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GC-MS Analysis of Bio-Active Compounds in Aqueous Extract of *Boerhaavia Diffusa, Euphorbia Hirta and Amaranthus Polygonoides*

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ABSTRACT

Medicinal plants have had a crucial role in human culture and civilization. The present study deals with the Gas Chromatography Mass Spectroscopy analysis of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides*, which have various medicinal properties. The aim of this study was to investigate and characterize the chemical composition of the different crude aqueous extracts of these plant samples using PerkinElmer Gas Chromatography–Mass Spectrometry. Our results show that the compounds in the extract revealed by GC-MS were the same as recorded in the National Institute of Standards and Technology (NIST) library. The identity and quantity of the measured active compounds was correlated with the expected therapeutic effects. The content of active principles was determined. The quantity of the active principles in the plants was also determined.

KEYWORDS: *GC-MS, Boerhaavia diffusa, Euphorbia hirta, Amaranthus polygonoides, medicinal plants*

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INTRODUCTION

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments (Bachrach, 2012). They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of successful drugs, and will continue to be important for screening of new lead compounds (Atanasov et al., 2015) The importance of medicinal plants has been recognized since time immemorial synthetic medicine were not available in the part only the herbal medicines were used to treat all diseases. From this we can understand that plants are rich in medicinal properties and hence are useful in human health and wellbeing. Therefore studies using experimental model are essential to identify more medicinal properties of the plants (RastogiandMehrotra, 1979).Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Sidhha and Unani) and unorganized (folk, tribal, native) forms of traditional medicine. With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has assumed great importance to understand the novel mechanism of action (Murthy et al., 2002; Badami et al., 2003; Meurer-Grimes et al., 1996).

Gas Chromatography Mass Spectroscopy is a hyphenated system which is a very compatible technique and the most commonly used technique for identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites, 1997).There are numerous reports on the GC MS analysis studies on many plants and plant parts. These studies were undertaken to ascertain the presence of active biomolecules which have therapeutic activities (Jayapriya and Gricilda Shoba, 2015; Kanthal et al., 2014).

Boerhavia diffusa is a species of flowering plant in the four o'clock family which is commonly known as punarnava (meaning that which rejuvenates or renews the body in Ayurveda), red spiderling, spreading hogweed or tarvine. It is consumed for pain relief as a herbal medicines in addition to other uses. The leaves of *Boerhavia diffusa* are often used as a green vegetable in many parts of India. *Euphorbia hirta* (sometimes called asthma-plant) is a pantropical weed, possibly native to India. It is a hairy herb that grows in open grasslands, roadsides and pathways. It is used in traditional herbal medicine. *Amaranthus polygonoides*is an annual plant glabrescent proximally,

pubescent distally and becomes glabrous at maturity. Stems erect-ascending to prostrate, branched mostly at base and in proximal 1/2, 0.1-0.5 m. Leaves: petiole ± equaling blade; blade ovate, obovate-rhombic to narrowly ovate, sometimes lanceolate, 1.5-3(-4) × 0.5-1.5(-2) cm, base cuneate, margins entire to undulate-erose, apex rounded, obtuse, or emarginate, mucronate

In this study the Gas Chromatogram Mass Spectrometric method (GCMS) was used determination of the compounds in the aqueous extract.The plants produce these chemicals to protect themselves but research has shown that they have the capacity to treat human diseases in an effective way (Dutta and Ghosh, 1947). The plant possesses various medicinal properties; the aim of this study was to identify the phytocompounds in the aqueous leaf extract of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* to identify each specific compound with their concentrations by Gas Chromatography – Mass Spectrum (GCMS) analysis.

Materials and Methods

Collection of Plant Materials

The fresh samples of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* were collected randomly from the Tiruvannamalai Dt, Tamil Nadu. Sample materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

Preparation of Extracts

Crude Sample extract was prepared by Soxhlet extraction method. About 20gm of powdered sample material was uniformly packed into a thimble and extracted with 250ml of aqueous extract separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºCtill all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC till future use.

The GC–MS analysis

The GC–MS analyses of bioactive compounds from the different leaves of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* were done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in asplitless mode. Relative quantity of the chemical compounds present in each of the extracts of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* was expressed as percentage based on peak area produced in the chromatogram.

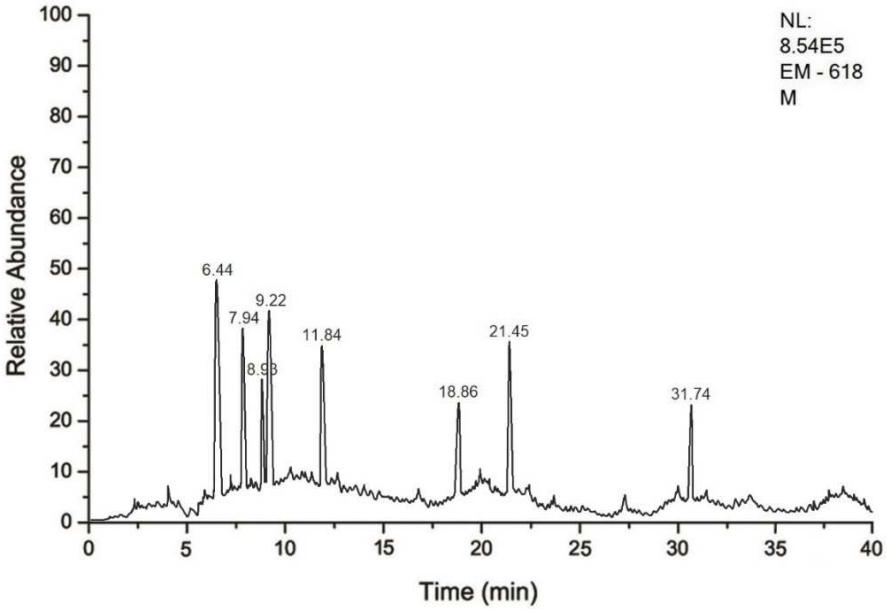
Identification of chemical constituents

Bioactive compounds extracted from different extracts of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Result and Discussion

GC-MS is the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds.

Figure 1: GCMS Analysis of *Boerhaavia diffusa*



The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented. The results pertaining to GC-MS analysis of the aqueous extract of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* lead to the identification of a number of compounds. These compounds were identified through mass

spectrometry attached with GC. The various components present in the entire herb of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* that were detected by the GC-MS are shown in (Table 1, 3 and 5).The biological activity of the compounds were studied and reported in (Table 2,4 and 6).

Table 1: GCMS Analysis of *Boerhaavia diffusa*

S. No	RT	Name of the Compound	Molecular Weight	Molecular Formula
1	6.44	1 – Butoxy -1- ethoxy ethane	146	$C_8H_{18}O_2$
2	7.94	Phenol, 2,6 – bis (1,1 – dimethyl ethyl) – 4 – methyl, methyl carbamate	277	$C_{17}H_{27}NO_2$
3	8.93	2,5 – Pyrrolidinedione, 3 – ethyl – 1,3 - dimethyl	155	$C_8H_{13}NO_2$
4	9.22	1,1 – Diethoxy – 3 – methylbutane	160	$C_9H_{20}O_2$
5	11.84	Tetrahydro furan – 5 – on – 2 – methanol alpha	260	$C_{11}H_{16}O_7$
6	18.86	2 – 0 – Methyl – D - mannopyranosa	194	$C_7H_{14}O_6$
7	21.45	N – Hexadecznoic acid	256	$C_{16}H_{32}O_2$
8	31.74	N – Cyanomethylpiperidine	124	$C_7H_{12}N_2$

Table 2: Biological activity for *Boerhaavia diffusa*

S. No	Name of the Compound	Biological activity
1	1 – Butoxy -1- ethoxy ethane	Antibacterial and Leishmanicidal activity
2	Phenol, 2,6 – bis (1,1 – dimethyl ethyl) – 4 – methyl, methyl carbamate	Antioxidant and Antibacterial activity
3	2,5 – Pyrrolidinedione, 3 – ethyl – 1,3 - dimethyl	Antibacterial and Anticancer Activity
4	1,1 – Diethoxy – 3 – methylbutane	Antibacterial Activity
5	Tetrahydro furan – 5 – on – 2 – methanol alpha	Antibacterial and Antifungal Activity
6	2 – 0 – Methyl – D - mannopyranosa	Synthesis, Antibacterial and Antifungal Activity
7	N – Hexadecznoic acid	Antimicrobial, Larvicide and anti- inflammatory activity
8	N – Cyanomethylpiperidine	Antimicrobial and Antioxidant activity

Figure 2: GCMS Analysis of *Euphorbia hirta*

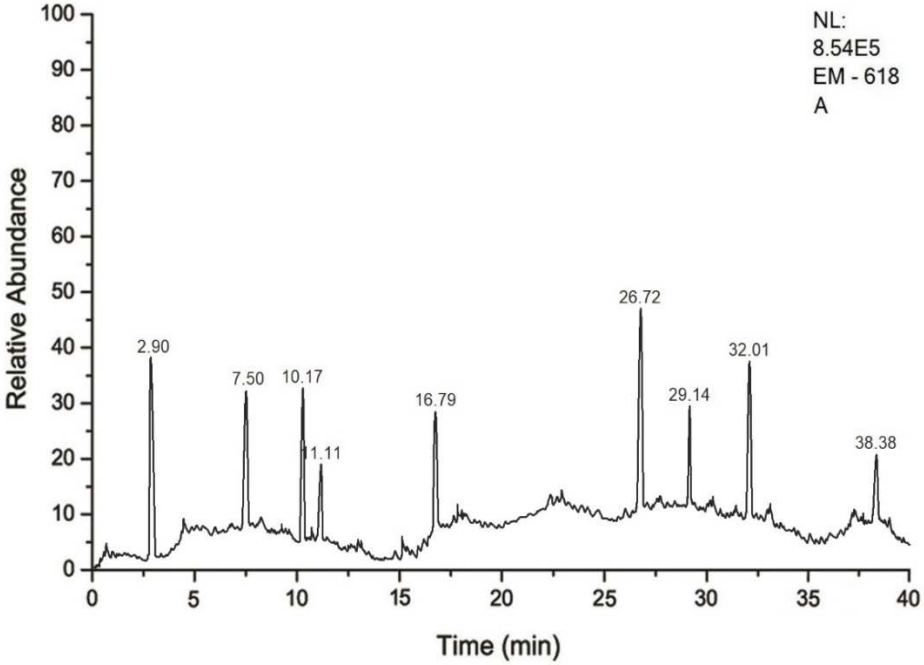


Table 3: GCMS Analysis of *Euphorbia hirta*

S. No	RT	Name of the Compound	Molecular Weight	Molecular Formula
1	2.90	1,3 – Two methyl Cyclohexane	112	C_8H_{16}
2	7.50	4 – Hexane – 3 – One, 4,5 - dimethyl	126	$C_8H_{14}O$
3	10.17	1,3,5 – Triazine – 2,4,6 – triamine	126	$C_3H_6N_6$
4	11.11	(S) – 5 – Hydroxy methyl – 2 [5H] - furanone	114	$C_5H_6O_3$
5	16.79	2 – Methyl pyrrolidone	81	C_5H_7N
6	26.72	Pinane	138	$C_{10}H_{18}$
7	29.14	Palmitic Acid	256	$C_{16}H_{32}O_2$
8	32.01	Phytol	297	$C_{20}H_{40}O$
9	38.38	Tetracosane	339	$C_{24}H_{50}$

Table 4: Biological activity of *Euphorbia hirta*

S. No	RT	Name of the Compound	Biological activity
1	2.90	1,3 – Two methyl Cyclohexane	Antimicrobial Activity
2	7.50	4 – Hexane – 3 – One, 4,5 - dimethyl	Antibacterial and Antifungal Activity
3	10.17	1,3,5 – Triazine – 2,4,6 – triamine	Antibacterial and Synthesis
4	11.11	(S) – 5 – Hydroxy methyl – 2 [5H] - furanone	Antimicrobial Activity
5	16.79	2 – Methyl pyrrolidone	Antimicrobial, Cytotoxicity Activity and PharmaceuticalApplications
6	26.72	Pinane	Antimicrobial and Antioxidant Activity
7	29.14	Palmitic Acid	Antimicrobial, Antioxidant and Cytotoxicity Activity
8	32.01	Phytol	Antimicrobial and Anti-asthematic activity
9	38.38	Tetracosane	Antimicrobial, Alleopathic, Antioxidant and Cytotoxicity Activity

Figure: GCMS Analysis of *Amaranthuspolygonoides*

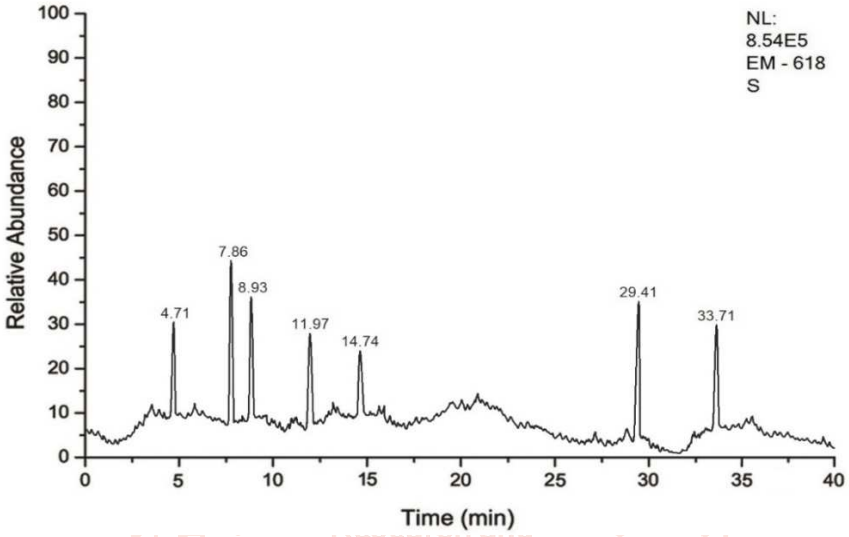


Table 5: GCMS Analysis of *Amaranthus polygonoides*

S. No	RT	Name of the Compound	Molecular Weight	Molecular Formula
1	4.79	1,2,4 – Butanetriol, trinitrate	241	$C_4H_7N_3O_9$
2	7.83	2,5 - Hexanedione	114	$C_6H_{10}O_2$
3	8.93	2,5 – Pyrrolidinedione, 3 – ethyl – 1,3 - dimethyl	155	$C_8H_{13}NO_2$
4	11.97	2 – (3 – oxo -3- phenyl propyl) – 3,5,6 – trimethyl pyrazine	254	$C_{16}H_{18}N_2O$
5	14.74	1 - Tetradecane	196	$C_{14}H_{28}$
6	29.41	9,12 – Octadecadienoic Acid, Methyl esters	270	$C_{17}H_{34}O_2$
7	33.71	Docosane	310	$C_{22}H_{46}$

Table6: Biological activity of *Amaranthus polygonoides*

S. No	RT	Name of the Compound	Biological activity
1	4.79	1,2,4 – Butanetriol, trinitrate	Synthesis activity
2	7.83	2,5 - Hexanedione	Antibacterial Activity
3	8.93	2,5 – Pyrrolidinedione, 3 – ethyl – 1,3 - dimethyl	Antimicrobial, Antioxidant and Cytotoxicity Activity
4	11.97	2 – (3 – oxo -3- phenyl propyl) – 3,5,6 – trimethyl pyrazine	Unknown
5	14.74	1 - Tetradecane	Antimicrobial and Anti-tumour activity
6	29.41	9,12 – Octadecadienoic Acid, Methyl esters	Phytochemical and Antimicrobial Activity
7	33.71	Docosane	Anticholinesterase and *Antimicrobial activities*

But only a limited number of plant research laboratories have gas chromatography mass-spectrometry. The identified compounds occupy many biological properties. GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical value of that plant. Thus, this type of GC-MS analysis is the first step towards understanding the nature of medicinal properties in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation is needed to identify the pharmacological importance and phytochemistry of *Boerhaavia diffusa, Euphorbia hira* and *Amaranthus polygonoides*.

Conclusion

The present GC MS results of *Boerhaavia diffusa, Euphorbia hirta* and *Amaranthus polygonoides* indicated the presence of some bio molecules which have important medicinal activities which correspond well with the reports of the medicinal activities of this plant. Further work is required to confirm the efficacy of this plant.

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