THINK INDIA JOURNAL

ISSN:0971-1260

Vol-22- Issue-14-December-2019

GC-MS Analysis Of Methanolic Extract Of *Boerhaviadiffusa L*

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Abstract

The present study was carried out to characterize the bioactive constituents present in leaf extracts of *Boerhaviadiffusa L* using GC-MS. The results of the GC-MS analysis provide different peaks determining the presence of 20 phytochemical compounds with different therapeutic activities. The From the results, it was observed that presence of l-(+)-Ascorbic acid 2,6-dihexadecanoate,cis-9-Hexadecenal, and Squalene were the major components in the extract and these compound having the properties of GC-MS chromatogram of the extract of *B. diffusa.*

Keywords: *Boerhaviadiffusa L*and GC-MS

INTRODUCTION

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties .The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world .It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants **(Nameirakpam Nirjanta Devil *et al*., 2014).**

Boerhaviadiffusa belongs to the family Nyctaginaceae commonly known as 'punarnava' and locally called 'thazuthama' which means rejuvenating the cells or renewing the body4 by eliminating excess water from it make it highly advantageous for the kidney

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and the liver. The plant acts on all important organ systems and rejuvenate them, giving a new life. In India it has a long history of use by indigenous and tribal people, and in Ayurvedic or natural herbal medicine. Ayurveda classified this plant as 'rasayana' herb which is said to possess properties like antiaging, reestablishing youth, strengthening youth, strengthening life and brain power, and disease prevention like jaundice (**Gaitonde*et al*., 1974**), all of which imply that they increase the resistance of the body against onslaught (**Lal *et al*., 1983**). Pharmacological studies have demonstrated that *B. diffusa*known to possess diuretic (**Mudgal, 1975**), antifertility (**Chopra, 1969**), antifibrinolytic (**Jana, 2007**), immunomodulatory (**Akah et al., 1993**), antidiabetic (**Shah *et al*., 1983**), antiviral (**Parveen*et al*.,2007**), antistress (**Agarwal *etal*.,2008**), antimicrobial (**Jaiswal, 2010**), antiinflammatory, hepatoprotective (**Ramachandra *et al*., 2011**), antioxidant (**Gupta *et al*.,2004**), antiurethritis (**Chandan,1991**) and ant metastatic (**Adesina, 1979**). The main aim of the present study was to evaluate anticancer activities of the methanolic extract of *Boerhaviadiffusa*.

MATERIALS AND METHODS

Plant Material

The plant material of *Boerhaviadiffusa*Lleaf used for the investigationwas collected fromin and around the village nearby Thanjavur. The plant was identified and authenticated by **Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai** and a voucher specimen is kept in the herbarium.

Preparation of the extract

300gm of coarsely ground powder was packed into soxhlet column and extracted with 250ml of 70% methanol for 48 hours (64.5-65.5°C). The extract was filtered and concentrated on water bath at reduced pressure (bath tem 50°C) to syrup consistency (yield: 15%). Then the dried extract was stored in air tight container for further use..

GC-MS analysis

Procedure:

GC-MS technique was used in this study to identify the components present in the extract. GC-MS analysis of this extract was performed using a Shimadzu QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused

ISSN:0971-1260

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silica capillary column (30 m × 1µl was Mdf. Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 2µl was employed (Split ratio of 10:1). Injector temperature was 250ºC. The oven temperature was programmed from 110ºC (isothermal for 2min.), with an increase of 10ºC/min to 200ºC, then 5ºC /min. to 280ºC, ending with a 9min. isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Compound identification was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from library data of the corresponding compounds. The given sample was extracted with ethanol and analyzed in GC-MS for different components **(Saravanan et al., 2014)**

RESULT

GC-MS ANALYSIS

The results of the GC-MS analysis of *Boerhaviadiffusa*Linn is presented in Table 6.The GC-MS analysis revealed presence of twenty compounds in crude methanol extract of *B.diffusa*L. The compounds with their Retention Time (RT), Molecular Formula, Molecular Weight (MW) and Peak Area (%) have been presented in Table 2. The major compounds had been identified on basis of the percentage Peak Area in the Chromatograph. On basis of the peak area percentage the present compounds are like; propane, 1, 1, 3-triethoxy;1- (1-Ethyl-2,3-dimethyl-2-cyclope; dodecanoic acid; 1, 2-Benzendicarboxylic acid, DIE; 4-(2,2,6-trimethyl 7 oxaBicyclop 4; Hexanoicacid,tridec 2ynylester; 2cyclohexon1one, 4hydroxy 3, 5, 5trimeth; 2, 6, 10Trimethyl,14, Ethylene 14, PE; 3, 7, 11, 15Tetramethy l2 hexadece Ethoxy4 methoxyphenethylamine;1,5Anhydrodmannitel; 1(+) Ascorbicacid2,6dihexadecanoate; Phthalic acid, 5-methylhex-2-yl butyl ester; octadecanoic acid, ethylester; Trans-geranyl geraniol; n-Nonadecanol-1; cis-9- hexadecenal; octadecanoic acid; farnesal(E)methyl ether; squalene;

GC-MS analysis was done using the organic solvent ethanol and it shows the presence of twelve different chemical compounds present in the plant sample. The sample was

ISSN:0971-1260

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extracted with ethanol because of the effect of antibacterial activity in this solvent. GC-MS analysis also provides the spectrum for the methanolic extract. The spectrum profile of GC-MS confirmed the presence of 20 major components with the retention time 2.69, 1.14, 1.15,1.74, 1.27,1.22, 1.40,2,22, 1.06, 1.14, 4.33, 26.70, 4.48, 2.38, 2.73, 1.43, 21.99, 7.80, 94, 12.20 respectively. This gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in *B.diffusa*L.The individual fragmentation patterns of the components were illustrated.

In this context, (**Okaka*et al*., 1992**) reported that the phytochemical screening showed the presence of alkaloids, tannins, steroids, flavonoids, saponins, phlobatannins and phenolics. In GC MS, the mass spectrum of the unknown component was compared and interpreted with the spectrum of the known components stored in the National Institute Standard and Technology (NIST) library. Alkaloids are beneficial chemicals to plants with predator and parasite repelling effects. As flavonoids having antioxidant property, it protects tissues against oxygen free radicals, thus have a role in prevention of atherosclerosis, cancer, chronic inflammation and may inhibit aging. The polyphenols possess anti parasitic activity, and monoterpenes have been reported to constitute anti-plasmodic, anti-neoplasmic and anti-viral activities. Presence of flavonoids, phenol and alkaloids in the study revealed that the species may be used as a basic medicinal agent for analgesic, antispasmodic, antibacterial, anti cancer, anti inflammatory and anti oxidant properties. The phytochemical profile of the species gives an insight into its value as a medicinal as well as highly nutritious one, safe for consumption both as a medicine and as a natural source for antioxidant and antioxidant promoting activities (**Sharma, 2006**).

In this context, (**JunaBeegam*et al*, 2014**) reported that the phytochemical screening showed the presence of alkaloids, tannins, steroids, flavonoids, saponins, phlobatannins and phenolics. In GC MS, the mass spectrum of the unknown component was compared and interpreted with the spectrum of the known components stored in the National Institute Standard and Technology (NIST) library.

In our study, GC-MS analysis of the methanolic extract of*B.diffusa* showed the presence of three phyto components. From the results, it was observed that presence of l-(+)-Ascorbic acid 2,6-dihexadecanoate (26.70%),cis-9-Hexadecenal (21.99%), and Squalene

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(12.20%) were the major components in the extract and these compound having the properties GC-MS chromatogram of the ethanolic extract of *B. diffusa*like anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective and antiarthritic activities

GC-MS chromatogram of the methanolic extract of *B. diffusa*(Figure 1) showed 20 peaks indicating the presence of 20 phytochemical constituents. All the constituents were characterized and identified by comparison of the mass spectra of the constituents with the NIST library. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 2. The prevailing compound was l-(+)-Ascorbic acid 2,6-dihexadecanoate (26.70%),cis-9-Hexadecenal (21.99%), and Squalene (12.20%) .Phytochemicals which contributes to the medicinal activity of the plant is listed in Table III. The presence of various bioactive compounds in *B diffusa*justifies the use of the whole plant for various ailments by traditional practitioners. In this context, (**JunaBeegam*etal*, 2014**) the leaves contains eugenol and caryophylline are considered mainly to be responsible for various antimicrobial properties GC-MS chromatogram of the ethanolic extract of *B. diffusa*. In our study, l-(+)-Ascorbic acid 2,6-dihexadecanoate is the main constituent and it is responsible for its repellent property. The presence of l-(+)-Ascorbic acid 2,6-dihexadecanoate attributes to its antioxidative property and is also thought to be responsible for inhibition of lipid peroxidation

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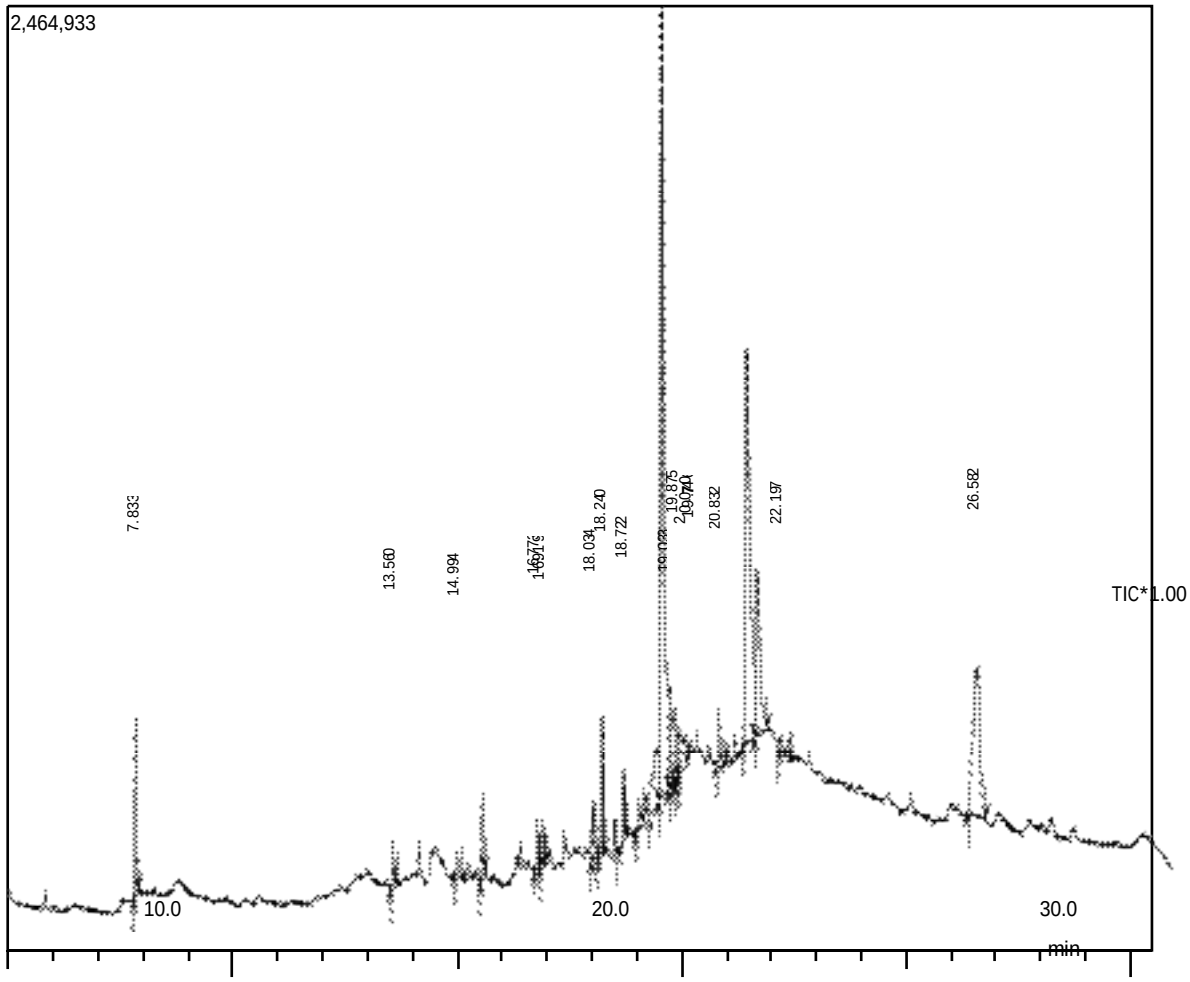

Figure 1: Chromatogram of GC-MS methanolic extract of *B.diffusa*L

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Table 1: Phytocomponents identified in ethanolic leaf extract of *Boerhaviadiffusa*L using GC-MS

S. No.	RT	Name of Compound	Area %	Biological activity
1	7.833	Propane, 1,1,3-triethoxy	2.69	Antifertility activity
2	13.560	1-(1-ethyl-2,3-dimethyl-2-cyclope)	1.14	Antifibrinolytic activity
3	14.994	Dodecanoic acid	1.15	Immunomodulatory activity
4	15.560	1,2-benzenedicarboxylic acid,DIE	1.74	Antidiabetic activity
5	16.773	4-(2,2,6-trimethyl-7-oxa-bicyclop4)	1.27	Antiviral activity
6	16.919	Hexanoic acid, tridec-2-ynyl ester	1.22	Antistress activity
7	18.034	2-Cyclohexen-1-one,4-hydroxy-3,5,5-trimeth	1.40	Antimicrobial activity
8	18.240	2,6,10-trimethyl,14-ethylene-14-PE	2.22	Antiinflammatory activity

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9	18.722	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.06	Hepatoprotective activity
10	19.033	3Ethoxy-4-methoxy phenethylamine	1.14	Antioxidant activity
11	19.417	1,5-Anhydro-d-mannitol	4.33	Antiurethritis activity
12	19.558	1-(+)-Ascorbic acid 2,6-dihexadecanoate	26.70	Antimetastatic activity
13	19.746	Phthalic acid, 5-methylhex-2-yl butyl ester	4.48	Antifungal activity
14	19.875	Octadecanoicacid,ethyl ester	2.38	Antioxidant activity
15	20.070	trans-Geranylgeraniol	2.73	Antibacterial activity
16	20.832	n-Nonadecanol-1	1.43	Antihistamic activity
17	21.451	cis-9-Hexadecenal	21.99	Cytotoxic activity
18	21.682	Octadecanoic acid	7.81	Antiasthmatic activity
19	22.197	Farnesol (E), methyl ether	0.94	Antiproliferative activity
20	26.582	Squalene	12.20	Anticonvulsant activity

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CONCLUSION

.*Boerhaviadiffusa*L. is a plant which is extensively used in folk medicine and little attention has been given to the characterization of secondary metabolites other than rotenoids and alkaloids. Various chemical constituents observed in the phytochemical profiling of *B.diffusa*suggests their significance in pharmacological actions. Study also recommended the species as a plant of phytopharmaceutical importance due to the presence of of biologically active compounds that may serve as candidates for new drugs in the treatment and prevention of many livestock diseases. GC-MS analysis was also carried out to identify the name, molecular weight and structure of the components of the test materials. To rationalise use of the plant however, more work needs to be carried out at molecular level.

ACKNOWLEDGEMENT

The authors are grateful thank to the Secretary, Correspondent, Trust member, principal and vice principal of RabiammalAhamedMaideen College for Women – 610001, Tamilnadu, India. for providing necessary laboratory facilities to complete this manuscript.

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