NAAS Score: 4.11; IC Value: 74.82; UGC-India Approved
The Journal of Phytopharmacology 2018; 7(2): 116-120
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(2): 116-120
March- April
Received: 30-01-2018
Accepted: 06-03-2018

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GC-MS Analysis and Antibacterial Activity of different Solvent Extracts of *Shuteria involucrata*

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ABSTRACT

The present study was focused to examine the presence of phytoconstituents in the ethanolic extract of *Shuteria involucrata* plant using GC-MS analysis and Antibacterial activity. The GC-MS analysis of *S. involucrata* leaf was performed using Agilent 6890-JEOL GC-Mate-II Mass Spectrometer. The result of the study showed the presence of six bioactive compounds in the ethanolic extract. The antimicrobial activity was carried out by disc diffusion technique against the four selected pathogens. Among the four, tested for Antibacterial Activity *Staphylococcus aureus, and Pseudomonas aeruginosa* and were more susceptible to the extract, whereas the others are less susceptible. Ethanol and methanol extracts of plant materials exhibited good antibacterial activity against gram positive, gram negative bacterias.

Keywords: *Shuteria involucrata*, GC-MS analysis, Antibacterial activity, Disc diffusion technique.

INTRODUCTION

In the recent years the use of medicinal plants in the controlling and treatment of diseases has gained great importance. Plant parts are considered as one of the important sources for biologically active compounds [1]. Medicinal plants are expensive gift from nature to human. The approval of traditional medicine as an alternative from of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds [2, 3]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the phytochemical pathway.

Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate [4]. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [5, 6]. Bacterial infections are one of the important causes of mortality and morbidity [7, 8]. These are second leading cause of death worldwide and biggest threat for child and adults [9]. Gram-positive bacteria are mainly responsible for various disease including nosocomial infection, skin infection, puritic eruption, endocarditis, pneumonia and etc. Gram negative bacterium such as *Escherichia coli* (*E. coli*) is present in human intestine and causes lower urinary tract infection, gastrointestinal tract, wound infections, bacteriaemia, pneumonia, septicaemia and meningitis [10].

Shuteria involucrata belongs to "fabaceae" is commonly known as "kodiveli" in solakadu tribals of kolli hills, Eastern Ghats Tamilnadu. Paste prepared from the leaf is taken once in a day for period of 2-3 weeks to treat dental diseases and boils appearing on the skin by the solakadars [11]. Taking into consideration of the medicinal importance of this plant, ethanol extract of whole plant of *S. involucrata* were analysed using GC-MS. Perusal of literature reveals that information on the GC-MS analysis of *S. involucrata* is totally lacking.

In this study, we discussed antibacterial efficacy of various organic extracts of *S. involucrata* and examined the chemical composition of ethanol extract from *S. involucrata* by gas chromatography and mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant material

Whole plant of *S. involucrata* was collected from the natural habits of Kolli hills, Namakkal District, Tamilnadu, India in February 2015. The plant identified was authenticated by Dr.S. Susairaj, Associate Professor, Department of Botany, St Joseph's college, Tiruchirappalli, Tamilnadu, India.

Preparation of Plant Extracts

Collected plant material were washed under running tap water, then distilled water and chopped into small pieces, and air dried under shade at room temperature for fifteen to twenty days. The dried plant materials were pulverized into the powder form. The different solvent extracts of the plant were prepared by hot continuous extracion method using soxhlet extractor. It was concentrated by using a rotary vaccum evaporator and subjected to dryness to yield crude residue. This residue was used for the investigation. Different extracts including chloroform, ethanol, hexane, methanol, petroleum ether and water were prepared from this powder at 37 ± 2 ^{0}C. The powdered materials were stored in air tight polythene bags until use.

Test Organism

The tested microorganisms such as *Staphylococcus aureus* (NCIM 2079) *(S. aureus), Bacillus subtilis* (NCIM 2063) *(B. subtilis), Klebsiella aerogenes* (NCIM 2098) *(K. aerogenes), Pseudomonas aeruginosa* (NCIM 2036) *(P. aeruginosa)* were used in this study, were obtained from National Chemical Laboratory (NCL) Pune, India.

Culture Media and Inoculums Preparation

The NCIM numbered strains bought from Chemical Laboratory (NCL) pune was sub cultured in nutrient agar and maintained in the laboratory. The plates were incubated 37 ± 2^0 C for 24 hours. During this period the drug diffuse the agar and inhibit the growth. The ATTC numbered strains bought from Madras Medical College was periodically sub cultured in Sabouraud dextrose agar maintained in the laboratory. The test sample about 100 µl was loaded to the sterile disc by using aseptic precautions. The plates were incubated at room temperature for 2 to 4 days. During this period the drug diffuse through the agar and inhibit the growth if the drug is potent.

Determination of Antimicrobial Activity

In vitro antibacterial activity assay was performed by disc diffusion technique [12]. Whatman No. 1 filter paper disc of 5.5 mm diameter were prepared by impregnated with extracts [chloroform, ethanol, hexane, methanol, petroleum ether and water]. Each disc contains 300 µg respective extract. The test microorganisms of *Staphylococcus aureus, Bacillus subtilis, Klebsiella aerogenes, and Pseudomonas aeruginosa* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and Fungi respectively.

GC-MS analysis

GC-MS analysis was carried out in Indian Institute of Technology (IIT), Madras. GC-MS analysis was performed by using JEOL GC MATE II (GC Model), quadruple double focusing detector. One microliter of extract was injected in splitless mode in injection port of GC column. The inlet temperature was set at 220 ^{0}C and oven temperature was programmed as 50 to 250 ^{0}C for 1min then 10 ^{0}C min^{-1}. Total run time was 60 min. Helium gas was used as the carrier gas at constant flow rate of 1.0 mL/min. The interface temperature (GC to MS) was set at 250 ^{0}C.

MS was set in scan mode. MS quad temperature was 250 ^{0}C, MS source temperature was 250 ^{0}C. Ions were obtained by electron ionization mode. Molecular ions (mass range) were monitored for identification which was set 50-600 m/z. Peak area denoted the relative percentage of constituents.

RESULTS

Antibacterial Activity of Plant Extracts

The in vitro antibacterial activities of various organic extracts of *S. involucrata* against the tested bacteria were assessed by the presence or absence of zone of inhibition. The organic extracts exhibited antibacterial activity against two Gram positive and two Gram negative bacteria (Figure 1). The highest zone of inhibition is measured by ethanol extract against *S. aureus* and *P. aeruginosa* (Table 1). Methanol and chloroform showed moderate antibacterial activity against *S. aureus* and *P. aeruginosa.* Ethanol and methanol extracts of *S. aureus* revealed great potential of antibacterial activity against all bacteria (Figure 2).

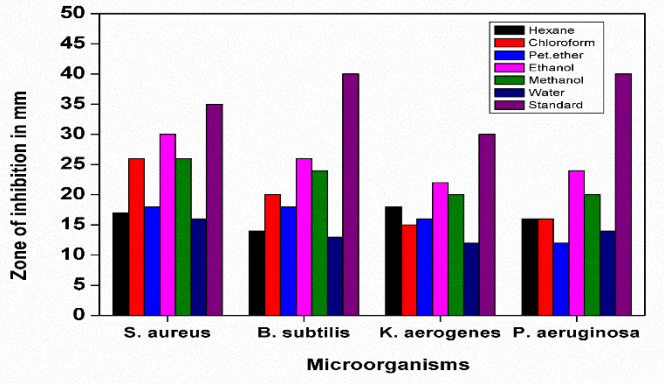

Figure 1: Antibacterial activity of various extracts of *S. involucrata.*

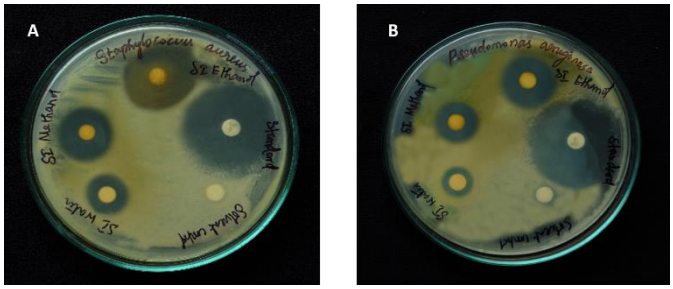

Figure 2: Different concentration of ethanol and methanol extracts of *S. involucrata* produce zones of inhibition against (A) *S. involucrata,* (B) *Pseudomonas aeruginosa.*

Table 1: Antimicrobial activity of different extracts of *S. involucrata* against test organisms

Microorganisms	Zone of inhibition in mm						
	Hexane	Chloroform	Pet.Ether	Ethanol	Methanol	Water	Std
Staphylococcus aureus	17	26	18	30	26	16	35
Basillus subtilis	14	20	18	26	24	13	40
Klebsiella aerogenes	18	15	16	22	20	12	30
Pseudomonas aeruginosa	16	16	12	24	20	14	40

GC-MS analysis

GC-MS analysis of ethanolic extract of *S. involucrata* identified of six different compounds. List of identified were listed in Table 2. GC-MS chromatograph ethanol extract showed six peaks, each peak indicating the presence of six compounds (Figure 3A-3F). The major compounds detected in ethanol extract were ethanol, 2-(9-octadecenyloxy)-(Z) (12 %), pentadecanoic acid, 13-methyl, methyl ester (7.6 %), 8-acetyl-5,5-dimethyl-nona-2,3,8-trienoic acid, methyl ester (4.2 %), hexadecanoic acid, ethyl ester (15.7 %), nonadecanoic acid 18-oxo-methyl ester (7.8 %), 14-oxononadec-10-enoic acid, methyl ester (10.8 %). The component in lower amount 8-acetyl-5,5-dimethyl-nona-2,3,8-trienoic acid, methyl ester and Pentadecanoic acid, 13-methyl-, methyl ester, were identified as minor compound.

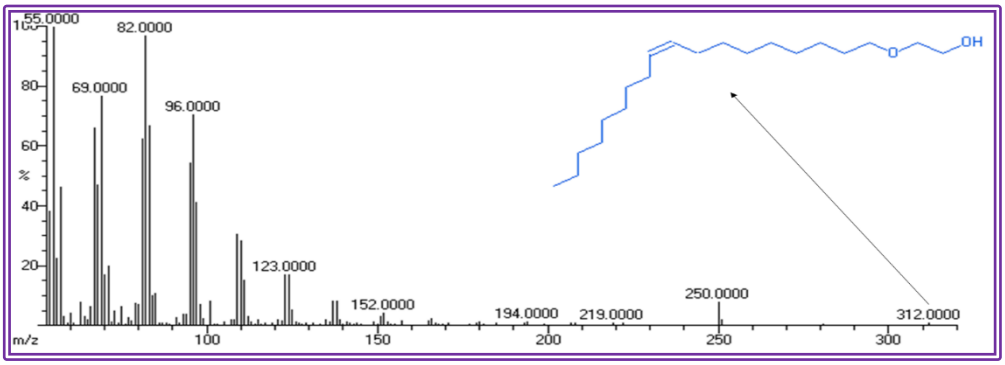

Figure 3A: ethanol, 2-(9-octadecenyloxy)-(Z)

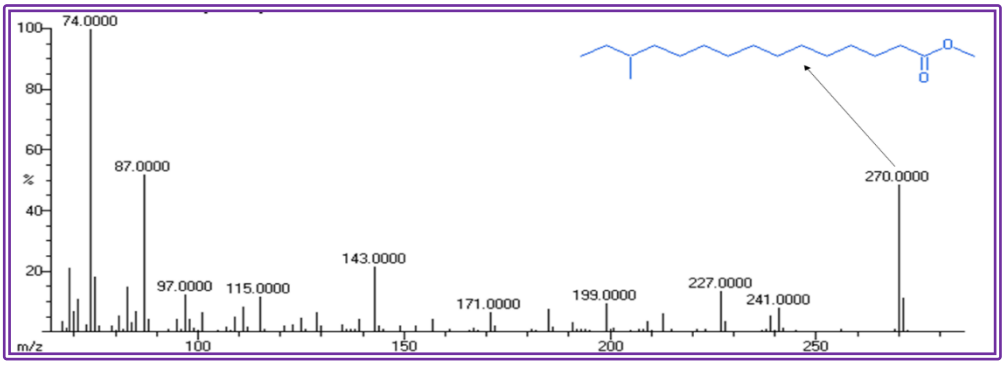

Figure 3B: pentadecanoic acid, 13-methyl, methyl ester

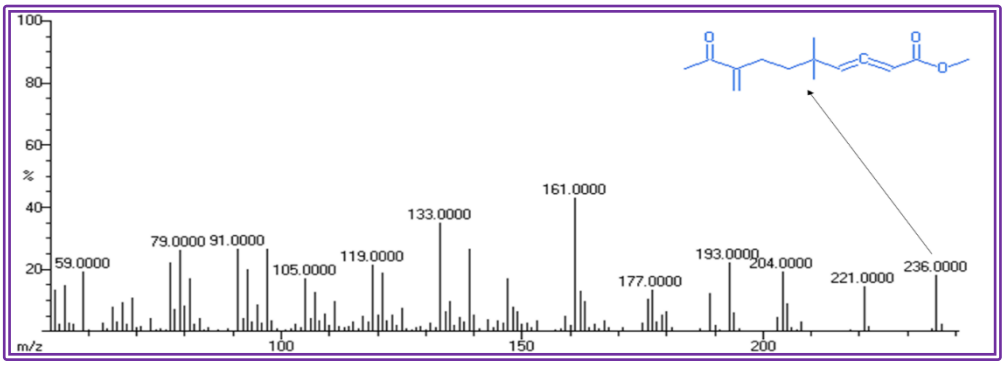

Figure 3C: 8-acetyl-5,5-dimethyl-nona-2,3,8-trienoic acid, methyl ester

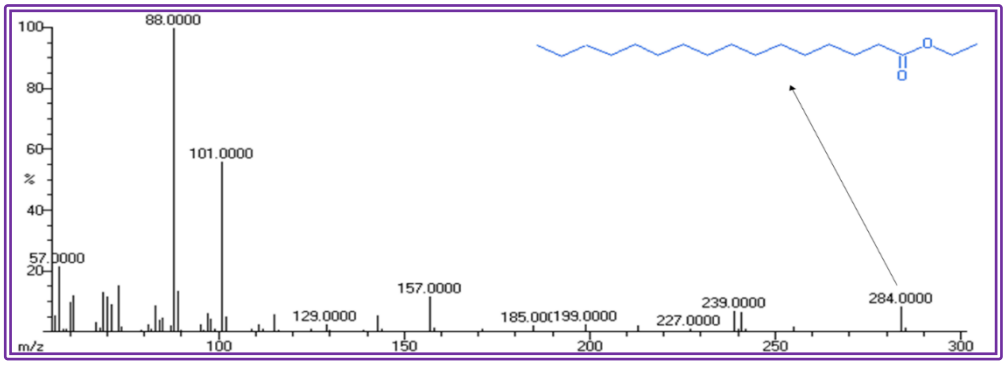

Figure 3D: hexadecanoic acid, ethyl ester

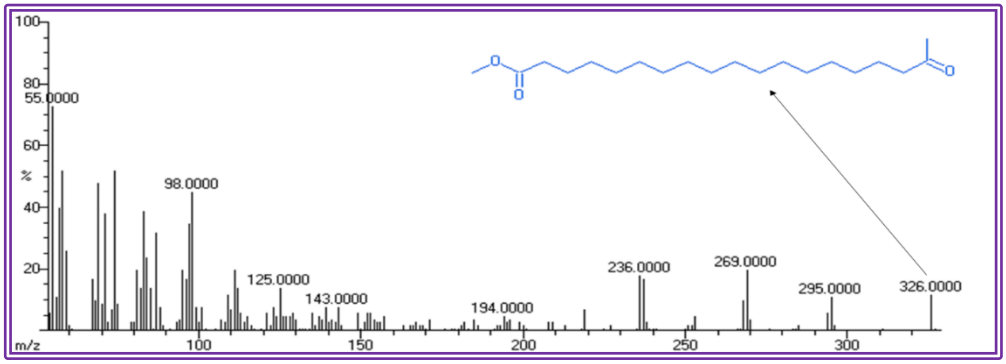

Figure 3E: nonadecanoic acid 18-oxo-methyl ester

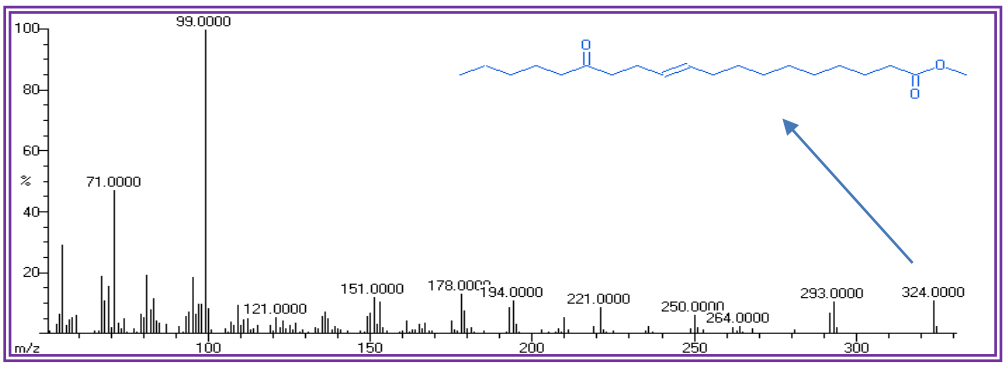

Figure 3F: 14-oxononadec-10-enoic acid, methyl ester

Table 2: Chemical composition of ethanol extracts of *S. involucrata*

Peak No	Retention Time	Area %	Name of Compound	Formula
1	18.90	12.0	Ethanol,2-(9-octadecenyloxy)-Z	$C_{20}H_{40}O_2$
2	17.18	7.6	Pentadecanoic acid, 13-methyl-, methyl ester	$C_{19}H_{40}O_2$
3	12.57	4.2	8-acetyl-5,5-dimethyl-nona-2,3,8-trienoic acid, methyl ester	$C_{14}H_{20}O_3$
4	17.83	15.7	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
5	18.22	7.8	Nonadecanoic acid, 18-oxo-, methyl ester	$C_{20}H_{38}O_3$
6	19.50	10.8	14-oxononadec-10-enoic acid, methyl ester	$C_{20}H_{36}O_3$

DISCUSSION

The result of this study suggests a fairly good correlation between traditional therapeutic use and antimicrobial activity. They show that the different extract of *S. involuctarata* has antibacterial effect on *Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Klebsiella aerogenes (K. aerogenes), Pseudomonas aeruginosa (P. aeruginosa).* The tested antibacterial potency of the extracts of ethanol, methanol and chloroform showed equipotential activity against *S. aureus, P. aeruginosa, C. albicans* [13]. The various extracts displayed moderate activity to the tested culture.

The GC-MS analysis of the ethanolic extract of the *S. involuctarata* showed the presence of six major compounds such as, ethanol, 2-(9-octadecenyloxy)-(Z), pentadecanoic acid, 13-methyl, methyl ester, 8-

acetyl-5,5-dimethyl-nona-2,3,8-trienoic acid, methyl ester, hexadecanoic acid, ethyl ester, nonadecanoic acid 18-oxo-methyl ester, 14-oxononadec-10-enoic acid, methyl ester. *S. involuctarata* is a plant used Ayurvedic medicine however there are no reports on the through phytochemical analysis of the plant [14]. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. The GC-MS analysis is the initial step towards understanding the nature of active constituents in this medicinal plant (*S. involuctarata)* and this type of work will be helpful for further detailed study on designing antibacterial drugs.

Acknowledgements

The authors are grateful to the Indian Institute of Technology (IIT), Madras, for providing the laboratory facilities for (GC-MS) analysis.

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HOW TO CITE THIS ARTICLE
Selvan NS, Isaiah S. GC-MS Analysis and Antibacterial Activity of different Solvent Extracts of *Shuteria involucrata*. J Phytopharmacol 2018; 7(2):116-120.