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GC-MS screening of active secondary metabolites present in the *Cleome gynandra*

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Abstract

The aim of present investigation was to analyze the bioactive compound from the five different medicinally important wild edible plants. The plant was *Cleome gynandra* was collected, washed, shade dried and powdered. Ethanol extract was prepared by simple soxhlation method. The extract was concentrated and analyzed using Gas Chromatography Mass Spectroscopy for the identification of biochemical components present in the *Cleome gynandra*. The GC-MS study different compound analyzed from these plants. Majority of the compounds were belonging to acid group. Common compound in this plant was hexadecanoic acid. Which are having anti fungal anti inflammatory antibiotic activity, skin conditioning property were already reported, so that it can be recommended as a plant of phytopharmaceutical importance.
Keywords: *Cleome gynandra*, Heavy metal, Plant root, GC-MS.

1.Introduction

In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha. The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products were of plant origin and their sales exceeded US $65 billion in 2003 [1]. Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides [2]. The search for new plant derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity [3]. In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites [4]. Cell suspension culture systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. The soil physiochemical and trace metal concentrations may alter the physiology of the living things [5-8]. The secondary metabolites from the biological things are very essential for pharmaceutical applications and some of the strains are resistant to drugs [9].

Bioactive compounds or plant secondary metabolites consist of low-molecular weight compounds that are regarded as not essential for sustaining life, but as crucial for the survival of the producing organism [10]. More than 50,000 structures have been identified in plants by NMR, MS and X-ray analysis. However, as only less than 20% of all plants have been studied, it is very likely that the actual numbers of secondary metabolites or bioactive compounds in the plant kingdom would exceed 100,000 structures [11]. Secondary metabolites are produced in specific pathway and sites of synthesis can differ between types of compounds and between plant species. Furthermore, some compounds can be produced by all tissues, whereas others are produced in a tissue or even cell-specific fashion. Plants produce a wide variety of bioactive metabolites which serve as plant defense mechanisms against pests. Some secondary metabolites give plants their odors (terpenoides), some are responsible for plant pigments (quinines and tannins) and others (e.g., some of terpenoids) are responsible for plant flavor. These antimicrobial bioactive compounds are divided into 5 main classes consisting [12]: Terpenoids and essential oils; phenolics and polyphenols; alkaloids; polypeptides and mixtures (crude extract). The antimicrobial and anticancer activity of secondary metabolites of the biological micro and macro organism were processed by disk diffusion ([13-16] and MTT assay [17,18].

The results obtained from this study will provide information for the background levels of metals in the water, soil and plants and at the same time, to find the source of metal pollutions and interaction between the environmental things.

2. Materials and methods

2.1 Study area and sampling

The plant materials were collected from from Manamedu village of Tiruchirappalli district, Tamil Nadu during summer 2015. The shade dried *Cleome gynandra* fine powder was sterilized at 121°C for 15 min.

2.2 Ethanol extraction of plant samples

Sterilized fine powder, 20 g each was taken each plant, mixed with 200 ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman No. 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C and it's used as test samples for basic preliminary study. The 250 g of sterile fine powder was processed in Soxhlet apparatus for attaining of ethanol extraction of the plant sample. The ethanol extraction of plant sample was used for analysis of bioactive compounds through several techniques.

2.3 Secondary metabolites screening

This ethanol extracts of plant sample were sonicated for 20 min in sonicator 20 µl from sonicated extracts was passed through 0.45 µm filter. Filtrate was used for GCMS analysis. Gas Chromatography Mass Spectrometry (GC-MS) is a technique for the analysis and quantitative of organic volatile and semi-volatile compounds. Gas chromatography (GC) is used to separates mixtures into individual components using a temperature-controlled capillary column. Smaller molecules with lower boiling points travel down the column more quickly than larger molecules with higher boiling points. The maximum allowable temperature for this method is 300ºC. The GCMS system (Agilent 7890A GC-MS QToF 7200 series) was used. Chromatographic analysis was carried out using an INNOWAX 30 m x 0.250 mm x 0.25 um column at temperature: ambient. Running conditions included: injection volume HS 2.5 mL syringe, HS SPME injection technique; mobile phase: Helium. Samples were filtered through an ultra-membrane filter (pore size 0.45 µm) prior to injection in the sample loop. Retention time and concentration of Metabolites were analyzed by using in-built GCMS software.

3. Result and discussion

The studies on the active principles in the whole plant *Cleome gynandra* ethanolic extract by GC-MS analysis clearly showed the presence of ten compounds. The active principles with their retention time (RT) and concentration (peak area %) are presented in Table-1. The GC-MS chromatogram of the eight peaks of the compounds detected was shown in Figure-2a and 2b. The compounds identified by the mass spectroscopy were presented. The total numbers of compounds indentified in ethanol extracts were the GC-MS. Retention time (RT) and percentage peak of the individual compounds. The results revealed that (E)-9-Octadecenoic acid, ethyl ester (21.81%) and Hexadecanoic acid, ethyl ester (11.23) was found as the 2 major component in the ethanol extract., the six minor compounds such as methyl[2]-5,11,14,17-eicosatetraenoate (10.28%) Diethyl phthalate (7.48%) phenol

1,3-pentadecyl (5.63%) 1-Hexadecanol,2-methyl (2.68%) Methyl [2]-5, 11, 14, 17-eicosatetraenoate (2.27%) and Eicosanoic acid, Phenyl methyl ester (0.64%).

Correa and Alcantara [19] in 2011 reported the ethanolic extract of *Mussaenda frondosa* has been subjected to GC-MS analysis. Twenty chemical constituents have been identified. The major chemical constituents are (-)-Quinic acid(32.87%),4- (IE)-3-Hydroxy-1-Propenyl)-2-methoxy phenol(8.30%), Naphthalene, decahydro-2-methoxy-(7.20%),1,2,3- Benzenetriol(7.70%) [20]. The bioactive compounds of *Polygonum glabrum* have been evaluated using-MS [21]. The chemical compositions of the whole plant ethanol extract of *P. glabrum* were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library [22]. GC-MS analysis of *P. glabrum* whole plant ethanol extract revealed the existence of the ether compound –Propane 1,1-diethoxy- (64.86%), alkane compound -2-Heptane, 5-ethyl-2,4-dimethyl- (13.51%), sulphur compound –Tiophene-2-Carboxamide, N-(2-furfuryl)- (8.!!%), alcoholic compound - 1,14-Tetradecanediol (5.41%), and plasticizer compounds -1,2-Benzenedicarboxylic acid, isodecyloctyl ester5.41%) and 1,2,3-Benzenetriol (2.79%). The results of this study offer a base of using *P. glabrum* as herbal alternative for the synthesis of antimicrobial agents [23].

Table 1: Phyto- components identified in the *Cleome gynandra*

Name	Retention Time (RT)	Area	Area %
Peak-1	13.77	39591976	7.48
Peak-2	17.88	59471752	11.23
Peak-3	19.55	115534688	21.81
Peak-4	19.77	154488624	29.17
Peak-5	20.75	14197288	2.68
Peak-6	22.83	29843688	5.63
Peak-7	23.27	3402560	0.64
Peak-8	25.22	12020560	2.27
Peak-9	25.58	46830192	8.84
Peak-10	30.37	54261968	10.25
Total		529643296	100.00

Figure 1: Sampling site of the study area

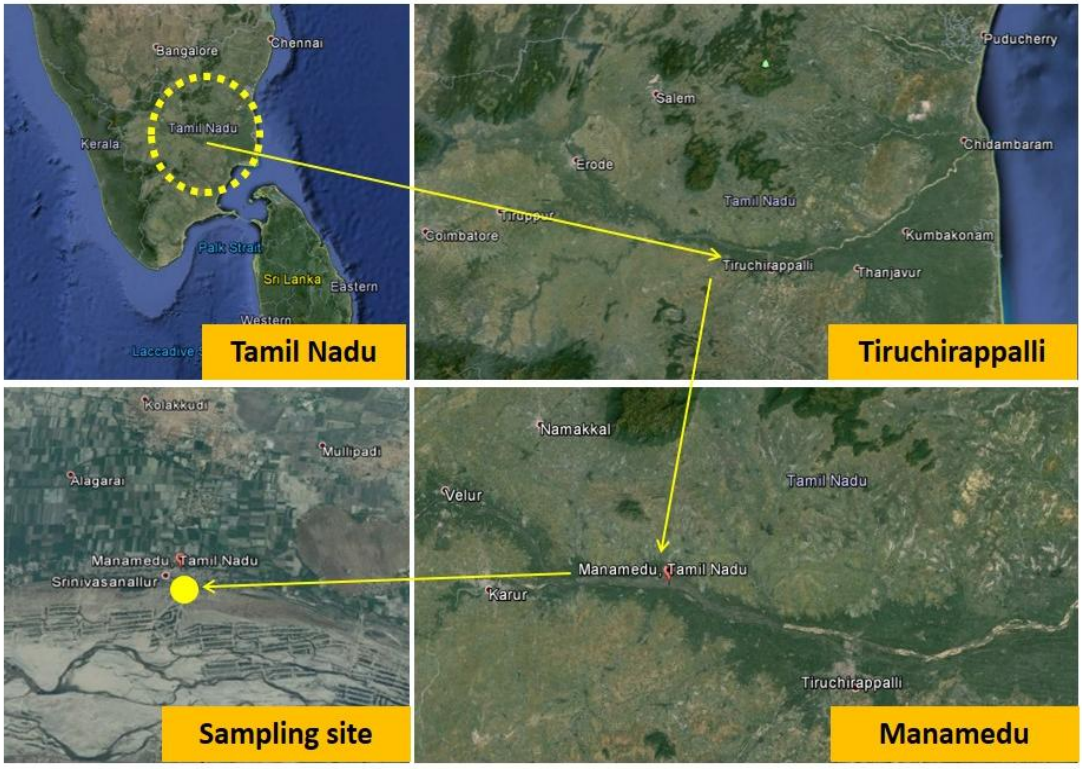


Figure 2a: GC-MS Spectrum of *Cleome gynandra*

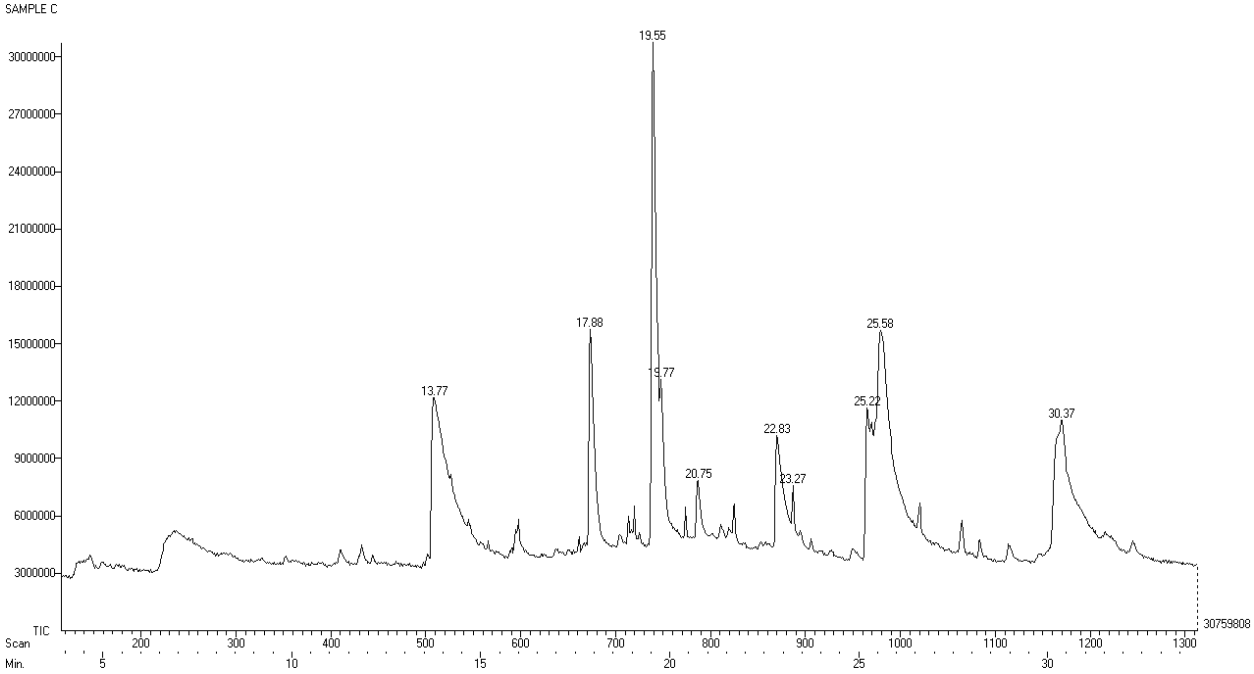
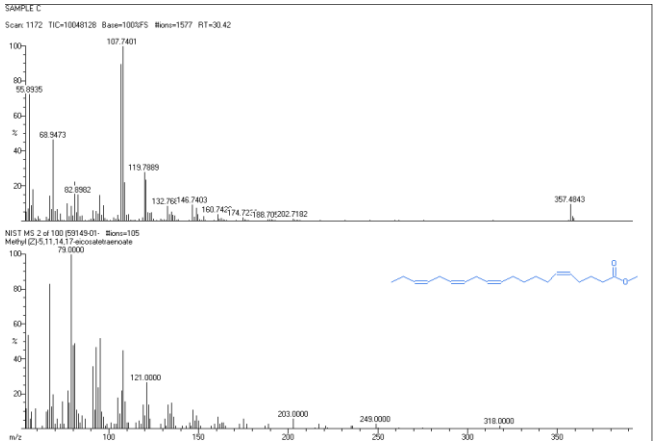
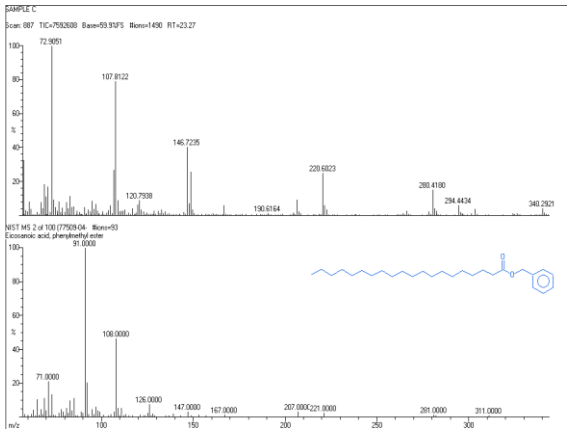


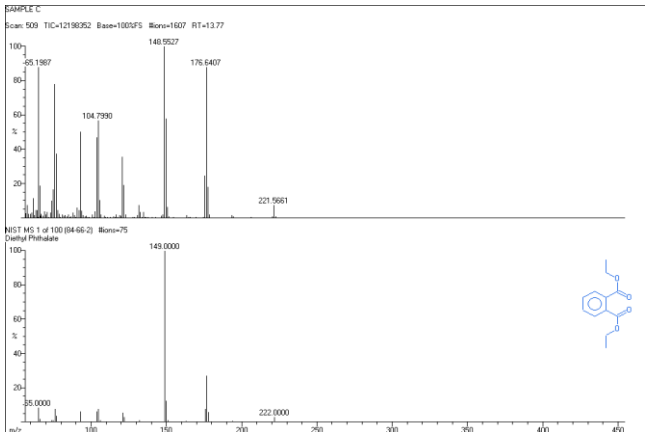
Figure 2b: GC-MS Spectrum fraction of *Cleome gynandra*



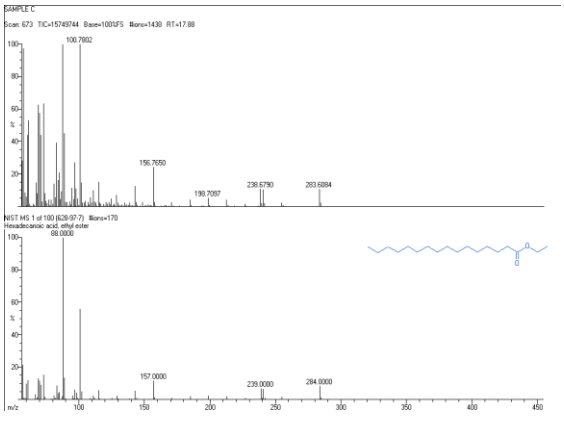
Methyl [2]-5, 11, 14, 17-eicosatetraenoate



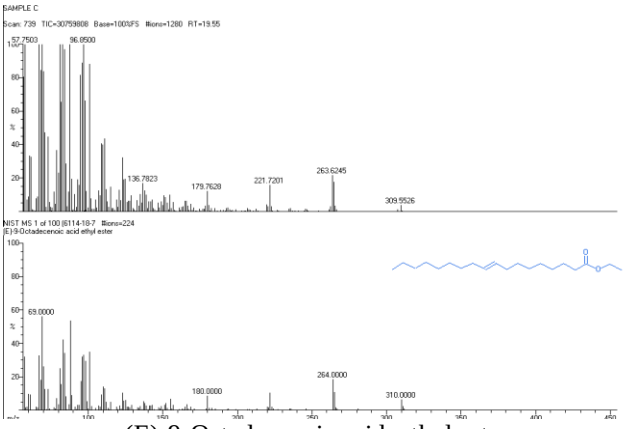
Eicosanoic acid, Phenyl methyl ester (0.64%)



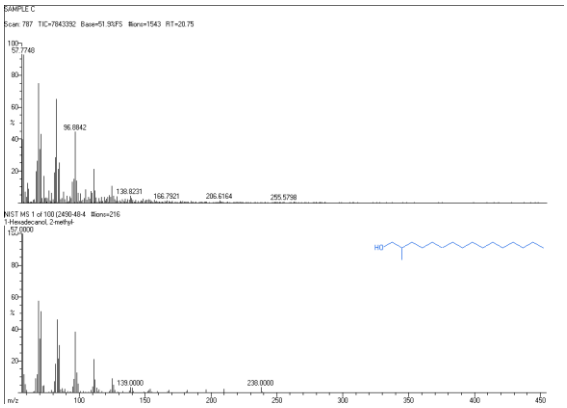
Diethyl phthalate



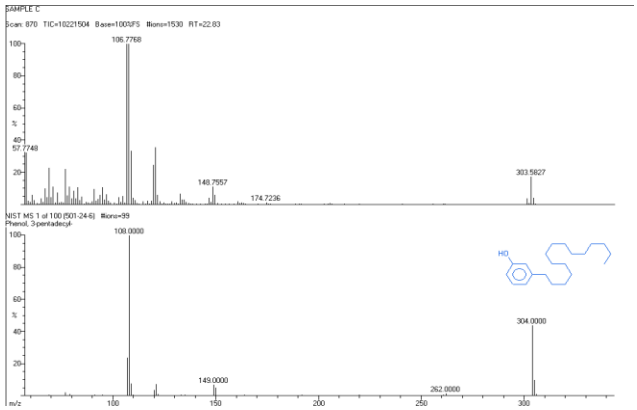
Hexadecanoic acid, ethyl ester



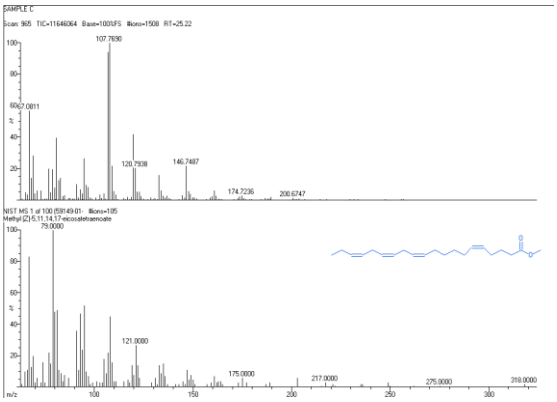
(E)-9-Octadecenoic acid, ethyl ester



1-Hexadecanol,2-methyl



Phenol,3-pentadecyl



Methyl [2]5, 11, 14, 17-eicosatetraenoate

4. Conclusion

The presence of so many phytocomponents in *Cleome gynandra* lends credence to its use by the local community as a plant with 'medicinal properties' and also holds promise for the production of novel pharmaceuticals as well as a nutraceutical. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for therapeutic roles.

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