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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation, Identification of Terpenoids and Antimicrobial Activity from Ethyl Acetate Extract of *Lantana camara* Leaves

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ABSTRACT
Terpenoids derived from Lantana camara leaves were isolated and tested for their antibacterial properties in this study. In this study, researchers used GC-MS to analyse Lantana camara leaves in a 1:1 Chloroform: Ethyl acetate fraction. The solid greenish residue was obtained by filtering, distilling, and condensing the ethanol extract at a concentration of 95%. N-hexane, chloroform, ethyl acetate, acetone, ethanol, and methanol were used to further fractionate the residue. For medicinal purposes, GC-MS study of the 1:2 fraction of Lantana camara leaves showed the presence of four substances. Oleanolic acid acetate was the first chemical found to have a shorter retention time (13.37 minutes). Column and thin-layer chromatography was used to isolate chemical molecules. The final isolated product was identified as oleanolic acid acetate and 4,4-Dimethylcholesta-8,14,24-trienol by means of several spectroscopic techniques. The well-diffusion method was used to evaluate the antibacterial activity. Antioxidant stress and bacterial-related disorders may benefit from Lantana camara. The isolation procedure is straightforward and time-saving.
Key words: *GC-MS analysis, antimicrobial activity, spectroscopic techniques, Lantana camara leaves, Oleanolic acid acetate, 4,4-Dimethylcholesta-8,14,24-trienol.*

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INTRODUCTION

Natural product research has long been a popular topic among scientists, particularly in the field of plants. For thousands of years, plants have been employed in traditional medicine [6]. Medicinal plant knowledge has been acquired over many years based on several medicinal systems such as Ayurveda, Unani, and Siddha. Traditional healers in India are said to use 2500 plant species, with 100 species serving as regular sources of medicine [7]. There has been a surge in interest in the study of medicinal plants and their traditional uses in various parts of the world over the last few decades [8]. It is critical to document indigenous knowledge through ethnobotanical studies in order to conserve and utilise biological resources. According to the World Health Organization (WHO), traditional medicine is used by up to 80% of the world's population for primary health care. The creation of indigenous medicines and the application of medicinal plants for the treatment of various ailments have significant economic benefits [9]. Most people are still obliged to use traditional medicines for their common daily problems due to a lack of communication, poverty, ignorance, and the lack of modern health facilities [10]. In locations where the usage of plants is still important, a broad understanding of how to employ plants against various illnesses is likely [11].

Plants contain pharmaceutically active metabolites, and a plant extract's activity is determined by the nature and concentration of these substances. The current or potential pharmacological activity of alkaloids, terpenoids, coumarins, flavonoids, and lignans is one of the most compelling reasons for studying natural products chemistry [12]. As a result, research of herbal plants are important not only for discovering active molecules, but also for determining how they work as medications to treat ailments. The research also revealed the general ingredients and effects that can support the use of herbal plants as "food" to improve health and avoid sickness.

Antimicrobial drugs either kill microbes (microbicidal) of prevent the growth of microbes (microbistatic). The discovery of antimicrobials in the previous century was followed by spectacular gains in human health

and life expectancy. The emergence of resistance to these "wonder drugs" is now so widespread that it threatens to undermine – or even reverse these gains.

Fungi are major destroyers of foods and grains during storage, leaving them unfit for human consumption by degrading their nutritional content and, in some cases, creating mycotoxins [13].

MATERIAL AND METHODS

Plant material authentication and treatment

Dr. John Britto, Rapinet Herbarium, St. Joseph's College, Tiruchirappalli, identified the leaves of the plant *Lantana camara* acquired in Thanjavur district. The leaves were cleaned, dried in the shadow, and pulverized.

Extraction

The powdered material was extracted with 95 percent ethanol for one week at room temperature using the cold technique. The 95 percent ethanol extract was filtered, distilled, and concentrated to acquire the solid greenish residue. Petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, n-butanol, and methanol were used to partition the 95 percent ethanol extract. Under lowered pressure, the solvents were recovered. The ethyl acetate fraction was tested for antibacterial activity, isolation, and GC-MS analysis.

Ethyl acetate fraction (Isolation)

The ethyl acetate fraction (4.8 g) is separated into three fractions by chromatography on a silica gel (100 - 200 mesh) column with chloroform, ethyl acetate, and methanol as eluents. On a column eluting gradiently with chloroform, ethyl acetate, 1:2 chloroform, ethyl acetate combinations, and methanol, fraction III was rechromatographed on a silica gel (100 - 200 mesh). Prior to employing the column, 1mL of 1:2 fractions were GC-MS analysed to determine the specific type of compound. After the fraction 1:2 chloroform: ethyl acetate combination was eluted with ethyl acetate 4.5:0.5, 4:1 ethyl acetate: methanol mixtures, and methanol, it was subjected to column chromatography using silica gel (100-200 mesh) eluted with ethyl acetate 4.5:0.5, 4:1 ethyl acetate: methanol mixtures, and Except for methanol and 4:1 elution, all of the eluted fractions were crystallised individually with methanol to produce LCEA1 (22 mg). TLC was used to separate the 4:1 fraction into four bands. The fourth band was scraped off and recrystallized with methanol to produce LCEA2 (14 mg).

Gas Chromatography-Mass Spectrometry analysis of 1:2chloroform: ethyl acetate fraction

The following settings were used to conduct GC-MS analysis using a Perkin Elmer GC clarus 500 system that included an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument using reported conditions.

Antimicrobial activity

The antimicrobial activity was measured using1:2 Chloroform: Ethyl acetate fraction from ethyl acetate extract of *Lantana camara* leaves using the well - diffusion method at a concentration of 1 mL and ethanol as the control. Antimicrobial activity was determined using known methods.

RESULTS AND DISCUSSION

GC-MS analysis 1:2 fraction

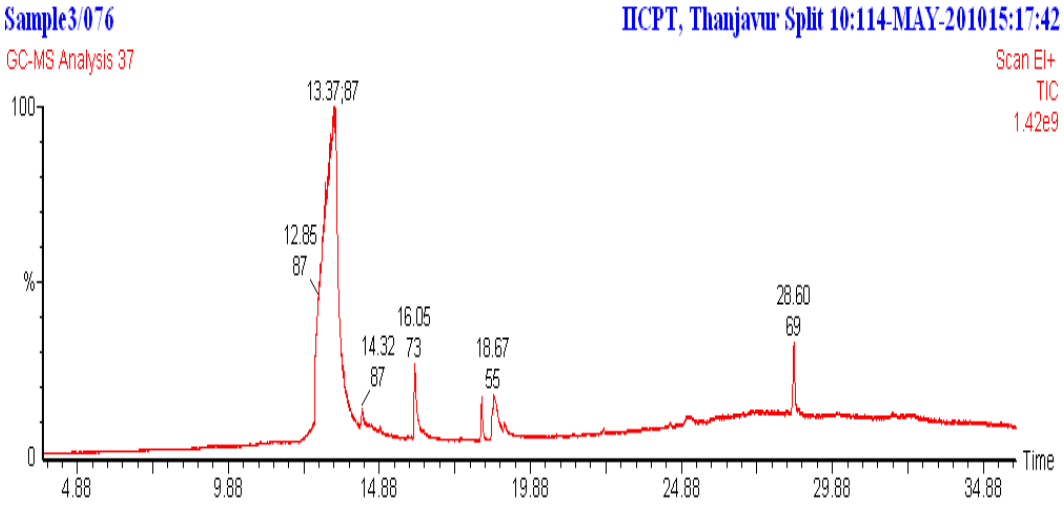

Fig 1: Analysis of a 1:2 portion of *Lantana camara* leaves using GC-MS

Table 1: GC-MS analysis of 1:2 fraction of ethyl acetate extract of *Lantana camara* leaves

S.No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	13.37	Oleanolic acid acetate	$C_{32}H_{50}O_4$	498	872.94
2.	16.05	Palmitic acid	$C_{16}H_{32}O_2$	256	33.06
3.	18.67	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	40.81
4.	28.60	4,4-Dimethylcholesta-8,14,24-trienol	$C_{29}H_{46}O$	410	84.56

The GC-MS chromatogram of the 1:2 (ethyl acetate: methanol) fraction of *Lantana camara* leaves ethyl acetate extract (Fig 1) revealed four peaks, suggests the existence of four chemicals. Table 1 lists the chemical components found in the 1:2 fraction of an ethyl acetate extract of *Lantana camara* leaves. The 1:2 fractions have triterpenoid type compounds oleanolic acid and squalene, as well as fatty acid ester type compounds palmitic acid and octadecadienoic acid, according to GC-MS analyses. According to GC-MS chromatogram oleanolic acid acetate (872.94 %) and squalene (84.56 %) were highly present in 1:2 fraction and acid (33.06 %) and acid ester (40.81) type of compounds were present in minimum amount. These phytochemicals have a variety of pharmacological effects, including antibacterial, antioxidant, and anti-inflammation properties. From a 1:2 fraction of ethyl acetate extract, this work provides a solid foundation for isolating oleanolic acid and 4,4-Dimethylcholesta-8,14,24-trienol. The implementation of massive screening processes is required by new scientific methodologies for the evaluation of phytochemicals with specific biological activity.

Isolation

Compound 1: The compound appears as a white crystalline solid with a retention factor of 0.63 (4.75:0.25 ethyl acetate: methanol) and a melting point of 267-269 ^{0}C.

Screening test for Terpenoids:The Lieberman-Burchard test for triterpenoids was positive for oleanolic acid acetate.

Elemental Analysis: The values obtained for $C_{32}H_{50}O_4$; C = 76.06 %, H = 10.10 %, O = 12.83 %, Molecular mass 498.

^{1}HNMR: The assigned NMR spectra were quite close to the values seen in the literature [10-15]. The chemical shift measured at 4.0 ppm in 1H NMR spectra suggested the (H-3) bonded with oxygen group. The presence of an olefinic proton was indicated by the presence of a signal at 5.3. The presence of the 'CH' group is responsible for the signal at 1.26, while the –CH2 group is responsible for the signals at 1.29, 1.34, 1.5, and 1.43. The –CH3 groups caused a shift in the 1HNMR at 0.857, 0.842, and 1.04. This has been observed previously using ethyl acetate extract [16]. The molecule was finally identified as Oleanolic acid acetate based on the preceding observations, GC-MS analysis of 1:2 fractions, and direct comparison with the authentic sample (co-TLC and MMP). [Figure 2].

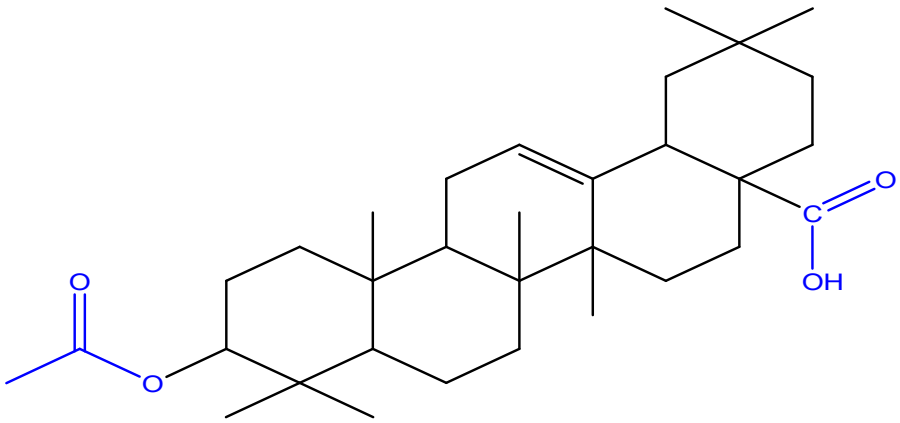

FIG 2: Oleanolic acid acetate.

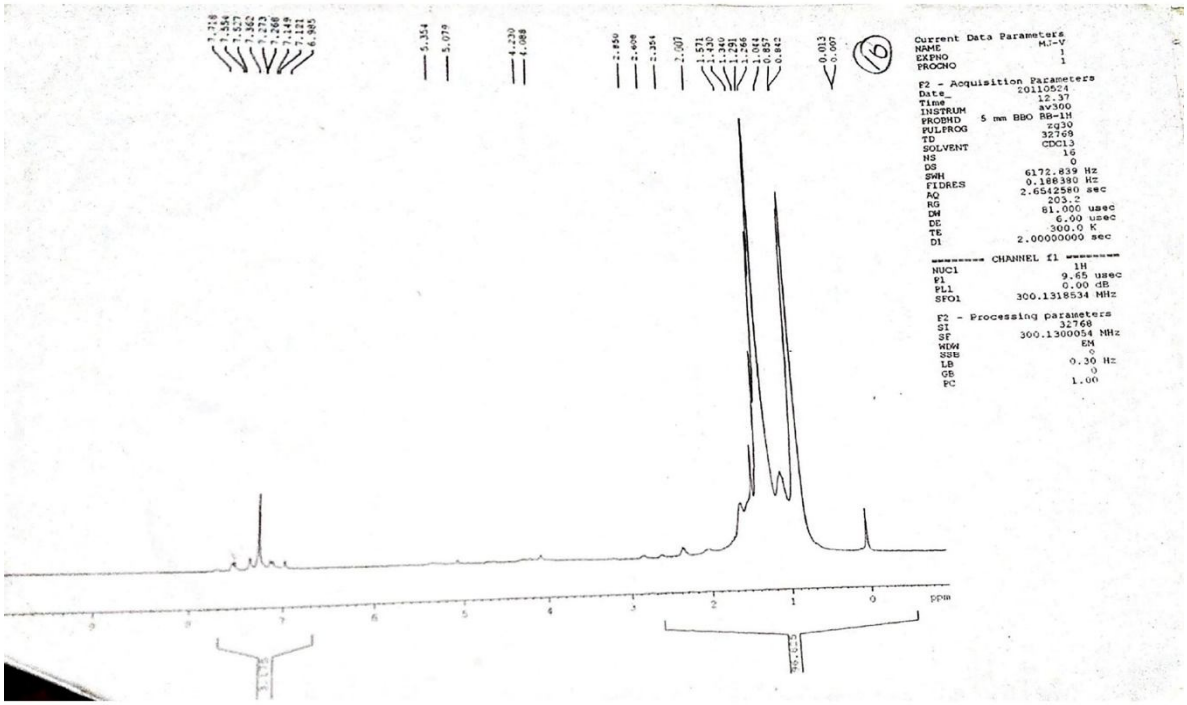

FIG 3: ^{1}H NMR Spectrum of LCEA1

Compound 2

Compound 2 has a white amorphous powder with a 0.82 retention factor (9.75:0.25 chloroform: ethyl acetate). The mass spectra of 1:2 fractions of GC-MS analysis were matched with the mass spectra of purified compound LCEA2. The molecular formula of the compound was $C_{29}H_{46}O$ and the Molecular mass was 410. From the NIST and Metlin library the compound was identified as 4,4-Dimethylcholesta-8,14,24-trienol [Figure 4].

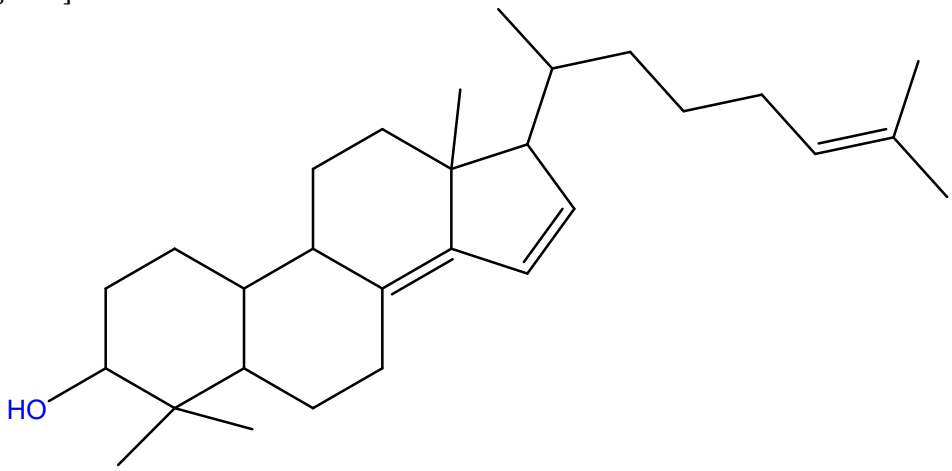

FIG 4: 4,4-Dimethylcholesta-8,14,24-trienol

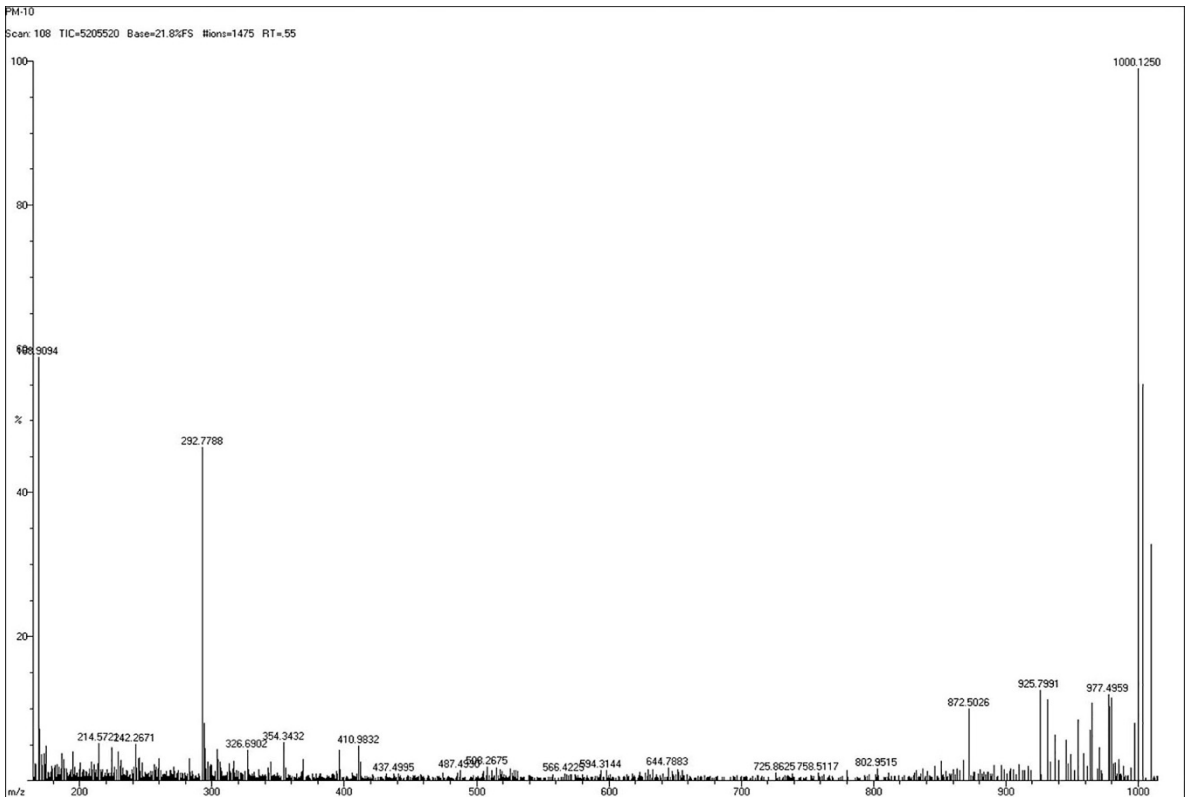

FIG 5: Mass spectrum of LCEA2

Antimicrobial activity

TABLE 2: Antimicrobial activity from 1:2 fraction of *Lantana camara* leaves

S.No	control	Antibacterial activity		Antifungal activity	
		Pathogens	1:2 fraction	Pathogens	1:2 fraction
1	0	*S. mitis*	12	*Candida albicans*	-
2	0	*B. asei*	14	*Cunninghamella bertholletiae*	-
3	0	*Lactobacillus sp*	15	*A.niger*	-
4	0	*E. aerogenes*	11	*A.indus*	-
5	0	*A. eucrenophila*	15	*A.flavus*	-
6	0	*M. morganii*	18	*Mucor hiemalis*	-
7	0	*E. faecalis*	15	*Cryptoccus*	-
8	0	*E. coli*	15	*Fusarium*	-

Table 2 shows the results of the antibacterial activity of the plant extracts. The 1:2 fractions were efficient against both gram-positive and gram-negative pathogens, according to the findings. M. morganii had the maximum activity (diameter of zone of inhibition 18 mm), whereas E. aerogenes had the weakest activity (diameter of zone of inhibition 11 mm) against complete pathogens.

Table 3 summarizes the antifungal activity of 1:2 fractions. In the 1:2 fraction, *Lantana camara* leaves showed no antifungal activity against most of the tested organisms, as shown in table (3). Plants are a valuable source of potentially beneficial structures for developing novel chemotherapeutic drugs. The in-vitro antimicrobial effects assessment is the first step toward the goal, and there have been various publications on the antibacterial activity of plant extracts in recent years.

One of the most frequent approaches for selecting plants for pharmacological research is to use an ethnobotanical approach. India is one of the twelve big biodiversity hotspots in the world, with over 45,000 plant species. Its diversity is unrivaled, with sixteen distinct agroclimatic zones, ten vegetative zones, and fifteen biotic provinces. Plants have long been used as a medicine source, constituting an important part of the healthcare system. Approximately 20% of all plants discovered on the planet have undergone pharmacological or biological testing. Systemic antibacterial screening of plant extracts could be a constant attempt to find new antibacterial chemicals. Given India's vast plant diversity, it is vital to screen plants for pests and diseases.

The presence of substances including Oleanolic acid acetate, Palmitic acid, 9,12-Octadecadienoic acid, and 4,4-Dimethylcholesta-8,14,24-trienol proved the antibacterial potential of *Lantana camara* leaves. Furthermore, phytochemical ingredients such as terpenoids and a variety of other aromatic compounds are secondary metabolites of plants that act as defence mechanisms against microbe predation. This could explain why the *Lantana camara* leaves from the 1:2 fraction showed antibacterial activity.

Though the exact mechanism of action of these chemical elements is unknown, it is apparent that the efficiency of the extracts is heavily influenced by the solvent utilized. Depending on their solubility or polarity within the solvent, different solvents are said to be capable of extracting different phytoconstituents. It's possible that this is one of the reasons for the plants' antibacterial activities differing. Furthermore, the efficacy of the extracts differs depending on their concentration and the pathogens utilized in the study.

The discrepancies in susceptibility to the 1:2 fraction across the test species could be attributable to differences in the pace at which active components permeate their cell walls and cell membrane structures. *Lantana camara* leaves were shown to be resistant to 1:2 chloroform: ethyl acetate, which is most likely related to the outer membrane of the plant. Nonetheless, the ability of the extracts' active principle to disrupt the permeability barrier of cell membrane structures and hence prevent bacterial and fungal development is what makes them so effective. For the first time, the 1:2 fraction has been studied, and the findings suggest that these *Lantana camara* leaves are promising candidates for additional research into the separation and characterization of the bioactive component responsible for antibacterial and antifungal activity.

CONCLUSION

Finding compounds Oleanolic acid acetate, 4,4-Dimethylcholesta-8,14,24-trienol was identified as the final isolated product utilizing several spectroscopic techniques, and it demonstrated outstanding antibacterial action. Isolation is straightforward, cost-effective, and combines the best.

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CONFLICTS OF INTEREST

There are no conflicts of interest among the authors.

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