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Identification of Components In *Boerhavia Diffusa* Plant Extract And Assessment Of Its Biological Activity By Gas Chromatography-Mass spectrometry

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ABSTRACT

Boerhavia diffusa is a medicinal plant used for various therapeutic benefits ant the plant can be found in tropics and subtropics regions. It has the pharmacological activities which include antifibrinolytic, immunosuppressive, diuretic, hepatoprotective, antidiabetic, anti-inflammatory, anticancer, immunomodulatory, antilympho proliferative, analgesic properties and also for the treatment of pulmonary tuberculosis. This study deals with the determination of possible phytocompounds present in the hydroethanolic extract of the plant *Boerhavia diffusa* using Gas chromatography- Mass Spectroscopy technique. Using GC-MS technique the phytocompounds present in the hydroethanolic extract of the plant *Boerhavia diffusa* was investigated and the mass spectra of the compounds found in the extract were matched with National Institute of Standards and Technology library. GC-MS Analysis of *Boerhavia diffusa* extract reveals that the existence of phytocompounds which includes 1, 2-Benzenedicarboxylic acid, diethyl ester, Hexadecanoic Acid, 1,2-Benzenedicarboxylic acid, dibutyl ester and 1,2-Benzenedicarboxylic Acid, Dioctyl Ester. This research paper helps to identify the phytocompounds which are present in the extract of *Boerhavia diffusa* and also prove the biological activities of the phytocompounds present. Furthermore, studies were made in-depth to find the mode of action of the phytocompounds.

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INTRODUCTION

Boerhavia diffusa is popularly known as Punarnava (Indian Medicine), which means rejuvenates or renews the body (Rajpoot and Mishra, 2011). The plants Boerhavia diffusa are seen in both tropical and subtropical regions all over the world (Struwig and Siebert, 2013). It is a medicinal herb in India and the roots used for the treatment of anasarca, ascites, inflammation, piles, and jaundice. They act as a diuretic and laxative agent (Mahesh *et al.*, 2012). *Boerhavia diffusa* has thick, deep penetrating and single taproot as shown in Figure 1. The root is stout and fusiform and they have creepy stem arise from the root, swollen at the nodes. They have slightly rounded leaves, as shown in Figure 2. Deep pink color flowers are seen in the plant *Boerhavia diffusa* (Agrawal *et al.*, 2011).

Most important bioactive Phytochemical constituents present in the plant *Boerhavia diffusa* are the glycosides, alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils and phenolic

compounds. The number of flavonoids and phenols are higher than the other phytochemicals and they are responsible for many pharmacological activities. Tissues are protected against oxygen free radicals due to its antioxidant property of flavonoids. Thus they prevent cancer, atherosclerosis and chronic inflammation (Beegum *et al.*, 2014).

Figure 1: The whole plant of *Boerhavia diffusa*

Scientific Classification

Botanical name : *Boerhavia diffusa*

Kingdom: Plantae

Order: Caryophyllales

Family: Nyctaginaceae

Genus: *Boerhavia*

Species: *diffusa*

In India six species were found, they are *B.diffusa, B.erecta, B.rependa, B.chinensis, B.repens and B.rubicunda* and commonly called as Mookarattai.

Figure 2: The Leaves of *Boerhavia diffusa*

Benefits of Mookarattai

Boerhavia diffusa used to cure a renal disorder, which may decrease the urea levels in the body. They plant able to cure arthritis and reduce pain and inflammation. They enhance erection, quality and quantity of semen. It increases appetite, reduces abdominal pain and relieves constipation. It acts as a diuretic which may cure kidney stone and also acts as a laxative and detoxifier, which may cure hepatitis. They regenerate the whole body (Kanagavalli *et al.*, 2018). *Boerhavia diffusa* can also cure stomach problems, fever, diarrhoea, dysentery and skin disease. All the parts of the plant have its unique medicinal property (Panda, 2014).

MATERIALS AND METHODS

Collection of the Plant sample

The samples of plant *Boerhavia diffusa* Linn was collected from their characteristic natural surroundings and from the market. The plant *Boerhavia diffusa* was washed in tap water, air-dried and made to a fine powder by a homogenizer. The dry powder acquired was maintained at room temperature in airtight containers.

Preparation of Plant Extract

5 g of dry powdered plant material was extracted with 100 ml of 70% of ethanol for maceration periods (24 hr). At 150 rpm the extraction was agitated and carried out at room temperature. Through Whatmann No. 1 filter paper, the soaked powder-solvent mixtures were filtered after the maceration periods. A Part of the filtrate was used for phytochemical analysis and the remaining solvent evaporated to dry for *in vitro* antioxidant studies.

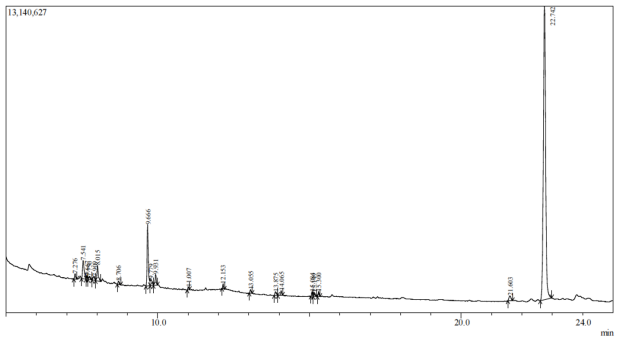

Figure 3: GC-MS Chromatogram of *Boerhavia diffusa* Ethanolic extract

GC-MS Analysis

By Shimadzu 2010 plus comprising AOC-20i autosampler GC MS analysis was carried out. Gas chromatograph employs the following conditions by interfacing mass spectrometer instrument: column RTX 5Ms with Column diameter is 0.32mm, column length is 30m, column thickness 0.50μm. It operates at 70eV electron impact mode; A carrier gas called Helium gas (99.999%) was used at a constant flow of 1.73 ml /min. An injection volume of 0.5 μI was employed with a ratio of 10:1.

Table 1: Identification of compounds in a sample using GC-MS

Peak #	R.Time	Area %	Molecular formula	Molecular weight	Name of the compound
1	7.276	0.63	$C_{14}H_{30}$	198	Tetradecane
2	7.541	3.35	$C_{10}H_{10}O_4$	194	1,2-Benzenedicarboxylic acid, dimethyl ester
3	7.667	0.44	$C_{17}H_{36}$	240	Tetradecane, 2,6,10-trimethyl
4	7.750	0.49	$C_{12}H_{24}$	168	Cyclododecane
5	7.909	0.86	$C_{13}H_{28}$	184	Nonane, 3-methyl-5-propy
6	8.015	2.34	$C_{18}H_{38}$	254	Heptadecane, 8-methyl
7	8.706	0.43	$C_{18}H_{38}$	254	Heptadecane, 8-methyl
8	9.666	8.44	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethyl ester
9	9.779	1.35	$C_{11}H_{23}BR$	234	1-Bromo-2-Methyl-Decan
10	9.931	1.56	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethyl ester
11	11.007	0.30	$C_{18}H_{38}$	254	Octadecane
12	12.153	0.47	$C_{18}H_{30}O$	262	2-tert-Butyl-4-(2,4,4-trimethylpent-2-yl)phenol
13	13.055	0.46	$C_{16}H_{34}$	242	1-Hexadecanol
14	13.875	0.59	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
15	14.065	0.58	$C_{16}H_{22}O_4$	278	1,2-Benzenedicarboxylic acid, dibutyl ester
16	15.084	0.50	$C_{22}H_{38}O$	318	2,4-Dioctylphenol
17	15.149	0.57	$C_{15}H_{32}O$	228	1-Pentadecanol
18	15.300	0.54	$C_{33}H_{48}O_4$	508	Bis(2,4-Ditert-Butylphenyl) Penta
19	21.603	1.05	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid, dioctyl ester
20	22.742	75.07	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid, dioctyl ester

The highest peak area % was 1,2-Benzenedicarboxylic acid, dioctyl ester (75.07%) while the lowest peak % was Octadecane (0.30%).

The temperature for the injector is 270 ºC and for ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150 ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV with a scan-interval of 0.5 seconds and fragments from 40 to 450 Da. The running time of total GC is 51.25min. The relative component of each component was calculated when it is compared to the average peak area to the total area. By adopted Software mass spectra was handled. And the chromatogram obtained was in Turbo Mass Version 5.2.0.

Identification of components

By using the database of National Institute Standard and Technology (NIST05s), the GCMS interpretation was done because it has more than 62,000 patterns. A comparison was made between the unknown component spectrum and the known component spectrum. The structure, molecular weight and name of the components of the test materials were determined.

RESULTS AND DISCUSSION

Identification of Bioactive compounds in Boerhavia diffusa Extract by GC-MS analysis

The GC-MS profile of plant *Boerhavia diffusa* showed the presence of 20 compounds. The active principles with their probability, molecular formula, molecular weight (MW) and area (%) are presented in Table 1 and Figure 3. The GC-MS output of *Boerhavia diffusa* showed two major peaks at retention times 9.66 and 22.74. The prevailing compounds are 1, 2-Benzenedicarboxylic acid, diethyl ester, Hexadecanoic Acid, 1, 2-Benzenedicarboxylic acid, dibutyl ester and 1, 2-Benzenedicarboxylic Acid, Dioctyl

Table 2: Activity of Components identified in sample using GC-MS

S.No	Molecular name	Biological activities**
1	Tetradecane	Antimicrobial, antifungal, Nematicidal
2	Heptadecane, 8-methyl	Antifungal, Antibacterial, Antioxidant
3	1,2-Benzenedicarboxylic acid, diethyl ester	Plasticizers
4	Hexadecanoic Acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
5	Octadecane, dibutyl ester	Used as a plasticizer for vinyl foams, which are often used as floor tiles. Other uses are in traffic cones, food conveyor belts, and artificial leather
6	Octadecane	Lubricants, anticorrosion agents
7	1,2-Benzenedicarboxylic Acid, Dioctyl Ester	Antimicrobial Antifouling

Ester were found in this plant *Boerhavia diffusa*. GC-MS is a cost-effective method but can able to cure many human diseases. The plant *Boerhavia diffusa* has many pharmacological activities since they act as steroidal lactones (Pooja *et al.*, 2017).

Biological activity

The biological activity of compound represented in Table 2. 1, 2-Benzenedicarboxylic acid, and diethyl ester was used in Plasticizers. 1, 2-Benzenedicarboxylic Acid reduces oxidative stress and able to cure neurodegenerative disorders (Choi *et al.*, 2009).

Hexadecanoic acid is palmitic acid and has some biological activities such as anti-inflammatory effect, antioxidants, hypocholesterolemic, nematicide, pesticide, lubricant, hemolytic inhibitor and antiandrogenic.

Hexadecanoic acid is a saturated fatty acid shows antibacterial and antifungal activities (Aparna *et al.*, 2012). Octadecane is the long-chain alkanes and acts as a lubricant and anticorrosion agents (Ghavipanjeh *et al.*, 2015).

Heptadecane acts as an antifungal and antimicrobial agents (Rao *et al.*, 2016). 1-Pentadecanol has antimicrobial activity which can be analysed by selected microorganisms (Stein *et al.*, 2000).

1, 2- Benzenedicarboxylic acid, dibutyl ester used as a plasticizer for vinyl foams, which are often used as floor tiles. 1, 2-Benzenedicarboxylic Acid, Dioctyl Ester used for Antimicrobial, Antifouling.

The presence of various bioactive compounds justifies the use of the *Boerhavia diffusa* for various ailments by traditional practitioners. Solid cyclic hydrocarbon at room temperature is the Cyclodecane and this compound has ability to break stone (Gielen *et al.*, 1989). Nonane shows increasing antitumor activity and has ability to cure cancer which is a dreadful disease (Kubo *et al.*, 1995).

CONCLUSION

20 different phytocompounds were found in the plant *Boerhavia diffusa* and their significance in pharmacological actions. Study also recommended the species as a plant of phytopharmaceutical importance due to the presence of biologically active compounds that my serve as candidates for new drugs in the treatment and prevention of many livestock diseases. A detailed study of the various compounds present in *Boerhavia diffusa* and their pharmaceutical importance requires to be carried out such that a drug with multiple effects can be made available in future studies.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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