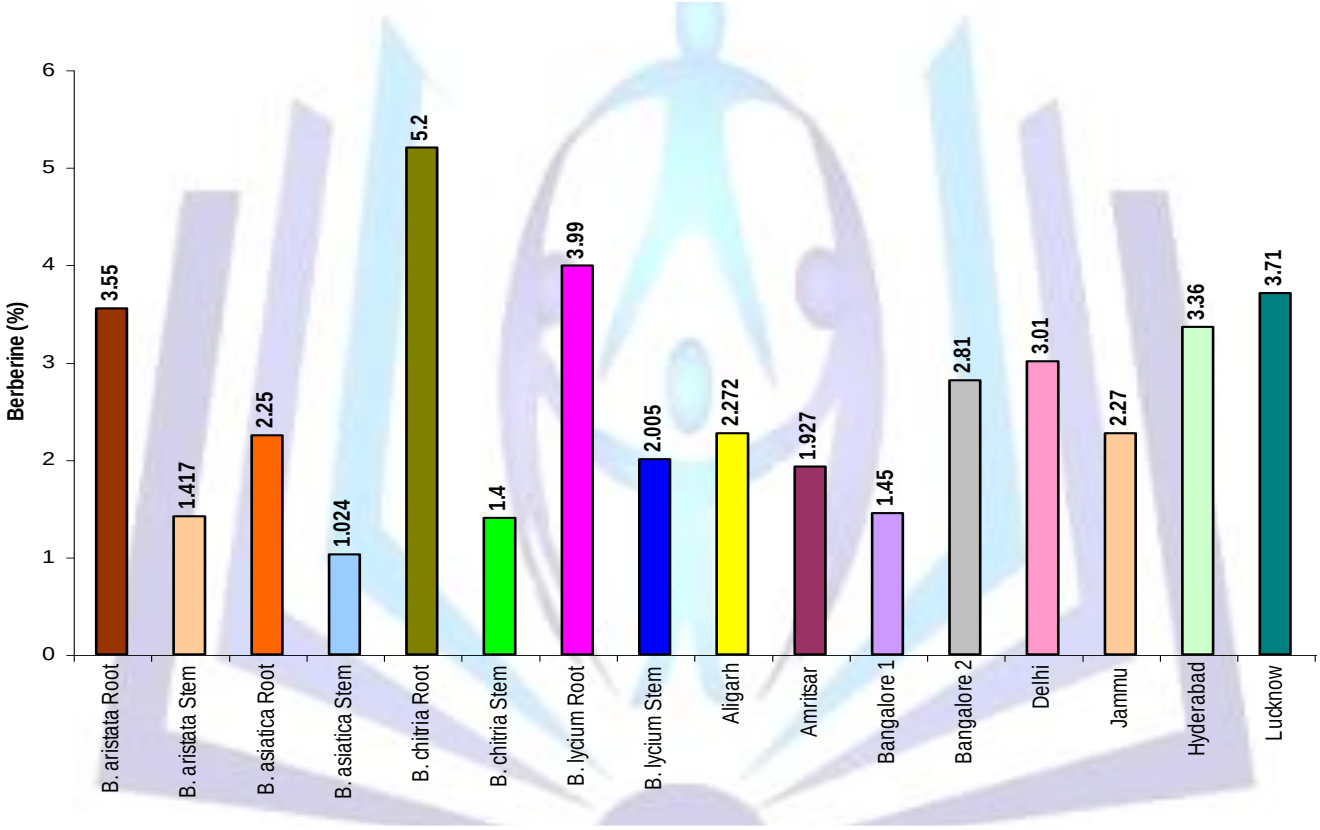

Fig. 22 Quantitative estimation of berberine in different species of *Berberis* and its commercial samples (mean value of three replicates)