

# Phyto screening of *Boerhavia diffusa* (Punarnava) as an antioxidant herbal drug

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**Abstract-** Medicinal importance of *Boerhavia diffusa* L. is mentioned as herb for treating various ailments like gonorrhea, internal inflammations, dyspepsia, jaundice menstrual disorder, anaemia, disorders of liver, kidney, gall bladder etc. in Indian Ayurveda, Unani, Chinese and Tibetan therapeutic system. Commonly called as Punarnava due to its rejuvenating Property. As oxidative stress is tremendously increasing among the biological organisms due to anthropogenic adulteration in the ecosystem, undoubtedly the human being at the top in the evolutionary hierarchy, are getting more affected. In this context, scientific validation of this plant was assessed by performing the biochemical tests of secondary metabolites and identification of potential functional groups through FTIR analysis in the various leaf extracts. Also, DPPH assay was carried out to judge the antioxidant property. Our biochemical analysis has revealed the presence of eight bioactive compounds exhibiting antioxidant ability to reduce the oxidative stress which will be presented in this communication.

#### Keywords: Boerhavia diffusa, secondary metabolites, antioxidant, FTIR analysis

### **INTRODUCTION**

Ayurveda of Indian scientific literature has girth of ethno-medicinal plants, among which popularly known "Punarnava" herb has been scientifically named as *Boerhavia diffusa* L.<sup>1</sup> belonging to genus *Boerhavia* in the honour of Dutch botanist Herman Boerhavia in eighteenth centuary and family Nyctaginaceae. It is also commonly referred as spreading hogweed and is found throughout the years as prostrate herb bearing stout roots, many diffused branches, pink flowers and short stalked sticky fruits containing one seed. In India this herb is highly recognized and vernacularly named in many ways (Table-1) in different Indian language. Being widely known as "Punarnava" it means renewal or rejuvenator which signifies the anti-aging property.<sup>2</sup>

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Languages/Regions/Countries	Names
1) Bengal,	Punarnava
2) Gujarati	Vakhakhaparo, Dholia-
	saturdo
3) Marathi	Tambadivasu, Ghetuli
4) Tamil	Mukarati Kirei
5) Hindi, Sanskrit & Telugu	Punarnava, Raktakunda,
	Shothaghni, Varshabhu
6) Kanarese	Kommegida
7) English	Hogweed
8) Assamese	Pananua, Ponounua

Globally, it is widely distributed in the tropical, subtropical and central part of the world.<sup>3,4</sup> In Asia, this herb is too common in Bangladesh Sri Lanka, Burma, Cambodia, China, India, Indonesia, Japan, Malyasia, Nepal, Pakistan, Phillipines, Taiwan, Thailand, Vietnam, Saudi Arab Emirates and yemen. In India many states have

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been documented for its distribution like Assam, Bihar, Gujarat, Jharkhand, Madhya Pradesh, Maharastra, Kerala, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh etc.

Each plant of Boerhavia diffusa has diffused branch of length about 90 to 158 cm with an orthotropic and superimposed axis. The axis bears pendulous and the renewable shoots on the upper surface while distal part of shoot becomes main axis. Having orthotropic shoot construction, it is almost prostrate due to rapid proliferation of rely axis over a length of the main axis. The creeping cylindrical stem is swollen at node from which arises alternate branch and appears purple or green. The sub tender leaves on the branches are arranged oppositely oriented while the rots are tapering and fusiform. The inflorescence has numerous buds with small pink bisexual flowers bearing short stalk, three stamens with two unequal lobed anther, glandular ovary secreting trichomes and comparatively small pistil with disc shaped stigma. Achne type fruit is 3-4 mm long, ovate, oblong, pubescent five ribbed and vicid on the ribs, The seed is visible through transparent seed coat.<sup>5</sup> Except flower all the parts of the plants are edible and posses bitter taste.

The plant B.diffusa is well documented in Ayurveda and Uninani literatures mentioning its pharmacological use. Its every part is medicinally important. For instance, the seeds are used as tonic, expectorant, carminative lumbago, scabies, scorpion-sting, blood purifier, and useful in muscular pain while roots are used for curing jaundice, internal inflammations, asthma, piles, pain etc. The juice of this plant is recommended as an antidote for ratpoisoning.<sup>6-9</sup> Brazil is known for using this plant's extract as an agent for curing albuminuria, beri-beri, bile insufficiency, cystitis, edema, gallstones, gonorrhea, guinea worms, hepatitis, hypertension, jaundice, kidney disorder, kidney stones, liver disorders, liver support, nephritis, renal disorders, sclerosis (liver), snakebite, spleen (enlarged), urinary disorders, urinary retention and gall bladder problems.<sup>10</sup> In Guatemala, it is used for erysipelas, guinea worms<sup>11</sup> while in Iran it is used for abdominal pain, anemia, ascites, asthma, blood purification, cancer, cataracts, childbirth, cholera, constipation, cough, debility, digestive sluggishness, dropsy, dyspepsia, edema, eye problems, fever, gonorrhea, guinea worms, heart ailments, heart disease, hemorrhages (childbirth), hemorrhages (thoracic), hemorrhoids, inflammation (internal), internal parasites, jaundice, kidney disorders, kidney stones, lactation aid,

liver disorders, liver support, menstrual disorders, renal insufficiency, rheumatism, snakebite, spleen (enlarged), urinary disorders, weakness, and as a diuretic and expectorant. In Iraq it is used for oedema, gonorrhea, hives, intestinal gas, jaundice, joint pain, lumbago, nephritis, and as an appetite stimulant, diuretic and expectorant. In Nigeria, it is used for abscesses, asthma, boils, convulsions, epilepsy, fever, guinea worms, and as an expectorant and laxative. In West Africa it is used for abortion, guinea worms, menstrual irregularities, and as an aphrodisiac. In tropical Africa the boiled roots are applied to ulcers, abscesses and to assist in the extraction of Guinea worm. The boiled roots and leaves are considered expectorant and febrifuge. A decoction of the aerial parts is also taken to treat gastro-intestinal pains, convulsions and intestinal worms and to regulate menstruation. In Mauritania the seeds are ground and made into cakes which are cooked and eaten as a remedy for dysentery.<sup>12-14</sup>

Owing to the ethno-medicinal propensity, leaves of *B. diffusa* were addressed as an alternative to the roots, shoots and seeds used conventionally. Root and shoots have been extensively investigated for the presence of many bioactive components like phenolic, flavanoids, fatty acids, proteins etc. contributing to incredible biopotency viz antidiabetic, antimicrobial and other similar.<sup>15</sup>

The unrevealed scientific validation for the medicinal values of the leaves of *B. diffusa* will be presented in this communication with respect to exploration of bioactive secondary metabolites, potential functional and antioxidant property.

#### **MATERIALS & METHOD**

#### Research plant: Punarnava (Boerhavia diffusa)

Punarnava (*Boerhavia diffusa*) was collected from the vicinity of KrishiVigyan Kendra, Hazaribag and identified by Dr. P. K. Verma., scientist at KVK, Hazaribag, an ICAR unit, Govt. of India. (Fig.1)

#### Preparation of the Punarnava leaves extract

Leaves of the Punarnava plant were plucked, washed and dried in shade. The shaded dried leaves were then grinded into powder and stored in a container for further use.

About 5 gram of the powder was weighed and dissolved separately in 50 ml of distilled water, ethanol and methanol to obtain the aqueous, ethanolic and methanolic extract respectively. (Fig.2)



Figure 1- Boerhavia diffusa at the collection site

# **Classification of the research plant:**

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Caryophyllales
Family	:	Nyctaginaceae
Genus	:	Boerhavia



Figure 2- Preparation of the Punarnava leaves extract A. Shaking of Plant Extract in Rotatory shaker B. Filtered extract

# Measurement of Extraction Yeild

The extraction yield was measured by pouring the obtained solvent fractionates in .pre-weighed petri-plates. By subtracting the weight of empty petri plates from their weight along with the phytoextracts, the resultant yield of the individual extract was calculated. The calculations were made based on the following formula.<sup>12</sup>

Yield (%) = 
$$\underline{X_1} \times 100$$
  
X<sub>0</sub>

Where,  $X_1$  refers to the weight of extract after evaporation of solvent and  $X_0$  refers to the dry weight of the plant powder before extraction.

# Phytochemical Analysis of the Plant Extract

The aqueous, ethanolic and methanolic extract of punarnava was subjected for preliminary qualitative

screening of various phytochemical groups in various solvent fractionates of *B.diffusa* viz. tannins, saponins, quinones, anthraquinones, alkaloids, flavonoids, phenols, steroids, terpenoids, carbohydrates and proteins under standard protocols described by Adegoke *et al.*, 2010. (Table 3; Fig.3)

Table2- Phyto- C	Chemical Analysis of screening	ıg
Protocols for	<b>Biochemical compositions</b>	

Name of Test	Vol. of Extract	Test Reagents/ Indicator/procedure	Observation	Result
Protein	1ml	Few drops' Biurets Reagent	Purple colour	Positive
Carbohydrate	1 ml	Few drops of Benedicts A & B and heated for 5 mins.	Colour changes to green, yellow/red	Positive
Phenol	1ml	Few drops of di potassium permanganate	Purple colour becomes discolour	Positive
Steroid	2ml	2ml of chloroform and 2 ml conc. sulphuric acid	A red colour develops in the chloform layer	Positive
Saponins	2 ml	Shaken vigoursely with water and warmed	Stable foam develops	Positive
Tannins	1ml Organic extract	1ml 5% FeCl <sub>3</sub>	Dark blue colour /green ppt	Positive
Flavanoids	2ml	1ml 2N Sodium hydroxide	Yellow colour appears	Positive
Coumarins	1 ml	10% Sodium Hydroxide	Appearance of Yellow	Positive
Quinones	1ml	Equal volume Con H <sub>2</sub> SO <sub>4</sub>	Appearance of red colour	Positive





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Figure 3. Biochemical tests for phytochemicals in leaf extracts of *B.diffusa* 

a. Quinone b. Trepenoids c. Flavanoids d. Coumarins e. Tannins f. Proteins g. Steroids h. Phenols

### FTIR analysis of leaf extracts of Boerhaavia diffusa

Interpretation through infra red absorption spectrum, various chemical bonds present in a molecule can be detected. Fourier Transform Infrared Spectrophotometer (FTIR) was then applied for detecting the types of chemical bonds (functional groups) present in the Leaf of *B.diffusa*. The wavelength of light absorbed is considered as the characteristic of the chemical bonds in the annotated spectrum. 10 mg of dried powder of different solvent extracts of leaf materials were used for FTIR analysis.10mg of the dried extract powder was encapsulated in 100 mg of potassium bromide to prepare translucent sample disc to be loaded in the FTIR spectroscope with a scan ranging in between 400 - 4000 cm<sup>-1</sup>

## ROS scavenging activity by DPPH assay

Standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is used to measure the reactive oxygen scavenging capacity of different solvent mixtures.<sup>17</sup> DPPH is a purple colored crystalline powder which tends to react with antioxidants as it is a free ROS generating agent. A 0.004% solution of DPPH in methanol was prepared by dissolving 4mg of DPPH in 100ml of methanol. It was stored in a dark bottle and covered with aluminum foil till use. It was treated as per standard protocol with different concentrations (20uL, 40uL, 60uL, 80uL, 100uL) of methanolic and aqueous extracts of Punarnava.

Simultaneously similar grades of standard i.e., Ascorbic acid were also treated with DPP.<sup>18</sup> A control was also kept in which only methanol was taken. All the test tubes containing the samples were kept for 20 minutes in dark chamber. Simultaneously similar grades of standard i.e., Ascorbic acid were also treated with DPPH.

A control was also kept in which only methanol was taken. All the test tubes containing the samples were kept for 20 minutes in dark chamber. Respective absorbance (OD) at 517 nm and ROS scavenging percentage were calculated using formula

## ROS Scavenging % = $[(A_{0} - A_{1})/A_{0}] \times 100$

where  $A_0$  was control absorbance and  $A_1$  was sample absorbance recorded at 517 nm by spectrophotometer



A.

B.

A. With methanolic extract B. With ascorbic acid RESULTS & DISCUSSION

Different phytochemical groups were detected in various solvent fractionates of *B.diffusa* leaves depending on the change in color or precipitate formation. Altogether eight phytochemicals in ethanolic extract except tannins & coumarins; seven in both methanolic & aqueous extracts except coumarins, saponions & quinones (in methanolic) and trepenoids, steroids and quinones (in aqueous) were detected indicating ethanolic extract as being more richer in terms of phytochemical composition.

SI	Phytochemicals	Ethanolic	Methanolic	Aqueous
No.	-	extract	extract	extract
1.	Tannins	Absent	Present	Present
2.	Flavonoids	Present	Present	Present
3.	Coumarins	Absent	Absent	Present
4.	Saponins	Present	Absent	Present
5.	Terpenoids	Present	Present	Absent
6.	Steroids	Present	present	Absent
7.	Quinones	Present	Absent	Absent
8.	Phenols	Present	Present	Present
9.	Carbohydrate	Present	Present	Present
10.	Proteins	Present	Present	Present

 Table 3- Qualitative analysis of the Phytochemicals

 present in *Boerhavia diffusa*

## FTIR Analysis of Boerhavia diffusa

FTIR spectroscopy provides a powerful approach for the structural and functional alternations induced by various factors due to its high sensitivity.<sup>19</sup> As per obtained spectrum (Graph-1), analysis was done with respect to standard references which revealed the presence of various functional groups as depicted in Table 5.



Figure 5 -FTIR Spectrum Courtesy: Department of Chemistry, BIT Mesra Table 4- Functional group corresponding to frequencies

Group frequencies (Cm <sup>-1</sup> )	Functional group
500-470	Polysulphides
705-570	Disulphides
710-685	Thiols
800-700	Aliphatic chloro compounds
1050-990	Aliphatic phosphates
1360-1250	Aromatic amines
1610-1550	Carboxylic acid salt
1680-1630	Amide
1690-1675	Quinone
1740-1775	Aldehyde
1750-1725	Ester
2100-1800	Transition metal carbonyls
2280-2240	Aliphatic cyanide
2800-2810	Methoxy
3400-3380	Aliphatic primary amine

Courtesy: Interpretation of Infrared Spectra, a Practical Approach John DPHH Assay

Table 5 -	Absorbance	and	ROS	scavenging	activities
	n	erce	ntage		

percentuge						
SI No.	Sample	Vol.	O.D.	ROS Scavenging		
				%		
1.		20uL	0.12	33.3		
2.	Methanolic	40uL	0.11	38.8		
3.	Extract of	60uL	0.09	50.0		
4.	Punarnava	80uL	0.08	55.5		
5.		100uL	0.08	55.5		
6.		20uL	0.16	11.1		
7.		40uL	0.15	16.6		
8.	Aqueous	60uL	0.14	22.2		
9.	Extract of	80uL	0.14	22.2		
10.	Punarnava	100uL	0.13	27.7		
11.		20uL	0.03	83.3		
12.	Ascorbic acid	40uL	0.03	83.3		
13.	as standard	60uL	0.02	88.8		
14.		80uL	0.02	88.8		
15.		100uL	0.01	94.4		
16.	Control	0uL	0.18	0		



Graph-1 -DPPH Assay -Reactive Oxygen Species Scavenging activities

Biogenically four broad classification of secondary metabolites: phenolics and polyphenolics, terpenes, nitrogen-containing alkaloids, and sulfur-containing compounds are there whose functional groups are accountable for delivering a variety of therapeutic applications. These functional groups not only directly involved in the normal growth, development, or reproduction of the plants but also display multiple effects beneficial for general well-being of human organism. The presence of amine bearing alkaloids in the present work exhibited antioxidant effectsimilar to that reported in the extracts of Lepidium meyenii.20 Likewise Thiol group was also which contains sulfhydryl group (-SH) and is found to be important in suppressing the oxidative stress<sup>21</sup> and contribute greatly in treatment of oxidative stress related disorders.

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A methoxy group is a functional group in organic chemistry that consists of a methyl group bound to oxygen (MeO). Methylated flavanoid has been reported to posses the antioxidant activities, that can be elevated, to a certain extent, by methylation.<sup>22</sup> This was confirmed in the present result where a methoxy functional group given rise a sharp isolated band near 2835 cm<sup>-1</sup>. Quinone functional i.e carbonyl was found in the extract seems to act as an antioxiodant as described by Minbyu Dong et al. (2024)<sup>23</sup> and his co-authors in their books Food & Functions. Thus, the methanolic extract B.difusa is confirmed to be the wealthy resource of phytochemicals constituents with high ROS scavenging activities that can be utilized for various pharmaceutical purpose to evaluate the quality and accuracy of formulating curative potentials and as antioxidant herbal drug.

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