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Phytochemical Screening of Main Classes of Secandary Metaboloties From *Boerhaavia diffusa* Growing In Three Major District Of Kumaun Himalaya.

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Abstract: *Boerhaavia diffusa belonging to the family Nyctaginace is is mainly a diffused perennial herbaceous creeping weed of India and well known for its traditional medicinal properties in Kumaun Himalaya. Keeping in the view the tremendous traditional use of B. diffusa as a medicine in Kumoun Himalaya. The study deals with the investigation of phytochamical screening of main classes of secondary metabolites from different parts (above ground components and below ground components) of B. diffusa collected from different study sites from foot hill to hot temperate valley of Kumoun Himalaya.*

Keywords: *Boerhaavia diffusa, Phytochemical Screening, Punarnava, Altitudinal gradient.*

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I. INTRODUCTION

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. 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 (Nadkarni, 1976). These pharmacological activities are due to the presence of marked chemical constituents in the plant *B. diffusa* previously been screened for chemical constituents by various authors. The plant contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins (Sahu *et al.,* 2008). Keeping in the view the tremendous use of *B. diffusa* as a medicine the study was aimed to carried phytochemical screening of different parts (above ground components and below ground components) of *B. diffusa* . 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.

II. 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.

Table1. Description of study sites used in phytochemical studies

S. No.	Study sites	Districts	Altitudes (M)
1	Kathgodam	Nainital	500
2	Jeyadi	Almora	620
3	Meena Bazar	Pithoragarh	560

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.

(II) **Phytochemical Screening of Main Classes of Secandary Metaboloties**

I. Alkaloids- Alkaloids are nitrogen containing metabolites of plants. The ethanolic extract of plant stirred with few ml of HCl and filtered. The alkaloid analysis was done by the precipitation reactions with the reagents of Wagner (Wagner, 1993), Mayer (Evans, 1997; Costa, 1994) and Dragendroff's reagents (Costa, 1994; Waldi, 1965).

A. Wagner's Reagent- 1.27g iodine + 2g KI are dissolved in 5ml distilled water and made up to 100 ml with distilled water.

Wagner's Test: - In a few ml of filtrate, few drops of Wagner's reagent is added by the side of test tube. A brown flocculent precipitate confirmed presence of alkaloids (Wagner, 1993).

B. Mayer's Reagent- 1.36g. of $HgCl_2$ (mercuric chloride) was dissolved in 60 ml distilled water (A) and 5g. KI was dissolved in 10 ml water (B). Solution A and B are mixed and allowed to stand. The resulting solution is made up to 100ml.

Mayer's Test: - In a few ml of filtrate, few drops of Mayer's reagent was added by the side of test tube. An orange red precipitate indicated the presence of alkaloid. (Evans, 1997; Costa, 1994).

C. Dragendroff's Reagent- 8g. of Bismith subnitrite was dissolved in 20 ml of concentrate HNO_3 (A). 27.2g KI was dissolved in 50ml of distilled water (B). Two solutions are mixed and allowed to stand when KNO_3 precipitates out supernatant solution is made up to 100ml.

Drangendroff test- In a few ml of filtrate 1 or 2 ml of Drangendroff reagent was added. Orange red precipitate confirmed the presence of alkaloid (Costa, 1994; Waldi, 1965).

II. FLAVONOIDS- Flavonoids are best known group of polyphenols, derived from parent substance flavones.

Screening of flavonoids- About 2g. of the powdered plant material was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while still hot. The filtrate was cooled and used (Ngbede *et al.,* 2008).

A. Sodium Hydroxide Test- Five ml of 20% Sodium Hydroxide was added to equal volume of the detanned water extract. A yellow solution indicated the presence of flavonoids (Ngbede *et al.,* 2008).

B. Shinoda test- Ethanolic solution of the plant extract (3ml) was dissolved in Magnesium and concentrated HCl (three drops), a red colour is obtained, indicated the presence of flavonoids (Shinoda, 1928).

C. Pew Test- Methanolic solution of plant extract was mixed with Zn dust and concentrated HCL which gave red colour (Pew, 1948).

D. $FeCl_3$ Test- Ethanolic solution of flavones was dissolved in alcoholic solution of $FeCl_3$ gave green to blue colour, indicated the presence of flavonoids (Horowitz, 1957).

III. Flavonol Glycoside- The extract (50mg) was dissolved in 5ml of alcohol and few fragments of Mg ribbon and concentrated Hydrochloric acid (drop wise) added. A pink to crimson colour developed; indicated the presence of flavonol glycosides is inferred (Horborne, 1998).

IV. Steroids- The steroids analysis was done by Salkowski test.

A. Salkowski test- Total 100 mg of plant material extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface is indicative of the presence of steroid ring (Sofowora, 1982).

V. Sugar- The presence of suger was done by Salkowski test.

A. Anthrone method (Benzidine – AcOH reagent)- 300mg of anthrone was dissolved in 10ml. of glacial acetic acid by warming. Followed by addition of 20ml of ethanol, 3ml of H_3PO_4 and 1 ml water. This reagent was added to the methanolic solution of plant extract. The appearance of yellow to green colours indicated the presence of sugars (Johanson, 1953).

III. RESULTS AND DISCUSSION

From different parts (Above ground and below ground components) and different study sites, phytochemical screenings of secondary metabolites like alkaloids, flavonoids, flavonol glycosides, steroids and sugars were identified from dichloromethane, EtOAC, BuOH and H_2O fractions of plant *B. diffusa*. Below ground samples collected from Nainital and Pithoragarh districts (Table 3 and 7) showed the presence of alkaloids in dichloromethane fractions and the samples collected from Almora district (table 5) showed

presence of alkaloids in Dichloromethane and EtOAC fractions where above ground samples did not give any alkaloid positive result (Tables 2, 4 and 5). Flavonoids and flavonol glycosides were present in both above ground and below ground sample of *B. diffusa* collected from Nainital, Almora and Pithoragarh districts. Above ground samples collected from Nainital district (Table 2) and below ground samples collected from Almora district(table 5) showed presence of flavonoids and flavonol glycosides in all tested fractions viz – Dichloromethane EtOAC, BuOH and H_2O while other samples gives positive results in two to three fractions (Table –3, 4, 6 and 7). Steroids were present in both above ground and below ground samples of *B. diffusa* collected from Nainital, Almora and Pithoragarh district. The above ground samples collected from Almora and Pithoragrah district (Table 4 and 6) showed presence of steroids in dichloromethane, BuOH and H_2O fraction whereas the sample collected from Nainital district showed presence of steroids in dichloromethane, and H_2O fraction (Table 3).The below ground sample collected from Nainital and Almora districts (Table 3 and 5) showed presence of steroids in BuOH fraction whereas the sample collected from Pithoragarh district (Table 7) showed presence of steroids in BuOH and EtOAC fractions. Sugars were also present in both above ground and below ground samples collected from Nainital, Almora and Pithoragarh districts. The above ground samples of these districts showed presence of sugars in H_2O fraction (table 2, 4 and 6) whereas below ground sample of these district showed presence of sugars in BuOH and H_2O fractions (Table 3, 5 and 7).

Table 2. Phytochemical screening of main classes of secondary metabolites from above ground components of *B. diffusa* collected from Nainital district

S. No.	Phytochemical test	Fractions			
		CH_2Cl_2	EtOAC	BuOH	H_2O
1.	Alkaloids a. Wagner's test	-	-	-	-
	b. Mayer's test	-	-	-	-
	c. Dragendroff's test	-	-	-	-
2.	Flavonoids a. NaOH test	+	+	+	+
	b. Shinoda test	+	+	+	+
	c.Pew test	+	+	+	+
	d. $FeCl_3$ test	+	+	+	+
3.	Flavonol glycosides	+	+	+	+
4.	Steroids a.Salkowski test	+	-	-	+
5.	Sugars a.Anthrone method	-	-	-	+

Table 3. Phytochemical screening of main classes of secondary metabolites from below ground components of *B. diffusa* collected from Nainital district

S. No.	Phytochemical test	Fractions			
		CH_2Cl_2	EtOAC	BuOH	H_2O
1.	Alkaloids a. Wagner's test	+	-	-	-
	b. Mayer's test	+	-	-	-
	c. Dragendroff's test	+	-	-	-
2.	Flavonoids a. NaOH test	+	-	+	-
	b. Shinoda test	+	-	+	-
	c. Pew test	+	-	+	-
	d. $FeCl_3$ test	+	-	+	-
3.	Flavonol glycosides	+	-	+	-
4.	Steroids a.Salkowski test	-	-	+	-
5.	Sugars a.Anthrone method	-	-	+	+

Table 4. Phytochemical screening of main classes of secondary metabolites from above ground components of *B. diffusa* collected from Almora district

S. No.	Phytochemical test	Fractions			
		CH_2Cl_2	EtOAC	BuOH	H_2O
1.	Alkaloids a. Wagner's test	-	-	-	-
	b. Mayer's test	-	-	-	-
	c. Dragendroff's test	-	-	-	-
2.	Flavonoids a. NaOH test	-	-	+	+
	b. Shinoda test	-	-	+	+
	c. Pew test	-	-	+	+
	d. $FeCl_3$ test	-	-	+	+
3.	Flavonol glycosides	-	-	+	+
4.	Steroids a.Salkowski test	+	-	+	+
5.	Sugars a.Anthrone method	-	-	-	+

Table 5. Phytochemical screening of main classes of secondary metabolites from below ground components of *B. diffusa* collected from Almora district

S. No.	Phytochemical test	Fractions			
		CH_2Cl_2	EtOAC	BuOH	H_2O
1.	Alkaloids a. Wagner's test	+	+	-	-
	b. Mayer's test	+	+	-	-
	c. Dragendroff's test	+	+	-	-
2.	Flavonoids a. NaOH test	+	+	+	+
	b. Shinoda test	+	+	+	+
	c. Pew test	+	+	+	+
	d. $FeCl_3$ test	+	+	+	+
3.	Flavonol glycosides	+	+	+	+
4.	Steroids a.Salkowski test	-	-	+	-
5.	Sugars a.Anthrone method	-	-	+	+

Table 6. Phytochemical screening of main classes of secondary metabolites from above ground components of *B. diffusa* collected from Pithoragarh district

S. No.	Phytochemical test	Fractions			
		CH_2Cl_2	EtOAC	BuOH	H_2O
1.	Alkaloids a. Wagner's test	-	-	-	-
	b. Mayer's test	-	-	-	-
	c. Dragendroff's test	-	-	-	-
2.	Flavonoids a. NaOH test	-	+	+	-
	b. Shinoda test	-	+	+	-
	c. Pew test	-	+	+	-
	d. $FeCl_3$ test	-	+	+	-
3.	Flavonol glycosides	-	+	+	-

4.	Steroids a.Salkowski test	+	-	+	+
5.	Sugars a.Anthrone method	-	-	-	+

Table 7. Phytochemical screening of main classes of secondary metabolites from below ground components of *B. diffusa* collected from Pithoragarh district

S. No.	Phytochemical test	Fractions			
		CH_2Cl_2	EtOAC	BuOH	H_2O
1.	Alkaloids a. Wagner's test	+	-	-	-
	b. Mayer's test	+	-	-	-
	c. Dragendroff's test	+	-	-	-
2.	Flavonoids a. NaOH test	+	-	+	+
	b. Shinoda test	+	-	+	+
	c. Pew test	+	-	+	+
	d. $FeCl_3$ test	+	-	+	+
3.	Flavonol glycosides	+	-	+	+
4.	Steroids a.Salkowski test	-	+	+	-
5.	Sugars a.Anthrone method	-	-	+	+

The phytochemical study of plant *B. diffusa* demonstrated the presence of alkaloids, flavonoids, flavonol glycosides, steroids and sugars. These metabolites are responsible for the high medicinal value in the plant *B. diffusa*. Alkaloids are beneficial chemical constituents and also affect glucagons and thyroids stimulating hormones, while some forms have been reported to be carcinogenic (Okaka *et al.,* 1992). The presence of flavonoids might be responsible for the antioxidant activity. Recently flavonoids potent free radical scavengers have attracted a tremendous interest as possible therapeutic against free radical mediated diseases (Darsini *et al.,* 2009). Plant steroids are known to be important for their cardio tonic activities, possess insecticidal and antimicrobial properties; they are also used in nutrition, herbal medicine and cosmetics (Callow *et al.,* 1936). In view of the various uses associated with these above described compounds found in *B.diffusa,* may be the reasons that the plant is used to cure number of diseases and consist of large number of pharmacological properties such as such as stomachic, antihepatotoxic, antiasthamatic, diaphoretic, anthelmintic, febrifuge, antiscabies and antiurethritis, diuretic and jauindice (Nadkarni, 197; Ramachandra *et al*.2012).

IV. CONCLUSION

Our study concludes presence of secondary metabolites in different parts (above ground components and below ground components) of *B. diffusa* which can provide various useful biological activities to this medicinal plant. 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 number of diseases. This study also provides biochemical base for the ethno-medicinal and traditional uses of *B. diffusa* in the treatment of various diseases.

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Plate 1: Profusely branched (above ground) *B. diffusa* growing on rocks

Plate 2: deep root penetration of (below ground) *B. diffusa* in the calcareous soil.

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