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Original article

Phytochemical and GC–MS analysis of bioactive compounds of *Sphaeranthus amaranthoides* Burm



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Somnath De^{a,*}, Akalanka Dey^b, A.M.S. Sudhakar Babu^c, Siddabathuni Aneela^a

^a Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur 523316, Andhra Pradesh, India
^b Annamalai University, Department of Pharmacy, Tamil Nadu 600 802, India
^c A.M. Reddy Memorial College of Pharmacy, Guntur 522601, Andhra Pradesh, India

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ABSTRACT

Objective: To isolate and analyze 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 with mottled degree. GC–MS results revealed the presence of 23 different phytocompounds viz., 2-Propenoic acid, 2-methyl-, 2-[[2,3,3a,4,7,7a(or 3a,4,5,6,7,7a)-hexahydro-4,7-methano-1H-indeny]oxy] ethyl ester (32.73%), Methanone, (1-hydroxycyclohexyl)phenyl – (13.71%), Methyl 2-bromomethyl-10-tetrahydropyranyloxy-2-decenoate – (7.84%),4,7-Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy) – (6.27%), Primidone – (4.50%), 2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo – (3.78%) and normorphine, bis(o-trimethylsilyl) – (3.65%) etc.

Conclusion: The presence of various bioactive compounds confirms the application of *S. amaranthoides* for various diseases by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

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1. Introduction

Medicinal plants have been used for centuries as remedies for human disease.^{1–3} In India plenty of plants are being used as drug due to their medicinal properties. The plant kingdom still holds many species of plant contain substance of medicinal values which are yet to be discovered.⁴ Extensive studies of the adverse effects of these herbal medicines and establishment of a good correlation between biomarkers and plants are essential for ensuring the efficiency and quality of the herbal medicines. Recently, there has been growing interest in exploiting the biological activities of flora and fauna owing to their natural origin, cost effectiveness and lesser side effects.^{5,6} Plant based natural constitutions can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seed, etc.⁷ The medicinal actions of the plants unique to

* Corresponding author. Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur 523316, Tarlupadu Road, Andhra Pradesh, India. Tel.: +91 (0) 8596 224045, +91 (0) 9618347303.

E-mail address: somnath.bankura@gmail.com (S. De).

particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct.⁸ 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.

The determination of phytoconstituents is largely performed by the relatively expensive and often laborious techniques such as gas (GC) and liquid chromatography (LC) combined with specific detection schemes.⁹ In the last few years, GC–MS has become firmly established as a key technological metabolic profiling in both plant and non-plant species.^{10–12} One of them is *Sphaeranthus amaranthoides*. *S. amaranthoides* Burm.f. is a small procumbent herb, with steam rooting and pubescent with appressed hair leaves palmately 3-foliolate. Features of the herb: low annuals with spreading branches, stem – erect, glabrous, sometimes as thick as the little finger, but short, branches – not winged and 8–12 inches, leaves – 2–4 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.¹³

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Table 1

Preliminary phytochemical screening of methanolic extract of *Sphaeranthus amaranthoides*.

Compounds	Methanol extract				
Steroids	+				
Alkaloids	+				
Sugar	+				
Phenolics	+				
Flavonoids	+				
Saponins	+				
Tannins	+				
Amino acids	+				

S. amaranthoides belongs to plant kingdom, Dicotyledon class, Gamopetalae sub class, Inferae series, Asterales order, and Asteraceae (Compositae) family. It is weed of paddy field of southern India particularly in Thoothukudi Dist., Tamil Nadu, India (Dec. 2012). Crude extracts and medicines manufactured of the principles of natural compounds even by pharmaceutical companies may lead to large scale exposure of humans to natural products. In order to promote the use of medicinal plants, it should be thoroughly investigated with their composition, activity and thus validate their use.¹⁴ The literature search reveals that still no work have been done on this plant. And nobody has isolated this crude extract from methanolic solvent and analyzed the crude extract by GC–MS. For this reason, the aim of this work was to isolate, investigate and characterize the bioactive chemical constitution in this organic crude extract by using photochemical test and GC–MS analysis.

2. Material and methods

2.1. Collection of the plant material

The plant *S. amaranthoides* was collected from the Thoothukudi Dist., Tamil Nadu, India and all the primary works done (washing, drying...etc.). The plant materials were identified and authenticated by Dr. V. Chelladurai, Retired Research Officer – Botany, Central Council for Research in Ayurveda and Siddha (C.C.R.A.S).

Table 2

Compounds present in the methanolic extract of Sphaeranthus amaranthoides using GC-MS analysis.

No.	RT	Name of the compound	MF	MW	Peak area %	Compound
						nature
1	3.10	(2RS,3aRS,7aSR)-2-(3-Hydroxy-1-methoxypropyl)perhydroindan-4-one	C14H24O4	256	1.08	Ketone
2	8.07	Dimethyl derivative of vitamin D3 – triol	C28H48O3	432	0.92	Sterol
3	9.33	7,8 Bi(trimethylsilyl)benzo(5,6-g)-1H,3H-quinazoline-2,4-dione	C18H24N2O2Si2	356	1.89	Alkaloid
4	12.61	1-Propanone, 2-bromo-1-phenyl – (CAS)	C9H9BrO	212	1.44	Ketone
5	13.23	1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazoline	C26H20Cl2N2	430	0.72	Alkaloid
6	14.34	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-	C13H20O2	208	3.78	Ester
7	17.12	2-tert-Butyl-4-isopropyl-5-methylphenol	C14H22O	206	0.49	Phenol
8	17.71	4,7-Methano-1H-indene, 3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy)-	C13H180	190	6.27	Ester
9	20.57	Methanone, (1-hydroxycyclohexyl)phenyl-	C13H16O2	204	13.71	Ketone
10	22.99	2-Propenoic acid, 2-methyl-, 2[[2,3,3a,4,7,7a(or 3a,4,5,6,7,7a)-	C16H22O3	262	32.73	Ester
		hexahydro-4,7-methano-1H-indenyl]oxy]ethyl ester				
11	25.71	Hexadecanoic acid, methyl ester	C17H34O2	270	0.75	Ester of fatty acid
12	29.01	Primidone	C12H14N2O2	218	4.50	Alkaloid
13	32.72	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester (CAS)	C19H36O5	344	1.80	Ester of fatty acid
14	33.40	4,4'-isopropylidene-bis-(2-cyclohexylphenol)	C27H36O2	392	0.83	Phenol
15	33.81	4,5-Bis(p-bromophenoxy)-1,2-dicyanobenzene	C20H10Br2N2O2	468	0.66	Aromatic
16	34.53	Epoxygedunin	C28H34O8	498	0.63	Saponin/steroids
17	36.00	6-(t-Butylimino)-8-(3'-trifluoromethylphenyl)-3,4-dihydro-2H,	C19H19F3N4S	392	0.87	Alkaloid
		6H-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile				
18	36.54	7a,9c-(Iminoethano)phenanthro[4,5-bcd]furan,4aà,5-dihydro-3-methoxy-12-methyl- 9(CAS)	C18H19NO2	281	0.67	Alkaloid
19	36.85	Diethyl2-(2-furyl)-4-hydroxy-4-methyl-6-oxo-1,3-cyclohexanedicarboxylate tbdms	C23H36O7Si	452	1.00	Ester
20	37.14	2,9-bis(2',6'-dimethoxyphenyl)-1,10-phenanthroline	C28H24N2O4	452	1.36	Alkaloids
21	37.61	6,7-Dihydro-6,6-dimethyl-2,3-diphenylindazol-4(2H,5H)-one	C21H20N2O	316	1.03	Alkaloids
22	37.98	Di-(2-ethylhexyl)phthalate	C24H38O4	390	2.54	Ester
23	38.58	Normorphine,bis(o-trimethylsilyl)	C22H33NO3Si2	415	3.65	Alkaloid

Govt. of India, Tirunelveli. The collected plant material was free from disease and also free from contamination of other plants.

2.2. Preparation of plant extract

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

2.3. Phytochemical screening

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

2.4. GC–MS analysis

The GC–MS analysis of methanolic crude extract of *S. amaranthoides* was performed using a GC–MS equipment Thermo GC-TRACE ultra ver: 5.0, Thermo MS DSQ II. Experimental conditions of GC–MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30 m, ID: 0.25 mm, Film: 0.25 μ m was used and flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature program (oven temperature) was 40 °C raised to 250 °C at 5 °C/min and injection volume was 1 μ L. Samples which dissolved in chloroform were run fully at a range of 50–650 *m/z* and the results were compared by using Wiley Spectral library search program. The mass spectra detected in 36 min.

3. Result & discussion

The phytochemical screenings of *S. amaranthoides* extract revealed that the methanolic extract contains steroids, alkaloids,

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