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

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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 phytopharmaceuticals 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-indenyl][ox] ethyl ester (32.73%), Methanone, (1-hydroxycyclohexyl)[phenyl — (13.71%), Methyl 2-bromomethyl-10-tetrahydropyranyloxy-2-decanoate — (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. 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. 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. Plant based natural constitutions can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seed, etc. The medicinal actions of the plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct. 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. In the last few years, GC–MS has become firmly established as a key technological metabolic profiling of both plant and non-plant species. 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.
S. amaranthoides belongs to plant kingdom, Dicotyledon class, Gamopetalaе 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. 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 analysis. 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). The collected plant material was free from disease and also free from contamination of other plants. The plant S. amaranthoides was 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.

2.2. 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, antraquinone 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 μm, Film: 0.25 μm 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 μL. 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,
sugars, phenolics, flavonoids, saponins, tannins and amino acids compounds except anthraquinone (Table 1).

The results pertaining to GC–MS analysis lead to the identification of number of compounds from GC fractions of the methanolic extracts of *S. amaranthoides*. They were identified through mass spectrometry attached with GC. GC–MS analysis of methanolic extract of *S. amaranthoides* was put into a (Table 2). The result revealed the presence of 23 different phytocompounds viz., (2RS,3aRS,7aSR)-2-(3-Hydroxy-1-methoxypropyl)perhydroindan-4-one (1.08%), Dimethyl derivative of vitamin D3 triol (0.92%), 7,8-Bis(trimethylsilyl)benzo[5,6-g]-1H,3H-quinoxaline-2,4-dione (1.89%), 1-Propanone, 2-bromo-1-phenyl (1.44%), 1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo|f|quinazoline (0.72%), 2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]|hept-2-yl ester, exo (3.78%), 2-tert-Butyl-4-isopropyl-5-methylphenol (0.49%), 4,7-Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-(2-propenyl)oxy (6.27%), Naphthalene (0.72%), Methanone, (1-hydroxycyclohexyl)phenyl (13.71%), 2-Propenoic acid, 2-methyl-, 2-[2,3,3a,4,7,7a(or 3a,4,5,6,7,7a)-hexahydro-4,7-methano-1H-indenyl|oxy|ethyl ester (32.73%), Hexadecanoic acid, methyl ester (0.75%), Primidone (4.50%), 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester (1.80%), 4,4'-isopropylidene-bis-(2-cyclohexylphenol) (0.83%), 4,5-Bis(p-bromophenoxy)-1,2-dicyanobenzene (0.66%), epoxypedunin (0.63%), 6-(1-Butoxy-1-methoxy-1,2,4-trioxolane-3,5-dicarbonyl)-1,2,4-trioxolane-2,5-dicarboxylic acid (0.67%), Diethyl 2-(2-furyl)-4-hydroxy-4-methyl-6-oxo-13-cyclohexanedicarboxylate (1.36%), 6,7-Dihydro-6,6-dimethyl-2,3-dihydrobenzofuran-4(3H)one (1.00%), 2,9-bis[2'-6'-dimethoxyphenyl]-1,10-phenanthroline (1.36%), 6,7-Dihydro-6,6-dimethyl-2,3-dihydrobenzofuran-4(3H)one (1.03%), Di-(2-ethylhexyl)phthalate (1.03%), normorphine, bis(o-trimethylsilyl) (3.65%). The GC–MS spectrum confirmed the presence of 23 components with the retention time 3.10, 8.07, 9.33, 12.61, 13.23, 14.34, 17.12, 17.71, 19.72, 20.57, 22.99, 25.71, 29.01, 32.72, 33.40, 33.81, 34.53, 36.00, 36.54, 37.14, 37.61, 37.98 and 38.58, respectively (Fig. 1).

In the present study, methanolic extract of the plant of *S. amaranthoides* is analyzed through GC–MS. Till date no reports exist on the GC–MS analysis of *S. amaranthoides*. In terms of percentage amounts epoxypedunin, hexadecanoic acid, Di-(2-ethylhexyl) phthalate and primidone predominant in the extract. These four major compounds have some important medicinal activity in future drug discovery system. Such as epoxypedunin having anticancer activity, on the other hand hexadecanoic acid had antimicrobial, nematicide, hemolytic 5-alpha reductase inhibitor and antioxidant activity. Hemophiliacs, kidney dialysis activity were present in Di-(2-ethylhexyl) phthalate compound. Primidone shows anti-convulsant activity. The individual fragmentation for few of the components is illustrated in Fig. 2A–D. The name, molecular weight, molecular formula and structure of the component of test material were determined. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. GC–MS analysis of methanolic extract of *S. amaranthoides* was tabulated in Table 2.

![Fig. 1. GC–MS chromatogram of methanolic extract of *Sphaeranthus amaranthoides*.](image-url)}
4. 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 authentic mother plants and differentiate from its adulterants.

Conflicts of interest

All authors have none to declare

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