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ISOLATION AND GCMS CHARACTERIZATION OF CERTAIN NON-POLAR COMPOUNDS FROM *SPILANTHES CILIATA*

Rahul Sharma, Masochon Zimik, Neelakantan Arumugam*

Department of Biotechnology, Pondicherry University, Pondicherry-605014, India

***Corresponding Author Email: arumugan.dbt@pondiuni.edu.in**

ABSTRACT

Spilanthes ciliata, commonly known as toothache plant, is a traditional medicinal plant. In the present study an attempt was made to analyze the oil obtained after solvent extraction of flowers of the plant. GCMS of the extract revealed presence of 15 different compounds in the oil. 11-tridecene-1-ol (18.72%), 2R-acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol (12.66%), Pentadecanoic acid (8.36%,), 1-hexacosanol (8.32%,), Nonadecane (7.35%) and Hexatriacontane (7.014%) were found to be the major constituents and the remaining were in trace amounts. Most of the compounds identified are found associated with various biological activities. The study presents a large-scale isolation of major compounds for their characterization and medicinal application.

KEY WORDS

GCMS; Fatty acids; sesquiterpenoid; Spilanthes ciliata

1. INTRODUCTION

Plants have been used as a reliable source of clinically important compounds that confer health benefits to humans since a long time. The plants continued to serve as cheap and best source of biomedicine, supplements and chemical entities to serve as source of drugs. Their mechanism of action generally imparts synergistic effects on human body making them efficient and safer as compared to the drugs that are synthetic ones. Recent reports from WHO estimate reveals nearly 80% of population rely on herbal medicines as primary healthcare in developing countries [1]. Genus *Spilanthes* is one such plant known since antiquity for several medicinal properties. Members of this genus, commonly known as toothache plants contain various bioactive molecules including alkaloids, sesquiterpenoids, flavonoids, tannins, coumarins, saponins, glycosides, phenolics, lignans, steroids *etc*. [2,3]. There are 300 species of *Spilanthes,* reported to be distributed widely in tropics and subtropics among which 6 species namely *S. calva*, *S. paniculata, S. radicans*, *S. ciliata*, *S. uliginosa* and *S. oleracea* are found in India [4,5]. Reports on occurrence of *Spilanthes ciliata (*syn: *Acmella ciliata)* indicate their distribution in Chhattisgarh, Kerala and some parts of Tamil Nadu [6]**.** Often the whole plant is used in the treatment of dysentery and rheumatism, while flowers in particular served as spice in Japanese food habits [7]. Though the phyto-constituents of many of the species of *Spilanthes* is described, the information on *Spilanthes ciliata* has not been completely described so far. Here we present our results on GCMS of the hexane fraction of the flowers extract of this important medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh flower heads of *Spilanthes ciliata* were collected from the plants that we maintain in the experimental garden of our department. The flower heads harvested were dried at 40°C in hot air oven until consecutive similar dry weight was achieved. The dry flowers were

made into powder in a warring blender to yield *S. ciliata* floral powder.

2.2 Preparation of hexane fraction

200g of floral powder was extracted with methanol (2L, 100%) by incubatiing for 72h at 28±2°C temperature. The methanol solution was then filtered through Whatman No.1 filter paper and the filtrate obtained was rotary evaporated to obtain the crude extract. The crude extract was then subjected to normal phase chromatography using silica (200-400) gel column with hexane as mobile phase. The hexane extract was evaporated, and a yellow oil was obtained. An aliquot of the oil was mixed with fresh hexane and subjected to GCMS.

2.3 GCMS Analysis

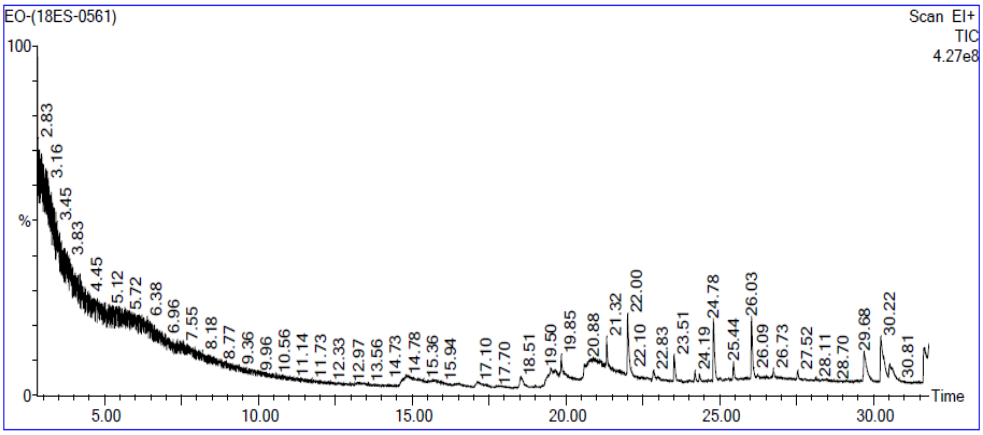
The mass analysis of the extracted oil was performed using Perkin Elmer GCMS (Model: Clarus 680). GC used in the analysis employed a fused silica column (30m x 0.25mm, ID: 250µm) packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane) and the components were separated using helium as carrier gas set at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. 1µL of the sample was injected into the instrument and oven temperature varied from 60°C (2 min) to 300°C with increment set at 10°C/min and a hold of 6 min at 300°C. The mass detector parameters include transfer line and ion source temperature both set at 240°C, ionization mode electron impact at 70 eV and a scan time 0.2 sec with a scan interval of 0.1 sec. The spectrums of the components were compared with the database of spectrum of known compounds available in the GCMS NIST (2008) library.

3. RESULTS AND DISCUSSION

A picture of the plant used in the current study is presented (Figure 1). The plant has several features common to its close relatives *Spilanthes paniculata* and *Spilanthes calva* [8]. Several species cultivated under the name of *Spilanthes* were differentially identified. For example based on phenotypic details and description, *S. ciliata* has been used as synonym for *S. acmella*, *S. mauritiana* and *S.calva* [8]. Similarly, *S. oleracea* is used as a synonym for *A. oleracea* and *S. acmella* [9]. We propose a systematic study of all the species under *Spilanthes/Acmella* at molecular and phytochemical level is needed for fine tuning the species confirmation. Nevertheless, the crop of the cultivated *Spilanthes* species is mostly used as toothache plants and the determination of phytoconstituents of the plant and their biological properties is a subject of extensive research in many labs around the globe [10,11].

Figure 1: *Spilanthes ciliata* crop at flowering stage growing in the experimental garden of Department of Biotechnology, Pondicherry University

Figure 2: GC chromatogram showing peaks for different compounds present in hexane fraction of flower extract of *S. ciliata*



A picture of chromatogram obtained after GC analysis is presented (Figure 2). An observation of the chromatogram reveals presence of many distinct peaks indicating presence of several compounds in the hexane fraction of the flower extract. Using GCMS coupled with NIST library search a total of 15 different compounds could be identified. The fragmentation pattern of the individual compounds along with their deduced structure is presented (Figure 3). The compounds identified by GCMS followed by library search with name, molecular formula, molecular weight and their potential biological properties is presented (Table 1). Among the compounds identified there were a number of fatty acids, esters, fatty alcohols, higher alkanes and terpenoids were present in the extract. Barring eicosanoic acid all other compounds are new report for *S. ciliata*. 11-tridece-1-ol constituted the most abundant (18.72%) component in the extract. This was followed by 2R-acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol, Pentadecanoic acid, 1-hexacosanol, Nonadecane, Hexatriacontane, 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one, Sulfurous acid, 2-propyl tridecyl ester, 3-O-Acetyl-6-methoxy-cycloartenol and 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl)-cyclohexene while others were in trace amount. This study revealed 14 new compounds

in *S. ciliata* of which 12 were oxygenated. Earlier in *S. acmella,* there were 20 compounds identified in the essential oil with only terpen-4-ol being oxygenated [12]. Later on, in *S. acmella* 44 compounds and in *A. oleracea* 71 compounds were identified [8,9]. In another study the hexane fraction of *A. oleracea* obtained following a similar procedure as ours was found to contain several fatty acids as is reported here [13]. But there were differences in number and type of compounds identified in the present and other studies cited above which might be attributed to the difference in isolation procedures followed. We infer that applying different procedures for isolation and fractionation would facilitate identification of diverse array of compounds with some of them becoming potential lead for developing new drugs. Often hydro-distillation method [7,8, 14-16] or gradient elution [13] have been used. The new compounds identified in our study appear to be associated with antineoplastic, anti-inflammatory, anti-microbial, anti-cancerous and other biological properties (Table 1). The sesquiterpenoid (2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1tcyclohexanol) found in the oil is a potential anticancer agent [17]. Another compound identified in the oil sample namely 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl)-cyclohexene satisfies the rule of five and indicates favorable bioavailability as determined by molinspiration calculation and is predicted to be a substrate for Cytochrome P_{450} 2J (CYP2J) enzyme which are expressed in heart tissues [18,19]. Moreover, similar esters have also been also reported in members of Malvaceae [20]. Thus, our study paves the way for large scale isolation of these compounds and subjecting them to validation for clinical application.

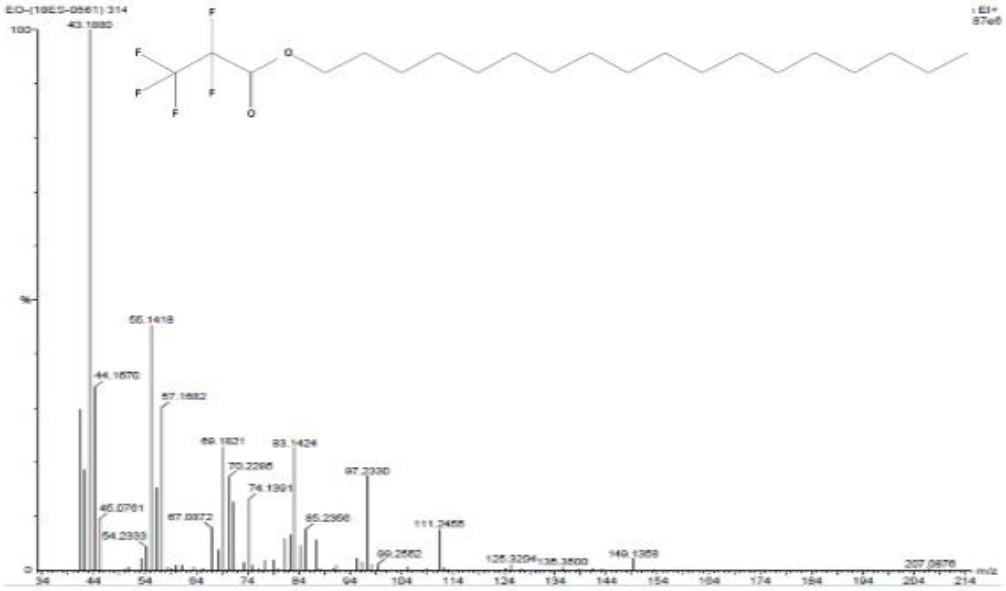
Table 1: List of compounds identified from flower extract of *S. ciliata* and their associated biological properties

S.No	RT	Area (%)	Compound Name	Chemical Nature	Molecular Formula	Molecular Weight	Bioactivity Properties	Reference
1	18.53	2.720	Pentafluoropropionic acid, octadecyl ester	Ester	$C_{21}H_{37}O_2F_5$	416	NR	NA
2	19.4	1.850	Eicosanoic acid	Fatty acid	$C_{20}H_{40}O_2$	312	Peroxisome proliferator activated receptor delta, alpha and gamma (PPAR-d/a/g) agonist	21
3	19.5	8.368	Pentadecanoic acid	Fatty acid	$C_{15}H_{30}O_2$	242	Peroxisome proliferator activated receptor delta (PPARd) agonist	22
4	19.84	8.320	1-hexacosanol	Fatty alcohol	$C_{26}H_{54}O$	382	Neuromuscular regeneration	23
5	19.94	2.446	Lauroyl peroxide	Peroxide	$C_{24}H_{46}O_4$	398	Bleaching agent, Polymerization catalyst, Plasticizer	24
6	20.58	2.849	Tetratetracontane	Higher alkane	$C_{44}H_{90}$	618	Anti-microbial	25

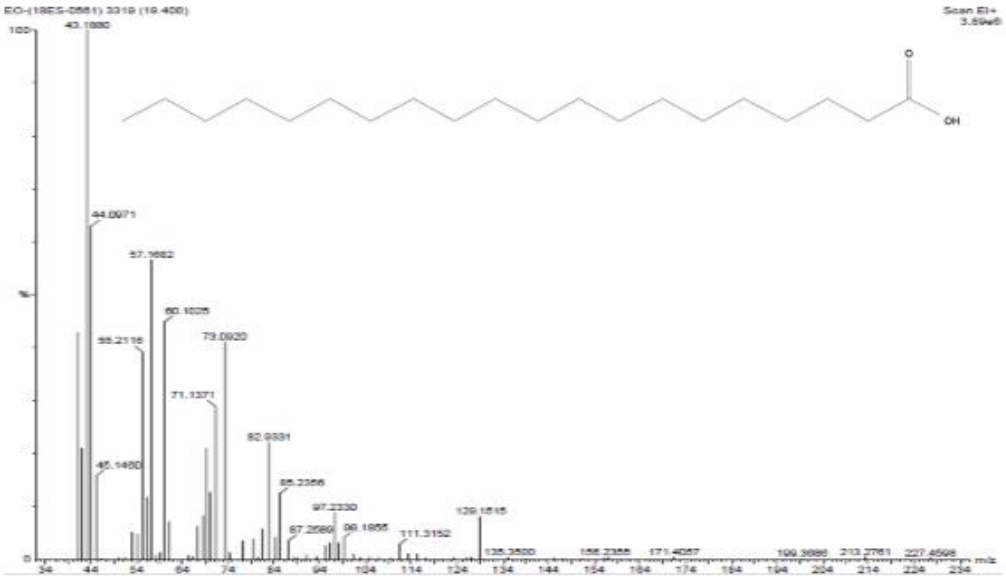
S.No	RT	Area (%)	Compound Name	Chemical Nature	Molecular Formula	Molecular Weight	Bioactivity Properties	Reference
7	20.88	18.724	11-tridecen-1-ol	Fatty alcohol	$C_{13}H_{26}O$	198	Detergents, Cosmetics, Leather processing	26
8	21.99	7.358	Nonadecane	Alkane	$C_{19}H_{40}$	268	Source of n-parafilms	27
9	23.50	2.853	Sulfurous acid, 2-propyl tetradecyl ester	Ester	$C_{17}H_{36}O_3S$	320	NR	NA
10	24.78	7.014	Hexatriacontane	Higher alkane	$C_{36}H_{74}$	506	Anti-microbial	28
11	26.02	6.608	Sulfurous acid, 2-propyl tridecyl ester	Ester	$C_{16}H_{34}O_3S$	306	NR	NA
12	29.68	6.850	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	Triterpenoid	$C_{30}H_{48}O$	424	Anti-neoplastic	29
13	30.22	12.662	2R-acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol)	Sesquiterpenoid	$C_{17}H_{30}O_3$	282	Anti-cancerous	30
14	30.52	6.327	3-O-Acetyl-6-methoxy-cycloartenol	Triterpenoid	$C_{33}H_{54}O_3$	498	Anti-inflammatory, anti-convulant	31
15	31.62	5.051	2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl)-cyclohexene	Organic Compound	$C_{15}H_{26}O$	222	Antibacterial	32

- NR – Not Reported, NA – Not Available

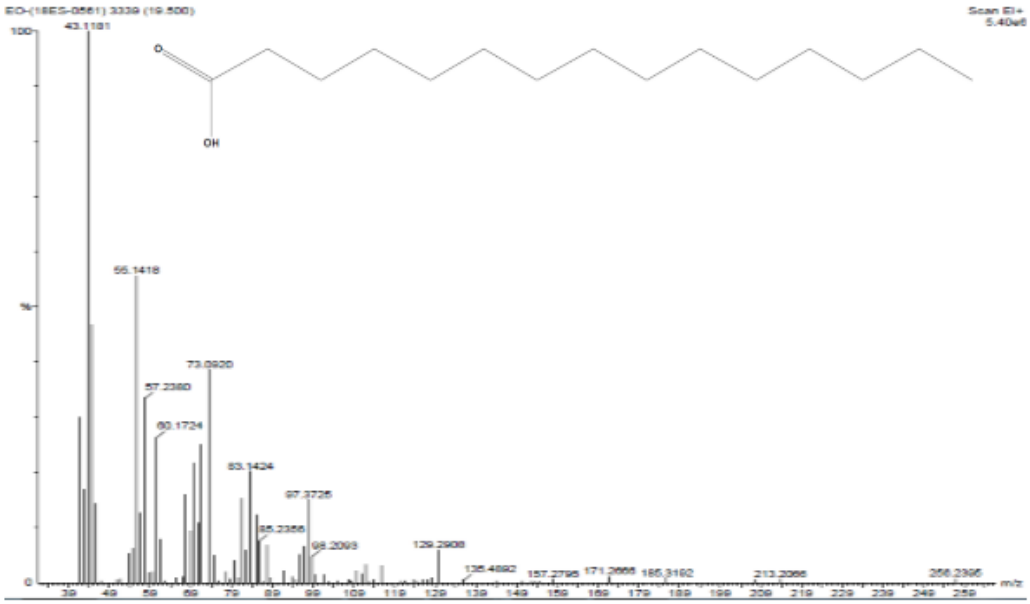
Figure 3: GCMS fragmentation pattern and deduced chemical structure of the identified compounds



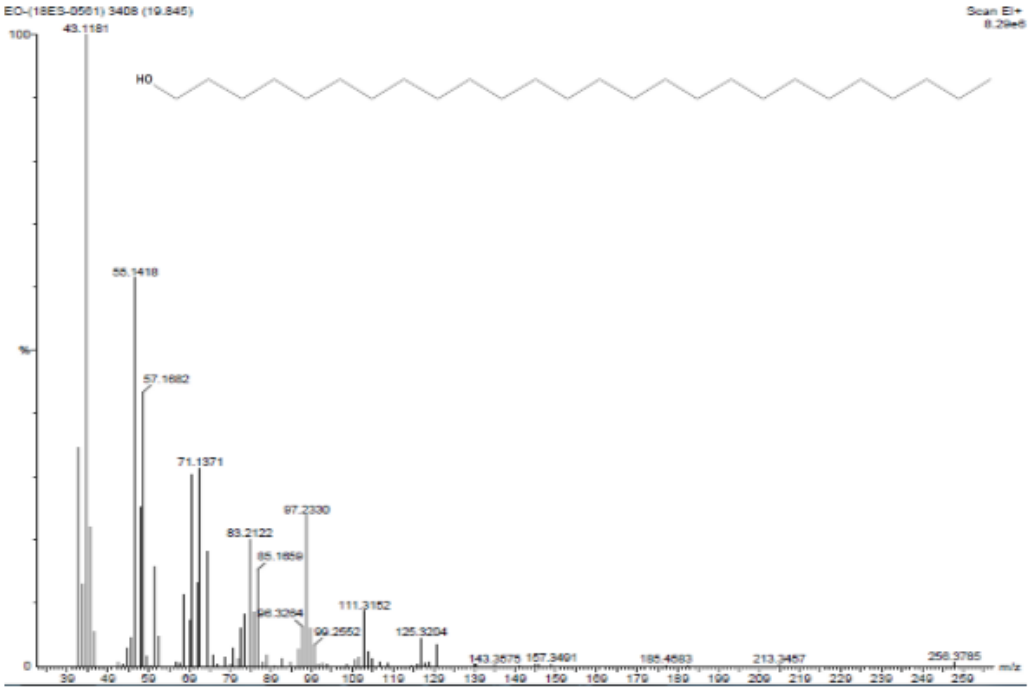
1. Pentafluoropropionic acid, octadecyl ester



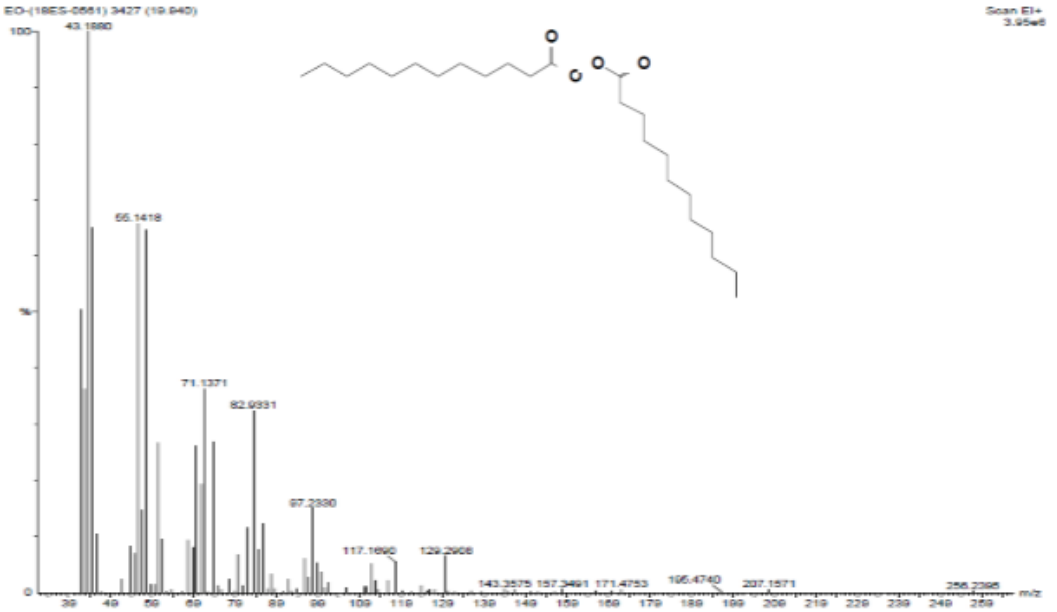
2. Eicosanoic acid



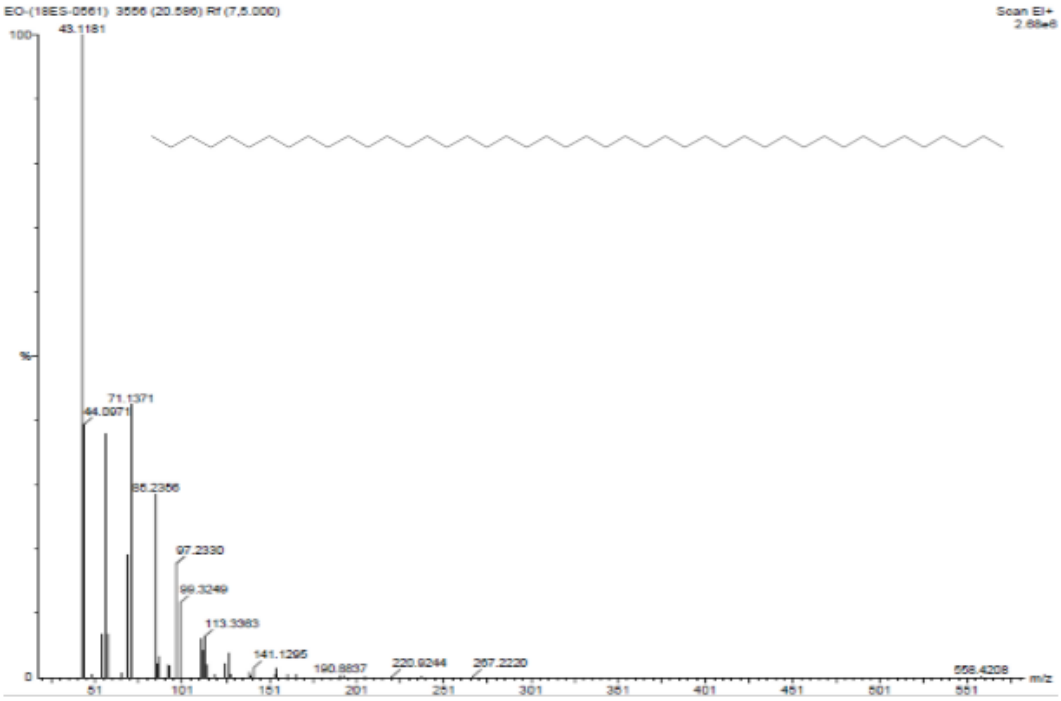
3. Pentadecanoic acid



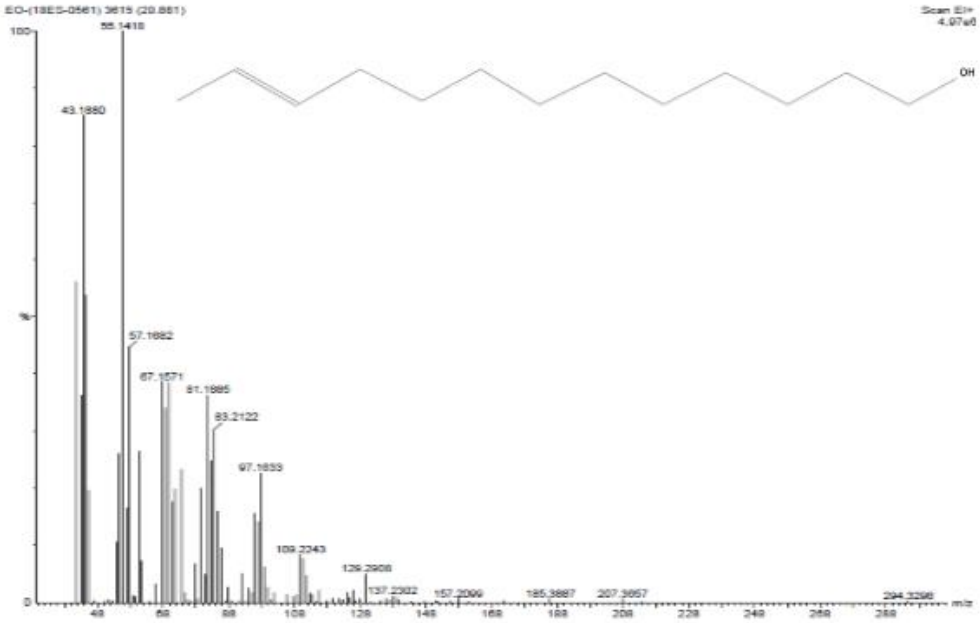
4. 1-hexacosanol



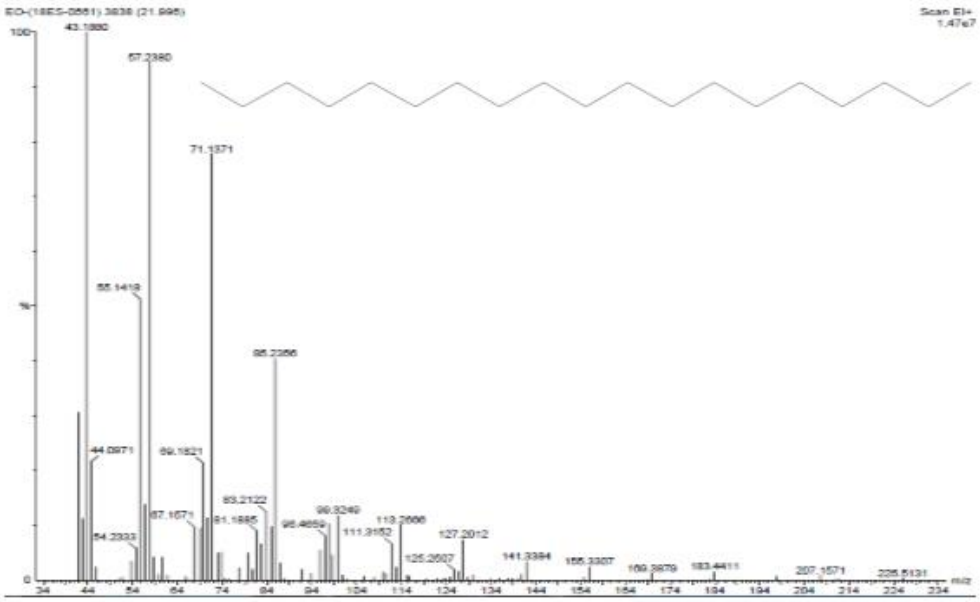
5. Lauroyl peroxide



6. Tetratetracontane

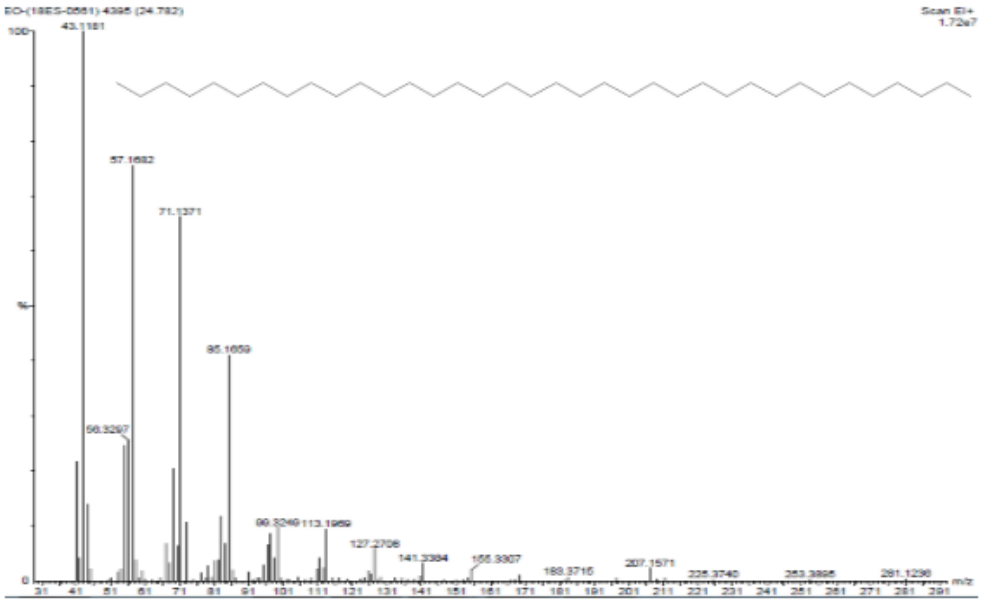


7. 11-tridecen-1-ol

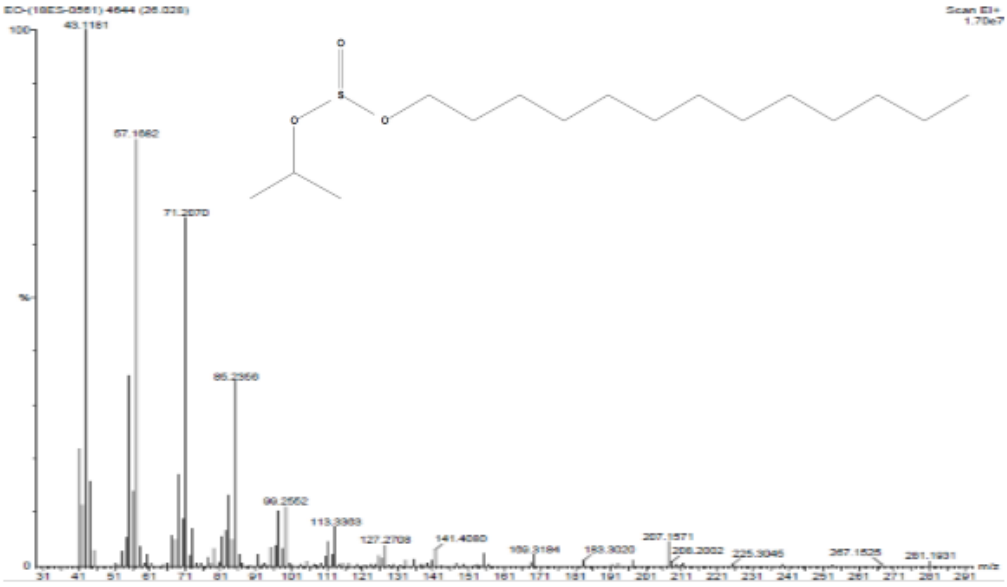


8. Nonadecane

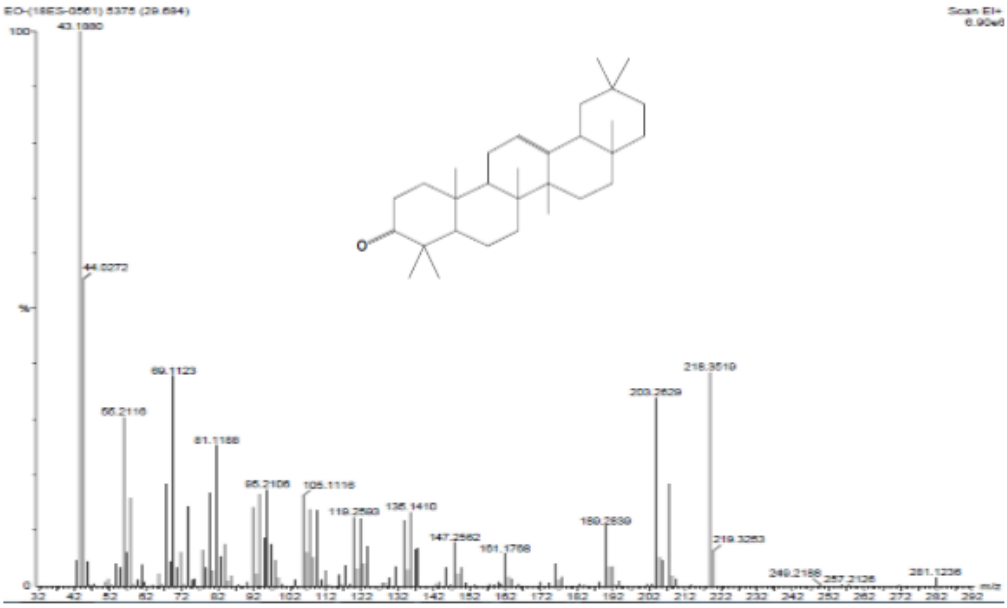
9. Sulfurous acid, 2-propyl tetradecyl ester



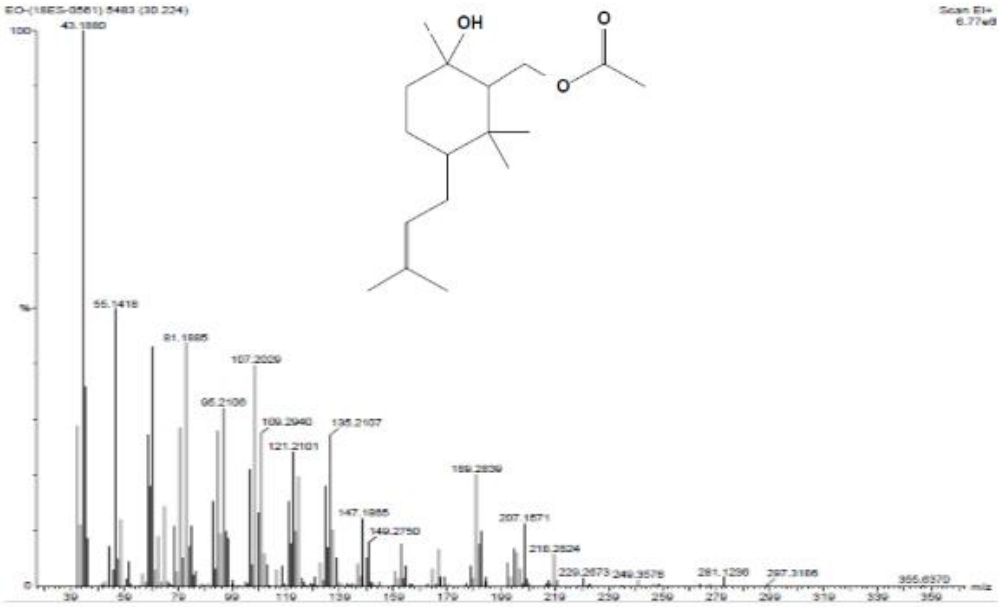
10. Hexatriacontane



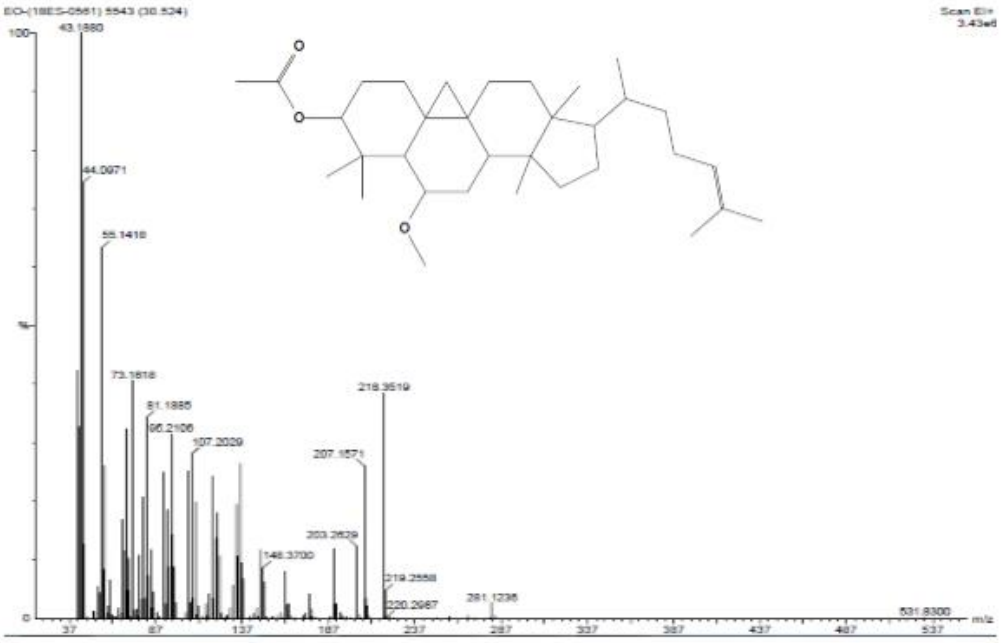
11. Sulfurous acid, 2-propyl tridecyl ester



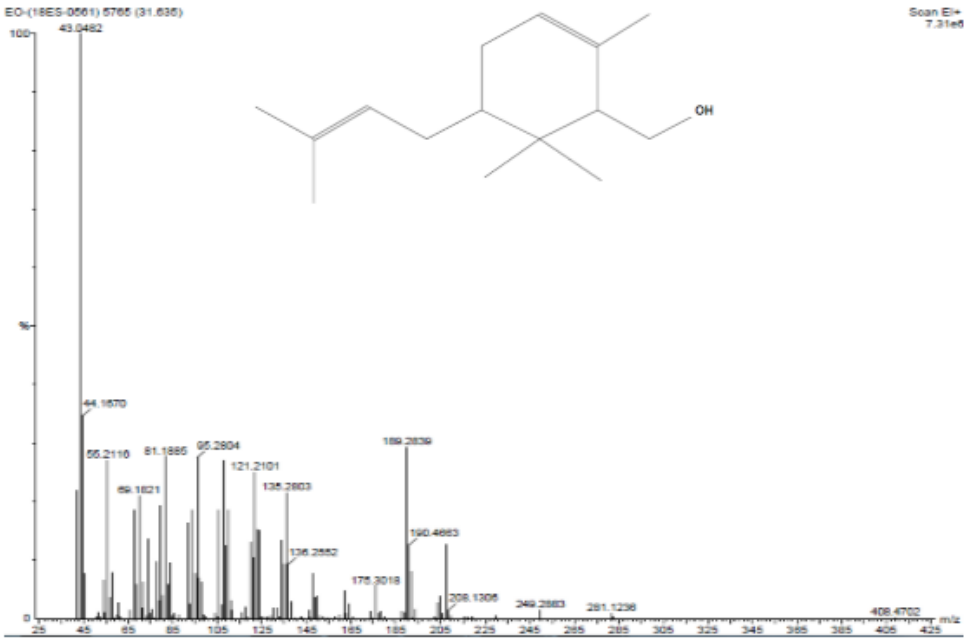
12. 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one



13. 2R-acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol)



14. 3-O-Acetyl-6-methoxy-cycloartenol



15. 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl)-cyclohexene

CONCLUSION

Traditional medicines have played vital role and remain as high value drugs even in the era of modern medicine. Phyto-constituents are responsible for medicinal value of any plant hence, in this regard a study on phyto-components in hexane fraction (oil) of *S. ciliata* flower extract was performed using GCMS technique. The hexane extract yielded a yellow oil which was found to be rich in fatty alcohols, sesquiterpenoid, esters, fatty acids and higher alkanes. Most of the compounds identified in the oil associated with anti-cancerous, anti-microbial, anti-inflammatory and other biological properties. This study thus facilitates large scale isolation of new compounds that may serve as lead molecules for discovery of new drugs and other products.

CONFLICTS OF INTEREST

The authors declare no conflict of interest in the publication of this manuscript.

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***Corresponding Author:**
Neelakantan Arumugam*
Email: arumugan.dbt@pondiuni.edu.in