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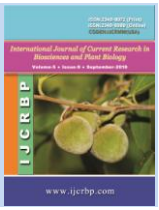

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GC-MS Analysis of Phytocomponents in the Ethanol Extract of *Sesamum alatum* Thonn. Leaves

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ABSTRACT

The leaves of *Sesamum alatum* Thonn. (were collected from Salem-Coimbatore Highways near Perundurai Bye-Pass road, Tamil Nadu, shade dried and powdered. The leaf powder was extracted with ethanol by cold extraction method and the final crude extract was stored until use. Ethanol extract of *Sesamum alatum* leaves was analyzed with the help of GC-MS analyzer (GC Clarius 500 Perkin Elmer) for the phytochemical compounds. Interpretation on mass spectrum GC-MS was done using the database of National Institute Standard and Technology (NIST). Among the various chemical compounds found, majority of the phytochemicals are cyanide compounds. Sulfathiozole was one among the phytochemicals found in the ethanolic leaf extracts of *Sesamum alatum*, which is a strong antibacterial agent as confirmed by the previous studies.

Introduction

Sesamum indicum L., commonly known internationally as sesame, is one of the oldest oilseed crops. Worldwide, it is used for its nutritional, medicinal, and industrial purposes. Through the use of various mechanism-based *in vitro* and *in vivo* assays, extracts and isolated compounds obtained from different parts of sesame were demonstrated to exhibit antioxidant, antimicrobial, antiinflammatory, antidiabetic, anticancer, antihyperlipidemic, hepatoprotective, anthelmintic, antileishmanial, gastroprotective, larvicidal, and vasorelaxant activities, among others. Potent pharmacological activities observed *in vivo* particularly highlight the need for further exploration at clinical levels and drug development of identified chemotherapeutic candidates (Amoo et al., 2017).

In addition, sesame plant is one of the richest food sources of phytoestrogenic lignans, a valuable

phytochemical known to man since the dawn of civilization (Thompson et al., 1991) and is now increasingly being incorporated into human diet worldwide because of their reported health benefits (Shittu, 2010).

Different species of *Sesamum* are reported to possess various biological activities. The methanolic and ethanolic extracts *Sesamum radiatum* have broad spectrum antimicrobial effect against various kinds of micro-organisms (Shittu et al., 2007). The extract of *Sesamum indicum* sprayed with $CuCl_2$ were the most inhibitory against the fungi, *Macrophomina phaseolina* and *Fusarium oxysporum* in case of leaves extract and against *Fusarium oxysporum* in case of stem extract (Syed et al., 2015). The antibacterial activities of *Sesamum indicum* leaf extracts have been well documented by Ogunsola and Fasola (2014). More recently, Sundarakumar and Karmegam (2018) reported the antibacterial activity of ethanolic leaf extracts of *Sesamum alatum*.

Sesamum alatum belongs to the family Pedaliaceae, is an erect annual herb up to 1.5-m tall; the fruit is a narrowly obconical capsule up to 5 × 0.7 cm. Seeds are 2.5 × 1.5 mm, pale to dark brown. Seed oil content is 28.9%. *Sesamum alatum* has renal protective and antidiabetic activities (Mariod et al., 2017).

Sesamum alatum grows predominantly as a road-side weed in Tamil Nadu, India and it spreads by its winged seeds very easily. Even though the young leaves are used for culinary purposes in African countries apart from oil yielding seeds, it is unbothered road-side plant in many parts of Tamil Nadu. Recent study shows that the leaf extracts of *Sesamum alatum* possesses antibacterial activity against different bacteria in disc diffusion assay (Sundarakumar and Karmegam, 2018). However, the phytochemical constituents present in the leaves are scanty. Hence the present study has been carried out to find out the phytochemical components in the ethanolic leaf extracts of *Sesamum alatum* by GC-MS analysis.

Materials and methods

Preliminary survey

Based on the preliminary survey made on the distribution of *Sesamum alatum* Thonn. (Pedaliaceae) Salem and adjacent districts Tamil Nadu, India along the highways, the plant was predominantly found in Salem-Coimbatore Highways near Perundurai Bye-Pass road (Fig. 1). The identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983).

Collection and of leaves

The leaves of *Sesamum alatum* were collected from Salem-Coimbatore Highways near Perundurai Bye-Pass road (Fig. 1-A and 1-B). The leaves collected were immediately transported to the laboratory of the Department of Botany, Government Arts College, Salem-7 for further processing.

Preparation of crude extract

The leaves of *Sesamum alatum* were washed with tap water, blotted with filter paper and spread over news paper for air drying under shade (Fig. 1-C). After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant was taken in a 250 ml conical flask and added with 100-200 ml of ethanol (95%) (Fig. 1-D).

Ethanol was used for the extraction of phytochemicals because it has the ability to dissolve the phytochemical compounds like tannins, polyphenols, flavonols, terpenoids and alkaloids (Habtenmariam et al., 1993).

After 48 hours, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

Fig. 1-A: *Sesamum alatum* Thonn.

Fig. 1-B: Collection of *Sesamum alatum* Thonn. leaves.

Fig. 1-C: Shade drying of leaves.

Fig. 1-D: Ethanol extract of *Sesamum alatum* Thonn. leaves

Gas chromatography-mass spectrometry (GC-MS) analysis

Ethanol extract of *Sesamum alatum* leaves was analyzed with the help of GC-MS analyzer (GC Clarius 500 Perkin Elmer). On Elite-1 column the data was generated. The carrier gas helium (99.999%) was used at flow rate of 1 ml per min in split mode (10: 1). 8 µl of acetone sample was injected to column at 250ºC injector temperature. Temperature of oven starts at 80ºC and hold for 2 min and then it was raised at rate of 10ºC per min to 200ºC without holding. Holding was allowed for 9 min at 280ºC at program rate of 5ºC per min. Temperature of ion source was maintained at 200ºC. The injector temperature was set at 230ºC and detector temperature was set at 260ºC. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode from 45 to 450 Da atomic mass units. A 0.5 seconds of scan interval and fragments from 45 to 450 Da was maintained. Total running time was 40 minutes. 2.1.1. Identification of Components Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the version (2005), software, Turbomas 5.2. This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extracting these compounds.

Results and discussion

The mass spectrum review active chromatogram and spectrum plots for four continuous run of ethanolic leaf extracts of *Sesamum alatum* are given below (Figs. 2, 3, 4 and 5).

The crude ethanolic extract of *Sesamum alatum* showed a wide range of phytochemical components namely, Butadiyne, Propanenitrile, Propargylamine, Ethenamine, N-methylene, Ethyl isocyanide Acetonitrile, Nitrogen trifluoride, Methane-isothiocyanato, Thiocyanic acid- methyl ester, Methyl isothiocyanate, Thiocyanic acid-2-propynyl ester, 4-Mercaptobutyl isothiocyanate, Propane-1-isothiocyanato-3-(methylthio)-, Sulfathiazole, O-Ethyl-O-(1,1-difluoro-2-chloroethyl)-N-isopropyl -phosphorothioamidate, O-Ethyl-O- (1,1-difluoro-2-chloroethyl) -N-propyl-phosphorothio amidate, O-Methyl-O- (1,1-difluoro-2-chloroethyl) -N-(1,1-dimethylethyl)-phospho-rothioamidate and Benzamide (pentafluoro-N-(pentafluorobenzoyl)-N-methyl-).

Among the various chemical compounds found, majority of the phytochemicals are cyanide compounds. Interestingly, sulfathiozole was one among the phytochemicals found in the ethanolic leaf extracts of *Sesamum alatum*. Sulfathiazole is an organosulfur compound used as a short-acting sulfa drug. It is an organic compound. Formerly, it was a common oral and topical antimicrobial, until less toxic alternatives were discovered. Wong et al. (1987) reported that sulfathiazole possesses antifertility effect in rats. Antimicrobial activity induced by a sulfathiazole derivative on *Staphylococcus aureus*, and *Vibrio cholera* have been reported by Valverde et al. (2013).

The ethanolic leaf extracts of *Sesamum alatum* is reported to possess antibacterial activity against different bacteria assessed by disc diffusion technique and resazurin dye reduction method (Sundarakumar and Karmegam, 2018). The results of their study reveal that the extract is effective in inhibiting the growth of *Micrococcus luteus* (NCIM-2103), *Staphylococcus epidermidis* (NCIM-2493), *Escherichia coli* (NCIM-2065), *Pseudomonas cepacia* (NCIM-2106) and *Bacillus megaterium* (NCIM-2187). The presence of an antibacterial compound, sulfathiazole in ethanolic

extract of *Sesamum alatum* leaves as per the GC-MS results of the present study, might be responsible for the antibacterial activity. Further studies on the isolation of the compound may confirm the exact antibacterial activity of ethanolic leaf extracts of *Sesamum alatum*.

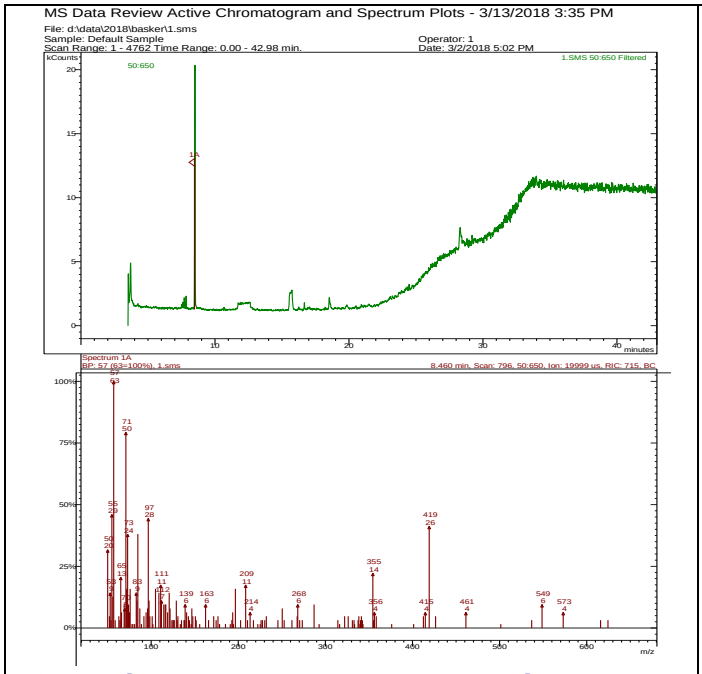


Fig. 2: The mass spectrum review active chromatogram and spectrum plots of ethanolic leaf extracts of *Sesamum alatum* – Run 1.

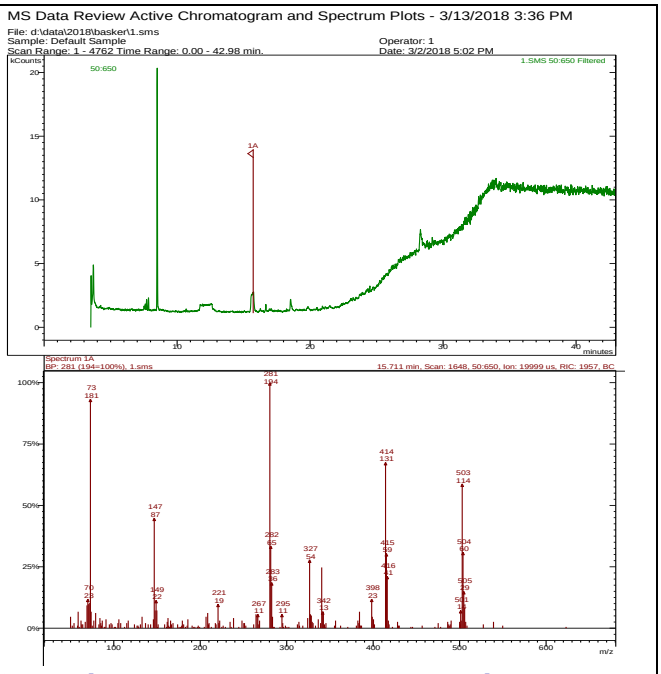


Fig. 3: The mass spectrum review active chromatogram and spectrum plots of ethanolic leaf extracts of *Sesamum alatum* – Run 2.

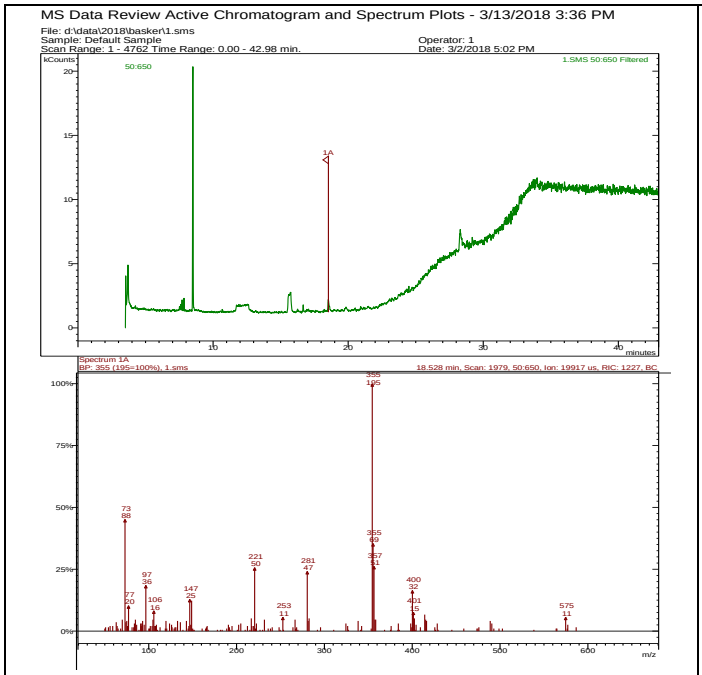


Fig. 4: The mass spectrum review active chromatogram and spectrum plots of ethanolic leaf extracts of *Sesamum alatum* – Run 3.

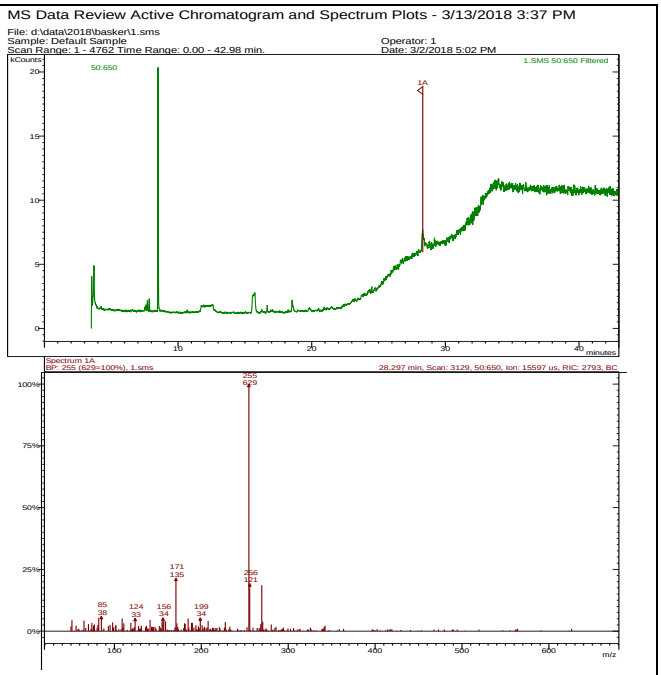


Fig. 5: The mass spectrum review active chromatogram and spectrum plots of ethanolic leaf extracts of *Sesamum alatum* – Run 4.

Table 1. Interpretation on GC-MS spectrum obtained for ethanol extracts of *Sesamum alatum* using the database of National Institute Standard and Technology (NIST).

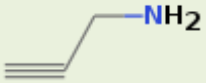
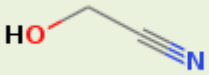
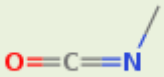
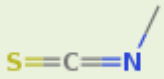
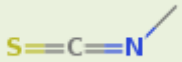
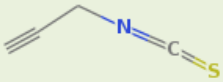
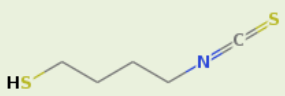
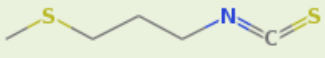
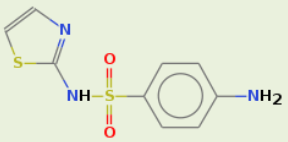
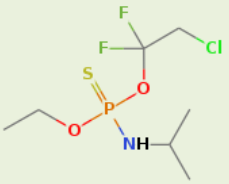
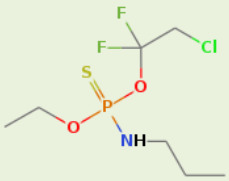
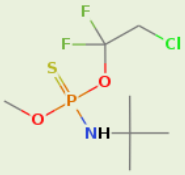
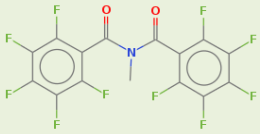
S. No.	Molecular weight	Name of the compound	Chemical formula of compound	Chemical structure of compound
1.	50.0587	Butadiyne	C_4H_2	
2.	55.0785	Propanenitrile	C_3H_5N	
3.	55.0785	Propargylamine	C_3H_5N	
4.	55.0785	Ethenamine, N-methylene	C_3H_5N	
5.	55.0785	Ethyl isocyanide	C_3H_5N	
6.	57.0513	Acetonitrile, hydroxy	C_2H_3NO	
7.	57.0513	Methane, isocyanato	C_2H_3NO	
8.	71.0019	Nitrogen trifluoride	F_3N	
9.	73.117	Methane, isothiocyanato	C_2H_3NS	
10.	73.117	Thiocyanic acid, methyl ester	C_2H_3NS	
11.	73.117	Methyl isothiocyanate	C_2H_3NS	
12.	97.138	Thiocyanic acid, 2-propynyl ester	C_4H_3NS	
13.	147.262	4-Mercaptobutyl isothiocyanate	$C_5H_9NS_2$	
14.	147.262	Propane, 1-isothiocyanato-3-(methylthio)-	$C_5H_9NS_2$	

Table 1. Cntd….

S. No.	Molecular weight	Name of the compound	Chemical formula of compound	Chemical structure of compound
15.	255.317	Sulfathiazole	$C_9H_9N_3O_2S_2$	
16.	281.688	O-Ethyl-O-(1,1-difluoro-2-chloroethyl)-N-isopropyl-phosphorothioamidate	$C_7H_{15}ClF_2NO_2PS$	
17.	281.688	O-Ethyl-O-(1,1-difluoro-2-chloroethyl)-N-propyl-phosphorothioamidate	$C_7H_{15}ClF_2NO_2PS$	
18	281.688	O-Methyl-O-(1,1-difluoro-2-chloroethyl)-N-(1,1-dimethylethyl)-phosphorothioamidate	$C_7H_{15}ClF_2NO_2PS$	
19.	419.1739	Benzamide, pentafluoro-N-(pentafluorobenzoyl)-N-methyl-	C_4H_3NS	

Conflict of interest statement

Authors declare that they have no conflict of interest.

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