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Research Paper

PHYTOCHEMICAL ANALYSIS OF *Boerhaavia diffusa* ALONG THE ALTITUDINAL GRADIENTS FROM FOOT HILL TO HOT TEMPERATE VALLEY OF KUMAUN HIMALAYA

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Abstract

On the basis of literature obtained from ethno medicinal documentation *Boerhaavia diffusa* is an important medicinal plant of Kumaun Himalaya, possess various pharmacological activities and used as a medicine with multi actions such as stomachic, diuretic, antihepatotoxic, antiasthamatic, diaphoretic, anthelmintic, febrifuge, antiscabies and antiurethritis these pharmacological activities are due to the presence of marked chemical constituents. Plant *B. diffusa*. previously been screened for chemical constituents by various authors, since no details previous studies deals with the quantification of principal chemical constituents along the altitudinal gradients. Keeping in the view the tremendous use of *B. diffusa* as a medicine the study was aimed to work out the active principle constituents of *Boerhaavia diffusa* along the altitudinal gradients from foot hills to hot temperate valley in Kumaun Himilaya.

Key words: *Boerhaavia diffusa, Punarnava, Phytochemical analysis, Altitudinal gradient*.

INTRODUCTION

Every herb is a complex mixture of biologically active compounds, some of them are synergistic, some antagonistic, some toxic and some inactive. There have been global interests in scientifically validating the therapeutic efficacy of the medicinal plants. The therapeutic properties of medicinal plants are due to the presence of some secondary metabolites. The secondary metabolites of plants are species specific and can be widely used in pharmaceutical industries.

Boerhaavia diffusa is an important medicinal plant of Kumaun Himalaya, possess various pharmacological activities and used as a medicine in Ayurvedic, Unani, Siddha and Homoeopathy Systems. The plant contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoprotein [1]. Therefore, authors have taken the objective to workout the active principle constituents of *Boerhaavia diffusa* from foot hills to hot temperate valley in Kumaun Himilaya. In the present study mineral elements like N, P, K and Ca have been quantified as well as crude protein was chemically investigated. Quantitative estimation of active principle flavonids (Gallic acid and Quercetin) of *Boerhaavia diffusa* was also

investigated. For this, studies were carried out from three ''major districts of Kumaun viz: Nainital, Almora and Pithoragarh districts from foot hills to hot temperate valley.

MATERIALS AND METHODS

(I) Plant materials collection, identification and preparation

Keeping in view the altitudinal range of its occurrence, the plant *B. diffusa* was collected from foot hills of Nainital to hot temperate valley of Almora and Pithoragrah districts.

The plant materials were collected when the plants were at matured stage. Verification and authentication of plant was made in the Department of Botany, Kumaun University, S.S.J. Campus, Almora. Samples collected from different study sites were brought to the laboratory, carefully washed through tape water, each and entire plant parts were separated into two major components:

1- Above ground component.
2- Below ground component.

Above ground component contains stem, leaves, flowers and fruits while below grounds component included roots only. Thereafter, the samples were shade dried until constant weight. Shade-dried samples were ground into fine powder using pestle and mortar and sieved through mesh with 10µ diameter. This communition gave a greenish (Above ground) and brown (Below ground) powdered sample that were used for entire analysis. The work was done in CIF (Central Instrumental Facility), National Botanical Research Institute (NBRI), Lucknow (U. P.).

(II) Proximate Analysis

[A]. Mineral Elements- Phosphorous and calcium content were estimated by Allen method [2] and Macro Kjeldahl procedure was used to estimate nitrogen content of sample [3]. Protein content was calculated using a conversion factor 6.25 [2].

[B]. Quantitative Estimation of Active Principles Flavonoids (Gallic Acid and Quercetin) of *Boerhaavia diffusa*

HPLC method was used for separation and quantitative determination of the active principles flavonoids (Gallic acid and Quercetin) from different parts of *B. diffusa*. The use of column and acidic phase enable the efficient separation of flavonoids compound. Methanolic extract of plant material was injected through HPLC column and presence of Quercetin and Gallic acid was detected with the help of authentic sample (COHPLC).

Analytical conditions:-

Instruments : Shimadzu
Software : Shimadzu class VP.
Detector : UV-VIS
Wavelength : 254hm
Column : Phenomenex RP- 18, 250 x 4.6mm ID, 5µ.
Flow rate : 0.6 ml/min
Mobile phase : Water acetic acid (99.0:1.0v/v) as solvent A and acetonitrile as solvent B using a gradient elution in 0-14 min with 20-35% of solvent B, 14-40 min with 35-50% of solvent B.
Injection Volume : 20 µl.
Reference Standard: Gallic acid (Sigma), Quercetin (Sigma).

RESULTS

The proximate composition of minerals from roots samples of *B. diffusa* collected from different study sites are presented in table-1 The root sample of *B. diffusa* collected from Almora district contains high percentage of nitrogen (0.75 %); phosphorus (83.6 mg/100g); potassium (1603 mg/100g) and calcium (467 mg/100g) followed by the samples collected from Pithoragarh district and least in Nainital district. The phosphorus, potassium and calcium (81.7, 1534, 302 mg/100g respectively) contents of roots samples collected from Pithoragarh district were found to be higher than root samples collected from Nainital district viz- phosphorus (72.8 mg/100g); potassium (1499 mg/100g) and calcium (288 mg/100g) except than nitrogen content i.e., 0.58 % in Nainital district whereas it was 0.49 % in Pithoragarh district.

The plant *B. diffusa* is traditional leafy vegetable and are highly recommended due to their relatively high macronutrients value. The plant *B. diffusa* is rich in vitamins, minerals and proteins [4]. The nutritive value of plant *B. diffusa* is due to the presence of number of minerals [5]. Looking on its high nutritional value lots of works have been done on its mineral composition [6; 7; 8; 4; 5].

As presented in Fig-1, the plant *B. diffusa* contains high mineral composition. Plant contained sufficient amount of nitrogen (0.49 - 0.75%) and phosphorus (72.8 - 83.6 mg/100g) contents. Nitrogen and phosphorus are best known as nutrients. The plant *B. diffusa* also contained high potassium (1499 – 1603 mg/100g) and calcium (288 – 407 mg/100g) contents. Potassium is known to decrease blood pressure. It plays a role in controlling skeletal muscle contraction and nerve impulse transmission as well as calcium is a major factor sustaining strong bones and plays a part in muscles contraction and relaxation, blood clotting and absorption of vitamin 12. The relatively high content of potassium and calcium (Fig-1) in *B. diffusa,* suggested that the plant may be of therapeutic value in calcium and potassium deficiency diseases as well as patients with soft bones problems are usually placed on high calcium and potassium meals [8]. The previous study revealed that the plant contained large quantities of potassium nitrate; high content of potassium in *B. diffusa* may be one of the reasons for its diuretic activities [9; 10; 11; 12; 13].

The proximate composition of calcium and phosphorus from plant and soil are presented in table-2. The calcium and phosphorus content of plant *B. diffusa* collected from different study sites viz Nainital (288, 72.8 mg/100g respectively); Almora (467, 83.6 mg/100g respectively) and Pithoragarh (302, 81.7 mg/100g respectively) district was found to be higher than the soil sample collected from Nainital (148, 14.4 mg/100g respectively); Almora (189, 17.97 mg/100g respectively) and Pithoragarh (173, 16.03 mg/100g respectively) districts.

The calcicole species are associated with high pH. The growth of plants may be stimulated by high calcium concentrations in soil as well as high calcium concentrations may be found in the xylem sap of calcicole species, because calcium is an important "**second messenger**" (e.g., in the regulation of stomatal conductance) [14]. Calcicole species are exclusively found in calcium rich soils (i.e., prefer calcareous soil) while calcifuge species in calcium deficient soils (i.e., avoid calcareous soil) [15]. The calcicole herb had also a significantly higher proportion of total P in their leaves [16]. In present investigation (Fig-2) plant *B. diffusa* also contained significant amount of (P) phosphorus (72.8- 83.6 mg/100g). In previous studies high calcium content 667.0 mg/100g (4); 251 mg/100g [17]; 174 mg/100g (3) from plant *B. diffusa* have been already reported. As presented in Fig-2 *B. diffusa* contains high calcium content (288 – 467 mg/100g) and prefers calcium rich soils (148 – 189 mg/100g). **It indicates the**

calcicole behavior of the plant *B. diffusa*. Previously Datta [18] reported that depending on the soil exchangeable calcium, *B. diffusa, Gomphrena celosioides* and *Mecardonia dianthera* will give rise to appropriate calcicolous and cacifugous ecotypes. Other reports on calcicole behavior of plant *B. diffusa* were not found in literature. Therefore, it is not possible for us to compare the present data with previous ones. Author (picture-1) also reported that *B. diffusa* are luxuriantly growing in sandy soil, especially along the railway embankments. *B. diffusa* can survives in very sandy soils, including sand dunes. The plant *B. diffusa* frequently encountered in the rainy season of a semi arid regions of India [19]. However, lots of works were done on calcicole and calcifuge behaviours of other plants [20; 16; 21]. Higher concentration of potassium together with calcium may be one of the reasons for rejuvenating function of *B. diffusa.* Other authors have also reported rejuvenating function of *B. diffusa* [22; 23].

Picture1-: *B. diffusa* growing along railway tract & on retaining rock wall

Table -1 Comparative account of proximate analysis of mineral nutrients from roots of *B. diffusa* collected from Kumaun Himalaya

Districts	Altitudinal range (M)	Nitrogen (%)	Phosphorus (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)
Nainital	500	0.58	72.8	1499	288
Almora	620	0.75	83.6	1603	467
Pithoragarh	560	0.49	81.7	1534	302

Table-2 Comparative account of proximate composition of Ca and P contents in *B. diffusa* and soil

Districts	Calcium (mg/100g)		Phosphorus (mg/100g)	
	B. diffusa	Soil	*B. diffusa*	Soil
Nainital	288	148	72.8	14.4
Almora	467	189	83.6	17.97
Pithoragarh	302	173	81.7	16.03

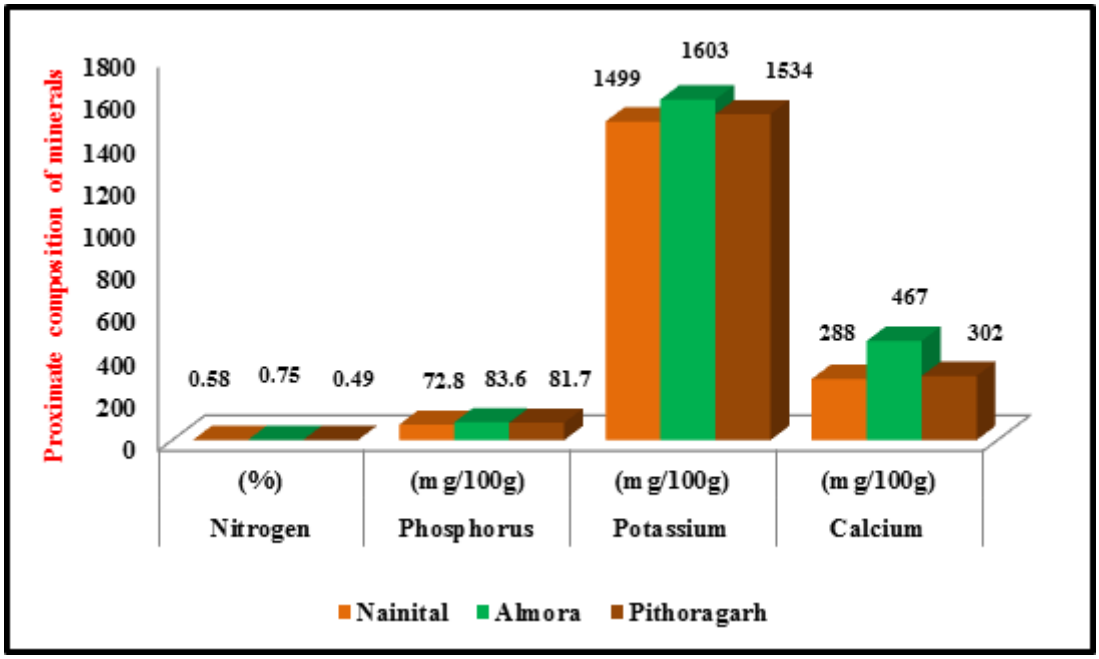


Fig.- 1. Comparative account of proximate analysis of mineral nutrients from roots of *B. diffusa* collected from different study sites

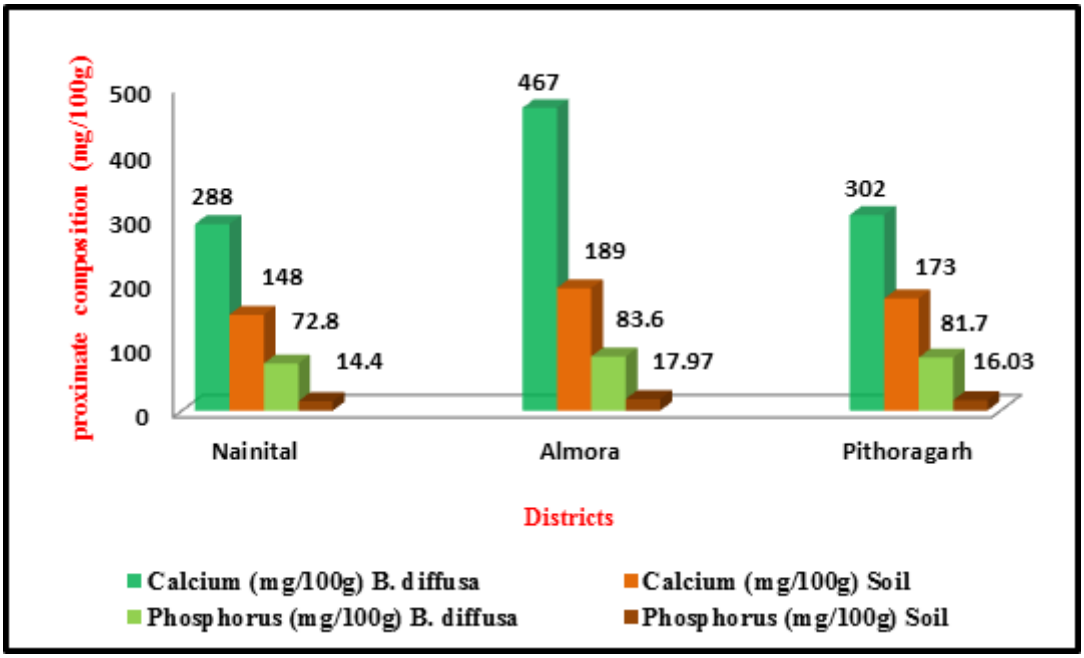


Fig.- 2. Comparative account of proximate composition of Ca and P in *B. diffusa* and soil

The comparative account of proximate composition of crude protein from different parts of *B. diffusa* is presented in table-3. The protein content in above ground components of B. diffusa was found to be higher in the sample collected from Almora (1.9%) district, followed by sample collected from Pithoragarh (1.4%) and least in the sample collected from Nainital (1.3%) district. Similarly, in below ground components the high protein content was reported for the sample collected from Almora (4.68%) district. The crude protein content in below ground component collected from Nainital district was found to be 3.62 % and least in the samples collected from Pithoragarh (3.06%) district.

The plant *B. diffusa* contains novel basic protein (30–34 kDa) capable of providing resistance/immunity to several susceptible hosts against commonly occurring viruses [24]. The herb *B. diffusa* and its roots are rich in protein [25]. The root of *B. diffusa* shows high viruse inhibitory activity [24]. Protein BD-SRIP endogenously occurring in the plant *B. diffusa* and this protein is responsible for systemic resistance inducing activity [26; 27], systemic resistance inducing proteins highly present in *B. diffusa* root extract [24]. As presented in Fig- 3 the percent of crude protein was high in below ground components i.e., roots (3.06 – 4.68 %) as compared to above ground components (1.3 – 1.9 %). Similar observations were also reported by various authors [28; 27; 25]. As the plant *B. diffusa* and its roots are rich source of protein, which is responsible for its nutritive value. The traditional value of plant *B. diffusa* has proven nutritive value in terms of having more proteins and mineral elements. The presence of high concentration of potassium, calcium and proteins proved the nutritive value of *B. diffusa.*

Table-3 Comparative account of proximate analysis of crude protein from different parts of *B. diffusa* growing in Kumaun Himalaya

Districts	Crude Protein (%)	
	Above ground components	Below ground components
Nainital	1.3	3.62
Almora	1.9	4.68
Pithoragarh	1.4	3.06

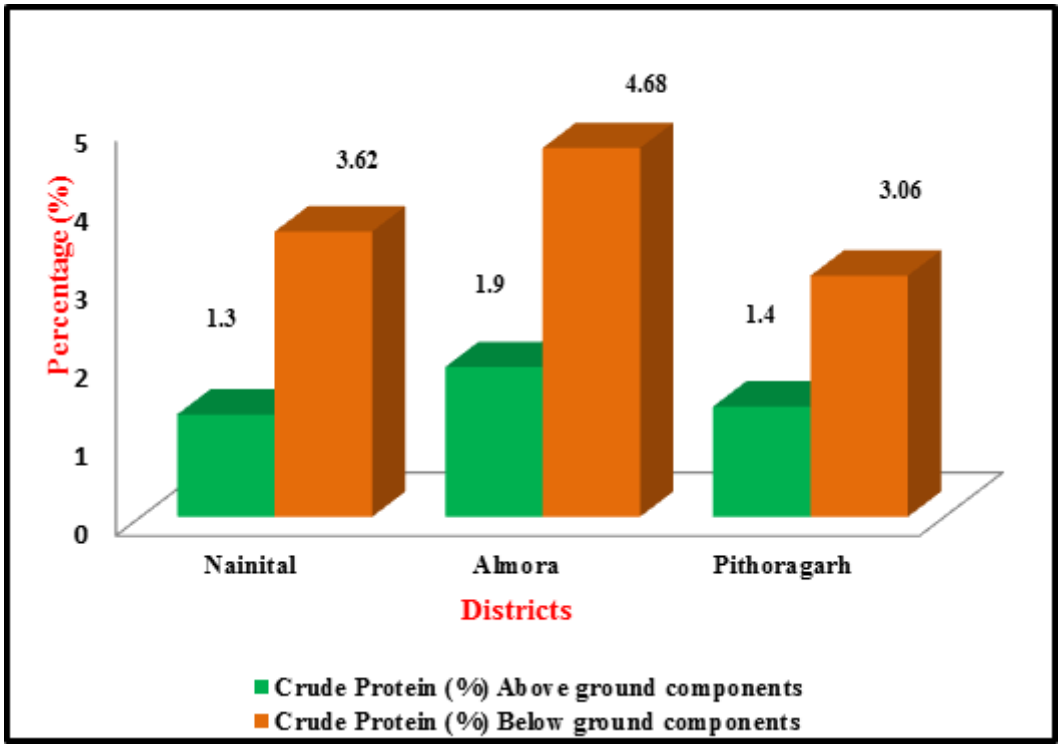


Fig.- 3. Comparative account of proximate analysis of crude protein from different parts of *B. diffusa* collected from different study sites

Quantitative analysis of falvonoids (Gallic acid and Quercetin) by High Performance Liquid Chromatography of different parts (above ground and below ground

components) of *B. diffusa* from different study sites were presented in table-4. Presence of Gallic acid and Quercetin from different parts and from different study sites were identified by CO-HPLC with their standards (plate-1).

The peak of Gallic acid was detected in all the samples collected from different study sites. In above ground samples the high intensity of the peak of Gallic acid (0.54%) was detected from the sample collected from Nainital and Almora districts (plate 2, 4) while low intensity of peak of Gallic acid (0.21%) was reported for the sample collected from Pithoragar district (plate 6) whereas in below ground samples the peak of Gallic acid is more or less similar (plate 3, 5 and 7). High intensity peak of Gallic acid (0.24%) is reported from the samples collected from Pithoragrah district (plate 7) followed by Nainital (0.21%) (plate 3) and Almora (0.20%) district (plate 5). High Performance Liquid Chromatography showed that high intensity of Gallic acid content (0.54%) was reported from the above ground sample of *B. diffusa* collected from Nainital and Almora district while low intensity of Gallic acid (0.20%) was also reported from the same below ground samples collected from the Almora district. However, Gallic acid content was always higher in above ground components of *B. diffusa* than below ground components. A very low intensity of the peak of Quercetin (0.0004%) was observed only in the below ground sample of *B. diffusa* collected from Almora district (plate 5) but not detected from above ground sample collected from the same district i.e Almora. Quercetin was also not detected from the other samples collected from different study sites. On the basis of above study it is hereby concluded that the Quercetin is not distributed frequently as a free aglycone in *B. diffusa* while it is widely been found as a flavonol-glycosides.

Thus, present study, showed variations (Fig-.4) within the composition of different parts of the same species as well as within the composition of same species along altitudinal gradients. All the parts of *B. diffusa* have medicinal properties which when used in the right proportion could be of tremendous benefits. For the first time quantitative estimation of Quercetin by HPLC method from nine leaf and three root samples collected from different locations were done by Ferreres *et al.* [29]. In India other reports on quantitative estimation of Gallic acid and Quercetin from different parts (above ground and below ground components) from different study sites were not found in literature, therefore, it is not possible to compare the present data with previous ones. However, numbers of flavonoids were identified from the plant *B. diffusa* [30; 31; 32; 33]. Flavonoids are responsible for the antioxidant activity of the plant *B. diffusa* [34]. The effect of *B. diffusa* on antioxidant status in liver and kidney of alloxan diabetic rats are reported by Satheesh and Pari [13]. The plant *B. diffusa* used in the treatment of jaundice may be due to the antioxidant activity [34]. The flavonoids present in *B. diffusa* may probably prevent the accumulation of excessive free radicals and protect the liver [35]. On the basis of the obtained results in this study it can be concluded that the plant *B. diffusa* can have important clinical implications in the future treatment of liver disorders and plant exert a protective effect against number of diseases.

Table 4. Quantitative analysis of Gallic acid and Quercetin from different parts of *B. diffusa* growing in Kumaun Himalaya

Districts	Altitudinal Range (M)	Gallic acid (%)		Quercetin (%)	
		Above ground components	Below ground components	Above ground components	Below ground components
Nainital	500	0.54	0.21	ND	ND
Almora	620	0.54	0.20	ND	0.0004
Pithoragarh	560	0.21	0.24	ND	ND

Key words:

ND: Not Detected

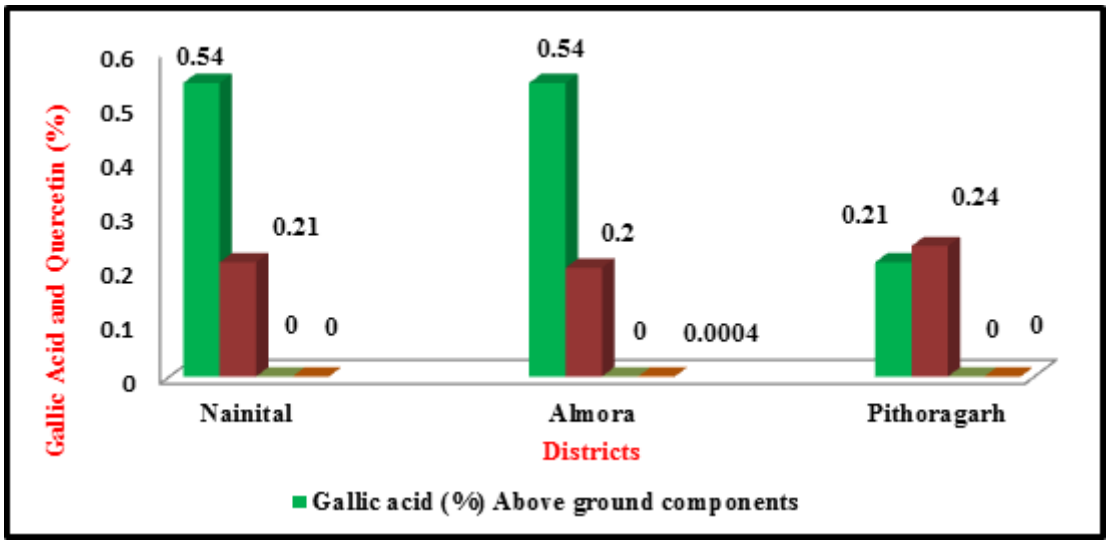


Fig.- 4. Quantitative analysis of Gallic acid and Quercetin from above ground components and below ground components of *B. diffusa*

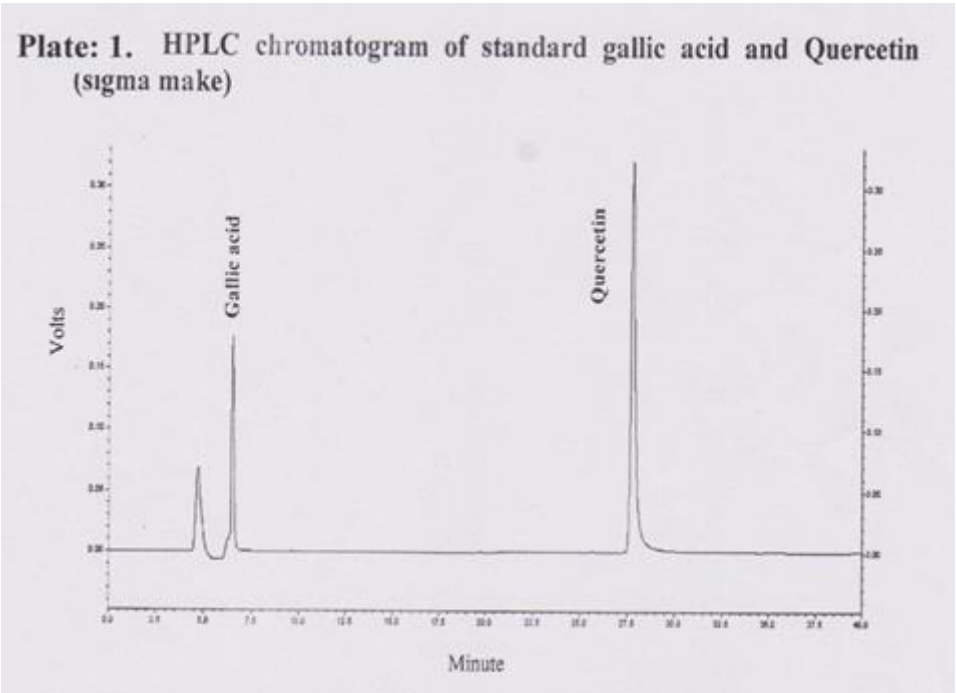


Plate: 2. HPLC chromatogram of above ground sample of *B. diffusa* collected from Nainital district

Gallic acid = 0.54%, Quercetin = Not Detected

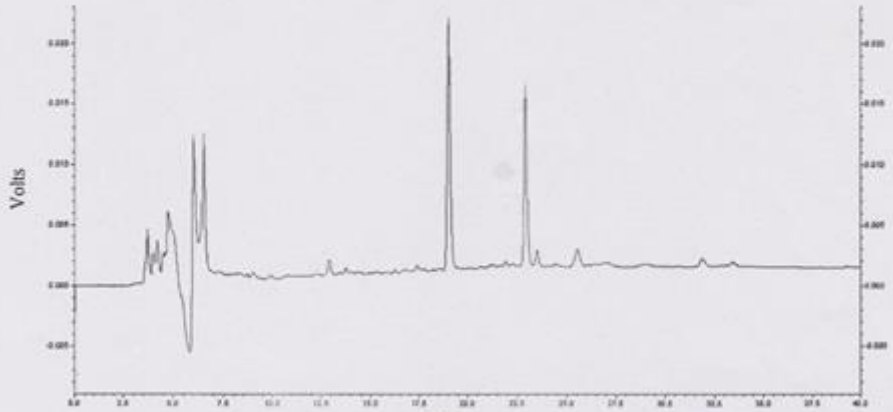


Plate: 3. HPLC chromatogram of below ground sample of *B. diffusa* collected from Nainital district

Gallic acid = 0.21%, Quercetin = Not Detected

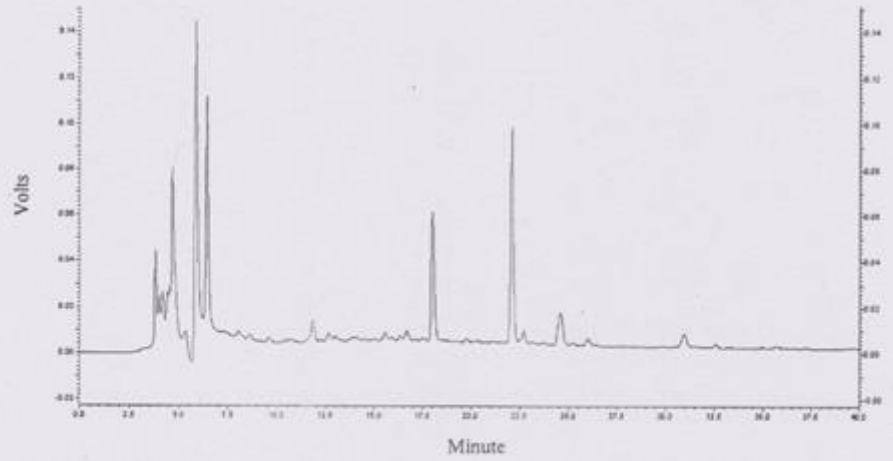


Plate: 4. HPLC chromatogram of above ground sample of *B. diffusa* collected from Almora district

Gallic acid = 0.54%, Quercetin = Not Detected

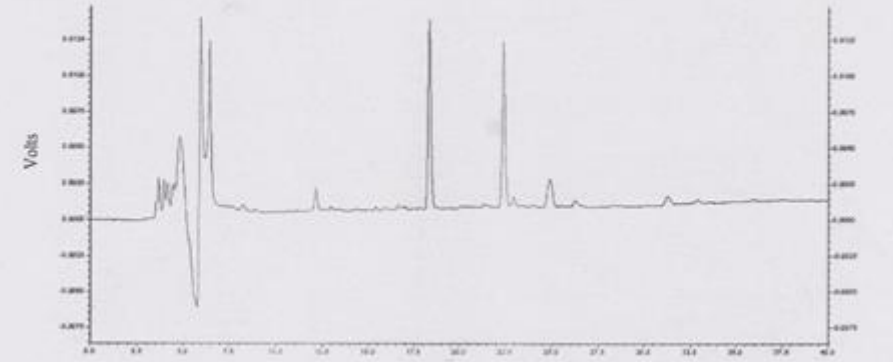


Plate: 5. HPLC chromatogram of below ground sample of *B. diffusa* collected from Almora district ↓

Gallic acid = 0.20 %, Quercetin = 0.0004%

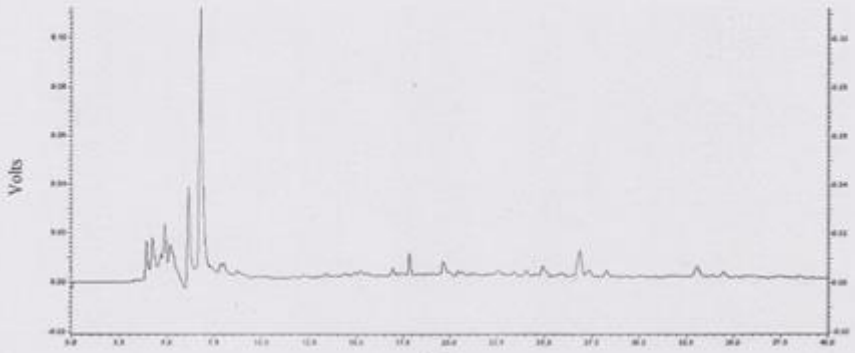


Plate: 6. HPLC chromatogram of above ground sample of *B. diffusa* collected from Pithoragarh district ↓

Gallic acid = 0.21 %, Quercetin = Not Detected

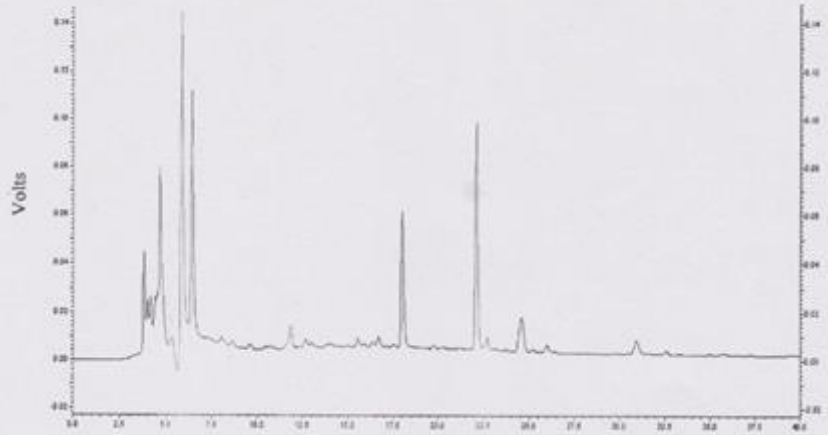
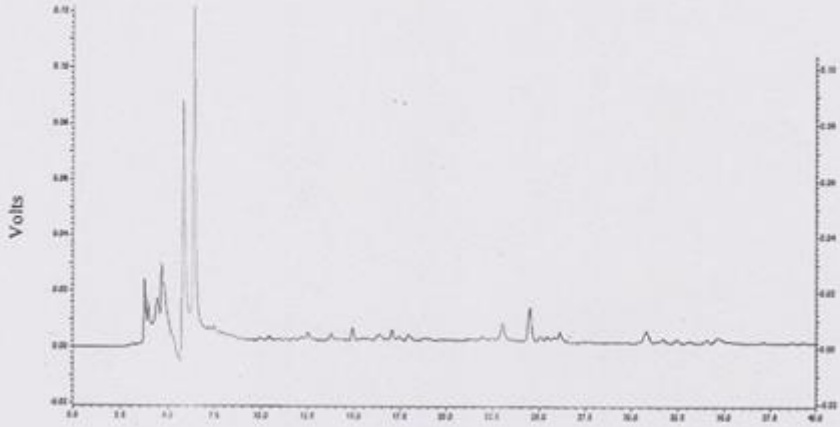


Plate: 7. HPLC chromatogram of below ground sample of *B. diffusa* collected from Pithoragarh district ↓

Gallic acid = 0.24 %, Quercetin = Not Detected



DISCUSSION

The presence of high concentration of potassium, calcium and proteins proved the nutritive value of *B. diffusa.* Current information on the nutritive and medicinal value of *B. diffusa* is not well documented and disseminated; this study may provide the biochemical base to prove the nutritive value as well as traditional value of plant *B. diffusa* in terms of having more proteins and mineral elements.

Plant *B. diffusa* has many ethno-botanical uses (the leaves are used as vegetables; the roots juice is used to cure asthma, urinary disorders and rheumatism, plant is widely used for the treatment of jaundice by villagers of Kumaun Himalaya) thus, the medicinal claims of the plant *B.diffusa* for the treatment of large number of diseases is due to the presence of various secondary metabolites. Thus the Quantitative Estimation of Active Principles of secondary metabolites is useful for the detection of various phytochemical compounds and are known to have beneficial use in the industries and medical sciences. This Quantitative Estimation also simplifies the process of qualitative separation of these pharmacologically active chemical compounds. This study also provides biochemical base for the ethno-medicinal and traditional uses of *B. diffusa* in the treatment of various diseases well as helpful in documentation of disseminated local medicinal uses of *Boerhaavia diffusa.*

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