

GC-MS ANALYSIS OF PHYTOCONSTITUENTS FROM THE ETHANOL EXTRACT OF WHOLE PLANT OF *SPHAERANTHUS AMARANTHOIDES*

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ABSTRACT

Sphaeranthus amaranthoides is a spreading aromatic herb distributed in the plains of India, Africa and Australia. The whole plant is used in the treatment of jaundice, fever, epilepsy, gastric complaints, swellings and diabetes. The plant is used as a substitute for the Ayurvedic drug Munditika for which the accepted botanical source is *Sphaeranthus indicus* Linn. The aim of the present study is to analyse and identify the various bioactive constituents present in the ethanol extract of *Sphaeranthus amaranthoides* by GC-MS analysis. Preliminary phytochemical screening of ethanol extract was carried out following standard procedures. GC-MS analysis of the ethanol extract of *S. amaranthoides* whole plant was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra version: 5.0, Thermo MS DSQ II using non polar column. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, proteins, saponins, flavonoids and tannins. The results showed the presence of 12 phytoconstituents namely (-) – Carvone, α -Eudesmol, 3, 7, 11, 15 – Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, 2, 3-dihydroxypropyl ester, phytol, 9, 12, octadecadienoic acid, Cis-10-Nonadecenoic acid, Eicosanoic acid, Eicosanoic acid methyl ester, Squalene, 1-Hepatacosanol and Heptacosane. To best of our knowledge this is the first report about the presence of β -eudesmol, phytol, Squalene in the whole plant of *Sphaeranthus amaranthoides*.

KEYWORDS : Ethanol extract, β -Eudesmol, GC-MS, Phytol, *Sphaeranthus amaranthoides*

INTRODUCTION

Medicinal Plants are integral part of human life and several civilizations from time immemorial. As per WHO nearly 80% of world's population relies on the use of traditional medicines, which is predominantly based on plants parts. Several distinct bioactive compounds derived from medicinal plants are important drugs currently used in different parts of the world¹. Discovery of useful medicines and isolation of therapeutically active phytoconstituents like quinine, reserpine and taxol has added impetus to the ongoing research on natural products^{2,3,4}. *Sphaeranthus amaranthoides* Burm.f. belonging to Asteraceae is a fragrant herb distributed in moist places throughout the plains of Southern India. It is considered as a substitute for the drug Munditika in Ayurveda⁵, the accepted source for which is *Sphaeranthus indicus* Linn^{7,8}. The plant is also used in Siddha system of medicine and referred as Sivakaranthai. Munditika is traditionally used to treat various diseases like jaundice (*kamala*), fever (*jwara*), epilepsy (*apasmara*), gastric disorders (*udararoga*), painful swellings (*sotha*)⁹, Urolithiasis¹⁰. Antioxidant¹¹, wound healing¹² and hepatoprotective activity¹³, anticancer¹⁴ activity has been reported for *S. amaranthoides*. However no work has been reported on the nature of phytoconstituents present in ethanol extract of whole plant of *S. amaranthoides*, hence the present work has been undertaken.

MATERIALS AND METHODS

Collection of plant material

The whole plant of *S. amaranthoides* in flowering condition was collected from Komaneri village of Thuthukudi district; Tamil Nadu in January 2011. The collected plant material was identified and authenticated by Dr. S.N. Yoganarasimhan, Taxonomist and Research coordinator, M.S.Ramaiah College of Pharmacy, Bangalore. Taxonomic identification was carried out using available literature¹⁵. A voucher herbarium specimen (PCog.No. 044) has been deposited in the Department of Pharmacognosy along with the sample of crude drug. The plant material was thoroughly washed with water to remove the adhering dirt and sandy material, cut into small pieces and shade dried.

Preparation of ethanol extract

Coarsely powdered whole plant material (100 g) was

extracted with 95 % (V/V) ethanol using soxhlet apparatus. The extract was filtered and concentrated using rotary evaporator under reduced pressure and temperature.

Preliminary phytochemical screening

The Preliminary phytochemical screening of ethanol extract was carried out following standard methods^{16,17,18,19}.

GC-MS analysis

The GC-MS analysis was carried out using GC-MS equipment, THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II with ZB 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN, dimension 30 Mts, ID - 0.25 mm, FILM: 0.25 μ m^{20,21}. Helium is used as the carrier gas with a flow rate of 1.0 ml/min. The initial temperature of the instrument was set to 70°C and raised up to a temperature of 250°C, with a raise in temperature of 6 °C /min. The ionization voltage was 70 eV. The injection volume is 1 μ l. Mass spectra were scanned in the range of 50-650 (*m/z*). The MS running time was 38.52 min.

Interpretation of MS

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components in the NIST library and the phytoconstituents present in the ethanol extract were confirmed.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Preliminary phytochemical screening of the ethanol extract showed the presence of carbohydrates, proteins, alkaloids, flavonoids, tannins, saponins and triterpenoids.

GC – MS analysis

GC-MS analysis of ethanol extract of *Sphaeranthus amaranthoides* whole plant was performed using THERMO GC - TRACE ULTRA VER: 5.0 (Fig 1). The GC-MS analysis revealed the presence of nearly 12 compounds. The name of the compounds with their retention time (RT), molecular formula, molecular weight, chemical nature and biological activity are shown in Table 1. It showed the presence of (-) – Carvone, α -Eudesmol (Fig.1a), 3, 7, 11, 15 – Tetramethyl-2-

hexadecen-1-ol, Hexadecanoic acid, 2, 3-dihydroxypropyl ester (Fig.1b), phytol, 9, 12, octadecadienoic acid (Fig.1c), Cis-10-Nonadecenoic acid, Eicosanoic acid (Fig.1d), Eicosanoic acid methyl ester, Squalene (Fig.1e), 1-Hepatacosanol (Fig.1f) and Heptacosane. This is the first report on the presence of α -eudesmol, phytol, Squalene in whole plant of *Sphaeranthus amaranthoides*. The compound like Phytol is reported to have anticancer, antioxidant and anti-inflammatory properties²². Hexadecanoic acid has the property of antioxidant activity²². α -eudesmol has been reported to have antioxidant and anticancer activity²³. Squalene is reported to treat gastroesophageal reflux²⁴. Hence based on the phytoconstituents identified, *Sphaeranthus amaranthoides* can be used as a substitute for *Sphaeranthus indicus*. Presence of these phytoconstituents substantiates the use of *S. amaranthoides* in Ayurveda as an alternate source for the drug, Munditika.

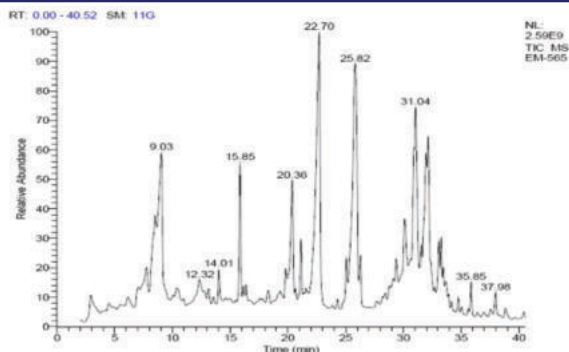


Figure 1: GC-MS of ethanol extract of *Sphaeranthus amaranthoides* Burm.f.

Table.1: Activity of bioactive compounds identified in the ethanol extract of *Sphaeranthus amaranthoides* Burm.f.

S. No	Name of the compound	Retention time (min)	Peak area %	Molecular formula	Molecular weight	Chemical nature of the compound	Biological activity**
1.	(-) – Carvone	8.51	5.30	C10H14O	150	Terpenoid	Flavouring agent, mosquito repellent
2.	α – Eudesmol	16.38	0.97	C15H26O	222	Sesquiterpene	Anticancer, antioxidant property
3.	3,7,11,15 – Tetramethyl-2-hexadecen-1-ol	19.79	0.91	C20H38	278	Terpene alcohol	Antimicrobial, Anti-inflammatory
4.	Hexadecanoic acid, 2,3-dihydroxypropyl ester	22.70	0.27	C19H38O4	330	Palmitic acid propyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Antiandrogenic flavor, Hemolytic, 5-Alpha reductase inhibitor
5.	Phytol	25.06	0.82	C20H40O	296	Diterpene	Anticancer, antioxidant, anti inflammatory, diuretic and antimicrobial agent
6.	9,12,octadecadienoic acid	25.84	13.7	C18H32O2	280	Polyunsaturated omega – 6-fatty acid	anti-inflammatory, acne reductive, and moisture retentive properties when applied topically on the skin
7.	Cis-10-Nonadecenoic acid	27.65	0.32	C19H36O2	296	Monounsaturated long chain fatty acid.	Antimutagenic and p53 inhibitory activity
8.	Eicosanoic acid, methyl ester	28.83	0.65	C21H42O2	326	Saturated fatty acid	Nutritional supplement, photographic materials and lubricants.
9.	Eicosanoic acid	29.37	1.37	C20H40O2	312	Saturated fatty acid	Production of detergents, photographic materials and lubricants.
10.	Heptacosane	33.77	5.34	C27H56	380	Hydrocarbon	-----
11.	Squalene	35.85	1.10	C30H50	410	Steroid	Anticancer, Antiageing, Analgesic, Antidiabetic, Anti-inflammatory, Antioxidant, Anti ulcer, Hepatoprotective, Hypocholesterolemic, Antispasmodic
12.	1 -Hepatacosanol	37.98	8.74	C27H56O	396	Fatty alcohol	Antihyperlipidemic

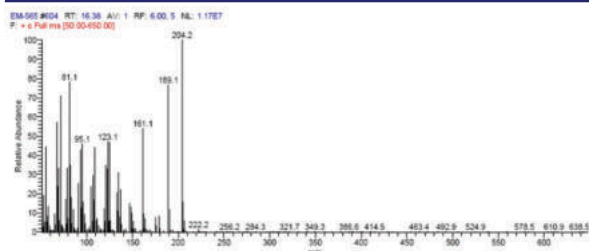
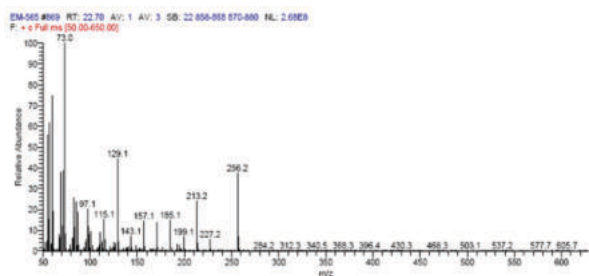
Figure 1a. Mass spectra of α -Eudesmol at 16.38 min

Figure 1b. Mass spectra of Hexadecanoic acid, 2, 3-dihydroxypropyl ester at 22.70 min

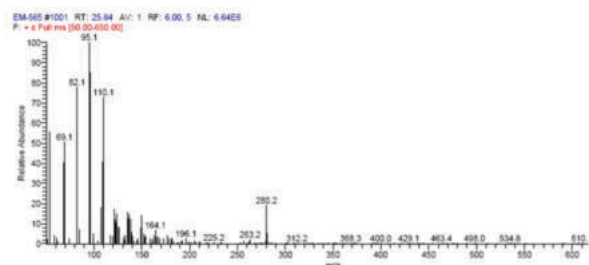


Figure 1c. Mass spectra of 9, 12, octadecadienoic acid at 25.84 min

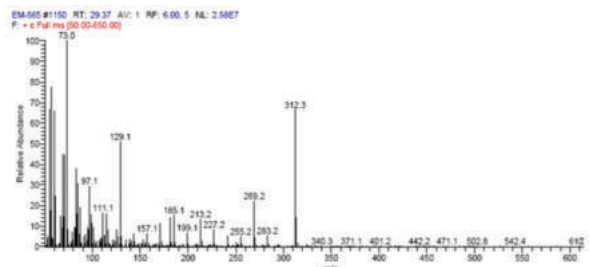


Figure 1d. Mass spectra of Eicosanoic acid at 29.37 min

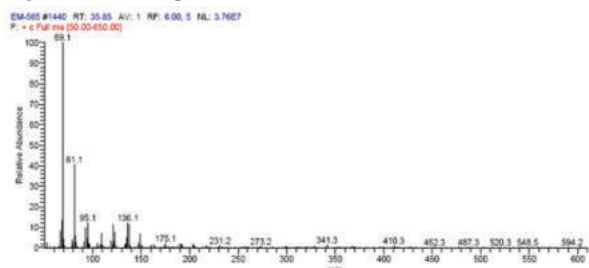


Figure 1e. Mass spectra of Squalene at 35.85 min

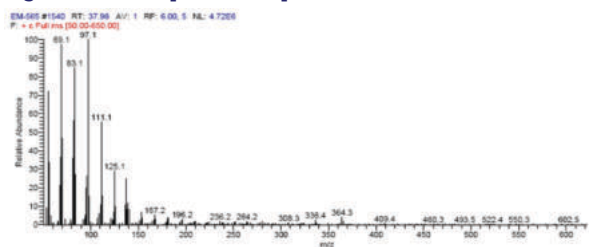


Figure 1f. Mass spectra of 1-Hepatacosanol at 37.98 min

CONCLUSION

Presence of these therapeutically valuable phytoconstituents in the ethanol extract of whole plant of *S. amaranthoides* substantiates the traditional use of plant in the treatment of jaundice and diabetes and also as an alternate botanical source for *S.indicus*. Further work on isolation of the identified phytoconstituents and establishment of its pharmacological activity can be undertaken.

Acknowledgement

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Conflicts Of Interest

The author does not have any conflicts of interest

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