

Phytochemical and GC-MS analysis of *Sphaeranthus amaranthoides* Burm

Kanimozhi.S^{1,2}, Elumalai Prithviraj³, Govindarajan Sumathy*

¹ Research Scholar, Sree Balaji Dental College & Hospital, BIHER, Chennai.

² Reader, Department of Anatomy, Sri Sairam Siddha Medical College and Research Centre, Chennai

³ Associate professor, Department of Anatomy, Sree Balaji Dental College & Hospital, BIHER, Chennai.

* Professor & Head, Department of Anatomy, Sree Balaji Dental College & Hospital, BIHER, Chennai.

Abstract

Objective: To isolate and evaluate the phytochemical constituents of *Sphaeranthus amaranthoides* using GC-MS.

Method: Preliminary phytochemical screening of the extract was carried out according to the standard method described by Brindha et al. GC-MS analysis was performed on the methanolic extract of *S. amaranthoides* to find out the chemical constituents.

Results: Phytochemical screening revealed the presence of steroids, alkaloids, sugars, phenolics, flavonoids, saponins, tannins, and amino acids to a spotted degree. GC-MS results revealed the presence of 15 different phytocompounds, viz., 3,4-Xylyl, 3,5-di-tert-butylbenzoate, n-Hexadecanoic acid, 17.β. -Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid, 3-Cyclopenten-1-one, 3-hydroxy-2-(1-hydroxy-3-methylbutylidene)-5-(3-methyl-2-butenylidene)-5, 17.β. -Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester 6, Indan, 6-tert-butyl-4-ethyl-1,1-dimethyl -7, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester 10(E),12(Z)-Conjugated linoleic acid, 9-Octadecenoic acid, (E)-Octadecanoic acid, 9.12-Octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester, 1,8, 11-Heptadecatriene, (Z,Z)-, 11-Methyltricosane, Nonane, 5-butyl-, 1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester etc.

Conclusion: The presence of various bioactive compounds confirms the application of *Sphaeranthus amaranthoides* for various diseases by means of a herbal system of treatments.

Introduction

The significance of plants is known to us well. Many Medicinal plants have been used for centuries as remedies for a number of human diseases and from these plants many potential drugs have been isolated. Discovery of such herbal drugs increases the awareness on Siddha system. Herbal drugs are easily available, less expensive, safe, and efficient. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [2]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body. Some of the important bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [6,7]. These bioactive substances are synthesized by primary or to a certain extent secondary metabolism of living organisms

Secondary metabolites are chemically and taxonomically diverse compounds with doubtful function. They are widely used in the veterinary, agriculture, scientific research and many other areas

Natural constituents which is native of plant origin can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seed, etc.[4] The medicinal properties of the plants unique to particular plant species or groups are depends on the concept that the combination of secondary

products in a particular plant is taxonomically distinct[5].The spectrometric and chromatographic screening method could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations[3].The determination of phytoconstituents is largely performed by the relatively laborious techniques such as gas (GC) and liquid chromatography (LC) combined with specific detection schemes. GC-MS has become firmly established as a key technological metabolic profiling in both plant and non-plant species.

Sphaeranthus amaranthoides. *S. amaranthoides* Burm.f. is a small procumbent herb, with appressed hairy leaves palmately 3-foliolate. The species are low annuals with spreading branches, stem erect, glabrous, sometimes as thick as the little finger, but short, branches are not winged and 7-13 inches, leaves 2-5 inches, linear, oblong narrowed at the base. This plant is well known for its medicinal value for the treatment of eczema, blood disorder, stomach worms, filarial, fever and as a remover of kapha, vata, and piles. It is also known to cure skin diseases.[8] In the present work, qualitative and quantitative phytochemical analysis were carried out in *Sphaeranthus amaranthoides*.

Material and methods

Collection of the plant material

The plant, *S. amaranthoides*, was collected from the Salem Dist., Tamil Nadu, India. The primary tasks, like washing, drying, etc., were done. The plant materials were identified and authenticated by DR.P.Murugan, M.Sc, Ph.D, Department of Medicinal Botany, Sri Siddha Medical College & Research Centre. The collected plant material was free from disease and also free from contamination of other plants.

Preparation of plant extracts

100 g of *S. amaranthoides* air-dried and coarsely powdered plant material was extracted with 500 mL of methanolic solvent by using a Soxhlet extractor. After extraction, the sample was kept in the dark for 72 h with intermittent shaking. Then the solvent was evaporated under reduced pressure using Rota-vapor to obtain viscous semi-solid masses.

Phytochemical analysis

The methanolic extract was tested for steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids. Phytochemical screening of the extract was carried out according to the standard method.[15]

GC-MS examination

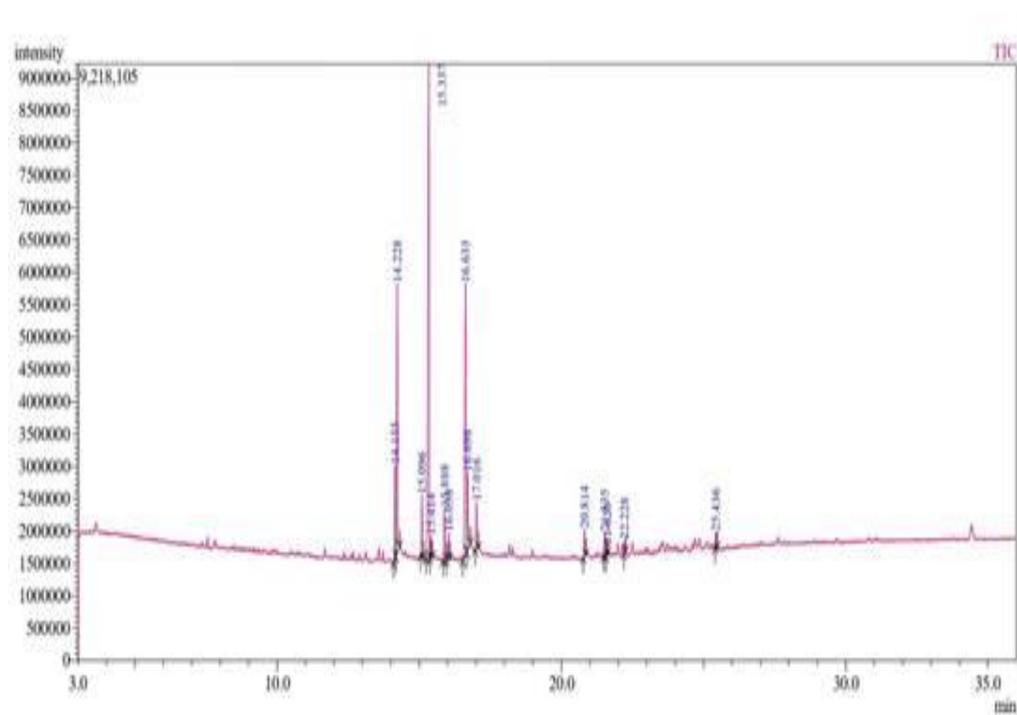
The Shimadzu GCMS QP 2020 was used in the analysis. The method was employed with a fused silica column, packed with SH-Rxi-%Sil MS (30 m 0.25 mm ID 250m df) and the components were separated using Helium as the carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 280°C during the chromatographic run. The 1L of extracted sample injected into the instrument at the oven temperature was as follows: followed by 280 °C at a rate of 10 °C min⁻¹ and 280 °C for 3 minutes. The mass detector conditions were: transfer line temperature of 280 °C; ion source temperature of 230 °C; and ionisation mode electron impact at 70 eV, a scan time of 0.2 sec, and a scan interval of 0.1 sec. The fragments range from 40 to 550 Da. The spectrums of the components were compared with the database of spectrums of known components stored in the GC-MS NIST (2017) library.

Results and discussion

The phytochemical screenings of *S. amaranthoides* extract revealed that the methanolic extract contains Steroids, Sugar, Flavanoids, Alkaloids, Phenolics, Saponins, Aminoacids, and Tannins except Anthraquinone (Table 1)

Table 1: Preliminary phytochemical screening of methanolic extract of *Sphaeranthus amaranthoides*.

S.NO	COMPOUNDS	METHANOLIC EXTRACT
1.	Steroids	+
2.	Sugar	+
3.	Flavanoids	+
4.	Alkaloids	+
5.	Phenolics	+
6.	Saponins	+
7.	Amino acids	+
8.	Tannins	+



The results pertaining to GC-MS analysis led to the identification of a number of compounds from GC fractions of the methanolic extracts of *Sphaeranthus amaranthoides*. They were recognised through mass spectrometry attached to GC. A GC-MS analysis of a methanolic extract of *Sphaeranthus amaranthoides* is shown in (Table 2). The result revealed the presence of 15 different phytocompounds viz.,(5.5%).3,4-Xylyl3,5-di-tert-butylbenzoate, (17.0%) Hexadecanoicacide, (3.5%) 17.beta.-Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester, (34.19%) 3-Cyclopenten-1-one, 3-hydroxy-2-(1-hydroxy-3-methylbutylidene)-5-(3-methyl-2-butenylidene)-, (1.14%) 17.beta.-Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester, (3.04%) Indan,6-tert-butyl-4-ethyl-1,1-dimethyl-,(1.38%) 9,12-

Octadecadienoic acid (Z, Z)-, methyl ester, (21.08%) 10 (E), 12 (Z)-Conjugated linoleic acid, (5.65%) 9-Octadecenoic acid, (E)- (2.66%) (1.51%) Octadecanoic acid 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester, (1.42%) 1,8,11-Heptadecatriene, (Z, Z)-, (0.77%) 11-Methyltricosane, [0.56%] Nonane, 5-butyl, (0.85%) 1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester respectively. In a study done by Geethalakshmi et al., the methanolic extract of *Sphaeranthus amaranthoides* showed the highest

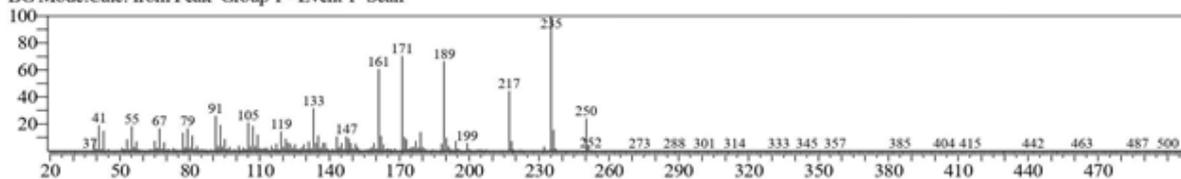
Peak #	R.Time	Area	Area%	Height	Height%	Name
1	14.155	3630368	5.50	1443174	6.22	3,4-Xylyl 3,5-di-tert-butylbenzoate
2	14.228	11232709	17.02	4172076	17.98	n-Hexadecanoic acid
3	15.096	2146580	3.25	950028	4.09	17.β.-Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester
4	15.337	22564641	34.19	7622054	32.85	3-Cyclopenten-1-one, 3-hydroxy-2-(1-hydroxy-3-methylbutylidene)-5-(3-methyl-2-butenylidene)-
5	15.414	752352	1.14	330452	1.42	17.β.-Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester
6	15.888	2003215	3.04	799939	3.45	Indan, 6-tert-butyl-4-ethyl-1,1-dimethyl-
7	16.038	912144	1.38	417711	1.80	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	16.633	13911518	21.08	4187155	18.05	10(E),12(Z)-Conjugated linoleic acid
9	16.698	3726515	5.65	1201783	5.18	9-Octadecenoic acid, (E)-
10	17.016	1754486	2.66	728746	3.14	Octadecanoic acid
11	20.814	993749	1.51	426771	1.84	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
12	21.535	936431	1.42	353713	1.52	1,8,11-Heptadecatriene, (Z,Z)-
13	21.620	509310	0.77	132960	0.57	11-Methyltricosane
14	22.228	366797	0.56	185721	0.80	Nonane, 5-butyl-
15	25.436	558223	0.85	247539	1.07	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
		65999038	100.00	23199822	100.00	

antioxidant activity of any other extract. 13th. *Sphaeranthus amaranthoides* methanolic extract is known to have antioxidant, antimutagenic, and antimicrobial properties. The rich antioxidant properties of *Sphaeranthus amaranthoides* might be due to the presence of carotenes, Neoxanthin, Chlorophyll a,

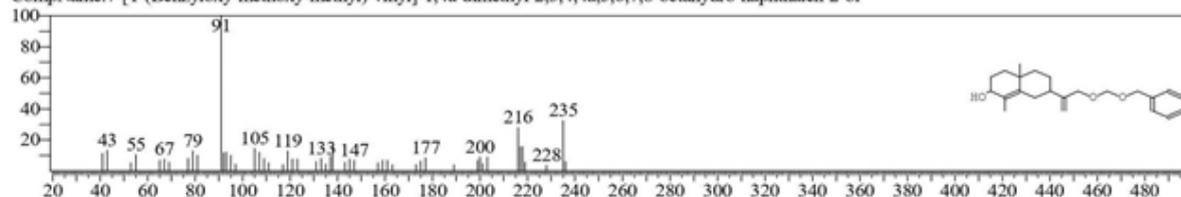
Chlorophyll b, lactein, vialaxanthin, and pheophytin in it (15). Individual diffraction of some dominating compounds is shown in fig.2.

<< Target >>

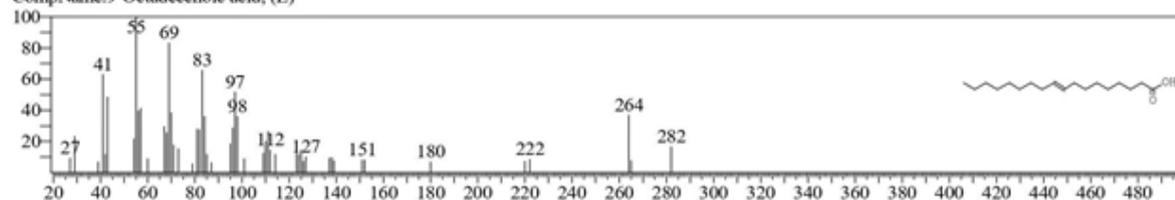
Line#:3 R.Time:15.095(Scan#:2420) MassPeaks:318
RawMode:Averaged 15.090-15.100(2419-2421) BasePeak:235.15(89822)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



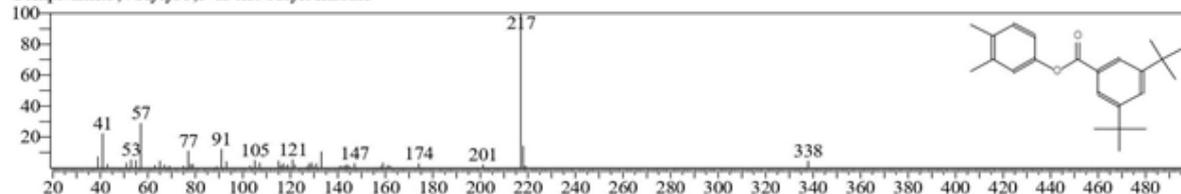
Hit#:2 Entry:234327 Library:NIST17.lib
SE:65 Formula:C23H32O3 CAS:0-00-0 MolWeight:356 RetIndex:2714
CompName:7-[1-(Benzyloxy-methoxy-methyl)-vinyl]-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydro-naphthalen-2-ol



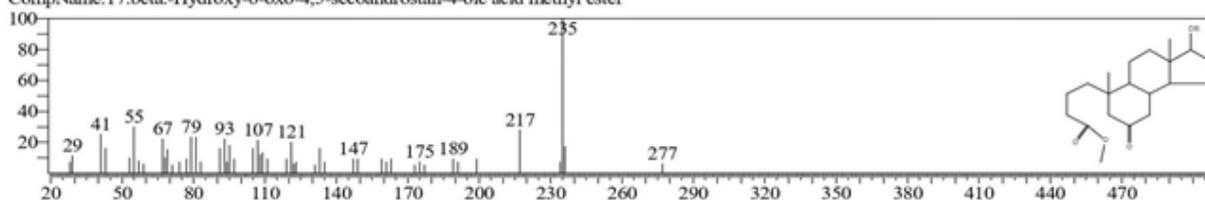
Hit#:1 Entry:157089 Library:NIST17.lib
SE:92 Formula:C18H34O2 CAS:112-79-8 MolWeight:282 RetIndex:2175
CompName:9-Octadecenoic acid, (E)-



Hit#:1 Entry:217240 Library:NIST17.lib
SE:63 Formula:C23H30O2 CAS:293761-66-7 MolWeight:338 RetIndex:2514
CompName:3,4-Xylyl 3,5-di-tert-butylbenzoate



Hit#:1 Entry:215159 Library:NIST17.lib
SE:70 Formula:C20H32O4 CAS:59252-01-6 MolWeight:336 RetIndex:2496
CompName:17.beta.-Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester



Conclusion

The present study results confirmed the presence of phenolics, alkaloids, steroids, saponins, tannins and flavonoids with varied degree. In addition to this, GC-MS profile can be used as biochemical markers in the pharmaceutical industries to identify the genuine mother plants and distinguish from its adulterants. Thus the presence of various bioactive compounds confirms the application of *Sphaeranthus amaranthoides* for various diseases by herbal system of treatments.

REFERENCES

1. RNS Yadav* and Munin Agarwala Phytochemical analysis of some medicinal plants Journal of Phytology 2011, 3(12): 10-14 ISSN: 2075-6240

2. Arunkumar, S., Muthuselvam . Analysis of phytochemical constituents and antimicrobial activities of aloe vera L. against clinical pathogens. World J. Agril. Sc., 2009, 5(5): 572-576.
3. Somnath De a,et al , Phytochemical and GC-MS analysis of bioactive compounds of *Sphaeranthus amaranthoides* Burm S Pharmacognosy Journal 5 (2013) 265e268
4. Gordon DM. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. Microbiology. 2001;147:1079e1085.
5. Wink DA, Vodovotz Y, Grisham MB, et al. Antioxidant effects of nitric oxide. Meth Enzymol. 1999;301:413e424.
6. Edoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemicals constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4(7): 685-688.
7. Mann, J.1978. Secondary Metabolism. Oxford University press, London, pp. 154.
8. V. Thanigavelan et al., Pharmacological Study of a Siddha Holistic Herb Sivakaranthai - *Sphaeranthus Amaranthoides* Burm for Analgesic and Anti-Inflammatory activities Journal of Applied Pharmaceutical Science 02 (01); 2012: 95-101 ISSN: 2231-3354.
9. Somnath De et al., In-vivo Hepatoprotective Activity of Methanolic Extracts of *Sphaeranthus amaranthoides* and *Oldenlandia umbellata* Pharmacognosy Journal,2017,9,1,98-101.DOI:[10.5530/pj.2017.1.16](https://doi.org/10.5530/pj.2017.1.16)
10. S. Gayatri, Assessment of *in vitro* cytotoxicity and *in vivo* antitumor activity of *Sphaeranthus amaranthoides* burm.f Pharmacognosy Res. 2015 Apr-Jun; 7(2): 198–202.doi: [10.4103/0974-8490.150544](https://doi.org/10.4103/0974-8490.150544)
11. Gc-MS Analysis Of Bioactive Compounds In Bryonopsis Laciniosa Fruit Extract International Journal Of Pharmaceutical Sciences And Research DOI: [10.13040/IJPSR.0975-8232.6\(8\).3375-79](https://doi.org/10.13040/IJPSR.0975-8232.6(8).3375-79).
12. Antimycobacterial Activity of Constituents from *Foeniculum Vulgare* Var. Dulce Grown in Mexico Patricia et al, *Molecules* 2012 doi:[10.3390/molecules17078471](https://doi.org/10.3390/molecules17078471) Evaluation of Antioxidant and Wound Healing Potentials of *Sphaeranthus amaranthoides* Burm.f. R. Geethalakshmi et al BioMed Research International doi.org/[10.1155/2013/607109](https://doi.org/10.1155/2013/607109).
13. K. M. Nadkarni, *Indian Materia Medica*, Popular Prakashan, Bombay, India, 1976.
14. Screening of Antioxidant, Antimutagenic, Antimicrobial Activities and Phytochemical Studies on *Sphaeranthus amaranthoides* (Burm).Prabakaran M et al , Asian J. Pharm. Tech. 1(4): Oct. - Dec. 2011; Page 125-12.
15. Brindha P, Sasikala B, Purushothaman KK. Pharmacological studies on Merugan kizhangu. Bull Medico Ethnobot Res. 1981;3:84e96.