Vol.02, Issue 03 (2024): 39-51
DOI: 10.58921/jse.02.03.057

Journal of Sustainable Environment
ISSN (p): 2710-2386, ISSN (e): 2957-9228

Published by
1885 SMI UNIVERSITY

GC/GC-MS analysis and biological activities of *Lantana Camara* Linn.

Riffat Abdul Ghafoor[1], Anjum Ayub[2], Syed Nawazish Ali[1*], Aneela Wahab[3], Sabira Begum[4], Bina Shaheen Siddiqui[4], Saima Tauseef[3], Muhammad Tariq Rajput[5]

[1] Department of Chemistry, University of Karachi, Karachi.

[2] Department of Chemistry, NED University of Engineering and Technology, Karachi, Pakistan

[3] Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

[4] HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi.

[5] Department of Zoology, University of Karachi, Karachi.

* Corresponding author: snali@uok.edu.pk

ABSTRACT

Medicinal plants have been a part of human history for thousands of years and are still used as healthcare throughout the world. The current research aims to explore the chemical constituents of the methanol soluble extract (LC-Me) and petroleum ether soluble fraction (LCM-PES) from the leaves of Lantana camara Linn by GC/ GC-MS. This chemical analysis revealed the existence of 16 and 23 phytoconstituents in LC-Me and LCM-PES respectively. The major constituents in LC-Me were found to beethyl 9,12,15-octadecatrienoate (31.9%), hexadecanoic acid, ethyl ester (12.6%), n-hexadecanoic acid (11.1%), linoleic acid ethyl ester (9.1%), squalene (8.7%), di-n-octyl phthalate (6.2%), 9,12-octadecadienoic acid (Z,Z)- (4.2%), (E)-9-octadecenoic acid ethyl ester (2.7%) andcyclopropanebutanoicacid,2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester (2.3%). The chief bioactive compounds in petroleum ether soluble fraction were found to beandrost-8-en-3-ol, 4,4,14α-trimethyl-17-(2-bromo-1-methylethyl (57.9%), 14,17-nor-3,21-dioxo-β-amyrin, 17,18-didehydro-3-dehydroxy- (13.0%), barringtogenol B (2.5%), olean-12-ene-3,16,21,22,28-pentol, 21-(2-methyl-2-butenoate), [3β,16α,21β(Z),22α]-(1.7%),perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl (1.7%), ethyl iso-allocholate (1.6%) and 1,2-benzenedicarboxylic acid, diisooctyl ester (1.6%). Both the extract and its fraction have exhibited very significant antibacterial, antifungal,mosquito repellent and larvicidal propertiesoriginated by numerous bioactive metabolites. Twenty eight (20 Gram-positive and 8 Gram-negative) bacteria were tested against LC.Me and LCM-PESwith noteworthy zone of inhibition.The significant in vitro antifungal activity was observed against fifteen fungi in LC-Me and LCM-PES. Very robust initial repellency was observed for LC-Me and LCM-PES (94% and 80% respectively) against the dengue-carrying mosquito (Aedes aegypti) at 2% concentration. The extract and its fraction were also found to be an efficient larvicidal agent against fourth-stage larvae of Aedes aegypti. The effective larvicidal property was noted in methanol soluble extract as compared topetroleum ether soluble fraction and standard with LC50value of 20 and 400 ppm respectively.

Keywords: Lantana camara Linn., medicinal plants, GC/GC-MS analysis, chemical composition, antibacterial, antifungal, dengue mosquito repellent, larvicidal activities.

INTRODUCTION

Lantana camara Linn. (Family: *Verbenaceae*) is classified as an intrusive weed, commencing from tropical America. Initially brought into in various regions, notably in the Australian Pacific area, as a hedge or ornamental shrub.The plant is traditionally used in treating a wide array of ailments including rheumatism, eczema, anemia,bilious fever, leprosy, toothache, swellings, influenza,stomachache, ulcers, malaria, and tumors. Additionally, it acts as an antiseptic to promote wound healing. This plant species has been documented to contain numerous terpenoids, flavonoids, steroids, esters, hydrocarbons, tannins, saponins, glycosides,fatty acids and nitrogen containing compounds(Ahmed et al., 1972; Ashford et al., 2000; Ayub et al., 2017; Begum et al., 2000; Desjeus et al., 2001; Dua et al., 1996; Sharma et al., 1988; Ganjwala et al., 2009). L. camara exhibits an extensive range of biotic attributes including the nematicidal, CNS-depressant, analgesic, anti-microbial, anti-inflammatory, anti-cancer, insecticidal, anti-convulsant, hepatotoxic, antimalarial, anti-hyperglycemic and anti-hypertensive (Ghisalberti, 2000; Jaipal et al., 1983; Kalita et al., 2012; Kensa, 2011). The extracts from aerial parts of L. camara eventually proved to be immensely effective against Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa thus revealing the anti-bacterial potential of this plant (Basu et al., 2005). These extracts effectively eradicated two multidrug-resistant strains of Escherichia coli and Staphylococcus aureus as well as other bacterial strains including Proteus vulgaris, Pseudomonas aeruginosa and Vibrio cholera (Pattnail et al., 2010).

The skin, nails, hair and subcutaneous tissues of humans and animals can be vulnerable to infection from various organisms, predominantly fungi known as dermatophytes. This fungal invasion leads to dermatophytosis (Marchisio, 1996; Tsang et al., 1996) which is primarily caused by three genera of molds, Epidermophyton, Trichophyton and Microsporum. (Chermette et al., 2018). Human dermatophyte infection due to rapidly growing geophilic distribution of Microsporumgypseumhas increased in the last decade (Ritsuko et al., 2002). This fungus is mainly responsible for sporadic dermatophytosis (Tinea capitis, Tinea incognito and Tinea corporis) in humans. Tinea corporis caused by M. gypseumhas also been reported in aids patients (Giudice et al., 2012). The plant is also found to be potent against numerous fungi responsible for dermatophytosis (Bokhari, 2009).

Dengue is a break-bone fever caused by viral infection from mosquitoes and it is commonly found in tropical and subtropical regions. The cure against break-bone fever (Dengue) is a matter of global concern as 5.2 million cases are reported to WHO in 2019(Bhatt et al., 2019). Extracts from *Lantana camara* Linn. have also demonstrated very significant efficacy against dengue-carrying mosquito *Aedes* mosquitoes (Bhuvaneswari et al., 2022).

The intriguing pharmaceutical outcomes mentioned earlier have directed our focus to obtain comparative substantial data on methanol soluble extract and petroleum ether soluble fraction of *Lantana camara* by GC/GC-MS analysis. The current research comprehensively exploresnot only the composition of phytoconstituents in methanol and petroleum ether soluble parts but also provide significant results of the antibacterial, antifungal, mosquito repellent and larvicidal activities of *L. camara*.

2. MATERIALS AND METHODS

2.1 Sample Collection

The collection of leaves of *Lantana camara* were achieved in the Karachi region and verified by a Senior Taxonomist (Mr. Abdul Ghafoor), is deposited inUniversity of Karachi. (Department of Botany). A voucher specimen (No. 63482 KUH) was also recorded in the Herbarium for future reference.

2.2 Extraction

The air-dried leaves of *Lantana camara*, were crushed and imperiled to repetitive extraction at room temperature using MeOH. The resulting extract (LC-Me) was obtained after evaporating the solvent under reduced pressure. This extract underwent partitioning into ethyl acetate and aqueous phases. To separate the neutral from the acidic fraction in the ethyl acetate phase, an aqueous solution of Na_2CO_3 (4%)was employed. The neutral fraction in the EtOAc layer was then treated with water. The moisture was removed by drying (Na_2SO_4) and passed over activated charcoal. EtOAc and MeOHwere used to wash the charcoal bed. These fractions were mixed on TLC basedresults. After eliminating the solvent from charcoal filtrate and washings, the residual substance was separated into petroleum ether insoluble portion and the petroleum ether soluble portion (LCM-PES).

3. BIOLOGICAL ASSAY

3.1 Screening of Antibacterial Activity

Disc diffusion method (Bauer et al., 1966) was employed for assessing antibacterial activity. Stock solutions of 100 mg/mL (LC-Me) and 50 mg/mL (LCM-PES) were prepared in DMSO. Antiseptic strainer discs, laden with 10 µL of the respective stock solutions, were utilized for screening. Iso Sensitest agar plates (Oxoid) were inoculated with a culture aged 24 hours (1-2 x 10^8 CFU/mL) cultivated in Mueller Hinton broth (Oxoid). Placed on various positions on the agar surfaces, the prepared discs were incubated at 37°C for a day. The results were observed by measuring the region of inhibition in millimeters, with DMSO (negative control). Antibacterial activity of methanol (LC-Me) and petroleum ether soluble (LCM-PES) parts of *L. camara* was studied against twenty Gram-positive and eight Gram-negative bacteria.

3.2 Antifungal Activity

Additionally, the *in vitro* effectiveness of LC-Me and LCM-PES against a range of fungi was assessed, encompassing seven filamentous fungi (*Aspergillus terreus, Aspergillus flavus, Penicillium sp., Rhizopus sp., Aspergillus niger, ATCC Rhizopus, ATTC Penicilium),* six dermatophytes (*T. mentagrophytes, Trichophyton rubrum,Microsporumcanis, T. tonsurans, Fusarium sp.M. gypseum,*) and two other fungi (*Saccharomyces cerevisiae*and *Candida albicans).*

To create a homogeneous suspension of fungal culture, a slightvolume of culture was transferred to 2-3 mL of purified water or normal saline in a screw-capped tube, along with a small assortment of glass beads (diameter of 1 mm), then vortexed for 5-10 minutes. Along with this suspension, Sabouraud dextrose agar (SDA) plates were immunized and sterilised filter discs fullwith concentrations of 1000 µg/disc of LC-Me or 500 µg/disc of LCM-PES were positioned on the surfaces by following disc-diffusion protocol (Bauer et al., 1966). Incubation occurred for one week at room temperature and results were examined based on the zones of inhibition measured in millimeters.

3.3 Screening of Mosquito Repellent Activity

Female pupae of the *Aedes aegypti* mosquito were obtained and confined within an isolated cage measuring 30×24×24 inches. Upon reaching adulthood, these mosquitoes were manually hand-fed for their initial blood meal. Due to their developmental progression from the larval phase to the pupal stage before adulthood, these mature female mosquitoes were found free of the dengue virus. Additionally, they were confined within the enclosure to prevent contact with any dengue-infected individuals. Following a three-day period, samples were individually tested on separate days.

To conduct the test, the substance (LC-Me and LCM-PES) in an amount equivalent to 10 drops) were applied to the palm, then gently spread on the uncovered part of the right hand, covering an area of 3×3 inches. The hand was inserted into the mosquito enclosure, and the number of visits and bites by mosquitoes was tallied over a 5-minute period. Mosquitoes that refrained from biting and returned due to the scent of the sample were categorized as repelled mosquitoes. Those that bit and fed on blood were recorded as biting mosquitoes.

Initial observation was considered the zero-time (zero hour) reading, without washing the hand, the same hand was revived into the cage precisely after 0.5 hours, repeating the observation. Following the same procedure, subsequent observations were made at intervals of 1.0, 1.5, 2.0, and 2.5 hours. Each sample underwent testing on separate days while the mosquitoes were sustained with a 5% sugar solution to support those that did not feed on blood(Tariq & Qadri, 2001).

The resulting data was tabulated) and analyzed using specific formulas for evaluation.

$$\text{Average biting\%} = \frac{\text{Total no. of biting in 5 minuts}}{\text{Total no. of visits in 5 minuts}} \text{ X } 100$$

$$\text{Repellency\%} = \text{Control biting (100\%)} - \text{Average biting\% (in 5 minutes)}$$

Repellency demonstrated an inverse relationship with biting behaviour. The outcomes were contrasted with Mospel, a commercially available product found in the local market, which contains 20% DEET.

3.4 Screening of Larvicidal Activity

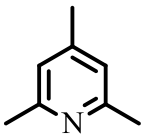
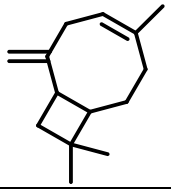
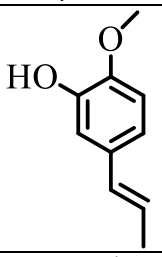
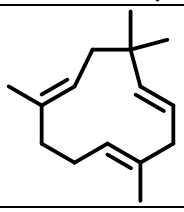
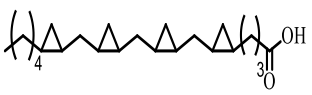
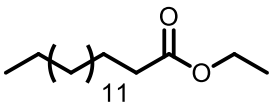
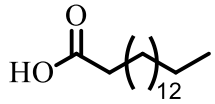
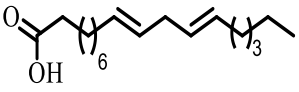
Ten larvae (4^{th}instar)of *A. aegypti* (comprising 10 females and 10 males), all of identical size and age, were introduced into 250 mL (beakers) employing the WHO technique(WHO Expert Committee on Insecticides & World Health Organization, 1973). The larvae underwent exposure to varying concentrations of both samples and the standard (biosal). For each sample, duplicates of seven beakers were prepared, 5 for distinct doses, 1 for the negative control (methanol) and another for the control (water).For the standard (biosal), 6 beakers were arranged, 5 allocated for 5 different doses and 1 for the control (water). However, the 7thbeaker(designated for the negative control) was omitted as biosal had been diluted in distilled water. The Mortality was recorded after twenty-fourhours, and this experiment was replicated five times. Larvae showing signs of near-death were considered as deceased. The gathered data underwent statistical analysis using Abbot's formula(Abbott, 1925).

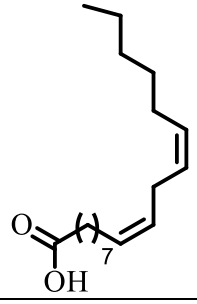
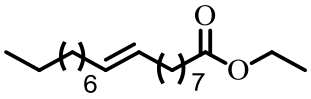
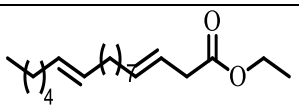
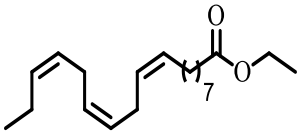
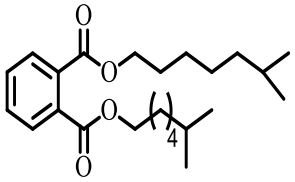
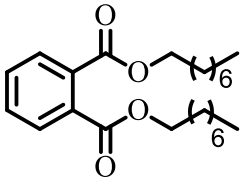
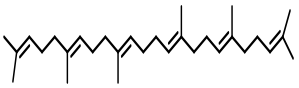
Mean values were computed and mortality curves were plotted on logarithmic graph paper to determine the LC_{50}. The doses were marked on the x-axis and the corresponding percentages of mortalities on the y-axis were plotted for each sample. Statistical analysis was performed on the data. The samples were contrasted with the biosal(Tariq & Qadri, 2002), a neem-based formulation with an LC_{50} of 400 ppm against the identical dengue vector mosquito.

4. ANALYSIS OF LC-Me AND LCM-PES VIA GC/GC-MS

In this study LC-Meand LCM-PES underwent analysis usingGC/GC-MS. Identification of compounds relied on retention time, fragmentation design, molecular formulaand matching with reported data by using data search of NIST library.Below are the tables 1 and 2 of all the compounds which are identified.

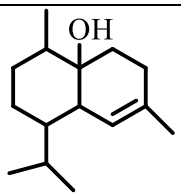
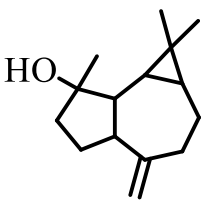
Table1. Compounds from the methanol soluble extract(LC-Me) of leaves(*L. camara)*

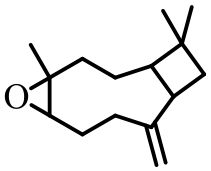
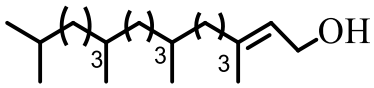
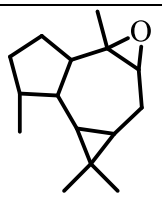
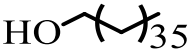
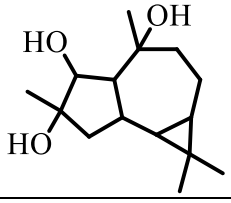
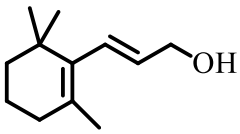
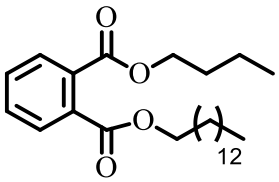
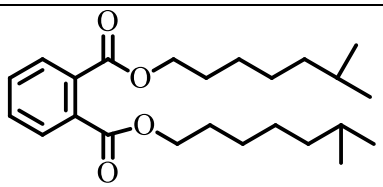
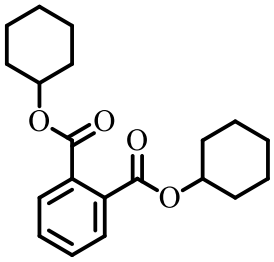
S.No	RI[a]	Retention Time[b](min)	Percentage of compounds	Name of compound/molecular formula	Structure	Mass Fragmentation
1	1014	9.9	2.0	Pyridine, 2,4,6-trimethyl ($C_8H_{11}N$)		121, 106, 79, 77, 51, 42
2	1494	18.3	1.6	Caryophyllene ($C_{15}H_{24}$)		204, 133, 105, 93, 69, 41
3	1410	18.8	1.9	Phenol, 2-methoxy-5-(1-propenyl)-, (E)- ($C_{10}H_{12}O_2$)		164, 149, 137, 91, 77, 55
4	1579	19.1	0.6	α-Caryophyllene ($C_{15}H_{24}$)		204, 189, 121, 93, 80, 69
5	2528	26.5	2.3	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester ($C_{25}H_{42}O_2$)		270, 227, 87, 67, 55, 43
6	1978	27.5	12.6	Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$)		284, 101, 88, 69, 57, 43
7	1976	27.7	11.1	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)		256, 213, 129, 61, 57, 41
8	2139	30.3	1.3	9,12-Octadecadienoyl chloride, (Z,Z)- ($C_{18}H_{31}ClO$)		294, 109, 95, 81, 67, 41
9	2183	30.8	1.7	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$)		292, 121, 108, 95, 79, 67

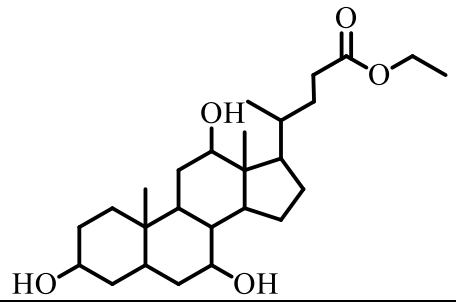
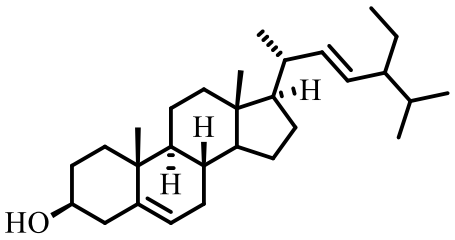
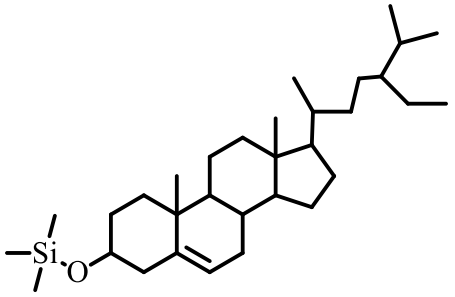
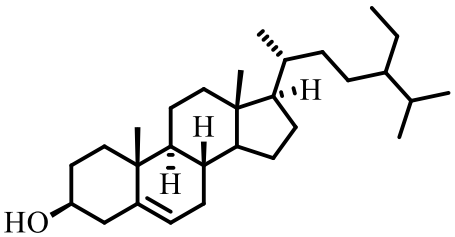
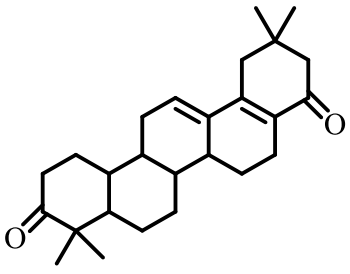
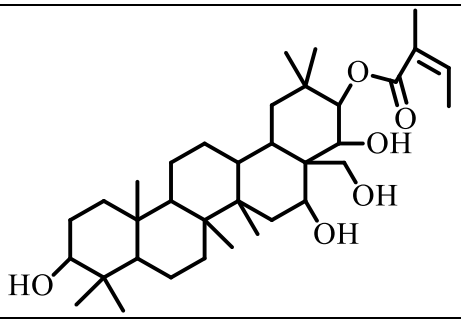
10	2185	31.7	4.2	9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$)		280, 109, 95, 81, 67, 55
11	2193	31.9	2.7	(E)-9-Octadecenoic acid ethyl ester ($C_{20}H_{38}O_2$)		310, 264, 101, 88, 69, 55
12	2201	32.6	9.1	Linoleic acid ethyl ester ($C_{20}H_{36}O_2$)		308, 109, 95, 81, 67, 55
13	2704	56.8	31.9	Ethyl 9,12,15-octadecatrienoate ($C_{20}H_{34}O_2$)		306, 101, 108, 95, 79, 67
14	2914	60.7	2.3	1,2-Benzenedicarboxylic acid, diisooctyl ester ($C_{24}H_{38}O_4$)		279, 167, 149, 70, 57, 41
15	2183	69.6	6.2	Di-n-octyl phthalate ($C_{24}H_{38}O_4$)		279, 167, 149, 70, 57, 41
16	3942	74.4	8.7	Squalene ($C_{30}H_{50}$)		218, 137, 121, 95, 81,69

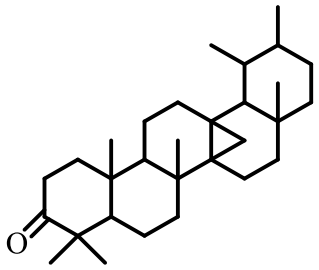
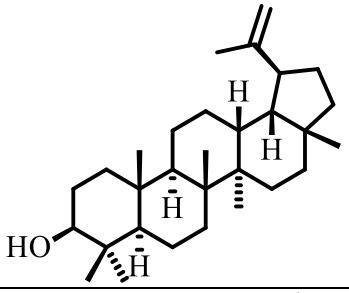
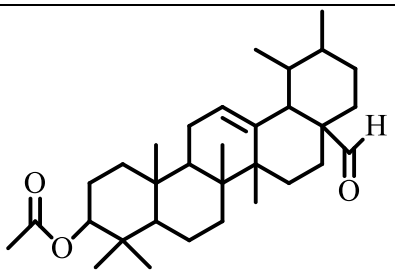
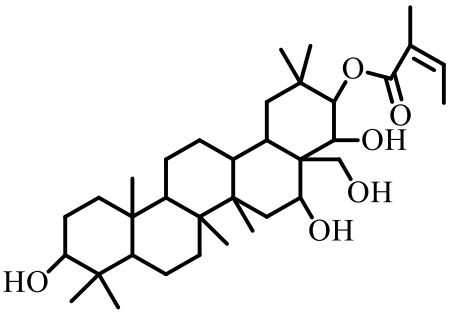
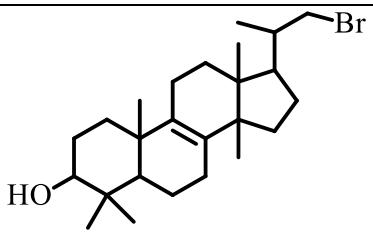
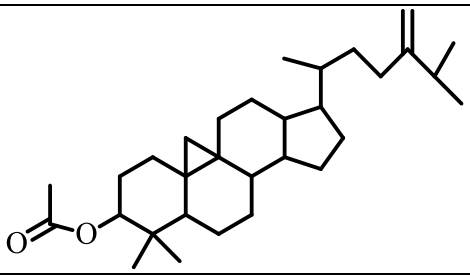
[a] The retention indices (RI), specifically non-isothermal Kovats retention indices, are determined on a ZB-5MS column in relation to C10-C30;
[b] compilation of compounds is arranged according to their elution sequence from a Zebron ZB-5 capillary column

Table 2: Compounds from the petroleum ether soluble fraction (LCM-PES) of leaves (*L. camara)*

S. No	RI[a]	Retention time[b] (min)	Percentage of compounds	Name of compound / Molecular formula	Structure	Mass fragmentation
1.	1580	20.3	0.16	3,7,11,15-Tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$)		123, 95, 82, 71, 68, 55, 41
2.	1536	22.0	2.2	Spathulenol ($C_{15}H_{24}O$)		205, 119, 105, 91, 79, 69

3.	1507	22.1	0.5	Caryophyllene oxide ($C_{15}H_{24}O$)		109, 91, 81, 69, 67, 55,43
4.	2045	24.1	0.5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$)		123, 95, 82, 71, 68, 57, 43
5.	1281	25.0	0.2	Isoaromaden drene epoxide ($C_{15}H_{24}O$)		107, 93, 81, 67, 55, 43,27
6.	3942	25.9	0.5	1-Heptatriacotanol $C_{37}H_{76}O$ 536		95, 91,81, 69, 55,43
7.	1869	26.0	1.7	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl ($C_{15}H_{26}O_3$)		163, 107, 93, 81, 69, 55,43
8.	1465	26.5	1.2	β-Citrylideneethanol ($C_{12}H_{20}O$)		147, 121, 119, 105, 91, 81, 55,41
9.	3031	29.2	0.9	Phthalic acid, butyl tetradecyl ester ($C_{26}H_{42}O_4$)		223, 150, 71 ,69, 56, 55, 43, 41
10.	2704	56.3	1.6	1,2-Benzenedicarboxylic acid, diisooctyl ester ($C_{24}H_{38}O_4$)		167, 149, 83, 71, 55, 57, 41
11.	2561	58.3	0.4	1,2-Benzenedicarboxylic acid, dicyclohexyl ester ($C_{20}H_{26}O_4$)		249, 167, 149, 104, 83, 76,67

12.	2739	63.9	0.2	Ethyl iso-allocholate ($C_{26}H_{44}O_5$)		83, 69, 57, 44, 41, 29, 17
13.	2789	66.1	0.8	Stigmasterol ($C_{29}H_{48}O$)		255, 133,95, 83, 69, 55, 43
14.	2731	66.7	5.2	β-Sitosterol trimethylsilyl ether ($C_{32}H_{58}OSi$)		485,396,381, 357, 129, 73, 57, 55
15.	2932	68.4	2.5	β-Sitosterol ($C_{29}H_{50}O$)		107, 105, 95, 81, 69, 55, 43
16.	4116	69.7	13.0	14,17-Nor-3,21-dioxo-β-amyrin, 17,18-didehydro-3-dehydroxy- ($C_{28}H_{40}O_2$)		409, 408, 203, 189, 69, 55
17.	2819	70.3	2.1	Barringtogenol B ($C_{35}H_{56}O_6$)		264, 215, 207, 297, 105, 95, 55

18.	3201	70.7	1.5	13,27-Cycloursan-3-one ($C_{30}H_{48}O$)		424, 205, 138, 123, 95, 69, 55.
19.	4116	73.8	1.5	Lupeol ($C_{30}H_{50}O$)		109, 95, 81, 69, 55, 43, 41
20.	2819	74.3	1.7	28-Oxours-12-en-3-yl acetate ($C_{32}H_{50}O_3$)		249, 232, 203,189, 175, 133, 119
21.	3201	75.0	1.7	Olean-12-ene-3,16,21,22,28-pentol, 21-(2-methyl-2-butenoate), [3β,16α,21β(Z),22α]- ($C_{35}H_{56}O_6$)		264, 215, 207, 197, 105, 81, 69,55
22.	4116	75.2	57.9	Androst-8-en-3-ol, 4,4,14α-trimethyl-17-(2-bromo-1-methylethyl ($C_{25}H_{41}BrO$)		423, 421, 405, 119,95, 69, 55
23.	2696	78.3	1.9	9,19-Cyclolanostan-3-ol, 24-methylene-, acetate, (3β ($C_{33}H_{54}O_2$)		422 , 407, 175, 95, 69, 55,43

[a] The retention indices (RI), specifically non-isothermal Kovats retention indices, are determined on a ZB-5MS column in relation to C10-C30;

[b] Compilation of compounds is arranged according to their elution sequence from a Zebron ZB-5 capillary column

Table3. Antibacterial activity of LC-Me and LCM-PES (zones of inhibition in millimeter).

BACTERIA TESTED	LC-Me	LCM-PES[a]
GRAM POSITIVE		
Bacillus cereus	14	12
Bacillus subtilis	10	10
Bacillus thurengiensis 1	15	10
Bacillus thurengiensis2	10	10
Bacillus thurengiensis3	10	10
Bacillus thurengiensis4	10	10
Micrococcus luteus	9	7
Micrococcus lysodekticus	0	10
Staphylococcus aureus	10	7
Staphylococcus epidermidis	0	10
Corynebacterium hoffmanii	0	10
Corynebacterium diphtherae	10	7
Corynebacterium xerosis	0	10
MRSA (Methicillin Resistant *Staphylococcus aureus*)	9	9
Streptococcus faecalis	7	0
Streptococcus faecalis 064	0	9
Streptococcus faecalis 2400	7	0
Streptococcus pyogenes	7	0
Streptococcus pneumoniae	0	0
Staphylococcus saprophyticus	0	0
GRAM NEGATIVE		
Salmonella typhi	0	7
Salmonella para typhi A	0	7
Salmonella para typhi B	0	9
Escherichia coli wild type	10	0
Escherichia coli 40 MT	10	0
Escherichia coli 5014	10	0
Shigella dysenterae	10	10
Shigella flexneri	12	0
Shigella boydii	7	7
Proteus mirabilis	9	0
Proteus vulgaris	9	0
Pseudomonas aeruginosa	9	20
Klebsiella pneumoniae	0	0
Enterobacter aerogens	0	7
Enterobacter aerogens ATCC 6541	0	9

[a] Conc.: 500µg/disc

Table 5. The repellent effect of LC-Me and LCM-PES on *Aedes aegypti* at 2%.

Sample	Duration of repellency (hour)						
	%R %B	0 h	0.5 h	1 h	1.5 h	2 h	2.5 h
LC-Me	R B	94 06	82 18	76 24	68 32	64 36	49 51
LCM-PES	R B	80 20	76 24	32 68	18 82	10 90	00 100
Mospel (20% DEET) (Positive control)	R B	100 00	100 00	100 00	100 00	100 00	100 00
MeOH (Negative control)	R B	00 100	- -	- -	- -	- -	- -

R=Repellency; B=Biting

Table 6. LC_{50} values of the LC-Me and LCM-PES were determined against 4th instar larvae of *Aedes aegypti*.

Sample	LC_{50} (ppm)
LC-Me	20
LCM-PES	400
Biosal (Neem formulation)*	400

* Positive control; Negative control: MeOH

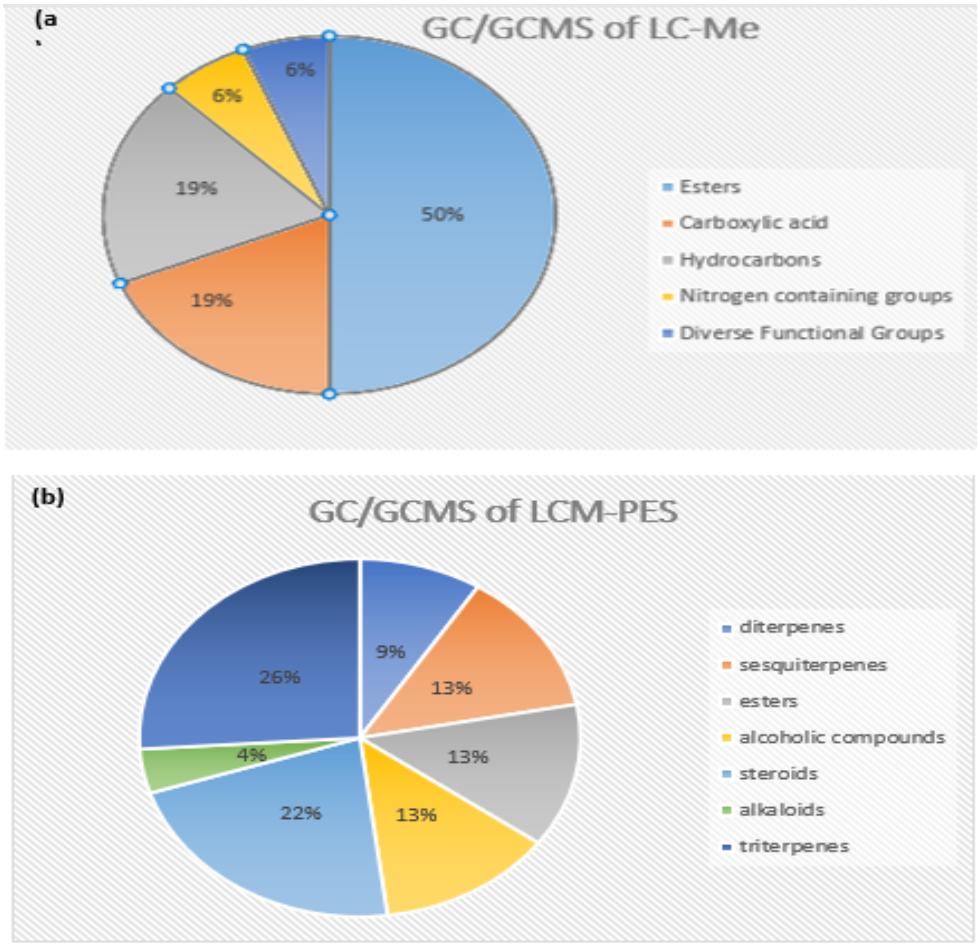


Figure 1; Distribution of natural products in **(a)** LC-Me and **(b)** LCM-PES by GC/GC-MS

5. RESULTS AND DISCUSSION

Sixteen compounds were identified in methanol soluble (LC-Me) extract (Table 1). The chemical profile (Figure 1a) is dominated by the presence of the esters (50 %), mainly ethyl 9,12,15-octadecatrienoate (31.9.%) and ethyllinoleate (9.1 %) with antimicrobial and mosquito repellent activities (Kumar et al., 2021). The overall percentage of carboxylic acids and hydrocarbons was found to be 38. The other classes of phytoconstituents were identified as 12%.The petroleum ether soluble (LCM-PES) fraction analysis by GC/GC-MS allowed to identify 23 compounds (Table 2), covering triterpenes (26%), steroids (22%), sesquiterpenes, alcoholic compounds and esters (13%), diterpenes (9%), alkaloids (4 %).The overall percentage of carboxylic acids and hydrocarbons are found to be 26 (Figure 1b).

The methanolic extract (LC-Me) was found operative against 13 out of 20 Gram-positive and 10 out of 16 Gram-negative microorganisms verified at 100mg/mL. The LC-Me has showed 15- and 14-mm zone of inhibition (ZOI) against *Bacillus thurengiensis 1* and *Bacillus cereus*. The antibacterial effect of petroleum ether soluble fraction (LCM-PES) was evaluated against 15Gram positive and 7 Gram negative microorganisms at 50mg/mL concentration. Significant results were obtained against *Bacillus thurengiensis 1* and *Bacillus cereus B* (10 and 12 mm ZOI).Exposure to *Bacillus thuringiensis* has led to respiratory, eye and skin irritation (Swandener, 1994). *B. cereus* is perhaps a universal soil bacterium, a cunning pathogen that is a mutual reason of food poisoning (Helgason et al., 2000). Our results reveal the potential use of *L. camara* as a remedy against *Bacillus thurengiensis 1* and *Bacillus cereus B* bacteria (Table 3).

The *in vitro* antifungal activity of LC-Me and LCM-PESwere determined against five dermatophytes, eight filamentous and two other fungi. The methanolic extract showed a broad-spectrum at 100 mg/mL. The petroleum ether-soluble part showed strongest activity (35 mm ZOIC) against *Microsporum gypseum*.In this study, both the extract and fraction have been found potent against sporadic dermatophytosis (Table 4).

The extract (LC-Me) and its fraction (LCM-PES) underwent assessment for their repellent effects against the dengue vector mosquito. LC-Me exhibited marvelous repellency which was up to 94% whereas LCM-PES have showed 80% effects against *Aedes aegypti*at similar concentration of 2% (Table 5).Additionally, the mosquito larvicidal tests of LC-Me and LCM-PES were conducted on the fourth instar larval stage (immature phase) of *Ae. aegypti*. The methanolic extract exhibited highest toxicity with an LC_{50} of 20ppm, demonstrating greater activity as compared to the standard biosal, a neem-based formulation(LC_{50} 400ppm)while its petroleum ether soluble fraction showed similar toxicity as for biosal(LC_{50} 400ppm). The recent findings indicate that the methanolic extract of *L. camara* and its constituents could serve as a promising origin for developing potent natural larvicides. This study also marks the initial documentation of the larvicidal potential of the methanolic extract and its petroleum ether-soluble fraction (Table 6).

6. CONCLUSION

In the present work, a complete GC/GC-MS analysis of methanol soluble extract of *Lantana camara* Linn.and its petroleum ether soluble fraction has been reported. This research comprehensively explored not only the percentage composition of chemical constituents in methanol and petroleum ether soluble parts but also provided useful results of the antibacterial, antifungal, mosquito repellent and larvicidal activities of the plant. Thus, the study also establishes the significance of plants used in Ayurvedic medicine which might be substantial attention to the development of innovative drugs.

5. REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide, Journal of Economic Entomology, 18(2), 265-267.

Ahmed, Z. F., Shoaib, A. E. M., Wassel, G. M. and El–Sayyad, S. M. (1972). Phytochemical study of Lantana camara I, Planta Indica,21(3), 282-288.

Ashford, R. W. (2000). The leishmaniases as emerging and reemerging zoonoses. International Journal for Parasitology,30 (12-13), 1269-1281.

Ayub, A., Tauseef, S., and Zehra, S. Q. (2017). Antimicrobial activity Lantana camara Linn. FUUAST Journal of Biology,7(1), 127-130.

Basu, S., Ghosh, A., and Hazra, B. (2005). Evaluation of the antibacterial activity of VentilagomadraspatanaGaertn., Rubia cordifolia Linn., and Lantana camara Linn.: isolation of emodin and physcion as active antibacterial agents, Phytotherapy Research, 19(10), 888-894.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method, American Journal of Clinical Pathology, 45(4), 493-496.

Begum, S., Wahab, A., Siddiqui, B. S., and Qamar, F. (2000). Nematicidal constituents of the aerial parts of Lantana camara, Journal of Natural Products, 63(6), 765-767.

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., and Hay, S. I. (2013). The global distribution and burden of dengue, Nature,496(7446), 504-507.

Bhuvaneswari, S., Sivakumar, J., Ragini, S., Rajkumar, M., and Venu, S. (2022). Effect of Lantana camara Leaf Crude Extracts on Third and Fourth Instar stages of Aedes Mosquito Larvae, International Journal of Research and Aalytical Reviews 9(4), 455-466.

Bokhari, F. M. (2009). Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia, Mycopath, 7(1), 51-57.

Chermette, R., Ferreiro, L., and Guillot, J. (2008). Dermatophytoses in animals, Mycopathologia, 166, 385-405.

Desjeux, P. (2001). The increase in risk factors for leishmaniasis worldwide. Transactions of the Royal Society of Tropical Medicine and Hygiene, 95 (3), 239-243.

Dua, V. K., Gupta, N. C., Pandey, A. C., and Sharma, V. P. (1996). Repellency of Lantana camara (Verbenaceae) flowers against Aedes mosquitoes, Journal of the American Mosquito Control Association, 12(3 Pt 1), 406-408.

Ganjewala, D., Sam, S., and Khan, K. H. (2009). Biochemical compositions and antibacterial activities of Lantana camara plants with yellow, lavender, red, and white flowers. EurAsian Journal of BioSciences, 3(10), 69-77.

Ghisalberti, E. L. (2000). Lantana camara L. (Verbenaceae), Fitoterapia, 71(5), 467-486.

Giudice, M. C., Reis-Menezes, A. A., Rittner, G. M. G., Mota, A. J., and Gambale, W. (2012). Isolation of Microsporumgypseum in soil samples from different geographical regions of Brazil, evaluation of the extracellular proteolytic enzymes activities (keratinase and elastase) and molecular sequencing of selected strains, Brazilian Journal of Microbiology, 43(9), 895-902.

Helgason, E., Økstad, O. A., Caugant, D. A., Johansen, H. A., Fouet, A., Mock, M., and Kolstø, A. B. (2000). Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensisone species based on genetic evidence, Applied and Environmental Microbiology, 66(6), 2627-2630.

Jaipal, S., Singh, Z., and Chauhan, R. (1983). Juvenile-hormone-like activity in extracts of some common Indian plants, Indian journal of Agriculture Sciences, 53(8), 730-733.

Kalita, S., Kumar, G., Karthik, L., and Rao, K. V. B. (2012). In vitro antioxidant and DNA damage inhibition activity of aqueous extract of Lantana camara L. (Verbenaceae) leaves, Asian Pacific Journal of Tropical Biomedicine, 2(3), S1675-S1679.

Kensa, V. M. (2011). Studies on phytochemical screening and antibacterial activities of Lantana camara Linn. Plant Sciences Feed, 1(5), 74-79.

Kumar, S. R., Chozhan, K., Murugesh, K. A., Rajeswari, R., & Kumaran, K. (2021). Gas chromatography-mass spectrometry analysis of bioactive compounds in chloroform extract of Psoralea corylifolia L, Journal of Applied and Natural Science, 13(4), 1225-1230.

Marchisio, V. F., Preve, L., and Tullio, V. (1996). Fungi responsible for skin mycoses in Turin (Italy). Mycoses, 39(3-4), 141-150.

Pattnaik, S., and Pattnaik, B. (2010). A study of Lantana camara Linn aromatic oil as an antibacterial agent, International Journal of Pharmaceutical Science, 32(1),1132-1135.

Ritsuko, H. A. G. A., and Suzuki, H. (2002). Tinea capitis due to Microsporumgypseum, European Journal of Dermatology, 12(4), 367-368.

Sharma, O. P., Makkar, H. P. S., and Dawra, R. K. (1988). A review of the noxious plant Lantana camara, Toxicon, 26(11), 975-987.

Swadener, C. (1994). Bacillus thuringiensis (BT), Journal of Pesticide Reform, 14(3), 13-20.

Tariq, R. M., and Qadri, S. S. (2001). Repellent activity of some local plant's oil, two commercial repellents, di-methyl phthalate and non-alcoholic bitter against dengue vector mosquitoes, Pakistan Journal of Entomology Karachi,16, 7-10.

Tariq, R. M., and Qadri, S. S. (2001). Repellent activity of some local plant's oil, two commercial repellents, di-methyl phthalate and non-alcoholic bitter against dengue vector mosquitoes, Pakistan Journal of Entomology Karachi, 16, 7-10.

Tsang, P., Hopkins, T., and Jimenez-Lucho, V. (1996). Deep dermatophytosis caused by Trichophyton rubrum in a patient with AIDS, Journal of the American Academy of Dermatology, 34(6), 1090-1091.

WHO Expert Committee on Insecticides, & World Health Organization. (1973). Safe use of pesticides: Twentieth report of the WHO Expert Committee on Insecticides [meeting held in Geneva from 10 to 16 October 1972]. World Health Organization.