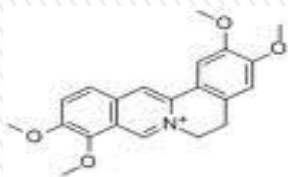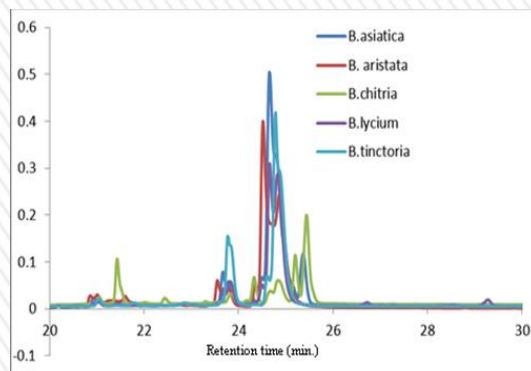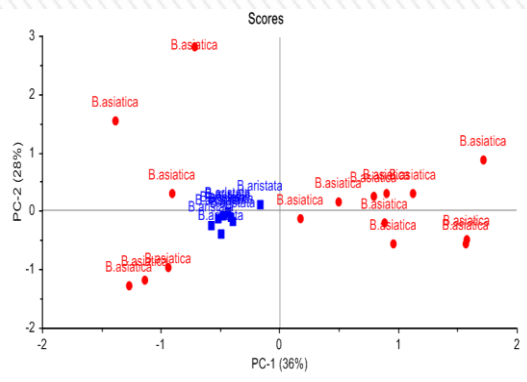# Interspecies variability assessment of *B. aristata* and its related species using HPLC fingerprinting and principle component analysis for standardization and quality control of their herbal drugs

**Daya Bhardwaj and
Nutan Kaushik
TERI University, Delhi**
daya.bhardwaj@teriuniversity.ac.in

Berberis: An Introduction

- A genus of spiny, deciduous, evergreen shrubs,

- with characteristic yellow wood and flowers,

- belongs to family Berberidaceae,

- distributed mainly in temperate and subtropical regions of Europe, Asia, Africa, North America and South America,

- around 500 species of *Berberis* with simple leaves (true *Berberis*) are distributed all over the world.

Medicinal uses of *Berberis* species

- » **Anti-bacterial, Anti-fungal, Anti-viral and Anti-protozoal**
- » **Antioxidant**
- » **Anti-inflammatory**
- » **Hepto-protective**
- » **Anti-hyperglycemic**
- » **Gynecological inflammation**

Distribution of *Berberis* species in India

» **77 species of *Berberis* are reported from India.**

» ***B. aristata* is the most important species as it is used as raw drug and in different Ayurvedic and Homeopathic formulations.**

» ***B. asiatica* is the most commonly distributed species of Indian Himalaya region (IHR).**

» ***B. wallichiana* is specifically distributed in North East Himalayan region**

» ***B. tinctoria* is exclusively exists in Southern part of India.**

Quality issues related to *Berberis* species

» **Three plants used as '*Daruharidra*'**

  > **In Northern India *Berberis aristata* DC. (Family-Berberidaceae),**

  > **In Southern part Coscinium fenestratum (Gaertn.) Colebr (Family- Menispermace),**

  > **In Eastern part *Coptis teeta* Wall. (Family- Ranunculaceae)**

» **Berberine is the active constituent present in all the three species thus on the basis of berberine all of these can be used as substitutes of '*Daruharidra*'.**

» **Over-exploitation of *B. aristata***

» **Adulterants of non plant origin**

Phyto-constituents present in *Berberis* species

Group	Compounds
Alkaloids (**In Most of the species**)	**Berberine, Oxyberberine, Berbamine, Aromoline, Karachine, Palmatine, Oxycanthine, Taxilamine, Jatrorrhizine etc.**
Flavanoids (**In *B. gagnepaini*)**	**Luteolin, 3-isorhamnetin galactoside, Hyperoside, Rutin, Quercitoside**
Anthocyanins (In *B. vulgaris, buxifolia, B. oblonga, thungerbii*)	**Peonodin, Cyanidin, Delphinidin, Leonidin-3-glucoside, Petunidin-3-rutinoside, Delphinidin etc.**
Carotenoids and Vitamins (Berberis spp. Fruits)	**α and βCarotene, Lutein, Zeaxanthin, Capsanthin, Phytofluene, Ascorbic acid etc.**
Phenolic Compounds (**In *B. vulgaris*)**	**N-(p-trans-Coumaroyl) tyramine, Cannabisin G, (±) – Lyoniresinol**

Various alkaloids found in *Berberis* species

Species	Parts examined	Alkaloids present
B. aristata DC	Roots	Berberine (chief alkaloid), Palmatine , Berbamine, oxyberberine, oxyacanthine, Jatrorhizine and Karachine
B. asiatica Roxb	Stems and roots	Berberine, Palmitine, Jatrorrhizine, Columbamine, Tetra Hydropalmitine, Oxyacanthine, Berbamine, Oxyberberine
B. asiatica, var. clarkeana	-	Berberine hydrastine and Neprotine
B. bhutanesis	Roots	Umbellatine and Oxyacanthine
B. insignis	Stems, bark and roots	Umbellatine (chief alkaloid)
B. lycium	Stems, bark and roots	Umbellatine (chief alkaloid)
B. orthobotrys Bienert ex Aitch	Stems, bark and roots	Berberine, Oxyacanthine
B. umbellate	Stem-bark	Umbellatine (chief alkaloid),
B. vulgaris	Root-bark	Berberine, Oxyacanthine and Berbamine
B. wallichiana	-	Umbellatine

Work flow

1. Development of analytical methods for the fingerprinting of *Berberis aristata* and their substitute species distributed in India.

2. Interspecies variability assessment of *B. aristata* and its related species using HPLC fingerprinting and principle component analysis for standardization and quality control of their herbal drugs.

Collection and authentication of B*erberis* species plants from known cultivated area

Collected species

- » *Berberis aristata*
- » *Berberis asiatica*
- » *Berberis umbellata*
- » *Berberis chitria*
- » *Berberis lycium*
- » *Berberis wallichiana*
- » *B. nepalensis*
- » *Berberis tinctoria*

Berberis aristata & its substituted species

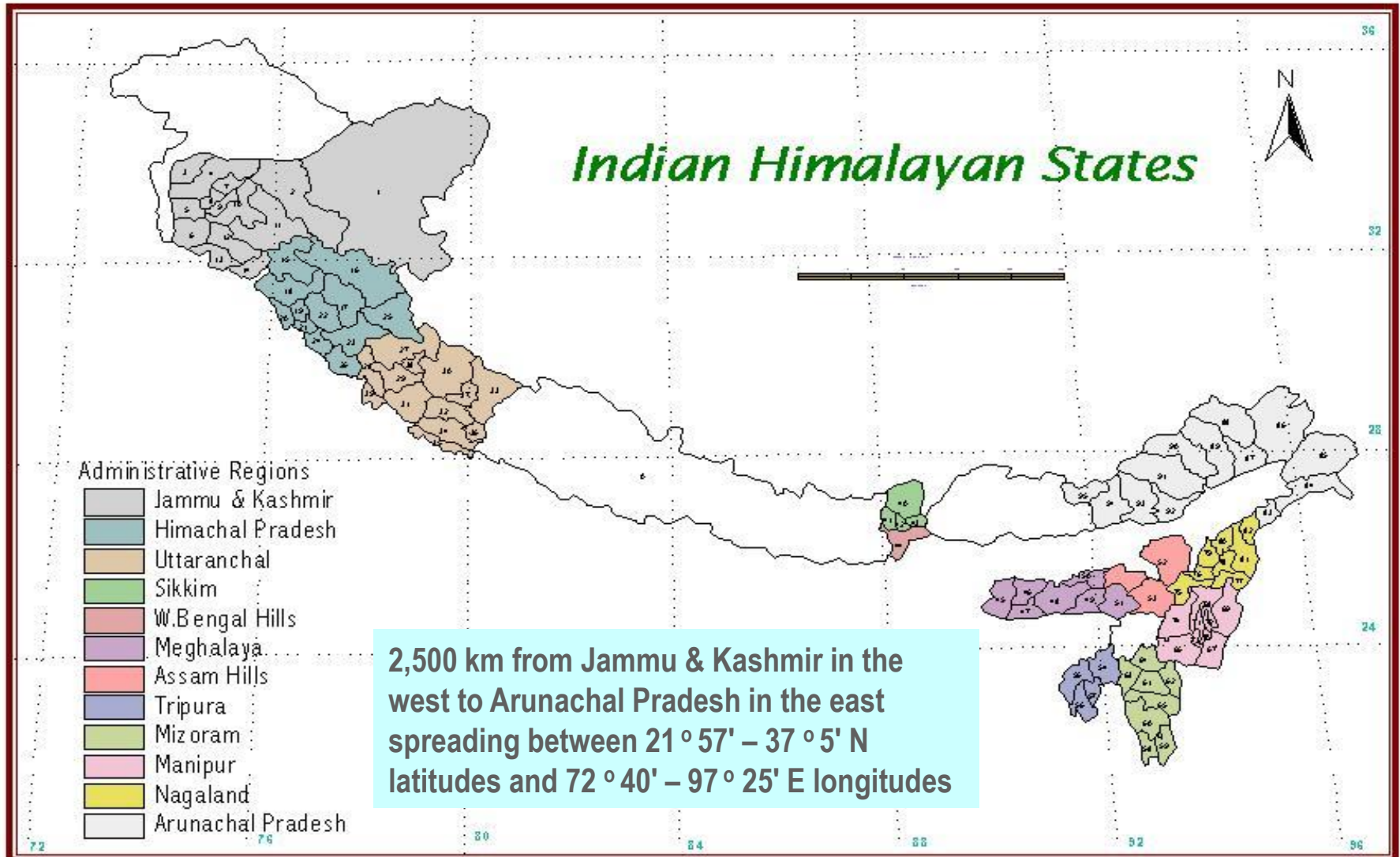**B. asiatica**

B. aristata

B. lycium

B. chitria

B. wallichiana

B. tinctoria

Locations: Indian Himalayan Region (IHR)

Indian Himalayan States

Administrative Regions

- Jammu & Kashmir
- Himachal Pradesh
- Uttaranchal
- Sikkim
- W.Bengal Hills
- Meghalaya
- Assam Hills
- Tripura
- Mizoram
- Manipur
- Nagaland
- Arunachal Pradesh

2,500 km from Jammu & Kashmir in the west to Arunachal Pradesh in the east spreading between $21°57' – 37°5'$ N latitudes and $72°40' – 97°25'$ E longitudes

Map Source: http://gbpihed.gov.in/envis/HTML/monograph3/introduction.html

Locations: South India

Details of *Berberis* samples collected from different locations across India

Species	Location	Altitude (m)	No. of plants collected
Berberis aristata	Shimla	2642	2
	NBPGR Regional Station, Bowali	1635	9
Berberis asiatica Roxb.ex DC	Dehradun	Site 1: 1120 Site 2: 1330	8
	Mukteshwar	Site 1: 2116 Site 2: 2120	4
	Champawat	1924	10
Berberis lycium Royal	Dehradun	Site 1: 1120 Site 2: 1330	4
	Srinagar	1590	1
	Shimla	1895	6
Berberis chitria Lindl.	Mukteshwar	Site 1: 2116 Site 2: 2120	10
	Shimla	1895	4
Berberis tinctoria	Kodaikanal	Site 1: 1779 Site 2: 2113	3
Berberis umbellata	Srinagar	1590	1
Berberis napalnesis	Shillong	967	3
Total plants collected			65

Authentication of plant materials

» Herbarium was prepared for each collected plant.

» Voucher specimens were deposited for identification and authentication to

> BSI, Dehradun

> FRLHT, Bangalore

> NISCAIR, New Delhi

Sample Processing

Separate the leaves, stem, flower, fruits and roots

⬇

Wash the roots thoroughly with tap water and then rinsed with distilled water

⬇

Dry the material except root in shade at room temperature till constant weight

⬇

Roots samples will be dried at 60°C in an oven till constant weight

⬇

Ground to powder form using a grinder

Dried *Berberis* Plant Roots

Leaves

Sample processing steps

Final protocol for sample extraction

Based on the results of optimization of various parameters, the final protocol for sample preparation of *Berberis* species involved extraction of 500 mg of the sample in 3x 15 ml of methanol and water mixture (3:1) by reflux method for an hour. All the samples were processed using this method.

Inter-species chemical variability of *Berberis aristata* and *Berberis asiatica* using HPLC fingerprinting and principle component analysis

In the present objective an attempt has been made to critically analyse the variability among the popular *Berberis* species on the basis of conventional approach and subsequently using the more advanced method of HPLC fingerprinting with principle component analysis.

The experiments were carried out to throw light on the variation in extractive value (%) and berberine content (%) with species, location, part of the plant and season and use of comprehensive HPLC DAD profile with PCA to access the variability among different species and effect of location, and stage of harvested plants (vegetative and reproductive).

Development of HPLC fingerprints of collected *Berberis* species samples

HPLC instrument:

The HPLC analysis was carried out on Water's HPLC system consisting of pump (Waters 600 controller), autosampler (Waters 717 plus) and PDA detector (Waters 2996).The separation was achieved using RP-18 column (4.6 x 250 mm, 5 μm). Empower 2 software was used for data processing.

Mobile phase for HPLC analysis:

Water (0.08% orthophosphoric acid) (A) and Acetonitrile (B) using a linear gradient program of 100% A in 0-5 min., 100% B in 5-45 min, 100 % A in 45-60 min.

Chemo-metric data analysis

The chemometric data analysis was done using multivariate software Unscrambler X (version 10, CAMO, USA).

Estimation of extractive value(%) and berberine content (%)

➢The maximum extractive value was found in leaves followed by the root and stem.

➢The maximum average berberine content (%) was obtained in root followed by the stem and leaves corresponding to 1.6%, 0.29%, and 0.098% respectively.
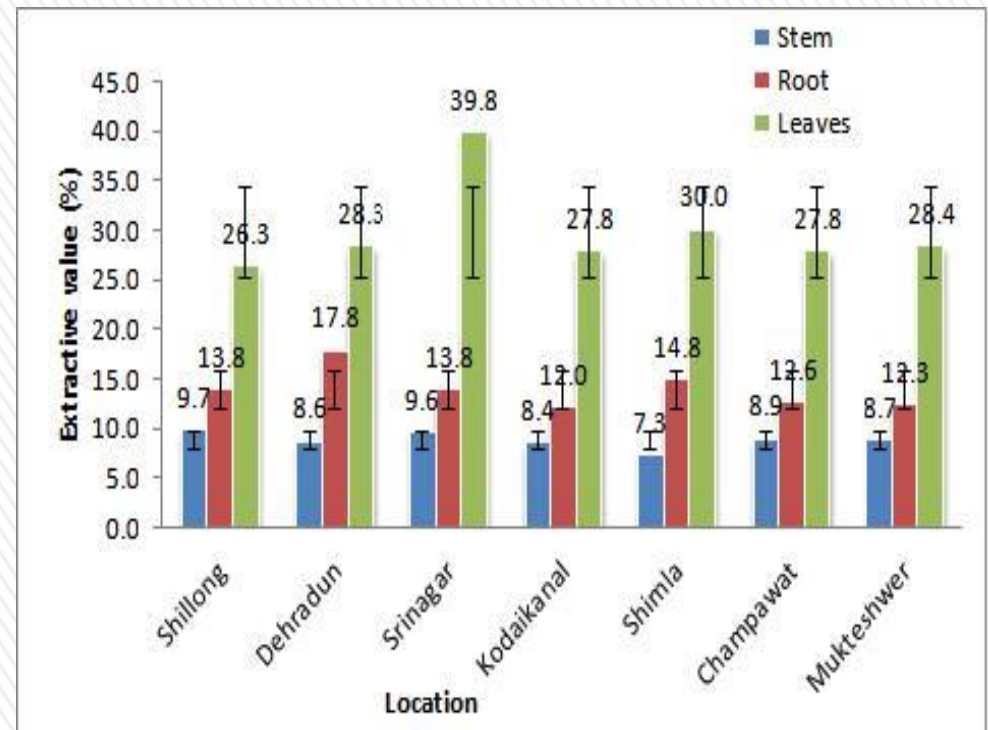
Bar graph of the average extractive values (%) of *Berberis* species collected from various locations of India

Estimation of berberine content (%)

Location	Average Berberine content (%) in stem±SD					
	B.asiatica	*B.lycium*	*B.chitria*	*B.tinctoria*	*B.umbellata*	*B. aristata*
Dehradun	0.34±0.62	0.39±0.13	NA	NA	NA	NA
Shimla	NA	0.53±0.23	0.05±0.01	NA	NA	NA
Champawat	0.08±0.02	NA	NA	NA	NA	NA
Mukteshwer	0.83±0.61	NA	0.24±0.18	NA	NA	NA
Kodaikanal	NA	NA	NA	0.35±0.03	NA	NA
Srinagar	NA	NA	NA	NA	0.02±0.01	NA
Kinnaur	NA	NA	NA	NA	NA	0.27±0.14
	Average Berberine content (%) in root±SD					
	B.asiatica	*B.lycium*	*B.chitria*	*B.tinctoria*	*B.umbellata*	*B. aristata*
Dehradun	2.37±0.23	2.94±0.54	NA	NA	NA	NA
Shimla	NA	1.05±0.67	1.05±0.02	NA	NA	NA
Champawat	2.72±0.40	NA	NA	NA	NA	NA
Mukteshwer	1.3±0.45	NA	o.58±0.24	NA	NA	NA
Kodaikanal	NA	NA	NA	1.37±0.03	NA	NA
Srinagar	NA	NA	NA	NA	1.39±0.34	NA
Kinnaur	NA	NA	NA	NA	NA	1.12±024

➢The maximum average berberine content (%) was obtained in root of *B. Lycium* Dehradun (2.94%) followed by *B. asiatica* of Champawat
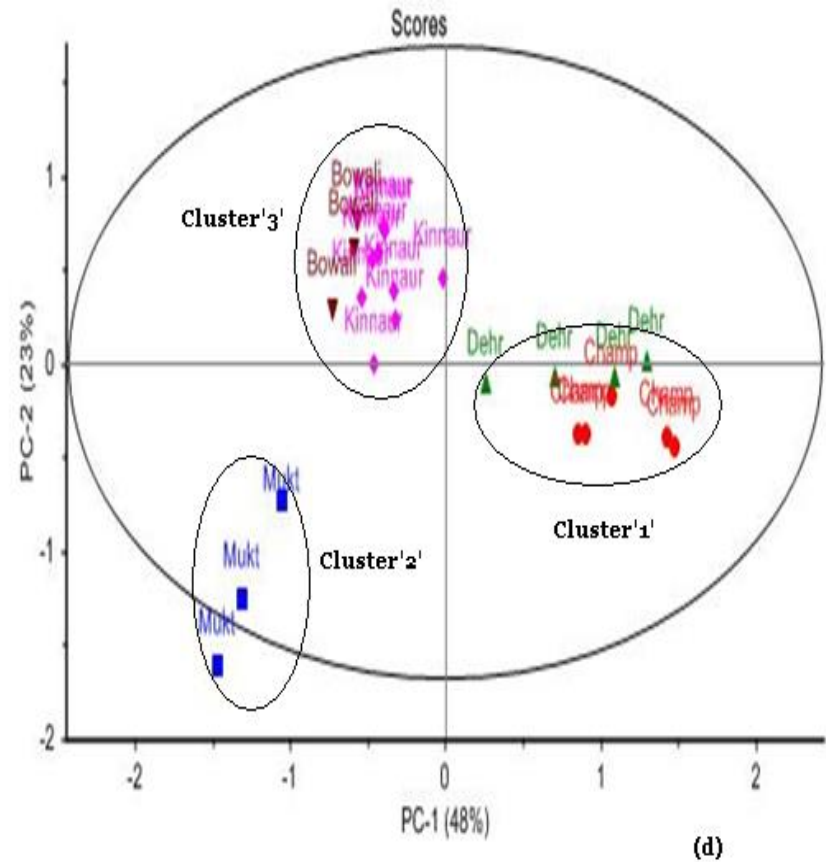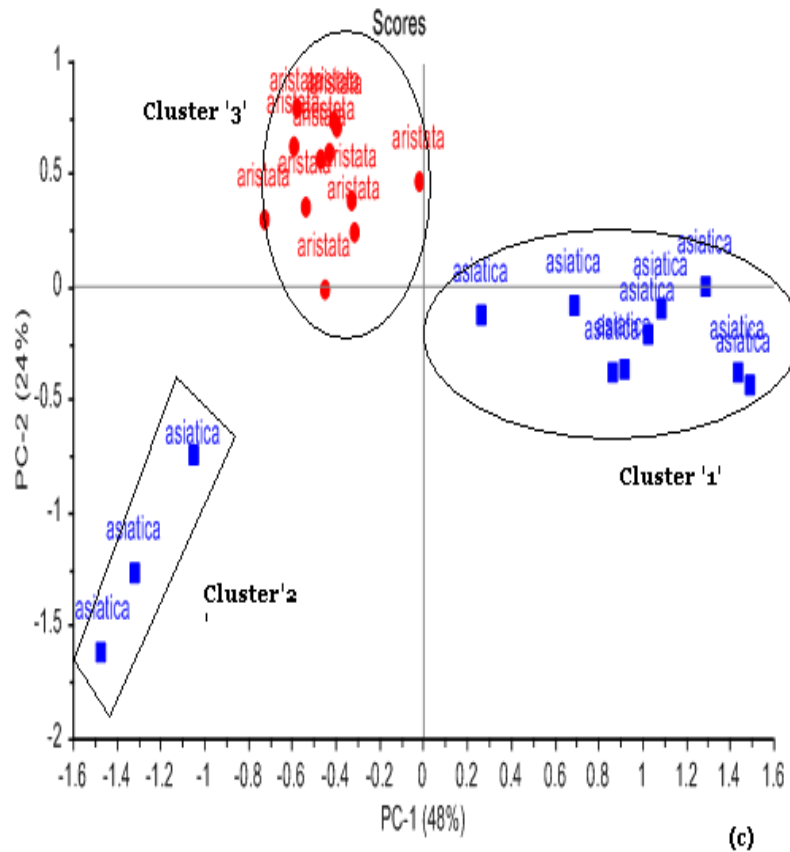
Differentiation of *B.aristata* and *B.asiatica* using Principle Component analysis
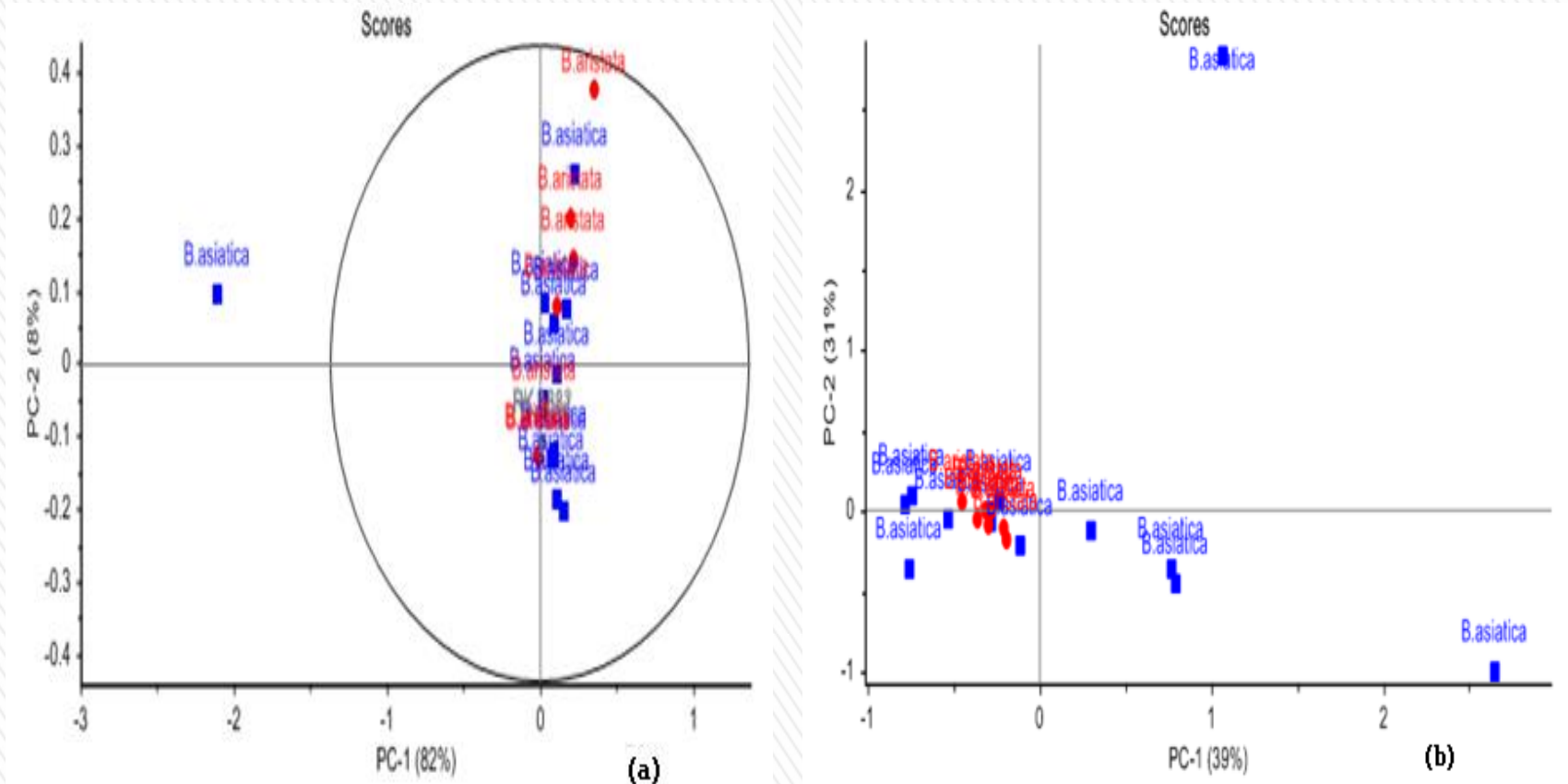
PCA score plot for the differentiation of *Berberis aristata* and *Berberis asiatica* (a) leaves and (b) stem extracts using HPLC fingerprinting
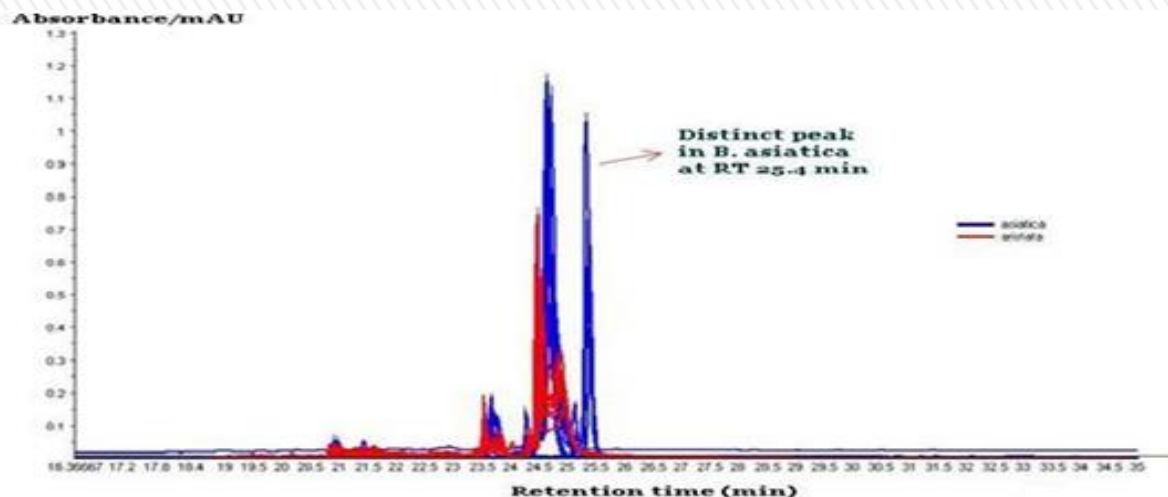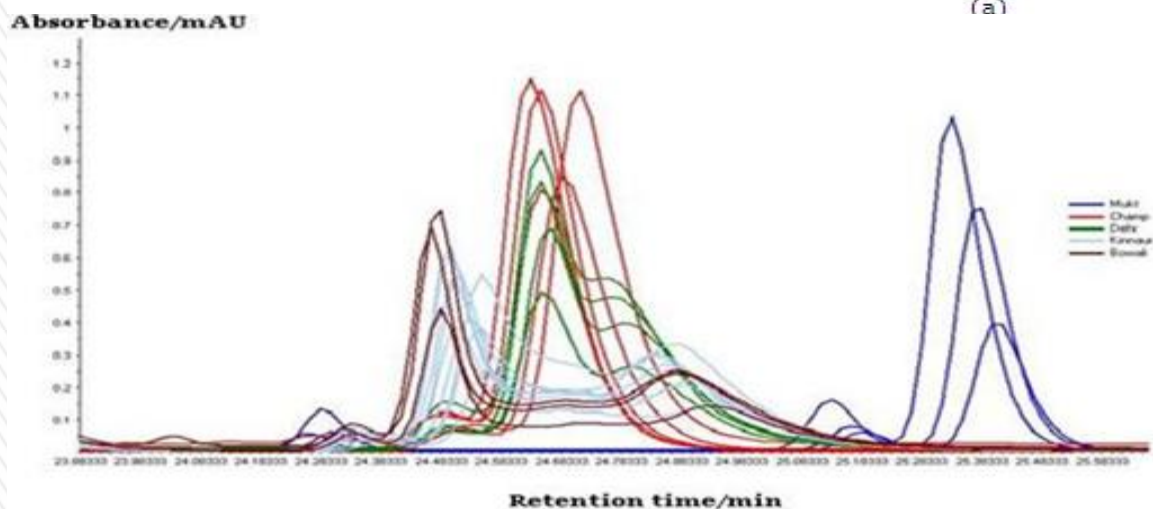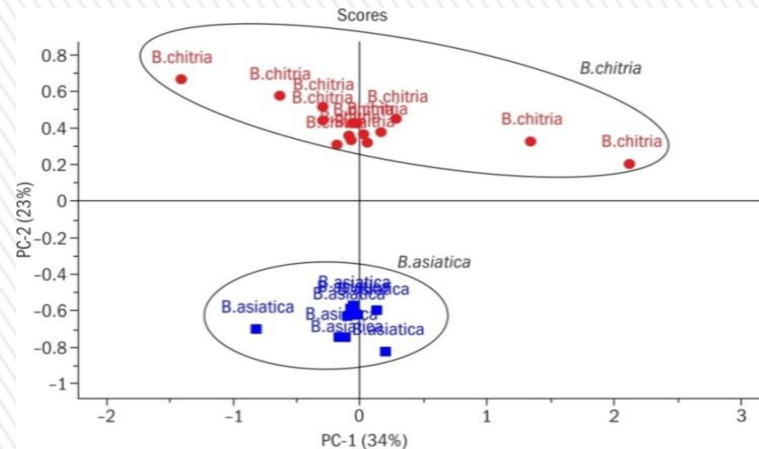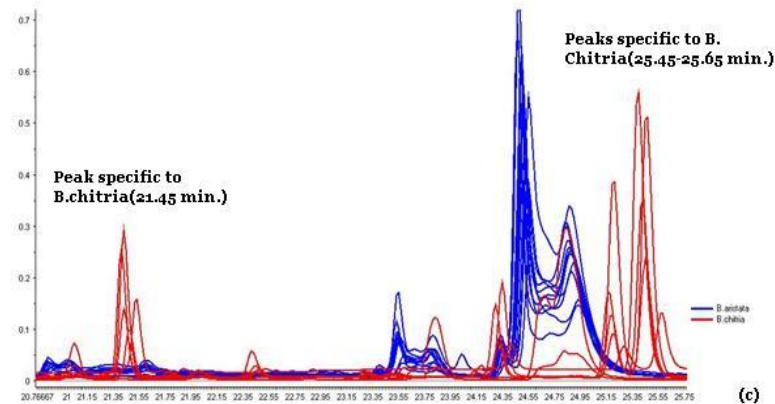
Differentiation of *B.aristata* and *B.asiatica*

PCA score plot for the differentiation of (c) *Berberis aristata* and *Berberis asiatica* and (d) location for root extract using HPLC fingerprinting

Differentiation of *B.aristata* and *B.asiatica*

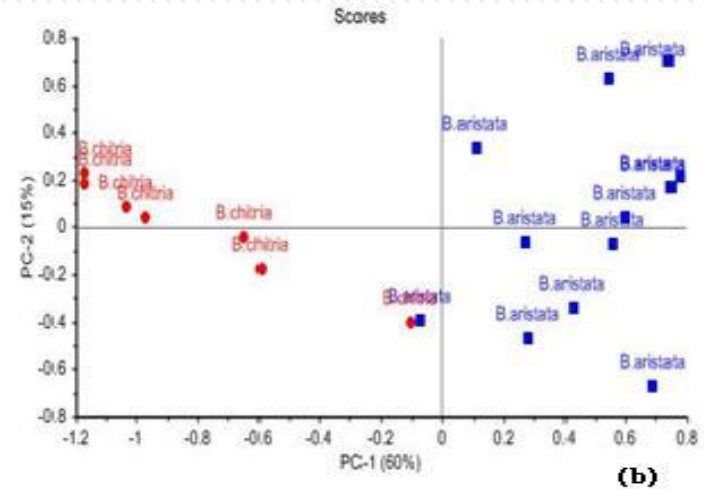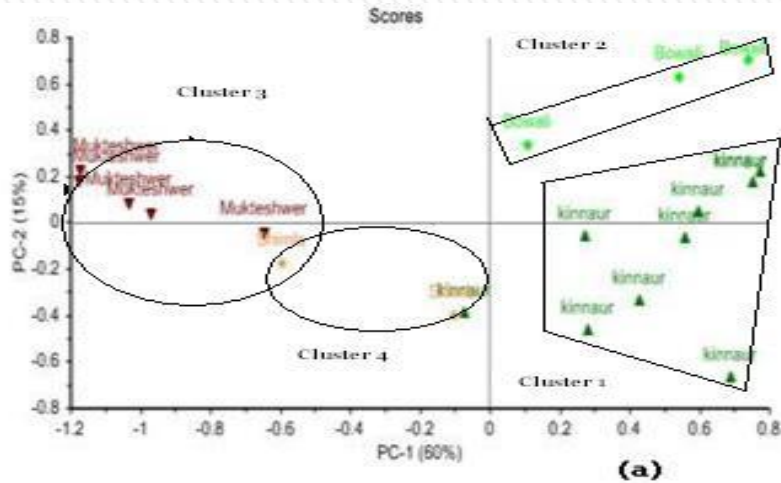> The peak at RT 24.5 min. impart variability to *B. aristata* samples.

Overlay chromatogram for the differentiation of *Berberis aristata* and *Berberis asiatica* root sample (a) between 0 to 60 min and (b) 15 to 35 min

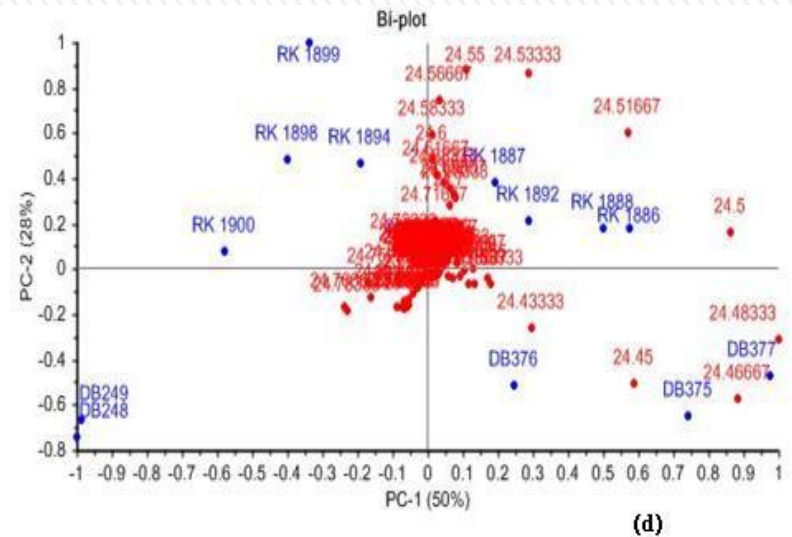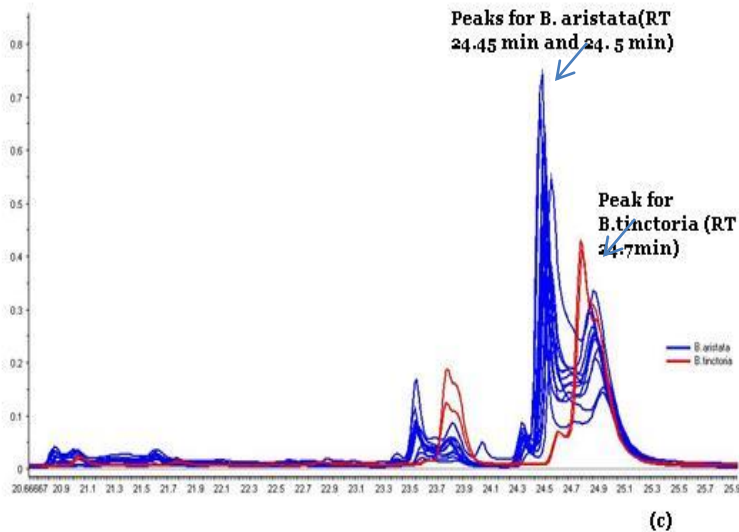# Differentiation of *B.aristata* and *B.chitria*

Differentiation of *B. aristata* and *B. chitria* (a) and (b) PCA score plots (c) overlay chromatogram (d) Differentiation of single location (Mukteshwer) species

Differentiation of *B.aristata* and *B. lycium*
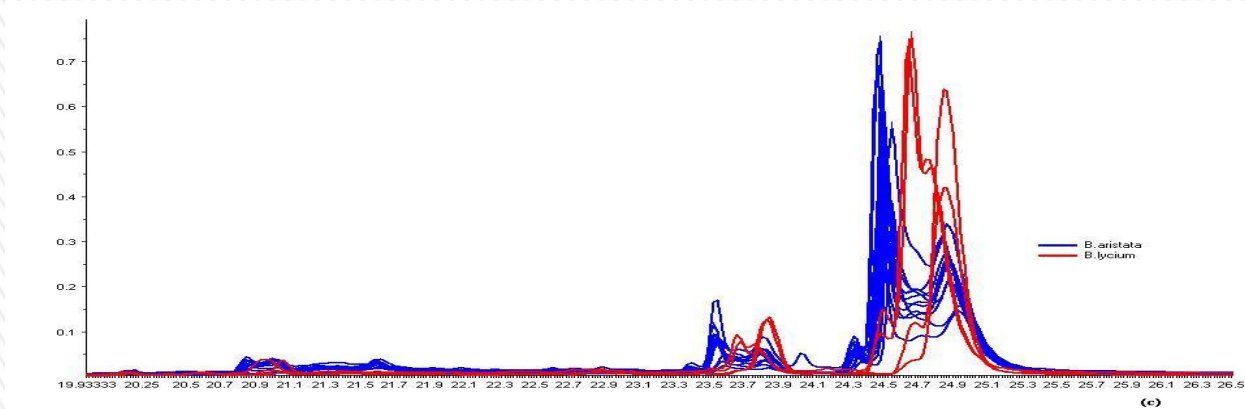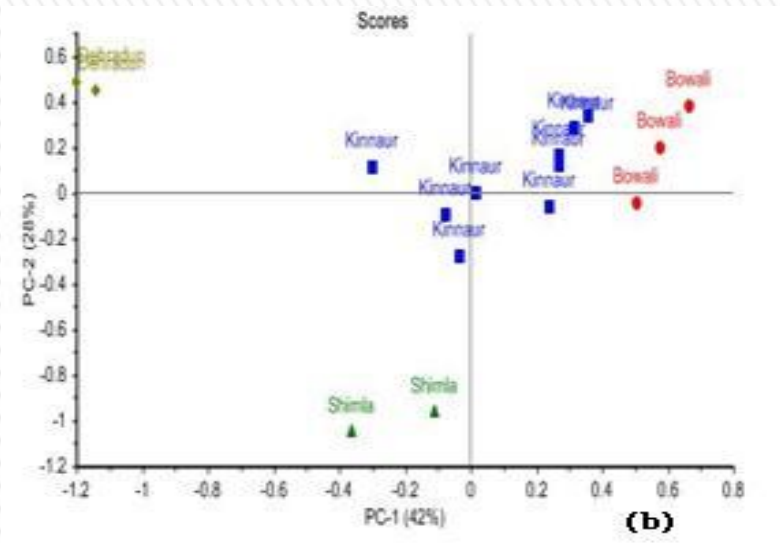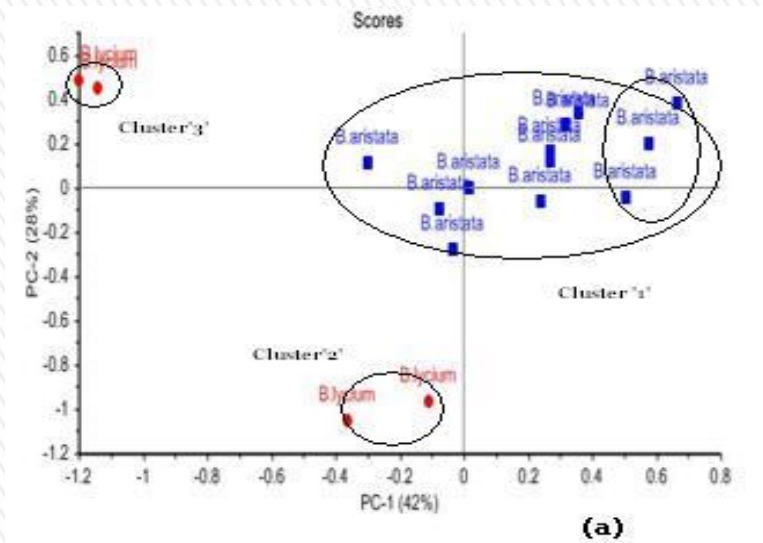
Differentiation of *B. aristata* and *B. lycium* (a) and (b) PCA score plots (c) overlay chromatogram

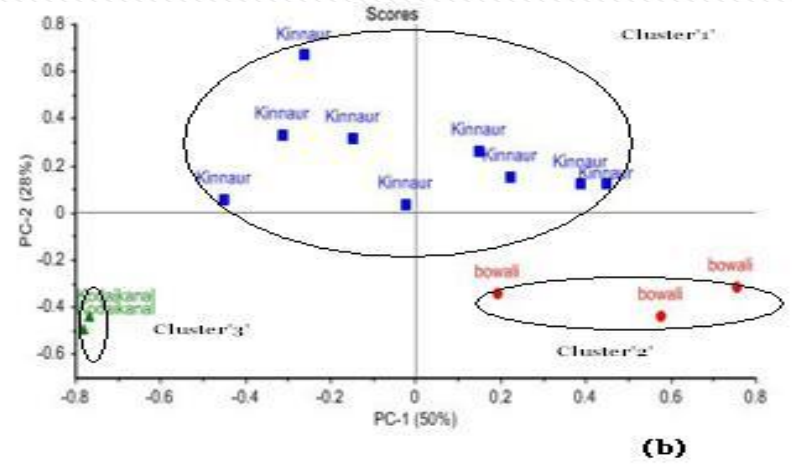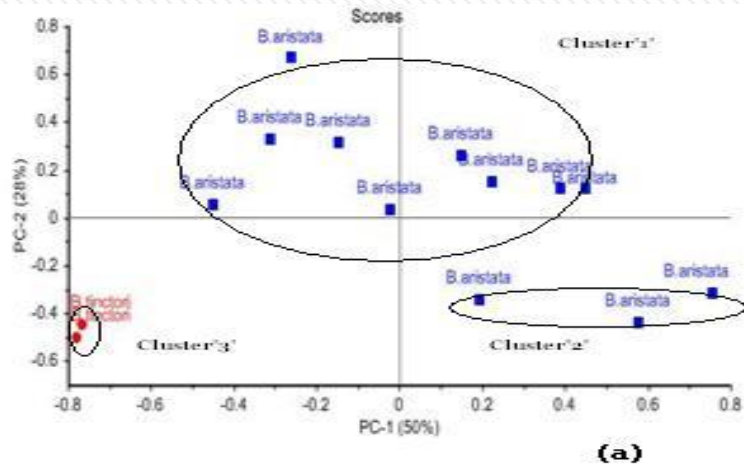# Differentiation of *B.aristata* and *B. tinctoria*

Differentiation of *B. aristata* and *B. tinctoria* (a) and (b) PCA score plots (c) overlay chromatogram (d) PCA bi-plot

Differentiation of multi-location root samples of *B. aristata* and its related species
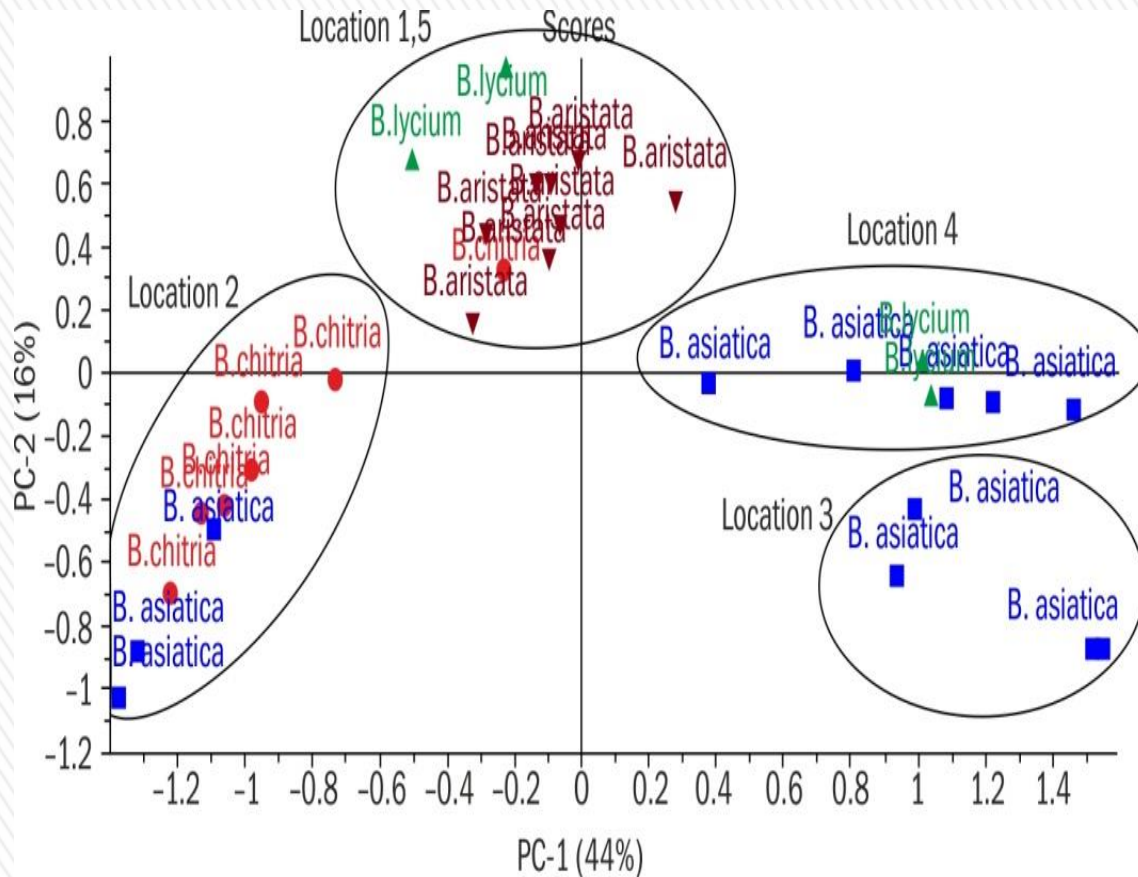
PCA Score plot for differentiation of multi location *Berberis* species developed using HPLC fingerprinting

Study of inter species chemical variability between *B. aristata* and *B. asiatica* by similarity analysis using HPLC fingerprinting

The mean standard HPLC fingerprinting chromatogram was generated for the stem, roots and leaves of *B. aristata* and *B. asiatica* and chemical variability or similarity was detected by estimating the correlation coefficient between the species and within the species.
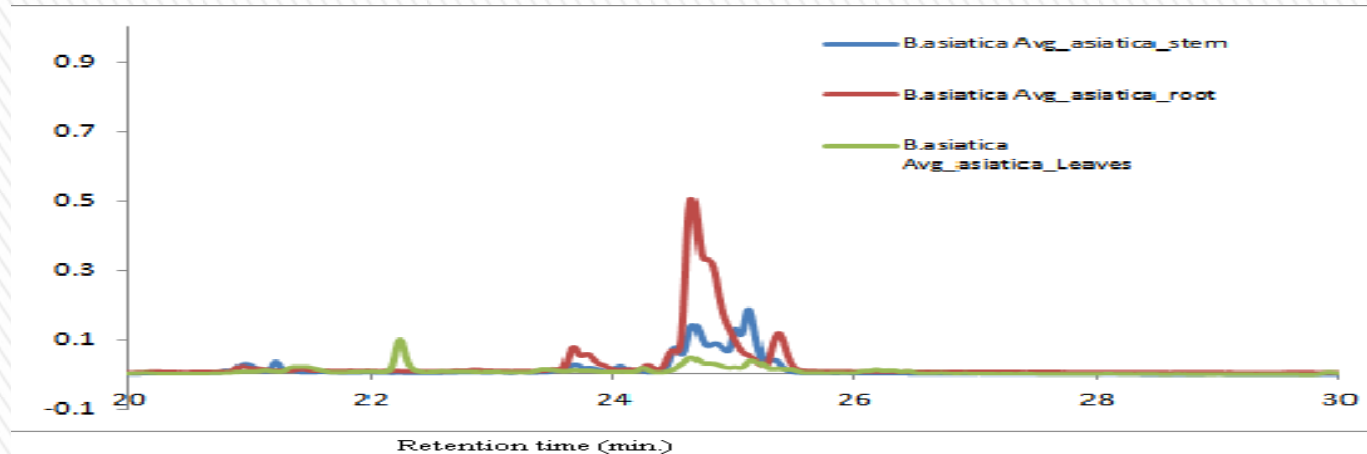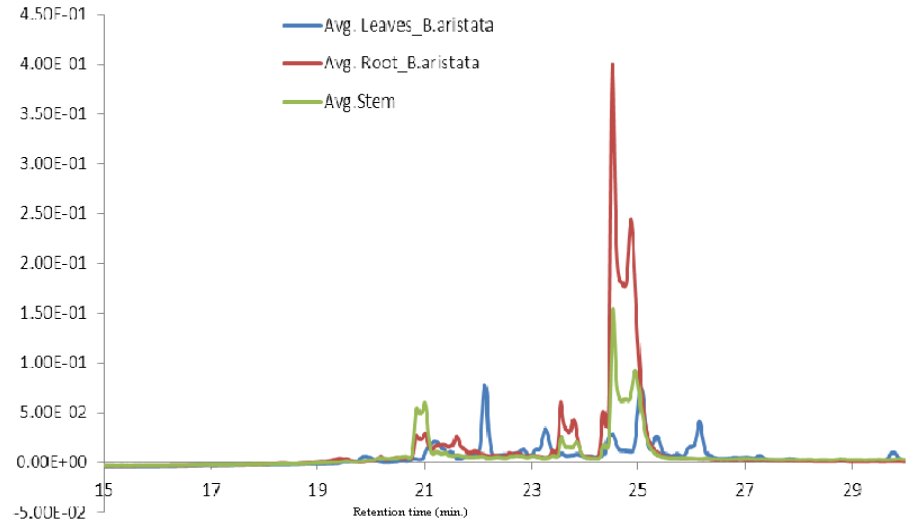
Study of inter species chemical variability between *B. aristata* and *B. asiatica* by similarity analysis using HPLC fingerprinting

Similarity analysis of *B. aristata* with the mean standard chromatogram of its root samples

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12
Location	Location 1									Location 2		
Correlation coefficient*	0.98	0.98	0.98	0.99	0.99	0.96	0.92	0.91	0.88	0.88	0.92	0.92

* Estimated with respect to the mean chromatogram of *B. aristata*

Similarity analysis of *B. asiatica* with the mean standard chromatogram of its root samples

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12
Location	Location 1					Location 2				Location 3		
Correlation coefficient*	0.79	0.84	0.79	0.79	0.74	0.94	0.94	0.95	0.95	0.68	0.75	0.72

* Estimated with respect to the mean chromatogram of *B. asiatica*

Identification of unknown species using similarity analysis

For verifying the ability of similarity analysis method for the identification and authentication of unknown *Berberis* species, raw HPLC data of five random samples were selected from identification on the basis of calculating their similarity index.

The chromatogram of these samples were compared with the mean chromatograms of all *Berberis* species under study and Pearson coefficient calculated, the sample species could be identified on the basis of maximum value of Pearson coefficient.

Inter species similarity index of *B. aristata* and its related species on the basis of Pearson correlation coefficient

Species	B.asiatica	B. aristata	B.chitria	B.lycium	B.tinctoria	B.nepalnesis
B.asiatica	1.00	0.74	0.40	0.95	0.72	0.61
B. aristata		1.00	0.33	0.78	0.67	0.62
B.chitria			1.00	0.36	0.34	0.42
B.lycium				1.00	0.84	0.61
B.tinctoria					1.00	0.65
B.nepalnesis						1.00

Identification of unknown species using similarity analysis

Sample no.	B. asiatica	B. aristata	B .chitria	B. lycium	B. tinctoria	B .nepalnesis
1	0.963*	0.715	0.300	0.932	0.683	0.534
2	0.738	0.676	0.346	0.854	0.996*	0.637
3	0.131	0.102	0.832*	0.078	0.063	0.219
4	0.796	0.947*	0.321	0.814	0.684	0.591
5	0.454	0.539	0.318	0.557	0.658	0.642

The sample no. 1, 2, 3 and 4 were the known samples of *B. lycium, B. tinctoria, B.chitria* and *B. aristata* respectively.

Whereas the sample no. '5' collected from Shimla could not be authenticated by NISCAIR, Delhi and has been declared as unknown species. The results of similarity analysis have been very promising, as could accurately identify the sample no. 2, 3, and 4. For sample no '1' though the maximum Pearson coefficient has been calculated with *B. asiatica* but the sample has been authenticated as *B. lycium.* As, it was evident from the results of interspecies similarity evaluation that *B. lycium* and *B. asiatica* has strong correlation and could be considered as substitute species of each other.

Very interestingly, sample '5' could not show strong correlation with either of species and confirms the results of morphological authentication results. The sample '5' may be a new species or some species not covered under the present study.

Concluding Remarks

➢ After critically analysing the variability among the popular *Berberis* species on the basis of conventional approach and subsequently using the more advanced method of HPLC fingerprinting with principle component analysis it could be concluded that significant variation in extractive value (%) and berberine content (%) exists with respect to selection of a particular species, location, part of the plant and stage of harvested plants (vegetative and reproductive).

➢ HPLC fingerprinting with PCA and similarity analysis were more appropriate method to assess the chemical variability between different *Berberis* species.