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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF *BOERHAVIA DIFFUSA L.*

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Abstract

Boerhaviadiffusa L. is widely used as a medicine to cure various diseases.The present study was aim to determine the various phytochemical compounds in *Boerhaviadiffusa* L. The qualitative an analysis revealed the presence of various secondary metaboloties such as tannins, saponin, flavonoids, terpenoids, phenol, emodin and carbohydrates. From the quantitative analysis it was found that phytochemical constitution with the highest quantity was flavonoid (0.013mg/g) followed by saponin (0.008mg/g), alkaloid (0.005mg/g), phenol (0.003mg/g), tannin (0.002mg/g) and terpenoid (0.001mg/g). From the GC – MS analysis of Methanolic leaves extract twenty compounds were identified. These compounds have various biological properties like antimicrobial, antioxidant, anti inflammatory anti tumour etc. Hence this plant is beneficial to use as herbal medicine to cure various diseases.

Keywords: *Boerhaviadiffusa L.*, phytochemical, ethanolicextract, GC-MS, biological activities.

Introduction

Plants are the richest resource of drugs of traditional systems ofmedicine, modern medicines, nutraceuticals, food supplements, folkmedicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). Traditional medical system has great value and also many medicinal plants have been identified from indigenous pharmacopoeias, because of which has many advanced (Adebolu *et al.*, 2005). The medicinal values of plants lie in their phytochemicals, which produce definite physiological actions on the human body. Phytochemical are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health (Alfolabi *et al.*, 2007). The genus Boerhavia comes under Nyctaginaceae family and is found in many part of the world like tropical and sub tropical regions so Asia, Africa and central and South America (Telci *et al.*, 2007). It is a source of essential oils and aroma compounds, a culinary herb and an attractive, fragrant or namental

plant. The whole plant had been used in several traditional medicine systems like Ayurveda, Greek, siddha, Roman, Unani for its range of therapeuticativities. The plant possesses anti inflammatory, antioxidant, anti tumoral, antifertility, antidiabetic, antifungal, antimicrobial, cardio protective, analgesic, antispasmodic and adap to genic properties and also effective in reducing the growth of a variety of cancer cellines invitro (Saravana kumar et al., 2018, Pattanayak *et al.*, 2010).

Materials and Methods

Collection of the plant material

Boerhaviadiffusa L. was collected from Naachikurichi, Tiruchirapalli District. The plants were washed with sterile water and dried in shades. Then the samples were powerd in mechanical grider.

Preparation of ethanolic extracts

An ethanolic extract of *B. diffusa L.* was prepared by drenched 40gm of the dried powder in 1 litre of ethanol by using a hotpercolation extractor for 24 hrs continuously. The extract was filtrerd through whatmann filter paper No. 1 (125mm). The filtered sample extract was concentrated and dried by using a rotator evaporator under reduced pressure.

Preliminary phyto chemical screening

Test for Tannins (Ferric chloride Test)

To 2 ml of extract, 2 ml of distilled water was added in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added to the filtrate. Green precipitate was regarded as positive for the presence of tannins.

Test for Phlobatannins (Precipitate Test)

To 2 ml of extract, 2 ml of 1% HCL was added and boiled. Red precipitate was regarded as positive for the presence of pholobatannins.

Test for Saponin (Foam test)

To 5ml of extract 5 ml of H_2O was added in test tube. The solution was shaken vigorously and observed for as table persistant froth. The frothing was mixed with 3 drop so live oil and shaken vigourously after which it was observed for the formation of an emulsion.

Test for Flavonoids (Alkaline Reagent Test)

To 1 ml of extract, a few drops of 10% lead acetate solution were added. A yellow colour indicates the presence of flavonoids.

Test for Steroids (Salkowski Test)

To 2ml of extract, 2 ml of glacial acetic acid, 1 ml of ferric chloride, 1 ml of distilled water and Con. H_2SO_4 were added. Formation of violet or brown ring was regarde as positive for the presence of cadiaglycosides.

Test for anthroquinnes (Kokate *et al.*, 1997)

To 2 ml of extract, 1 ml of benzene and 2 ml of fammonia solution were added. Pink, violet or red colouration was regarded as positive for the presence of anthroquinones.

Test for Xantho protein (Trease and Trease 1989)

To 1 ml of extract, 1 ml of conc. H_2SO_4 was and boiled. White precipitation was regarded as positive for the presence of proteins.

Test for Coumarins (Liu *et al.*, 2013)

To 2 ml of extract, 3 ml of 10% sodium hydroxide was added. Yellow colour was regarded as positive for the presence of Coumarins.

Test for Phenols (*Liu et al.*, 2013)

To 1 ml of extract, few drops so ammonia solution were added. Reddish orange precipitate formation was regarded as positive forth presence of phenols.

Test for Glycosids (Liebermann's Test)

To 2 ml of extract, 2 ml of chloride and 2 ml of acetic acid were added. Violet to blue green colour was regarded as positive for the presence of glycosides.

Test for Alkaloids (Hager's Test)

To 2ml of extract, few drops of picric acid were added and shaken gently to extract the alkaloids base, yellow precipitate was regarded as positive for the presence of alkaloids.

Test for carbohydrates (Molisch's test)

To 2 ml of extract, 2ml of distilled H_2O, 2 drops of ethanolic alphanapthol and 2 ml of Con. H_2SO_4 were added. Formation of reddish violet ring was regarded as positive for the presence of carbohydrates.

Quantitative Phytochemical Analysis: Determination of total phenols (Keay *et al.*, 1964)

1 gm of powder sample was boiled with 2 ml of diethylether and taken 5 ml of extract, added with 10 ml of distilled water and then added 2 ml of ammonium hydroxide and 5 ml of concentrated amyl alcohol. Plant sample was undisturbed to react for 30 minutes for colour formation. It was measured at 505 nm.

Alkaloid determination (Harborne 1989)

5gm of plant sample was boiled with 10 ml of 10% acetic acid in ethanol and covered, and allow to stand for 2 hours. Ammonium hydroxide was added to the plant extract. The solution was allowed to settle and precipitate was collected and washed with dilute ammonium hydroxide and then filtered, dried and weigh.

Tannin determination (Harborne 1989)

3 gm of powdered sample was extracted and mixed with 10 ml of distilled water. The solution was filtered through the filter paper. 5 ml of extracted sample was taken into a test tube and added with 1 ml of 0.1 M FeCL in 0.1N HCL and 0.008 M potassium ferrocyanide. Obsrvations was measured at 320 nm with in 10 min.

Determination of Saponin (Obadoni and Ochuko 2001)

1 gm of powdered sample and 20 ml of condensed ethanol extract were prepared. The concentrated samples were transferred into 250 ml separate funnel and 1 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and ether layer was discarded. Add few pinch of butanolin 10 ml of fremaining solution and the sample was heated in water bath, after bath, after desertion sample dried in crucible to constant weigh.

Flavonoid determination (Boham and Kocipai 1994)

3 g of the powdered sample was added with 10ml of 80% aqueous methanol. Extraction was filtered through the filter paper. Filterate was transfer into crucible and discarded sample dryness over water bath and weigh.

Determination of Terpenoid

10 g of sample was soaked in alcohol (50ml) for 24 hours Filtered sample was extracted with petroleum ether (40ml) for 24 hours.

Gas chromatography – Mass spectroscopy (GC - MS) Analysis

Ambling of plant materials for GC-MS analysis 10gm of powdered material was soaked in 20 ml of absolute alcohol over night and then filtered through whatmann No. 1 filter paper along with 2gm sodium sulfate to remove the sediments and trace of water in the filtratrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogengasin to the solution and was concentrated 1 ml. The extract contains both polarand non polarphyto components. GC- MS analysis was carried out on a GC clarus 500 perk in Elmersystem and gas chromate graph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions

interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions.

Column Elite – 5 MS fused silica capillary column (30mmX 0.25mm ID X 1 µM df, composed of 5% Diphenyl / 95% Dimethylsiloxane), operating in electron impactmodeat 70eV; Helium (99.999%) was used as carrier gasataconstant flow of 1 ml/min and an injection volume of 2 µl was employed (split ration of 10:1); Injector temperature 250°C . The oven temperature was programmed from 110° C (isothermal for 2 min.) with an increase of 10oC/min, to 200oC, then 5oC/min to 280oC ending with a 9 min. isothermal at 280oC. Mass spectra were taken at 70eV; as can interval of 0.5 seconds and fragments from 45 to 450 Dia. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing it saver age peak area to the total areas. The mass – detecter used in this analysis was Turbo – Mass Gold Perkin – Elmer, and the software adopted to handle mass spectra and chromatograms was a turbo – Massver – 5.2.

Identification of Components (Adams, 1995)

Interpretation on mass spectrum GC – MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components to red in the NIST library. The Name, Molecular weight and structure of the components of the test materials were as curtained.

Result

Qualitive screening revealed that the presence of various constituents such as tannin, pholobatannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leuco anthocyanin, anthcyanin, anthroquinone, protein, coumarin, glycoside, phenol, alkaloids, xanthoprotein, modine and carbohydrate in the ethanoloic leaves extract *B. diffusa L*. (Table 1).

Table 1: Qualitative phyctochemical analysis of ethanolic extact to *Boerhaviadifusa L.*

S.No.	Phytochemicals	Samples
1	Tannin	+++
2	Phlobatannin	++
3	Saponin	++
4	Flavonoids	+++
5	Steroids	-
6	Terpenoids	++
7	Cardiac glycosides	+++
8	Leucoanthocyanin	+
9	Anthocyanine	+
10	Anthroquinone	++
11	Protein	+++
12	Coumarin	+++
13	Glycoside	-
14	Phenol	+++
15	Alkaloids	+++
16	Xanthoprotein	+
17	Emodine	++
18	**Carbohydrate**	+

Strongly present = +++ , Moderate Present = ++ . Slightly present = + , absence = -

Quantitative phytochemical analysis

Quantative test revealed that the presence of various pytochemical constituents in Boerhaviadiffusa L. The phytochemicals with different quantities were mentioned in table – 2

Table: 2 Quantitative phytochemical analysis of *Boerhaviadiffisa L.*

S.No.	Phytochemical constituents	Samples (Mg/G)
1.	Flavonoid	**0.013**
2.	Tannin	**0.002**
3.	Saponin	**0.008**
4.	Alkaloid	**0.005**
5.	Phenol	**0.003**
6.	**Terpenoid**	**0.001**

GC – MS analysis

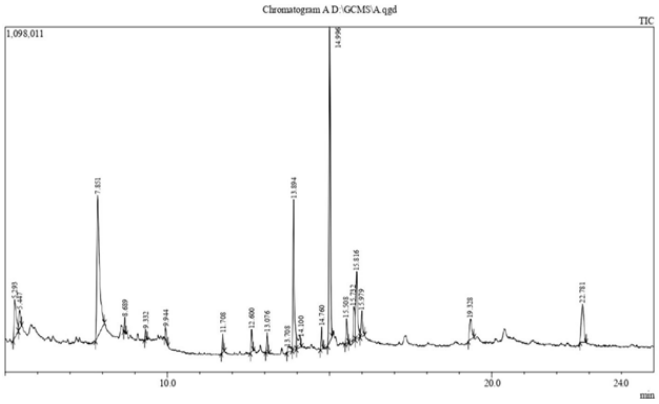
The studies on the bioactive components in the methanolic extract of Boerhaviadiffusa L. by GC – MS analysis clearly showed the presence of twenty bioactive compounds. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (pealarea %) were presented in table -3. The GC – MS chromatohram of the twenty peaks of bioactive compounds detected were showed in fig. 1. The bioactive compounds identified in ethanolic leaves extracts were 4 – Hepten – 3- one, 4 – methyl – (CAS) 4-Methyl – 4- Hepten – 3- one (5.55%), 1,2,3 – Propanetriol, diacetate (CAS) Diacetin (1.83), cytidine (CAS), Cyd (23.55%), Zingiberene (0.87%), Hexadecanoic acid (CAS), Palmitic acid (1.27%), Cyclopentane, heneicosyl – (CASH eneicosane, 1 – cyclopentyl (1.51), 2 – Nonen – 1 – o1, (E) – (CAS) trans – 2- Nonenol (1.48%), 2 – (4 – hydroxyl – 2-butenyl) – 2-nitro cyclo heptanone (1.21%), Hexadecanoic acid (21.33%), dl – Citronellol (1.82%), 1, E – 11, Z – 13 – octadecatriene (3.22%), 9, 12,15 Octadecatrienoic acid, methyl ester (7.04%), 9 – Octadecenoic acid (Z) – (CAS) Oleic acid (2.91%) and 1, 2 – Benzenedi carboxylic acid, bis (ethyl hexyl) ester (6.11 %).

Table 3: Phyto components identified in ethanolic extract of *Boerhaviadiffusa L.*

Peak	Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area%	Bioactivities
1	5.293	4-Hepten-3-one,4-methyl-(CAS)4-METHY	C_8H1_4O	126	5.55	Unknown
2	5.447	1,2,3-Propanetriol,diacetate(CAS)Diacetin	$C_7H_{12}O_5$	176	1.83	Unknown
3	7.851	Cytidine(CAS)Cyd	$C_9H_{13}N_3O_5$	243	23.55	Anti depressant(Machado-Vieira*et al.*, 2010)[17]
4	8.689	Zingiberene	$C_{15}H_{24}$	204	0.87	Antioxidant activity, anti-inflammatory,antinociceptive,Immunomodulatory ,antirheumatic,hypo- analgesic agents, anti-cancer (Rasna Gupta*et al.*, 2016)[18]
5	9.332	Hexadecanoicacid(CAS)Palmiticacid	$C_{16}H_3O_2$	256	0.69	Anti-inflammatory, anti-antioxidant,antitumor, antimicrobial, haemolytic,hypocholesterolemic,immunostimulant,hepatoprotective,antiacne,antiarthritic,antiandrogenic&anticoronary (Morenike*et al.*, 2018)[19]
6	9.944	1,2Benzoldicarbonsaeure, Di-(He	$C_{20}H_{26}O_4$	330	0.85	Anti-allergic&antibacterialactivity (PonnuchamyKumar*et al.*, 2013)[20]

7	11.708	Hexadecanoicacid(CAS)Palmiticacid	$C_{16}H_{32}O_2$	256	1.27	Anti-inflammatory, anti-antioxidant,antitumor, antimicrobial, haemolytic,hypocholesterolemic,immunostimulant,hepatoprotective, antiacne, antiarthritic,antiandrogenic&anticoronary.
8	12.600	Cyclopentane, heneicosyl-(CAS)Heneicosane	$C_{26}H_{52}$	364	1.51	Unknown
9	13.076	2-Nonen-1-ol,(E)-(CAS) trans-2-Nonenol	$C_9H_{18}O$	142	1.48	Unknown
10	13.708	2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon	$C_{11}H_{17}NO_4$	227	1.21	Unknown
11	13.894	Hexadecanoicacid(CAS)Palmiticacid	$C_{16}H_{32}O_2$	256	12.50	Anti-inflammatory, anti-antioxidant,antitumor, antimicrobial, haemolytic,hypocholesterolemic,immunostimulant,hepatoprotective,antiacne,antiarthritic,antiandrogenic&anticoronary.
12	14.100	Phthalicacid,butylester, ester with butyl glyc	$C_{18}H_{24}O_6$	336	2.28	AntimicrobialActivity (Lakshmi and Viji Stella Bai 2015)
13	14.760	Eicosa-5,8,11,14-tetraynoic acid	$C_{20}H_{24}O_2$	296	1.49	Unknown
14	14.996	5,8,11,14-Icosatetraynoicacid	$C_{20}H_{24}O_2$	296	21.33	Unknown
15	15.508	dl-Citronellol	$C_{10}H_20$	156	1.82	Anticancer,antimicrobial,antispasmodic,anticonvulsantactivities and anti-inflammatoryAnaDžamić*et al.*, 2014)
16	15.732	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	248	3.22	Unknown
17	15.816	9,12,15-Octadecatrienoicacid,methylester,	$C_{19}H_{32}O_2$	292	7.04	AntiInflammatory, Hypocholesterolemic, Cancerpreventive, Hepatoprotective, Antimicrobial,Anti- arthritic, anti-asthma, diuretic (SunitaArora*et al.*, 2017)
18	15.979	9-Octadecenoicacid(Z)-(CAS)Oleicacid	$C_{18}H_{34}O_2$	282	2.51	Cancerprevention,flavor,hypercholesterolemic5-alphareductaseinhibitor,antiandrogenicperfumery, insectifuge,anti- inflammatory, anemiagenic,dermatitigenic,choleretic (Rajalakshmi and Mohan, 2016)
19	19.328	Trans-2-phenyl-1,3-dioxolane-4-m	$C_{28}H_{40}O_4$	440	2.91	Unknown
20	22.781	1,2-Benzenedicarboxylicacid,bis(2-ethylhexy	$C_{24}H_{38}O_4$	390	6.11	Antifoulingandantimicrobialactivity,Insecticides,Plasticizer (Reddy*et al.*, 2017)

Fig. 1: Chromatogram obtained from GC – MS with the ethanolic extract of Boerhaviadiffusa L.



Discussion

The phytochemicals are the chemical compounds produced by plants either the product of plant metabolism or synthesized for defense purposes. In the present investigation, the results of phytochemical analysis of *B.diffusa*L. revealed the presence of various secondary metabolities such as tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides, leucoanthocyanins, anthocyanins, anthroquinones, phenols, proteins, coumarins, alkaloids, xanthoproteins, emodine and Carbohydrates. The quantitative analysis of *B. diffusa*L.showed highest amount flavonoid (0.013mg/g) followed by saponin (0.008mg/g), alkaloid(0.005mg/g), and phenol (0.003mg/g), tannin (0.002mg/g), and terpenoids (0.001mg/g).

The study on the phytochemical analysis by SangeetaSankhalkar, and VrundaVernekar, (2016)who investigated the quantitative and qualitative analysis of phenolic and flavonoid content in *Moringaoleifera* L. and *B. diffusa*L. and the results showed the total flavonoid in *B. diffusa*L. leaf was 4.47 mg/ml. Total phenolic content in a leaf of*B. diffusa*L. was 2.28 mg/ml. Balasaheb Kale, (2017)who investigated the phytochemical analysis for various chemical constituents of *B. diffusa*L. and he found out various chemical constituents such as carbohydrates, flavanoids, alkaloids, saponins, glycosides, cardiac glycosides, anthraquinone, tannins and steroids. Praveen Garg and Rajesh Garg, (2019) investigated the phytochemical screening and quantitative estimation of total flavonoids of *B. diffusa*L. in different solvent extract and the results shows the occurrence of flavonoids, alkaloids, glycosides, saponins, tannins, phenolics, amino acid and diterpenes. The quantitative estimation showed total flavonoids content in methanolic leaves was 4.75 mg/ 100g.

GC-MS chromatogram of the methanolic leaves extract of *B. diffusa*L. showed the presence of twenty phytoconstituents and possessed various biological activities. Cytidine (CAS) Cyd, 5,8,11,14,-Icosatetraynoic acid and Hexadecanoic acid (CAS) Palmitic acid are the major compounds detected through GC-MS analysis. The identified known compounds exhibits various biological functions such as antioxidant activity, anti-inflammatory, antitumor, antimicrobial, immune stimulant, hepatoprotective, antiarthritic, antiandrogenic etc. GC-MS analysis of *B. diffusa*L. by SenahDohare*et al.*, (2012) determined twenty two phytoconstituents such as Eugenol, Bornyl acetate, Camphor, Selinene, α-Pinene etc., Balasubramanian*et al.*, (2014) worked on GC-MS analysis of volatile compounds of the essential oil of Leaves of *B. diffusa*L. and they findout three compounds such as Benzene, 1, 2- dimethoxy4-(1-propenyl) , Caryophyllene and Eugenol and they possessed various biological activities like antibacterial, anti-inflammatory, antioxidant, Cancer Preventive, Fungicide, antispasmodic, antiviral, insecticide etc.

Conclusion

In the present study, the ethanolic leaves extract so *B. diffusa L.* revealed the presence of more than ten phtocompounds and the GC – MS analysis showed the presence of twenty bioactive compounds. The presence of various bioactive compound justifies that the leaves of *B. diffusa* L. used for various ailments by traditional practitioners. The bioactive compounds found in this plant are being used for the pharmacological work especially the presence of antimicrobial properties can be used for the synthesis of new drug. However, the isolation of individual photochemical constituent subjecting it to biological activity will definitely give fruitful results. It could be concluded, that presence of various bioactive compound justifies the whole plant is used as are various ailments by traditional practitioners. So it is recommended as plant of pharmaceutical importance.

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