Research Article

Inter-Specific Variation Studies On The Phyto-Constituents Of *Boerhaavia Diffusa* L. And *Cichorium Intybus L.*Using Phytochemical Methods

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ABSTRACT

The preliminary phytochemical screening of *Boerhaavia diffusa* leaves in the different solvent extract shows the presence of Alkaloids, Carbohydrates, Saponins, Pseudotannins, Tannins, Anthocyanin, Steroidal Glycosides, glycosides, Flavonoids, Flavones, Coumarin, Resins, Phenol, Xantho proteins , oil and fats. *Cichorium Intybus* leaves in different solvent extracts shows the presence of Alkaloids, Carbohydrates, Saponins, Pseudotannins, Tannins, Anthocyanin, Steroidal Glycosides, glycosides, Flavonoids, Flavones, Coumarin, Oil and fat, Resins, Phenol, Xantho proteins. Both plants are examined for antioxidant properties using DPPH method. Both plants show the presence of antioxidant activity. So both plants possess many bioactive medicinal compounds and also posseses antioxidant properties. Further isolation and characterization will reveal which bioactive principle are possessing antioxidant properties.

Keywords: Antioxidant activity, *Boerhaavia diffusa* , *Cichorium intybus*, Physico phyto-chemical properties.

INTRODUCTION

Knowledge of the chemical constituent of plant is desirable for the discovery of therapeutic agents and in discovering the actual value of folklore remedies. Traditionally, screening methods have been used to study the pharmacological effects of phytochemical compounds.[1,2]

Boerhaavia diffusa L. commonly known as punarnava in Sanskrit, Hog weed in English, Mukurattai in Tamil. It is a herbaceous plant of the family Nyctaginaceae. The whole plant or its specific parts (leaves, stem, and roots) are known to have medicinal properties and have a long history of use by indigeous and tribal people in India. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka samhita and sushrita samhita.[3,4] It was used in renal ailments as diuretic and to treat seminal weakness and blood pressure and also used in the treatment of stomach ache, anemia, cough, cold and as a diaphoretic , laxative , expectorant and a potent antidote for snake and rat bites in the treatment of nephritic syndrome, hepatitis, gall bladder abnormalities and urinary disorders. The flowers and seeds are used as contraceptive.[5,6] The leaves are cooked and eaten as vegetable. The root and leaves are considered to have an expectorant action to be emetic and diuretic in large doses and are used in the treatment of asthma.

Cichorium Intybus L is a diploid species belonging to the family Asteraceae with blue, lavender or occasionally white flowers is also known as blue sailors, (or) Kasini. It is native to the mid Asia and northern Africa.[7] The tuberous root of this plant contains number of medicinally important compounds such as inulin, bitter sesquiterpene lactones, Coumarins, flavonoids and vitamins.[8]

The plant is being used traditionally to cure various ailments in Ayurvedic and Unani systems are found to have enormous application in food industry as well. The leaves are used for easing skin inflammations and swellings. The whole plant extracts was reported to have antidiabetic,[9] antioxidant,[10,11] antibacterial,[12] immunotoxic,[13] antihepatotoxic,[14,15] and cardio protective properties.[16] Above two plants are widely used by the traditional healers to cure many diseases. But only little data available for the antioxidant activity by these two plants. The present study will provide information on chemical marker and interspecific variations of two study plans as well as to find out whether the two medicinal important study plants possesses antioxidant potential.

MATERIALS AND METHODS

Healthy, disease free entire plants of *B.diffusa* are collected from near palliaghrakaram, Thanjavur Dt, Tamil Nadu, India, *C.Intybus* plants are collected from local market, Trichirrappalli, Tamil Nadu, India. The collected specimens are authenticated by Dr. S.John Britto, The Director, The Rabinat Herbarium and Centre for Molecular Systematics, St.Joseph's College (Campus), Tiruchirrappalli 620002 Tamil Nadu, India. The fresh leaves are washed in tap water for 5 min and are dried using blotting papers. The washed plant leaves are air and shade dried for two weeks and pulverized to powder using mortar. The dried and powdered leaves material (150g) are extracted using ethyl acetate, hydro alcohol, methanol, toluene and water individually using soxhlet extractor for 18 hrs at a temperature not the boiling point of the respective solvent. The extracts are concentrated

in vacuum at 40 using rotatory evaporator. The residues obtained are stored in a freezer until further test. The samples of the two extracts are subjected to various analysis such as organoleptic characters[17], fluorescence studies on daylight and UV light[18], qualitative and quantitative phytochemical analysis[19], physio-chemical properties[20-21], Phytochemical screening of the extracts are carried out according to the standard methods [22,23] and antioxidant potential are carried out using DPPH method.[24-26]

Determination Of Antioxidant Activity

DPPH Assay

Free radical scavenging activity of different extracts is tested against a methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1-1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity.

The sample of different extract is prepared in various concentrations viz. 25, 50, 75, 100, 125 µg/ml in AR grade methanol. 1ml samples if above concentrations are mixed with equal volume of 0.1mM methanolic solution of DPPH (0.39mg in 10ml methanol). An equal amount of methanol and DPPH is added and used as a control. Ascorbic acid solution of various concentrations viz. 25, 50, 75, 100, 125µ g/ml in distilled water are used as standard. After incubation for 30 minutes in dark, absorbance is recorded at 517 nm. Experiment is performed in triplicates. % scavenging is calculated by using the following formula.

Calculation Percentage of anti - radical activity= [A - B/A] x 100

Where,

A: absorbance of control

B: absorbance of sample

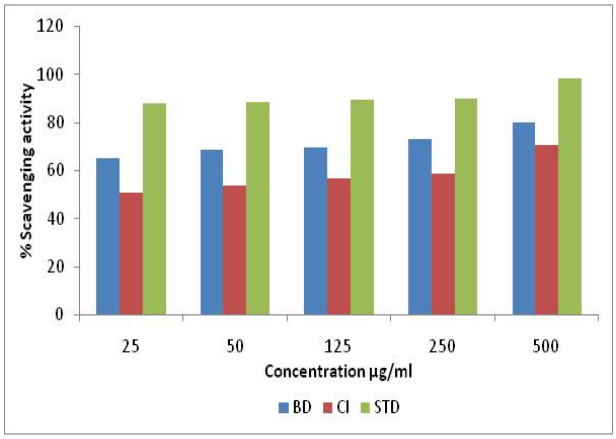

Figure 1: Antioxidant activity of *Boerhaavia diffusa* and *Cichorium Intybus* leaves

Table 1: Data on Organoleptic characters of *Boerhaavia diffusa* and *Cichorium Intybus*

Character	Leaves of BD	Leaves of CI
Colour	Dark green	Dark green
Odour	Not Discernible	Not Discernible
Taste	Tasteless	Tasteless
Texture	Soft	Fiber

RESULTS AND DISCUSSION

In the present study, the phytochemical screening is preformed with ethyl acetate, hydroalcohol, methanol, benzene, toluene and, aqueous extracts of the leaves of *B diffusa* and *C intybus*. A total of two plants and 10 extracts are examined for the phytochemical screening.

Table 1-3 present the results of organoleptic characters, flurescent characteristics, physico chemical parameters of the leaves of BD (leaves), and CI (leaves). Both plants leaves are appeared dark green in colour and their odor not discernible. Both plant leaves are tasteless. The leaves of BD texture are soft whereas leaves of CI are fibrous. There powder when viewed under UV light at 365nm appear dark green (BD) and dark green (CI), under normal light dark green (BD) and dark green (CI) respectively. Generally these powder when treated with sodium nitro prusside BD exhibits light green whereas CI exhibit sandal colour under visible light. After treating with various biochemical reagents they displayed narrow ranging colour variation.

Table 3 shows the physico-chemical parameters of BD and CI leaf powder. The total ash value, water soluble ash, acid insoluble ash and moisture content values are higher of BD when compare to CI whereas sulphated ash value is higher in CI.

Table 4,5 shows the preliminary phytochemical screening of BD and CI indicate the presence of the major constituents in methanolic extract and hydro alcoholic extract whereas other extracts many of the constituents show their absence. In BD methanolic and hydro alcoholic extract, alkaloids, carbohydrates, tannins, pseudo tannins, chlorogenic acid, steroidal glycosides, flavonoids, flavones, xanthoproteins are present, whereas saponin, saponin glycosides, anthrocyanin, coumarine, oil and fat, resins are absent. In CI methanolic extracts alkaloids, tannins, pseudo tannins, chlorogenic acids, flavonoids, flavones, and resins are present whereas carbohydrates, saponins, anthrocyanins, steroidal glycosides, saponin glycosides, coumarin, oil and fat and xanthroproteins are absent.

The quantitative extractive values are given in the Table 6 for two plants for different extracts.

Both plants show high extractive values for methanol solvent.

Table 2: Data on fluorescence studies of *Boerhaavia diffusa* and *Cichorium intybus*

Reagents used	BD	BD	CI	CI
	Visible light	UV light	Visible light	UV light
Powder as such	Dark green	Dark green	Dark green	Dark green
P + 1N NaoH	green	Dark green	Dark green	Dark green
P + 1N HCl	Pulp green	brown	Dark green	Dark green
P + 1N H_2SO_4 (50%)	green	green	Dark green	Dark green
P + 1N HNO_3	Brown	green	Brown	Dark green
P + 1N $FeCl_3$	Yellowish green	green	Dark green	Dark green
P + 1N NH_3	Dark green	green	green	green
P + Sodium nitroprusside	Light green	green	sandal	Florocent green
P + 1N KOH in H_2O (5%)	Dark green	Dark green	Dark black	Dark green
P + 1N KOH in Alcohol (5%)	Dark green	green	Dark green	Dark green
P + Picric acid	Dark green	Spring green	Dark green	Dark green
P +Acetic acid	Brown	green	Brown	Dark green

The *in-vitro* of antioxidant of BD and CI are studied using DPPH radical with different concentration of methanolic extract and the percentage of scavenging activity are shown in the Table 7. The antioxidant activity is compared with standard ascorbic acid at different concentration. The flavonoids behave as a potent free radical scavenges and also therapeutics against free radical mediated diseases.[27] The results of the present study clearly indicates that the both extracts possesses a good antioxidant potential. Among them BD shows a strong antioxidant activity than CI. But these two plants antioxidant potential even though good but lesser than that of standard Ascorbic acid.

Table 3: Physiochemical characteristics of leaves of *Boerhaavia diffusa* and *Cichorium intybus*

Parameters	BD	CI
Total ash	0.630g	0.356g
Water soluble ash	0.310g	0.232g
Acid insoluble ash	0.340g	0.225g
Sulphated ash	0.625g	0.827g
Moisture content	0.150g	0.131g

Table 4: Preliminary phytochemical analysis of BD

Name of the Test	Phytochemical constituents	Methanol	Ethyl acetate	Water	Hydro alcohol	Toluene
Mayer's test	Alkaloids	+	+	-	-	-
Dragendroff's test		+	+	+	+	+
Wagner Test		+	+	+	+	+
Molish Test	Carbohydrates	+	-	-	-	-
Fehling Test		+	-	-	-	-
Benedicts Test		+	-	-	-	-
Foam Test	Saponins	-	-	-	-	-
Lead Acetate	Tannins	+	-	-	-	+
Ferric chloride	Pseudo tannins	+	+	-	-	-
Ammonia	Chlorogenic acid	+	+	-	-	-
H_2So_4	Anthocyanin	-	-	-	-	-
Liebermann's Burchard Test	Steroidal Glycosides	+	+	-	+	-
H_2So_4	Saponins glycosides	-	-	-	-	-
Ammonia	Flavonoids	+	-	-	+	-
Shinoda's Test	Flavones	+	-	-	-	-
Sodium chloride	coumarin	-	-	-	-	-
Spot test	Oil and fat	-	-	-	-	-
Acetone	Resins	-	+	-	-	-
Nitric acid	Xantho protein	+	-	-	-	-

+ : Present - : Absent

Table 5: Preliminary phytochemical analysis of CI

Name of the Test	Phytochemical constituents	Methanol	Ethyl acetate	Water	Hydro alcohol	Toluene
Mayer's test	Alkaloids	+	-	-	-	-
Dragendroff's test		+	-	+	+	+
Wagner Test		+	+	+	+	+
Molish Test	Carbohydrates	-	-	-	-	+
Fehling Test		-	.-	-	-	+
Benedicts Test		-	-	-	-	+
Foam Test	Saponins	-	-	-	+	+
Lead Acetate	Tannins	+	-	+	+	+
Ferric chloride.	Pseudo tannins	+	-	-	+	+
Ammonia	Chlorogenic acid	+	-	-	+	+
H_2So_4	Anthocyanin	-	-	-	+	+
Liebermann's Burchard Test	Steroidal Glycosides	-	-	-	+	+
H_2So_4	Saponins glycosides	-	-	-	+	+
Ammonia	Flavonoids	+	-	-	+	+
Shinoda's Test	Flavones	+	-	-	+	+
Sodium chloride	coumarin	-	-	-	+	+
Spot test	Oil and fat	-	-	-	+	+
Acetone	Resins	+	+	+	+	+
Nitric acid	Xantho protein	-	-	-	+	+

+ : Present - : Absent

Table 6: The Plant Drugs of BD and CI leaf showing different extractive values in various solvents

Extractive values	BD (g)	CI (g)
Methanolic extract	1.62	1.90
Ethyl acetate	0.64	1.05
Water	1.34	1.8
Hydro alcohol	1.55	1.16
Toluene	0.26	0.60

*Estimation values for 5g of the sample

Table 7: Antioxidant Results (DPPH Method)

Samples	Concentrations (µg/ml)				
	25	50	75	100	125
BD	65.2 ± 0.21	68.70 ± 0.14	69.5 ± 0.21	73.10 ± 0.28	80.1 ± 0.28
CI	50.90 ± 0.21	53.6 ± 0.14	56.6 ± 0.07	59.0 ± 0.21	70.5 ± 0.35
Ascorbic acid (Standard)	88.2 ± 0.14	88.7 ± 0.28	89.6 ± 0.28	90.1 ± 0.14	98.8 ± 0.07

CONCLUSION

The results of the study revealed both the plants posses many important bioactive principles like flavonoids, alkaloids, terpenoids, steroids etc. Both plants methanolic extractive values are more than other extractive values it indicates methanol has a more power in extracting the bioactive principles for both plants than other solvents. The results are also revealed both plants have strong antioxidant activity achieved by quenching capacity against DPPH radical. Among two plants BD has more antioxidant activity than CI. Further investigation is needed to identify the active components which are responsible for the antioxidant activity.

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