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Original Article

Physicochemical and HPTLC analysis method for differentiation of *Boerhavia diffusa* from *Boerhavia verticillata* and *Boerhavia repanda* with application of PCA

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ABSTRACT

Boerhavia is one of the highly polymorphic genus in Nyctaginaceae family. *Boerhavia diffusa* is a well-known *Ayurvedic* plant useful in many diseases like diabetes, inflammation, renal disorders, obesity, anemia etc. It is deliberately adulterated with other species like *Boerhavia repanda* and *Boerhavia verticillata* in pharmaceutical field.The study was planned to authenticate *B. diffusa*(BD) from other two species *B. Verticillata*(BV) and *B. repanda*(BR) with the help of its physicochemical and high performance thin layer chromatographic (HPTLC) profiling along with data mining technique as Principal Component Analysis (PCA).The whole plant powders of respective samples were subjected to physicochemical analysis and chromatographic study. The physicochemical results were analyzed by PCA. HPTLC analysis was performed in solvent system Toluene: Ethyl acetate: Methanol (7:1:2%v/v).The present findings suggest that physicochemical analysis can distinguish *B. diffusa* from *B. verticillata* and *B. repanda* by PCA on favor of thermal and solubility category variables. In HPTLC profiling 0.24 and 0.58 Rf spectra may be considered as marker spectra to identify the genuine BD. Physico-chemical, chromatographic fingerprinting along with PCA is developing the unorthodox reliable analytical method to quality monitoring of respective phylogenetic plant.

Key words: *B. diffusa, B. verticillata, B. repanda,* Physicochemical profiling, HPTLC, PCA, Discrimination.

INTRODUCTION

Boerhavia is an important genus with wide range of medicinal uses. The genus Boerhavia is extensively used by local peoples and medicinal practitioners for treatments of hepatitis, urinary disorders, gastro intestinal diseases, inflammations, skin problems, infectious diseases and asthma.[i] About 40 species are distributed in tropical, subtropical and temperate regions.[ii] Among these, 6 species are reported in India and *Boerhavia diffusa* is indigenous.[iii] *Boerhavia diffusa* (Family: Nyctaginaceae) is commonly known as *Raktapunarnava*, *Shothaghni*, *Kathillaka*, *Kshudra*, *Varshabhu*, *Raktapushpa*, *Varshaketu* and *Shilatika*. The plant is also called "*Punarnava*," due to its ability to regenerate in rainy season with the help of perennial roots after the aerial parts get dried up completely in summer.[iv] BD is an important herbal constituent of various *Ayurvedic* formulations. In *Ayurvedic* texts, more than 35 formulations of different types contain it as major ingredient. It has been used in various formulations meant for inflammation, jaundice, asthma, rheumatism, nephrological disorders, ascites, anemia, and gynecological disorders.[iii] *B. diffusa* is extensively adulterated with other species like *Boerhavia erecta*, *Boerhavia repanda, Boerhavia coccinea* and *Boerhavia verticillata*.[ii] This study was planned to discriminate *B. diffusa* from its two adulterants species i.e. *B. verticillata* and *B. repanda* by means of physicochemical evaluation and HPTLC study. In this paper, we present PCA tool that discriminates *B. diffusa* from *B. verticillata* and *B. repanda* using physicochemical parameters. PCA relies on the linear transformation of the original set of measurements into a substantially smaller set of orthogonal variables while retaining as much information present in the original data set as possible. This method easily discriminates the three nearest species of Boerhavia by applying well-known higher order PCA on Physico-chemical profiling.

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MATERIAL AND METHODS

Procurement of raw materials: The whole plant of *B. diffusa* was collected from Gujarat Ayurved University campus, Jamnagar. *B. verticillata* was collected from Sasoi, Jamnagar and *B. repanda* was collected from Sapada, Jamnagar. All samples were authenticated by Pharmacognosy department, Gujarat Ayurved University with reference number– Phm. 6145/18/06/2015 for *Boerhavia diffusa*, Phm. 6152/22/11/2015 for *Boerhavia verticillata*, Phm. 6153/22/11/2015 for *Boerhavia repanda*. Drugs were shade dried and powdered for further use.

Physicochemical study: Physicochemical study was carried out at the Pharmaceutical Chemistry Laboratory of the institute. Preliminary phytochemical investigations such as Molisch's test, Salkowski test, Keller- Killiani test, Flavonoid test, Dragendorff's test and test for tannins were performed.[v] Physico-chemical parameters like loss on drying, ash value, acid insoluble ash, water and alcohol soluble extractive value were carried out in triplicate by following standard procedure as mentioned in Ayurvedic Pharmacopoeia of India for BD.[vi]

HPTLC study: HPTLC study was carried out with methanolic extract. Each of 5µl methanol extract of BD, BV and BR were spotted on pre-coated Silica Gel GF_{254} plates by means of Camag Linomat V sample applicator. The mobile phase consisted of Toluene:Ethylacetate:Methanol in a ratio of 7:1:2%v/v. After development, the densitometric scan was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under the control of Wincats software. Further spectral detection was also performed.

Principal Component Analysis: All the physicochemical data were tabulated in two way matrix form. One way is respective sample and another way thermal and solubility category parameter (LOD, AV and AIA, WSE and MSE). The single table data were executed for PCA with help of Unscrambler Camo ® student version.

RESULTS

Phytochemical screening: Preliminary phytochemical screening was carried out. Qualitative tests revealed presence of alkaloids, carbohydrates, glycosides, flavonoids, steroids, tannins, proteins and amino acids in all 3 samples. [Table 1]

Physicochemical analysis: Physicochemical studies were done on samples. All measurements were taken in triplicate. The list of parameters performed and observational values obtained are listed in [Table 2].

Table 1: Preliminary Phytochemical Screening of plant extract

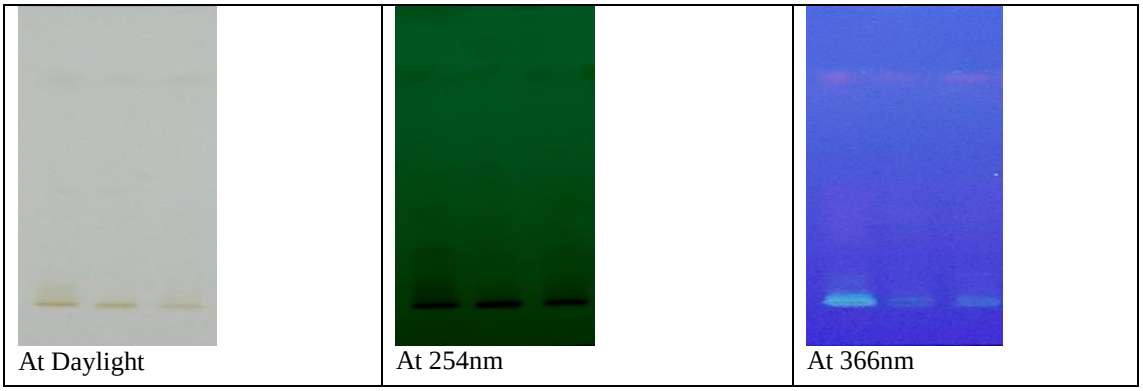
Functional group	*B. diffusa*	*B. verticillata*	*B. repanda*
Alkaloids	+	+	+
Carbohydrates	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Proteins	+	+	+
Amino acids	+	+	+

Table 2: Physicochemical parameter of BD, BV and BR

Sample	LOD (%w/w)	AV (%w/w)	AIA (%w/w)	WSE (%w/w)	MSE (%w/w)
BD1	7.3	11.24	3.82	18.24	12.8
BD2	6.5	9.78	3.68	17.84	12.4
BD3	6.3	9.48	3.54	17.3	10.16
BV1	8.8	7.62	4.17	16.88	11.44
BV2	8.7	7.32	4.59	16.84	12.32
BV3	8.6	7.47	4.38	16.79	11.88
BR1	8.6	9	3	8.8	8.24
BR2	8.3	9.68	2.8	10.83	7.76
BR3	8.5	10	2.6	11.2	8.32

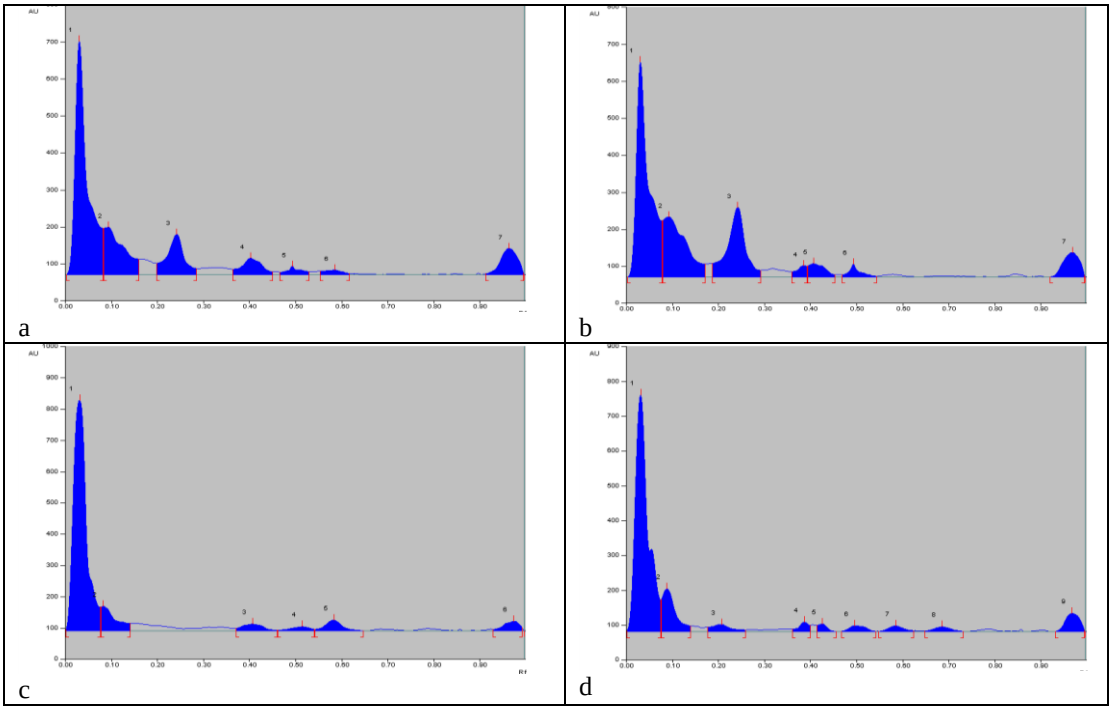
BD1=Reading 1 of BD, BD2= Reading 2 of BD, BD3=Reading 3 of BD, BV1= Reading 1 of BV, BV2=Reading 2 of BV, BV3=Reading 3 of BV, BR1=Reading 1 of BR, BR2= Reading 2 of BR, BR3= Reading 3 of BR, LOD=Loss on Drying, AV=Ash Value, AIA=Acid Insoluble Ash, WSE=Water Soluble Extractive, MSE=Methanol Soluble Extractive

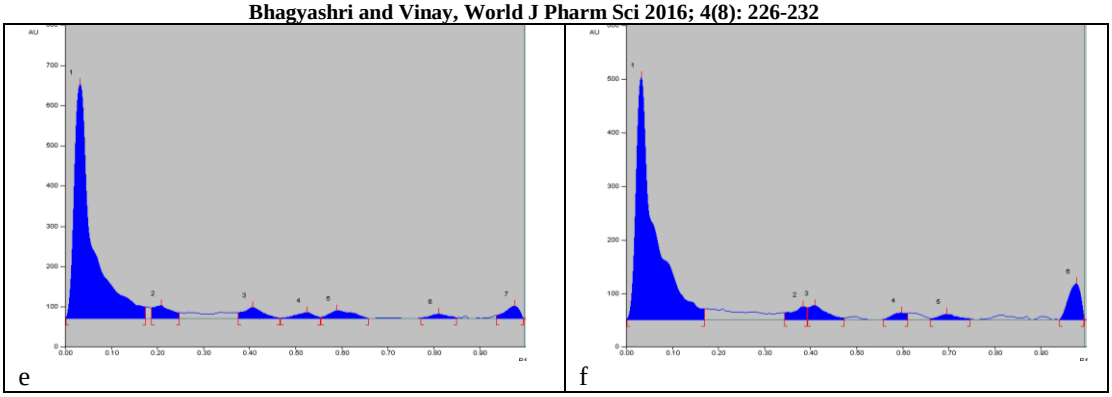
HPTLC analysis: Plate was scanned under short UV (254 nm) and long UV (366 nm). After scanning, further spectral detection was performed. In which following results were obtained in HPTLC study. Figure 1 shows images of HPTLC plates under daylight, 254 nm and 366 nm. Figure 2 depicts densitograms of samples at 254 nm and 366 nm. Figure 3 presents spectral comparison of overlapping spectra. The resulted peak table is given in table 3.



Track 1: BD, Track 2: BV, Track 3: BR

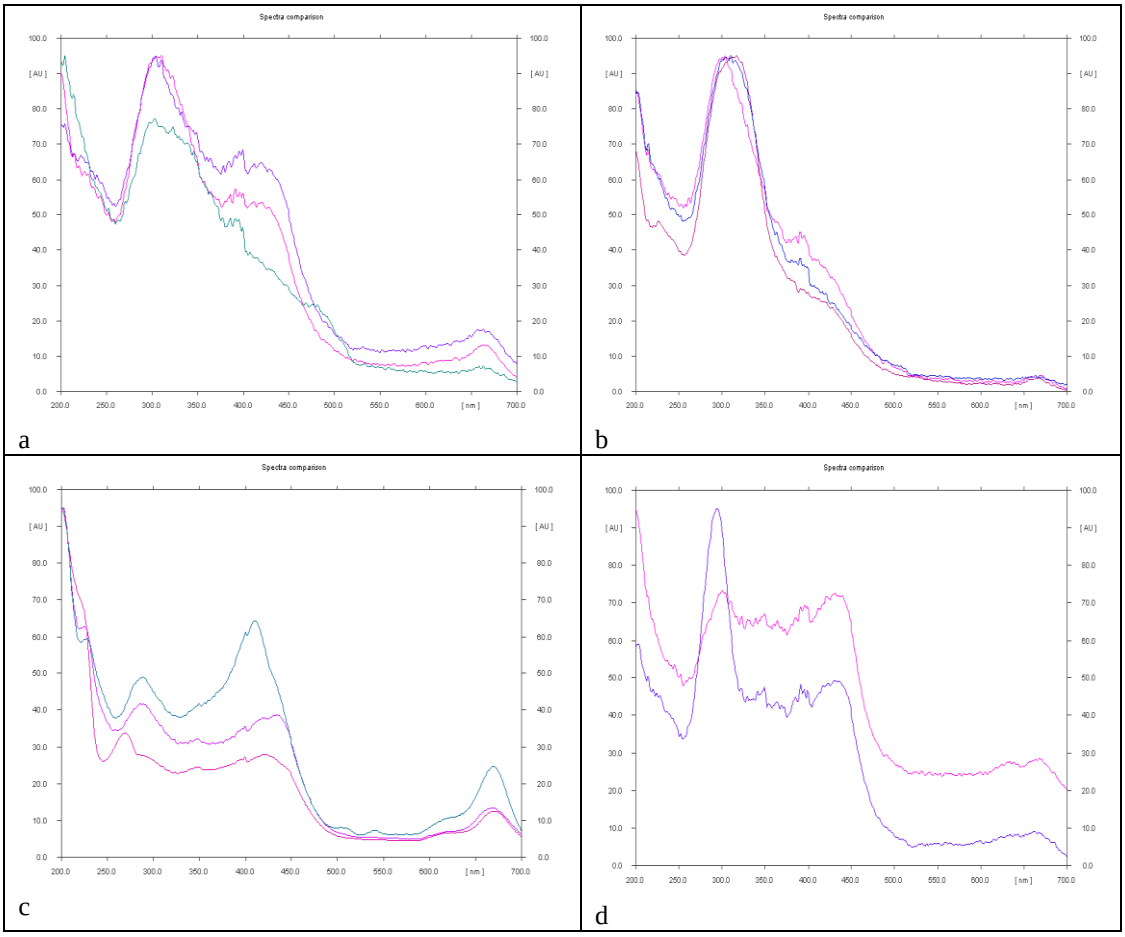
Figure 1: HPTLC photo documentation





e f

Figure 2: Densitogram of (a) BD at 254nm (b) BD at 366nm (c) BV at 254nm (d) BV at 366nm(e) BR at 254nm (f) BR at 366nm



a b c d

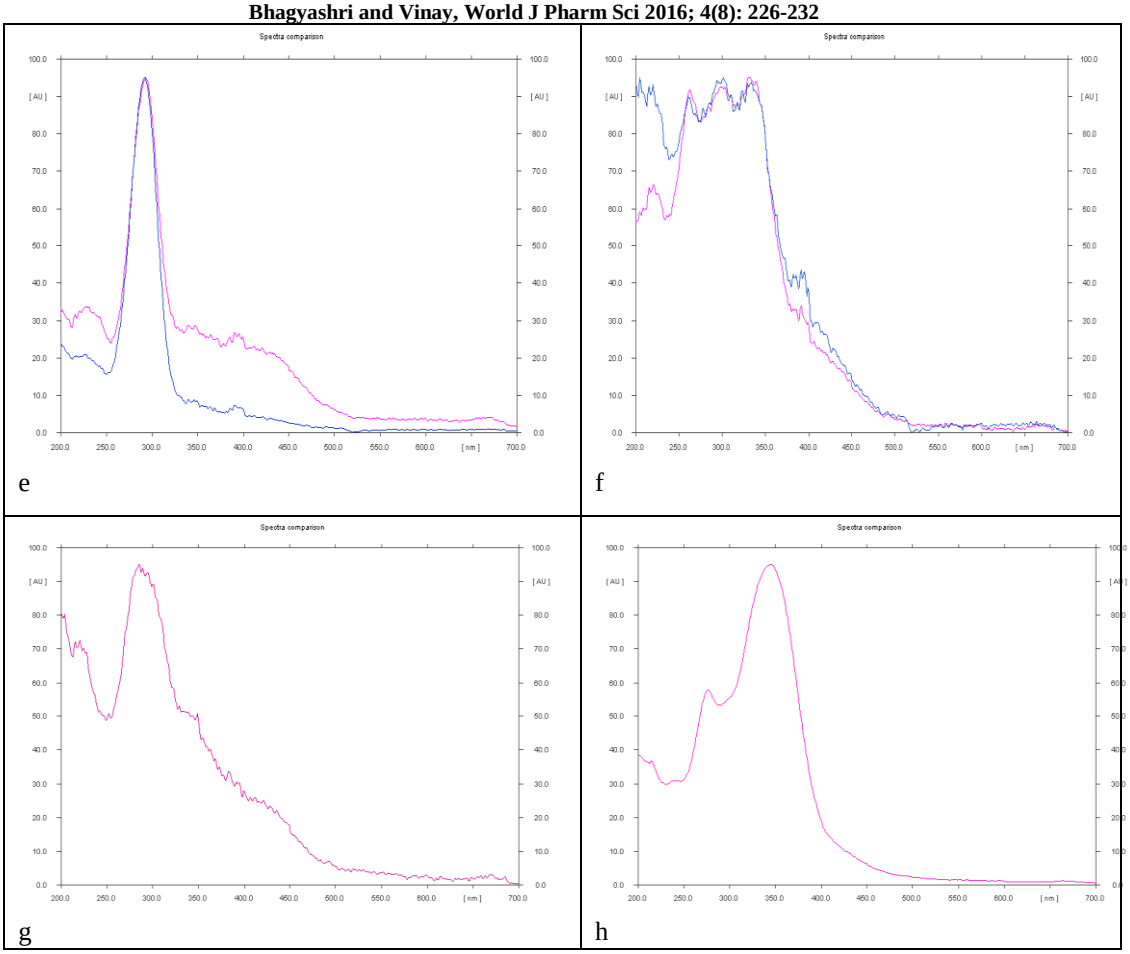


Figure 3: Spectral comparison at (a) 0.38Rf in BD, BV and BR, (b) 0.41Rf in BD, BV and BR, (c)0.96 Rf in BD, BV and BR and (d) 0.49Rf in BD and BV, (e) 0.51Rf in BV and BR and (f) 0.58Rf in BV and BR (g) BD at 0.58 Rf (h) BD at 0.24 Rf

Table 3: Rf values under 254nm and 366nm

Sample	Rf values at 254nm		Rf values at 366nm	
	No. of peaks	Rf value	No. of peaks	Rf value
BD	7	0.03, 0.09, 0.24, 0.40, 0.49, 0.58, 0.96	7	0.03, 0.09, 0.24, 0.39, 0.41, 0.49, 0.97
BV	5	0.03, 0.08, 0.41, 0.58, 0.97	9	0.03, 0.09, 0.21, 0.39, 0.43, 0.50, 0.58, 0.69, 0.97
BR	7	0.03, 0.21, 0.41, 0.52, 0.59, 0.81, 0.98	6	0.03, 0.38, 0.41, 0.60, 0.70, 0.98

PCA study: Principal Component Analysis was performed on tabulated triplicate of physicochemical analysis results [see table- 2]. Following results were obtained in PCA [Figure 4]. In score plot, BD, BV and BR found in different quadrant. When score and load plot were correlated then in upper right quadrant differentiating attributes observed were WSE in BD, in lower right quadrant MSE and AIA in BV, in upper left quadrant AV and in lower left quadrant LOD in BR.

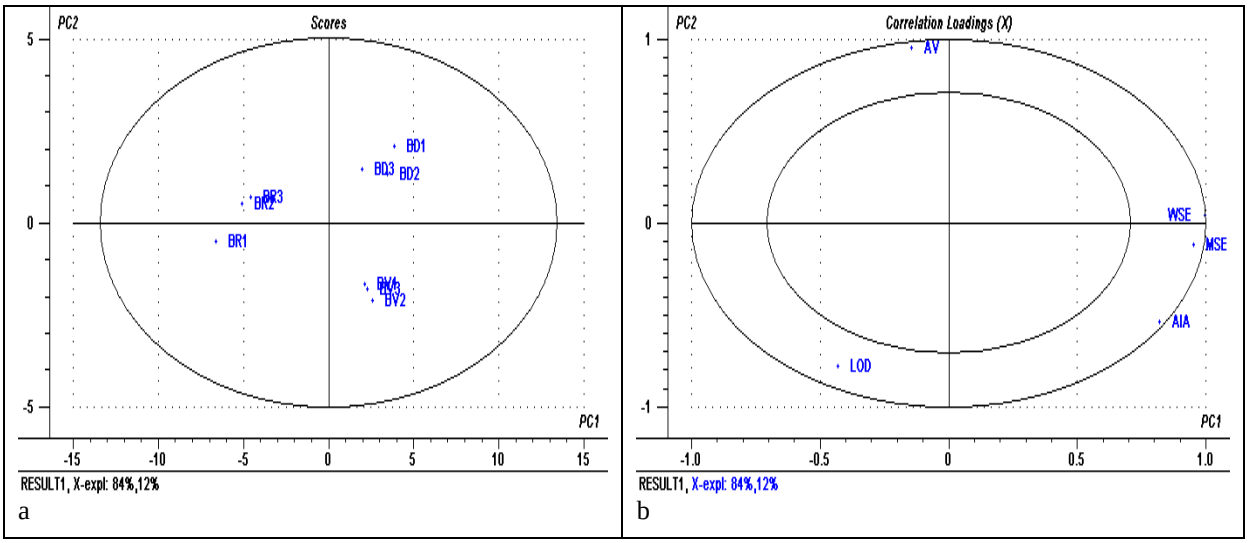


Figure 4: (a) PCA scores plot and (b) loading plot obtained from Physico-chemical constant data of BD, BV and BR. The ellipse represents the Hotelling T2 with 96% confidence in score plot.

DISCUSSION

In present work a very important *Ayurvedic* plant *B. diffusa* was identified from its adulterant species by its physicochemical and HPTLC profiling. In this work we also performed phytochemical screening in which BD, BV and BR shows presence of same phytochemical class with no difference may be due to same genus. Studies of physicochemical constants can serve as a valuable source of information and are useful in judging the purity and quality of the drug. Percent weight loss on drying content was found to be less in BD compared to other two species may be due to less moisture content. The ash value determines the earthy matter or inorganic composition and other impurities present along with the drug.

The percent of ash value was found higher in BD compared to other two samples which may be due to high content of inorganic and salt materials in the sample. Acid insoluble ash provides information about non-physiological ash produced due to adherence of inorganic dirt and dust to the crude drug. Percent of acid insoluble ash was greater in BV than BD and BR due to silica or silicates present in the drug. Water soluble extractive represents the percent of water soluble active constituents such as tannins, sugars, plant acids, mucilage, glycosides, proteins etc. In BD, it was high in amount than BV and BR. Alcohol is an ideal solvent for extraction of various chemicals like resins, alkaloidal bases, volatile oils, tannins, coloring matter, flavonoids etc. It was found quite near in BD and BV whereas low amount was found in BR. HPTLC study showed 7 spots in BD, 5 spots in BV and 7 spots in BR at 254 nm. While at 366 nm BD showed 7, BV showed 9 and BR showed 6 spots. Number of spots at 254 nm was similar in BD and BR while BV showed less number of spots. But at 366 nm, BV showed more number of spots. On further spectral detection, overlapping spectra were observed at 0.38, 0.41 and 0.96 Rf in all 3 samples. While only 2 samples BD and BV showed overlapping spectra at 0.49 Rf. BV and BR both showed overlapping spectra at 0.51 and 0.58 Rf. Although BD showed different spectra at 0.58 Rf and also at 0.24 Rf. The data matrix corresponding to the Physico-chemical data was submitted to PCA in order to show possible trends in their values and emphasize the similarities and differences among samples on a score plot.

The score plot in Fig-4 (a) showed that the authenticated BD, BV and BR samples were clearly discriminated in PC1 vs. PC2 space. The sample BR was scattered in upper left quadrant in which only BR1 appears below the horizontal line of the score plot. While BD was found in upper right quadrant and authenticated BV samples were well separated from the BD samples which scattered in the lower right quadrant. From the loading plot in Fig-4(b) it appeared that LOD, AV, AIA, WSE, MSE were came out as separating Physico-chemical constant contributing to the grouping of sample, and that these attributes corresponded to the PC1 and PC2 which explained about 96% of total variance. When correlating score and loading plot then BD and BV found in different quadrants but were quite near in loading plot due to 2 variables MSE and WSE due to its less variation in the results. However BR can be easily differentiated by WSE, MSE and AIA which are far away from BD in loading plot and same way BV can be easily separated from BD by 2 variables LOD and AV.

CONCLUSION

Boerhavia diffusa is the most valuable plant of Indian system of medicine which can be successfully identified from its adulterant species by means of physicochemical and advanced HPTLC analysis. From PCA analysis of Physicochemical parameters it can be conclude that BD can be differentiated from BR with WSE, MSE and AIA and like it BD and BV can also be separated by 2 parameters LOD and Ash value. The HPTLC analysis showed similar spectra at 0.38, 0.41 and 0.96 Rf in all 3 samples and also at 0.49 Rf in BD and BV. At Rf 0.51 and 0.58, BD has no similar spectra with BV and BR. Only BD shows one spectra at 0.24 Rf value. So spectra of 0.58 and 0.24 Rf is helpful in discrimination of the BD from other species. So we can assume that basic physicochemical profiling and HPTLC study can likewise serve as powerful discriminational tool for identification of genuine drug from adulterated ones in the field of *Ayurveda*. In this way we developed a reliable, cheapest and easily assessable data-mining realm Principal component analysis combined with Physico-chemical constant.

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