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IN-VITRO BIOLOGICAL ACTIVITIES AND CHARACTERIZATION OF AERIAL PART OF BOERHAAVIA DIFFUSA

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ABSTRACT

In the present examine of the biological activities and characterization of Aerial part of Boerhaavia diffusa from Govulapuram village, Villupuram District. The plant samples were extracted and polarity for screening radical scavenging activity by using DPPH assays method. In the qualitative analysis of phytochemical profile revealed the presence of highest amount of phenolic compounds from the three extract of Boerhaavia diffusa. From that the highest radical scavenging capacity followed by n-hexane (BDHE) and methanol (BDEA) extracts. The antibacterial activity has evidenced average zone of inhibition ranging from 3-7 mm, Ethyl acetate extract presented highest inhibition with a zone size of 7mm for E. fecalis, while E.coli and Staphylococcus aureus showing average zone sizes. According to the GC-MS results, ethanol extract of Boerhaavia diffusa was subjected to study of medicinal properties were presence in the phytocomponents in the plants material. So the present study, concluded to expose that the plant has quite a number of chemical constituents, which may be responsible for many pharmacological actions and have protective or disease preventive properties.

KEYWORDS: Biological activity, Boerhaavia diffusa, Phytochemical, Medicinal properties.

INTRODUCTION

The existence of traditional knowledge on medicinal plants and their uses are more common among rural people throughout the world because of their effectiveness. Boerhaavia diffusa, commonly known as Punarnava in Sanskrit is known from time immemorial as an important medicinal herb. Its genus consists of 40 species belonging to the Family: Nyctaginaceae, Order: Thimble, Group: Dicotyledons and Phylum: Angiosperms and are widely distributed in tropical and sub-tropical areas.[1] The whole plant of B. diffusa or parts (leaves, root, and stem) of it have a long history of uses by the indigenous and tribal people and in Ayurvedic and Unani medicines. Out of the 40 species of this genus, 6 species are found in India – B. diffusa, B. erecta, B. repens, B. rependa, B. chinensis and B. rubicund.[2,3]

The medicinal significance of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. It has many ethno botanical uses. Raw root extract of this plant cures urinary disorders, rheumatism, leucorrhea, asthma, and encephalitis, while leaves can be used as a vegetable. Besides, B. diffusa plant being reported to posses many Pharmacological, clinical, and antimicrobial properties; it acts as Ayurvedic medicine in India and Unani medicine in Arab countries. Recently, the authors observed potent antiviral efficacy of this plant against phytopathogenic viruses. The antiviral agent isolated from this plant was found to be a glycoprotein with a molecular weight of 16– 20 kDa.

Pharmacological study analysis reveals that B. diffusa acts as an anticonvulsant,[4] potent antidote for rat and snake bites,[5] antidiabetic,[6] antiurolithiatic,[7] immune-modulation,[8] anthelmintic,[9] febrifuge, anti-leprosy and also used to treat stomach ache, cough, cold. It also posses adaptogenic and antistress activity.[10] As per Ayurvedic, Punarnava is a coolant, bitter to taste but acts as astringent to bowels, heart diseases, useful in biliousness, blood impurities and inflammations etc. According to Unani system of medicine, leaves are alexiteric, appetizers, used to treat ophthalmic conditions and joint pains. Seeds are promising blood purifiers.[11] Furthermore roots of B. diffusa have been used for the treatment of abdominal tumors and cancers, enlargement of spleen, jaundice and dyspepsia, corneal ulcers and night blindness.[12,13]

Boerhaavia diffusa is a creeping weed characterized with thickened stems with fleshy hairy, green and glabrous

leaves while the underneath is white. Flowers are minute, subcapitate, having small bracteolate umbels. Perianth is present in the place of calyx and corella that is tubular shaped and funnel shaped at the top. These plants are found predominantly during rainy season. Chemical composition of B. diffusa contains flavonoids, steroids, triterpenoids, lipids, alkaloids, proteins.[14,15,16] ursolic acid.[17] punarnavoside.[18] and several other compounds have been isolated and studied for their biological activities.

The plant based traditional knowledge has become a recognized tool in search for new sources of drugs.[19] A large number of publications, review and research works on the chemistry, pharmacology, and several other aspects of B. diffusa have been made, but homogenous form of the plant as contemporary standardized drug is yet to be introduced, hence there is no formula to claim its position in herbal market. Also, Limited reports are available on phytochemical screening and in-vitro biological activity of B. diffusa collected from Villupuram District. Present study was carried out to investigate phytoconstituents, radical scavenging ability, antimicrobial activity of n-hexane, ethyl acetate and methanol extracts of aerial part.

MATERIALS AND METHODS

Sample collection and identification

Aerial parts of the plant were collected from Govulapuram village, Villupuram District during January, 2014 in a brown paper cover to prevent denaturation of phytochemicals. The authentication of the plant was made using available literature and taxonomic confirmation was carried out at the Department of CARISM, Sastra University, Thanjavur, Tamil Nadu as Boerhaavia diffusa.

Extraction of plant material

Fresh leaves were washed using tap water to remove any foreign organic matter and shade dried. The pulverized plant material (20g x3) was extracted using various solvents like ethyl acetate (BDEA), n-hexane (BDHE), aqueous (BDAQ) chloroform (BDCL) and methanol (BDME) by constant hot extraction at the temperature not beyond the boiling points of solvents.[20,21] All chemicals used for this work were purchased from Percision scientific suppliers, Trichy. The standard technique involves extraction of phytochemicals by polar solvent directing towards non-polar solvents. Cold percolation method was adopted for aqueous extraction.[22] The extracts were concentrated with a rotary evaporator (IKA, Germany). The extracts (BDEA: 6.45 g, BDHE: 5.43 g, BDAQ: 4.23 g, BDCL: 5. 34 g and BDME: 3.00 g) were stored at 4°C until further procedure. A brown coloured gummy crude substance was obtained.[23]

DPPH radical scavenging assay

DPPH assay is a simple, quick and convenient method independent of sample polarity for screening radical scavenging activity.[24] It makes use of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical and its specific absorbance properties. If the radicals are reduced by antioxidants the absorbance decreases. Stock solution of DPPH was prepared using methanol which showed absorbance of 0.197. Stock solution 100 µL was added to 5 mL of B. diffusa extracts of different concentrations (20-100 µg/mL). Decrease in the absorbance of methanol solution of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) representing scavenging activity of B. diffusa leaves extracts were measured. The solutions were then mixed well and set aside in dark for 20 minutes and the absorbance was measured at 517 nm. Scavenging activity was expressed as the percentage inhibition calculated using the following formula: Then % inhibitions were plotted against respective concentrations used and from the graph IC50 was calculated. Ascorbic acid, a potential antioxidant was used as positive control.[25,26]

$$\% \text{ of free radical scavenging activity} = \frac{\text{Absorbance of control} = \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Antibacterial susceptibility test

Primary investigation to check for anti-bacterial activity was carried out by well diffusion technique. Blank wells filled with solvents followed by evaporation were used as positive and negative control respectively. Microorganisms obtained from Pondicherry Center for Biological Sciences (PCBS), Pondicherry, were used for the study. Culture isolates including Staphylococcus aureus, E. coli, Bacillus subtilis, Klebsiella pneumonia and Enterococcus fecalis were screened. Fresh subcultures were used. A lawn culture of each bacterial strain (0.5 McFarland turbidity standards) was made on Muller Hinton agar plate and wells measuring 8 mm diameter, 2 cm apart were bored using a sterilized borer. 100 µl of B. diffusa extracts made from different solvents (BHEA, BHCL, BHAQ, BHHE and BDME) was carefully loaded into the well and pre-incubated at 4°C for half an hour to facilitate uniform diffusion of bacterial suspension into the agar. The plates were then incubated at 37°C for 24 h. Presence of inhibition zones surrounding each well evidenced antimicrobial activity. Each experiment was repeated three times and the mean of inhibitory zones was recorded.

Spectroscopic characterization using GC-MS Chromatography

The active extract was subject to spectral. GC-MS analysis were performed using Perkin-Elmer clauses 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-I fused silica capillary column (30m x 0.25 mm ID x 1µdf), composed of 100 % Dimethyl polysiloxane. For GC-MS detection an electron ionization system with ionizing energy of 70 e V was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate 1ml/min and an injection volume of 2µL was employed split ratio of 10:1 injector, temperature 2500C: ion-source

temperature 2800C. The oven temperature was programmed from 110°C (isothermal for 2 mins) with an increase of 100°C/min to 2000°C, then 50°C/min to 2800°C, ending with a 9 min isothermal at 2800°C. Mass spectra were taken at 70 eV: a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass. Interpretation of mass spectrum was conducted by using the data base of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of unknown components compared with the spectrum of known components in the NISt library. Molecular weight and structure of the test components ascertained.

Statistical analysis

Statistical comparisons were performed with Student's tests using Microsoft Excel 2007. A P value of 0.05 and 0.001 or less was considered to be significant. Mean values ± SD.

RESULTS

Phytochemical screening

Qualitative analysis of phytochemical profile revealed the presence of phenolic compounds like alkaloids, flavanoids, terpenoids, reducing sugars, saponins, tannins, cardiac glycosides, antroquinones, in varying amount in the B. diffusa extracts. Among the three extracts, the ethyl acetate extract showed the highest amount of phenolic compounds followed by the methanol extract and the n-hexane extract. These results expose that the plant has quite a number of chemical constituents, which may be responsible for many pharmacological actions and have protective or disease preventive properties.

Table 1: Phytochemical components of B. diffusa leaves.

S. No.	Constituents	Name of the test	BDHE	BDAQ	BDME	BDEA	BDCL
1.	Alkaloids	Hagers test	-	+	-	+	+
2.	Flavonoids	Ammoniatest (modified)	-	-	-	-	-
3.	Terpenoids	Salkowski test (modified)	-	+	-	-	+
4.	Reducing sugars	Fehling's test	-	-	-	-	+
5.	Saponins	Frothing's test	-	-	-	-	-
6.	Tannins	FeCl test	-	+	-	-	-
7.	Cardiac glycosides	Killer-Killer's test	+	+	+	+	+
8.	Anthroquinones	Chloroform layer test	-	-	-	-	-

Note: (+) Positive (-) Negative

DPPH radical scavenging activity

From the analysis of Figure 1, it is shown that ethanol extract of B. diffusa (BDEA) showed highest radical scavenging capacity followed by n-hexane (BDHE) and methanol (BDEA) extracts. Also, it was concluded that the scavenging activity of B. diffusa increases as the concentration increases.

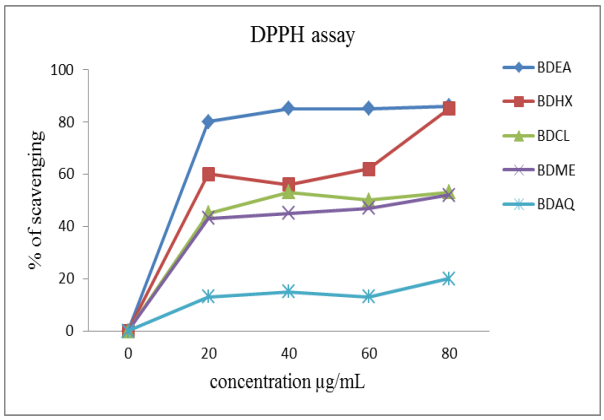

Figure 1: DPPH scavenging activity of the BDHE, BDEA, BDME, BDCL and BDAQ extracts of B. diffusa.

Antibacterial activity

Antimicrobial activities of the B. diffusa extract were tested against five pathogenic microorganisms and the results are presented in Figure 2. Extracts evidenced average zone of inhibition ranging from 3-7 mm, Ethyl acetate extract presented highest inhibition with a zone size of 7mm for E. fecalis, while E.coli and Staphylococcus aureus showing average zone sizes. Extracts of water and chloroform showed considerable activity. Methanol and hexane did not show antimicrobial activity. Both Gram positive and Gram negative organisms included in the study reveals that the ethyl acetate extract of B. diffusa has broad spectrum of activity.

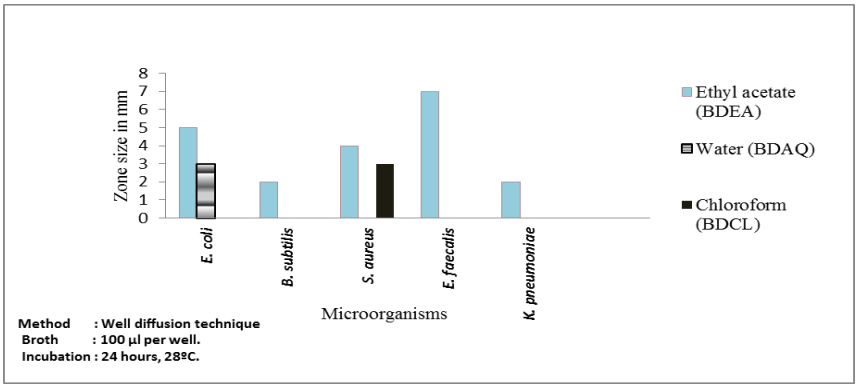

Figure 2: Antimicrobial activity of Boerhavia diffusa.

Table 2: Antibacterial activity of B. diffusa against Gram positive and Gram negative microorganisms.

Microorganism	Zone of inhibition in mm, Extract 5mg in 100 µl of solvent		
	Ethyl acetate	Water	Chloroform
E. coli	5	3	0
B. subtilis	2	0	0
S. aureus	4	0	3
E. faecalis	7	0	0
K. pneumoniae	3	0	0

Note: Microorganisms - 0.5 Mc Farlands / 0.5 ml turbidity in 0.85% saline

GC-MS studies

The ethanol and extract of the leaves of Boerhaavia diffusa leaves was subjected to CG-MS studies. The various plant phytochemical compounds found in the plant of Boerhaavia diffusa ethanol extract list in table - 3.interpretatin on mass spectrum GC-MS was conducted using the data base of (IICPT) .the name , molecular weight, and molecular formula of the components of the test material were ascertained in table-3.ethanol extract of Boerhaavia diffusa was subjected to GC-MS study of medicinal properties, According to the result, the phytocomponents are screened, and most of the medicinal properties of the plant is may be the presence of these following phytoconstituents 2,2-Dimethy locta-3,4-dienal (peak value is 4.07%), 2-Tridecen-1-01, (E)-(peak value is 4.71%),3,7,11,15- Tetramethy 1-2-hexadecen-1-01 (peak value 18.24%), Cyclohexanol, 5-methy11-2-(1-methylethy1)-,[1R-(1‘a,2’a,5’a)]- (peak value is 18.63%),E-7-Teradecenol (peak value is 17.61%), ‘a-D-Glucopyranoside, O-‘a-D-glucopyranosy 1-(1.fwdaw.3)-‘a-D-fructo furanosy1 (peak value is 13.21%), Teradecanoic acids (peak value is 20.03%), 7-Hexadecenoic acids, Methy1 ester, (Z)-(peak value is 2.75%), pentadecanoic acids,2,6,10,14-14-tetramethy1, methy1 ester (peak value is 0.75%).

Table 3: GC-MS study showing components identified in Boerhaavia diffusa extract (Code: 432).

S. No	RT	Name of the compound	Molecular formula	MW	Peak area %
1.	10.48	2, 2-3-Dimethylocta-3,4-dienal	$C_{10}H_{16}O$	152	4.07
2.	10.67	2-Tridecan-1-ol, (E)	$C_{13}H_{26}O$	198	4071
3.	10.95	3,7,11,15-Tetramethyl1-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	18.24
4.	11.16	Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-,[1R-(1á,2á,5á0]	$C_{10}H_{20}O$	156	18.63
5.	11.47	E-7-Tetradecenol	$C_{14}H_{28}O$	212	17.61
6.	12.27	á-D-Glucopyranoside, O-á-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	$C_{18}H32O16$	504	13.21
7.	12.93	Tretradecanoic acid	C14H28O2	228	20.03
8.	14.45	7-Hexadecenoic acid. Methylester, (Z)-	C17H32O2	268	2.75
9.	14.73	Pentadecanoic acid, 2, 6,10,14-tetramethyl-, methyl ester	C20H40O2	312	0.75

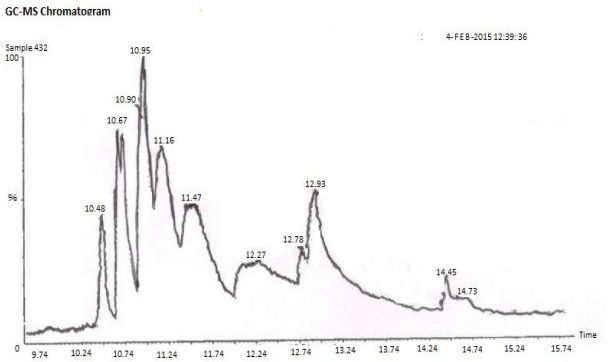

Figure 3: GC-MS analysis showing Peak value of components identified in Boerhaavia diffusa.

DISCUSSION

In the present study investigations revealed that the leaves extract of Boerhaavia diffusa showed the presence of phytoconstituents such as alkaloids, glycosides, carbohydrates, reducing sugar, amino acids and presented significant broad spectrum of anti-bacterial activity. The secondary metabolites of B. diffusa produced biological activity and responsible for their use as medicinal herbs. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some given plant their odors and some still are responsible for their pigment. Thus further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity. For a long period of time, plant has been a valuable source of natural products for maintains human health, especially in the natural therapies. Under this experimental study the extract was active for bactericidal action. The finding revealed the extract capability to penetrate the cell wall with hydrophilic environment (Gram-positive) and hydrophilic environment (Gram-negative) bacteria responsible for the bactericidal action which can be isolated and identified by some analytical techniques.

In antibacterial testing, the ethylacetate, chloroform, and Aqueous extract of Boerhaavia diffusa were tested against five different human pathogenic bacteria (show in table2). Among these extract, ethylacetate, choloroform, will possess antibacterial activity against Enterococcus faecalis Water extract showed activity against Bacillus substilis Staphylococcus aureous showed maximum inhibition in aqueous extract. The ethanol and extract of the leaves of Boerhaavia diffusa leaves was subjected to CG-MS studies. The various plant phytochemical compounds found in the plant of Boerhaavia diffusa ethanol extract list in table - 3.interpretatin on mass spectrum GC-MS was conducted using the data base of (IICPT). The name, molecular weight, and molecular formula of the components of the test material were ascertained intable-3.ethanol extract of Boerhaavia diffusa was subjected to GC-MS study of medicinal properties, According to the result, the phytocomponents are screened, and most of the medicinal properties of the plant is may be the presence of these following phytoconstituents 2,2-Dimethy locta-3,4-dienal (peak value is 4.07%), 2-Tridecen-1-01, (E)-(peak value is 4.71%) 3, 7, 11, 15- Tetramethy 1-2-hexadecen-1-01 (peak value 18.24%), Cyclohexanol, 5-methy11-2-(1-methylethy1)-,[1R-(1‘a,2’a,5’a)]- (peak value is 18.63%),E-7-Teradecenol (peak value is 17.61%), ‘a-D-Glucopyranoside, O-‘a-D-glucopyranosy 1-(1.fwdaw.3)-‘a-D-fructo furanosy1 (peak value is 13.21%), Teradecanoic acids (peak value is 20.03%), 7-Hexadecenoic acids, Methy1 ester, (Z)-(peak value is 2.75%), pentadecanoic acids,2,6,10,14-14-tetramethy1-,methy1 ester (peak value is 0.75%).

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