RESEARCH ARTICLE

Insecticidal and Antimicrobial Activity of Ethanolic Extract of *Lantana camara* and GC-MS Analysis

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Abstract

Weeds are still a major problem all over the world. People are not able to remove all the weeds from the earth. It is a demand of time that we must move towards a positive aspect of weeds. This work follows the same approach. The *Lantana camara* leaves are used to develop a herbal insecticide product. The *L. camara* leaves possess insecticidal, fungicidal, and antibacterial properties. During the process of development of the product, ethanolic extract of leaves was analyzed for its antimicrobial analysis, secondary metabolite analysis and activity against insects. Antimicrobial activity was tested against three pathogenic bacterial strains, i.e., *Micrococcus luteus*, *Citrobacter freundii*, *Staphylococcus aureus* and two fungal strains, i.e., *Aspergillus niger* and *Paecilomyces sinensis*, *P. sinesisis*, which is an Entomopathogenic fungus, infect and kill harmful insects. In some cases, it is also found as Endophytic fungi. The maximum zone of inhibition was found in *C. freundii* and *A. niger* when tested with 10% concentration of ethanolic extract of *L. camara*. GC-MS was used to analyze secondary metabolites, and the results show 27 compounds. The research reveals that diethyl phthalate has the maximum area percent in this ethanolic extract, which is mainly used in insecticides. The plant *Calotrope gigantea* was tested for its anti-insecticidal properties against the insect *Oleander aphid*, and on the *Hibiscus rosa-sinensis* against the insect *Aphis fabae*.

Keywords: Anti-insecticidal, Antimicrobial activity, Ethanolic extract, GC-MS, *Lantana camara*, Secondary metabolites, zone of inhibition.

Highlights

- GC-MS analysis of various ethanolic extracts of *Lantana camara* was examined, in which 27 compounds were detected, amongst which 4 are novel compounds.
- Antioxidants, anticancer, antifungal, antibacterial, antiviral and anti-inflammatory activities have been found earlier in the leaves of *L. camara*.
- Insecticidal activity of ethanolic extract was tested.

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Introduction

Weeds are unwanted, persistent, and harmful plants that hinder the growth of other agricultural plants and negatively impact human activities, agriculture, natural processes, and the national economy. The Cannabaceae family of plants includes the weed plant, which has been used for both therapeutic and recreational purposes for thousands of years. Chemical insecticides are used to control pests or stop them from acting unfavorably or destructively (Mariajancyrani *et al.*, 2014). Chemical insecticides pose a risk to humans and have the potential to destroy more creatures than planned. Additionally, they affect aquatic creatures when they interact with water sources due to leaching, drift, or runoff. Birds die when they consume contaminated insects and drink contaminated water. Chemical pesticides are determined to be extremely harmful to the environment, humans, and animals over the long term. As a result, there is increasing social pressure to reduce pesticide use and progressively replace it with biopesticides, which are safe for both humans and non-target creatures (Swamy *et al.*, 2015).

The most widely distributed species of the genus *Lantana* is *Lantana camara L.,* which is a member of the Verbenaceae family and is commonly referred to as wild or orange sage. It is a very famous evergreen weed that may be found almost everywhere on the earth. It is also considered as a garden ornamental plant. It is commonly utilized to treat a variety of health issues in many traditional medical systems. Many human diseases are cured using various plant parts. *L. camara's* essential oil and plant extracts have a variety of bioactivities, including antibacterial properties. The presence of several bioactive phytocompounds in the plant is what gives it its medicinal potential. For the past ten years, experts and researchers from all around the world have thoroughly investigated the chemical makeup of the entire plant of *L. camara* as well as its biological functions (Saraf *et al.*, 2011).

The word "Raimuniya" is the Hindi name for *Lantana camara* (Fig. 1). A thorny shrub reaching a height of 2 to 3 m, *L. camara* grows vertical, partially climbing, or rarely more or less drape. Angled stems and branches with curved spines are positioned along the edges. The leaves are straightforward, opposite, decussate, oval, regularly dentate, and have an acute tip. An inflorescence is a hemispherical head that can be axillary or

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Fig. 1: *Lantana camara*

terminal, and it can be yellow, pink, or orange in color. It is made up of several little tubular flowers. The fruits are tiny, meaty drupes with a diameter of around 3 mm with colors ranging from blue to black. A stalk with long hairs covering it that is between 5 and 7 mm long carries the cotyledons. The lamina is 5 mm long and 6 mm wide, oval in shape, light green, and emarginate at its base. Simple, opposite decussate leaves are linked by a 2 to 3 mm long, short, hairy petiole (Ganjewala *et al.*, 2013). The blade is small, oval, hispid, and has a ragged border. It measures 10 to 12 mm long by 6 to 8 mm wide. Its superficial root system, which can grow to a height of 2 to 4 m or more, is made up of a small taproot and lateral roots that branch out to create a carpet of roots. *L.camara* seeds can grow all year long if the right circumstances for moisture are present. In the first wet season, thickets develop after slow development and establishes a solid root structure. The dry season's flowering lasts until the end of the rainy season when the fruits are fully developed. L. camara is an evergreen perennial plant that reproduces via seeds, flowers, and fruits. Birds, monkeys, and rivers washing downstream all spread seeds. Bisexual *Lantana* species have functional male (androecium) and female (gynoecium) gonads, as well as stamens, carpels, and ovaries (Negi *et al.*, 2019).

As a result, the current study was conducted to see if the ethanolic extract of *L. camara* could substitute chemical pesticides. Leaves of *L. camara* possess insecticidal, fungicidal, and antibacterial properties. The leaves are broadly circular, opposite, and plain. When crushed, they release a potent odor (K, 2017). The *L.camara* fruit is a bean that appears dark purple as it matures and it resembles to berry. Both people and animals are unable to eat green, unripe fruits. Birds and other animals consume the mature, dark purple fruits, which disperse the seeds over great distances and aid in the spread of the plant.

The ethanolic extract of *L. camara* has great potential for inhibiting damage caused by various types of insects to horticulture and agricultural crops. It is an organic substitute for chemical insecticides. It shows a significant impact on crop plants and flowering plants. Consequently, the purpose of this work is to minimize the use of chemical Insecticides by plant extracts from natural sources to control insects, and also to protect farmers and gardeners from harmful chemical exposure. The ethanolic extract of *L. camara* was named "Lantocide."

Materials And Method

Collection and Preparation of Plant Material

The leaves of *L. camara* were collected from the reserve forest area of Jabalpur district, Madhya Pradesh. Leaves were first washed thoroughly with tap water and then dipped in sterile distilled water to remove the fine dust particles. After washing, leaves were oven-dried at the temperature 100°C for 45 minutes to 1 hour and, finely powdered by grinding in pestle mortar and then strained with the help of a strainer.

Extract preparation from leaves of *Lantana camara*

Three different concentrations of ethanolic extract of *L. camara* were prepared to evaluate the best concentration. 1, 5 and 10 g of dried powdered leaves of *L. camara* were dissolved in three different flasks containing 100 mL of 70% ethanol. Then, the solution was boiled on a hot plate for 40 to 45 minutes at 100 to 150°C. After boiling the solution was cooled up to room temperature and then filtered with the help of Whatman filter paper no. 1. Filtrates of different concentrations (1, 5 and 10 g) were then applied to analyze their antimicrobial activity against three human pathogenic bacterial strains, one fungal strain and one Entomopathogenic fungal strain.

Bacterial strains for antibacterial activity

Pure cultures of *Micrococcus luteus* [Causing agent of sepsis, or endocarditis - an infection of the lining of the heart], *Citrobacter freundii* [Leading pathogens of nosocomial infections], *Staphylococcus aureus* [Causing agent of skin infections, food poisoning and septic arthritis] were used for analyzing the antibacterial activity of different ethanolic extracts of *L. camara*. The bacterial suspensions of the above pure cultures were prepared by inoculating the lyophilized form of the pure strains into the nutritional broth. These cultures were maintained on sterile nutrient agar media (NAM) slants and stored at 4°C until further use.

Fungal strains for antifungal activity

Pure cultures of *Aspergillus niger* [causing agent of black mold on certain fruits and vegetables, several allergies, infections in lungs and other organs], *Paecilomyces sinensis* [causing many types of infections in humans and other animals] were employed to examine the antifungal properties of several *L. camara* ethanolic extracts. The fungal suspensions of the above pure cultures were prepared by inoculating the lyophilized form of the strains into the nutritional broth. These cultures were maintained on sterile potato dextrose agar media (PDA) and stored at 4°C until further use.

Antimicrobial tests of ethanolic extract of *Lantana camara* by well diffusion method

The antimicrobial activity of the ethanolic extract of *L.camara* was tested by the well diffusion method. 20 to 25 mL of melted agar media was poured into each petri plate. Nutrient agar media (NAM) was used for antibacterial and potato dextrose agar (PDA) was used for antifungal tests, respectively. About 15 µL of pure cultures were inoculated on solidified agar plates. Four wells in each plate were created using a cork borer. Three wells were filled with 50 µL of ethanolic extract of *L. camara*, each well with a different concentration (1, 5 and 10 g) and one well was kept as a control (Ethanol). The plates were then kept in an incubator for 24 hour at 35°C for analyzing antibacterial activity and at 28°C for the antifungal activity.

Measurement of zone of inhibition against pathogenic bacteria and fungi

After incubation, the antimicrobial activity was evaluated by measuring the zone of inhibition around each well for each bacteria and fungi. The zone of inhibition was measured in millimeters. One well is created as a control in each petri plate.

GC-MS analysis of various ethanolic extracts of *L.camara*

Gas chromatography-mass spectrometry (GCMS) analysis of ethanolic extract of *L.camara* was carried out at the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, Delhi. The analysis was performed on a Shimadzu GC-MS-QP-2010 ultra system.

Following operating conditions: oven temperature program from 60°C with holding time of 2 minutes, 250°C at 10°C/min with holding time of 5 minutes, and the final temperature was 280°C kept for 12 minutes at 15°C/min. The injector temperature was maintained at 260°C, pressure 73.3 k Pa, total flow 16.3 mL/min, column flow 1.21 mL/min, linear velocity 40.1 cm/sec, purge flow 3.0 mL/min, split ratio 10.0, ion source temperature 220°C, scan mass range 40 to 650 m/z, and interface line temperature 270°C. The identification of compounds was performed by comparing the mass spectra with data from NIST14.lib (National Institute of Standards and Technology, US) and WILEY8.LIBlibraries.

Insecticidal activity of ethanolic extract of *L.camara*

The insecticidal activity of *L. camara* was tested on the infected plants *Calotrope gigantean* against the insect *Oleander aphid* and *Hibiscus rosa-sinensis* against the insect *Aphis fabae*. The names of the insects were confirmed by the Department of Zoology, St. Aloysius' College, Autonomous, Jabalpur, M.P. Ethanolic extract of *L. camara* was sprayed on the leaves of infected plants where insects and larvae were present. It was sprayed about 1 to 2 mL in every three days. Observations were made after every third day of spray.

Results And Discussion

The findings of the antibacterial activity of ethanolic extract of *L.camara* in three different concentrations (1, 5 and 10 g) are shown in Table 1. The best results of antibacterial and antifungal were revealed by the ethanolic extract of 10 g/100 mL, after which GC-MS analysis was documented.

Antibacterial activity of leaf extract of *L.camara*

Ethanolic extracts of the leaf of *L.camara* showed strong antibacterial activity against pathogenic bacterial strains. A clear, prominent zone of inhibition was obtained against *Micrococcus luteus* and *Citrobacter freundii* in 5 and 10% concentrations. In the case of *M. luteus*, the zone of inhibition measured around 11.66 mm in 5% extract and 11.0 mm in 10% extract (Fig. 2). zone of inhibition was obtained around 16.6 mm in 5% extract, and it was increased with increasing concentration of leaf extract (10%), i.e.,

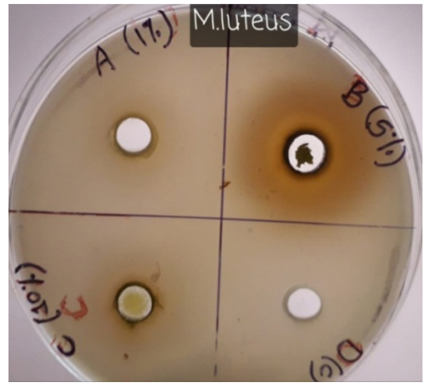


Fig 2: Antibacterial activity of extract of *L. camara* against *M. luteus*

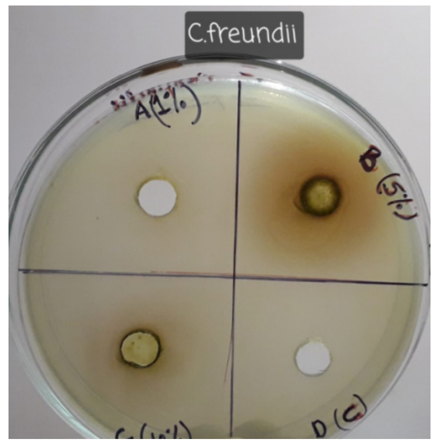


Fig 3: Antibacterial activity of extract of *L. camara* against *C. freundii*

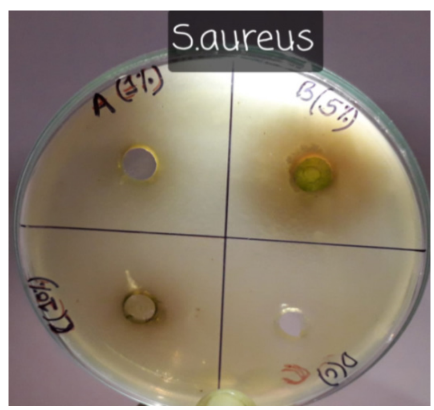


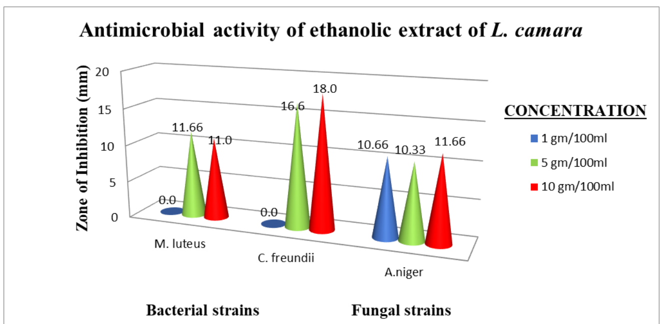
Fig 4: Antibacterial activity of extract of *L. camara* against *S. aureus*

18.0 mm against *C. freundii* (Fig. 3). When *Lantana* leaves extract concentration increases against *M. luteus* and *C. freundii*, the zone of inhibition increases, indicating increased antibacterial activity. However, *Lantana* leaves extract did not exhibit any action against *S. aureus* (Fig. 4). The highest zone of inhibition was observed against *M. luteus* (Graph 1). The antibacterial potential of extracts was evaluated in terms of the inhibition zone (Table 1).

For food and water-borne diseases pathogenic bacteria are one of the major causative agents. Pathogenesis refers both to the mechanism of infection and to the mechanism by which

Table 1: Antimicrobial activity of different concentrations of ethanolic extract of leaves of *L. camara*

S. No.	*Microbe's Name*	*Concentration of ethanolic extract of L. camara (gm/100 mL) (%)*	*Zone of inhibition (mm)*
A.	*Bacterial strains (Bacteria)*		
1.	M. luteus	1	No zone
		5	11.66
		10	11.0
2.	C. freundii	1	No zone
		5	16.6
		10	18.0
3.	S. aureus	1	No zone
		5	No zone
		10	No zone
B.	*Fungal strains (Fungi)*		
1.	niger	1	10.66
		5	10.33
		10	11.66
2.	P. sinensis	1	No zone
		5	No zone
		10	No zone



Graph 1: Antimicrobial activity of ethanolic extract of *L. camara*

disease develops (Kishore, 2021). They cause damage to the cells and tissues by releasing toxins and spread through skin contact, body fluids, air, contaminated items and water (Váradi *et al.*, 2017). Earlier, it was studied that *Pseudomonas aeruginosa* can cause pneumonia and blood infections; *Mycobacterium tuberculosis* causes tuberculosis; *Listeria* causes food-borne infections and flu-like symptoms; *Salmonella* causes typhoid and *E. coli* causes intestinal infections with symptoms like fever and diarrhea (Pandey *et al.*, 2014). Methanolic extract of *L. camara* was used against *Escherichia coli, Staphylococcus aureus, Enterobacter fecalis, Proteus vulgaris, Micrococcus luteus* and *Salmonella typhi* (Anand *et al.*, 2018); (Swamy *et al.*, 2015).

In our studies, the antibacterial activity of the ethanolic extract of *L. camara* was examined against two gram-positive bacteria, *M. luteus* causes brain abscess, native valve endocarditis, bacteremia, and septic arthritis in immune suppressive patients (Erbasan, 2018); (Ianniello *et al.*, 2019). *S. aureus* causes skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis

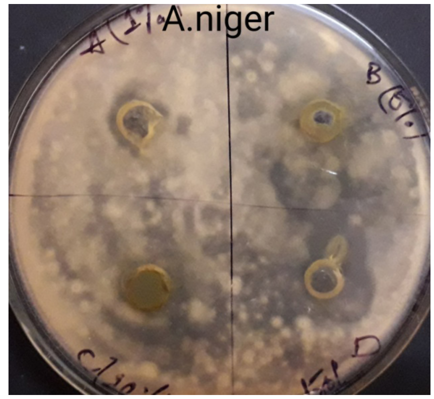


Fig. 5: Antifungal activity of extract of *L. camara* against *A. niger*

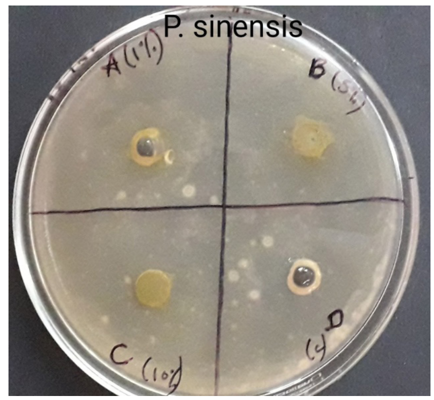


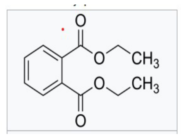
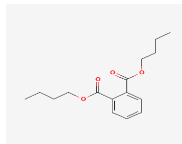
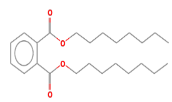
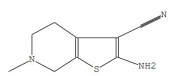
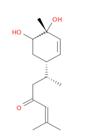
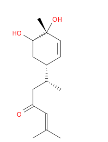
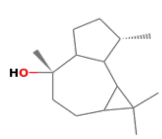
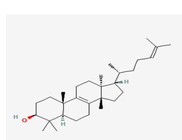
Fig. 6: Antifungal activity of extract of *L. camara* against *P. sinensis*

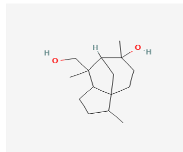
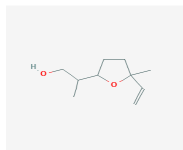
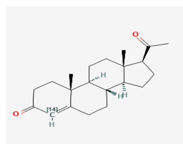
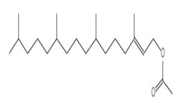
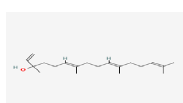
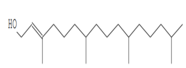
(Ghalehnoo, 2018). *C. freundii* is a gram-negative bacterium that causes infections in the urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, wounds, soft tissue, meninges, and the bloodstream (Liu *et al.*, 2018). These strains have been chosen based on their potential for use in further formulation research. Industrial use of *L. camara* is, it is used to activate carbon prepared from the stems of *L. camara* plant, which is investigated as an adsorbent for the removal of chromium (VI) from polluted water using batch methods of extraction (Ravulapalli & Kunta, 2018). The decoction made from *L. camara* leaves is mostly used in herbal therapy to treat fever, cough, influenza, stomach aches, malaria and wounds. It has also been reported to be effective in treating ulcers, rheumatism, chickenpox, and cancer (Gillela *et al.*, 2024).

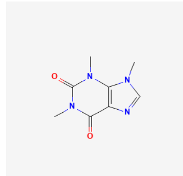
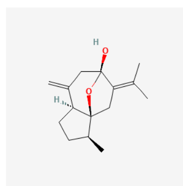
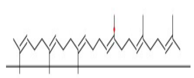
Antifungal activity of leaf extract of *L.camara*

Ethanolic extracts of the leaf of *L. camara* showed strong antifungal activity against pathogenic fungal strains. A clear, prominent zone of inhibition was obtained against *Aspergillus niger*, in 1, 5 and 10% concentrations of ethanolic extract of *L. camara.* Zone of inhibition was obtained around 10.66 mm in 1%, 10.33 mm in 5% and 11.66 mm in 10% against *A. niger* (Fig. 5). Enhancement in the size of zone of inhibition is an indication of increasing antifungal activity with increasing concentration of *Lantana* leaf extract against *A. niger, Lantana* leaves extract was not screening any activity against *P. sinesisis* (Fig. 6). Maximum zone of inhibition was observed against *A. niger* (Graph 1). The

Table 2: GC-MS analysis of ethanolic extract of *L. camara* leaves

S. No.	*Functional group*	*Name of compound*	*Chemical structure*	*Rt (retention time)*	*Area%*	*Applications*
1.	Benzoic acid	Diethyl Phthalate $C_{12}H_{14}O_4$		14.053	81.40	Making plastics, insecticides, cosmetics and aspirin. (https://pubchem.ncbi.nlm.nih.gov/compound/Diethyl-phthalate)
		Dibutylphthalate $C_{16}H_{22}O_4$		17.897	0.50	Used as a plasticizer. Also used in some cosmetic products like nail polishes. Used in printing inks and ectoparasiticide. (https://pubchem.ncbi.nlm.nih.gov/compound/Dibutyl-phthalate)
		Di-n-octylphthalate $C_{24}H_{38}O_4$		23.419	0.15	Colorless viscous liquid, use in plastics, coating products cosmetics and pesticides. (https://pubchem.ncbi.nlm.nih.gov/compound/Dioctyl-phthalate)
2.	Carboxy group	2-amino-3-cyano-6-methyl-4,5,6,7-tetra hydropyrido(3,4-b) thiophene $C_9H_{11}N_3S$		14.457	0.06	Primary bile acid, insoluble in water, white crystalline substance. (https://pubchem.ncbi.nlm.nih.gov/compound/Acetophenone-thiosemicarbazone)
3.	*Carbonyl group*	2-Pentadecanone,6,10,14-trimethyl $C_{18}H_{36}O$		16.655	0.25	Used by several species of insect as a pheromone. (https://pubchem.ncbi.nlm.nih.gov/compound/6_10_14-Trimethylpentadecan-2-one#section=Depositor-Supplied-Synonyms)
		8-Octadecanone $C_{18}H_{36}O$		14.823	0.25	-
4.	*Hydroxyl group +* alcohol	(S, E)-6-Hydroxy-6-methyl-2-((2S,5R)-5-methyl-5-vinyltetr $C_{15}H_{24}O_3$		15.121	0.11	Taken from turmeric. In vitro, it has several effects including anti-inflammatory, antioxidant, and anti-metastatic properties.(https://pubchem.ncbi.nlm.nih.gov/compound/Walleminone)
		(S, E)-6-Hydroxy-6-methyl-2-((2S,5R)-5-methyl-5-vinyltetr $C_{15}H_{24}O_3$		15.199	0.43	Taken from turmeric. In vitro, it has several effects including anti-inflammatory, antioxidant, and anti-metastatic properties. (https://pubchem.ncbi.nlm.nih.gov/compound/Walleminone)
		Ledol $C_{15}H_{26}O$		16.077	0.44	Poisonous sesquiterpene that can cause cramps, paralysis, and delirium. Caucasian peasants used Rhododendron plants for these effects in shamanistic rituals. (https://en.wikipedia.org/wiki/Ledol)
		1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl- $C_{30}H_{50}O$		28.802	0.06	It has several potential medicinal properties, like anticancer and anti-inflammatory activity. found in a variety of plants. It has a complex pharmacology, displaying antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemopreventive properties. (https://pubchem.ncbi.nlm.nih.gov/compound/5366014)

		(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]d/ Humulene epoxide $C_{15}H_{24}O$		14.275	0.64	Useful for its antioxidant properties, anti-inflammatory activity. a food additive.(https://pubchem.ncbi.nlm.nih.gov/compound/Humulene-epoxide)
		Cedran-diol,(8S,14)- $C_{15}H_{26}O_2$		35.347	1.61	-
5.	Oxolanes	LilacalcoholD $C_{10}H_{18}O_2$		15.404	0.52	Found in many flowers and spice plants. pleasant smell. an antimicrobial agent, and an aroma compound. used in manufacturing of soaps, fragrances, food additives as flavors, household products, and insecticides. (https://pubchem.ncbi.nlm.nih.gov/compound/Lilac-alcohol)
6.	Methyl group	Pregn-4-ene-3,20-dione / beta.-progesterone $C_{21}H_{30}O_2$		16.402	0.37	Popular herbal dietary supplement. Used in treatment of cancer. it acts as an antagonist. (https://pubchem.ncbi.nlm.nih.gov/compound/Pregn-4-ene-3_20-dione-4-14C)
		Neophytadiene $C_{20}H_{38}$		16.569	0.23	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. (https://pubchem.ncbi.nlm.nih.gov/compound/Neophytadiene)
		Phytol, acetate $C_{22}H_{42}O_2$		16.823	0.07	Phytol has been investigated for its potential anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects. (https://pubchem.ncbi.nlm.nih.gov/compound/Phytyl-acetate)
		Geranyl linalool isomer b $C_{20}H_{34}O$		18.509	0.11	Colorless waxy solid. It also used in the post-translational modification known as geranylgeranylation. Geranylgeraniol is a pheromone for bumblebees and a variety of other insects. (https://pubchem.ncbi.nlm.nih.gov/compound/5365872)
		Undec-10-ynoicacid, tridec-2-yn-1-ylester $C_{24}H_{40}O_2$		14.531	0.63	Used by predators to locate blood or prey. (https://pubchem.ncbi.nlm.nih.gov/compound/Undec-10-ynoic-acid_-tridec-2-yn-1-yl-ester)
		Phytol $$ 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- $C_{20}H_{40}O$		19.303	1.88	Potential anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects. (https://pubchem.ncbi.nlm.nih.gov/compound/Phytyl-acetate)

7.	Alkaloid group	1H-purine-2,6-dione, 3,7-dihydro-1,3,7-trimeth / coffein $C_8H_{10}N_4O_2$		17.197	0.46	Applied to various insects (milkweed bugs, caterpillars, mosquito larvae, etc.) causes agitation, reduce appetite, inhibits reproduction, and can even lead to death. Caffeine-treated mosquito larvae, for example, become so poorly coordinated that they can no longer swim and end up drowning. (https://laidbackgardener.blog/2017/11/29/caffeine-a-powerful-organic-insecticide/#:~:text=Caffeine%20extracts%20applied%20to%20various,swim%20and%20end%20up%20drowning.)
8.	Alkane hydrocarbon	Octadecane,1-isocyanato / Isocyanic acid, octadecyl ester $C_{19}H_{37}NO$		21.479	0.06	Applications as auxiliary materials for wool fabric softeners and intermediates for synthetic auxiliaries. Also used for matrix interactions,to investigate in vitro hemocompatibility assay using freshly drawn human whole blood. (https://www.sigmaaldrich.com/IN/en/product/aldrich/305405)
9.	Monocarboxylic acid	5Beta.-guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-/ isocurcumenol, (+)- $C_{15}H_{22}O_2$		36.240	0.77	Treating gastrointestinal disorders, pain, inflammatory conditions, wounds, and for cancer prevention and antiaging. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6164907/)
10.	Triterpenoids	Squalene $C_{30}H_{50}$		27.602	0.64	It contains properties of anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient activities. (https://pubmed.ncbi.nlm.nih.gov/22361190/#:~:text=Several%20studies%20exhibited%20results%20that,models%20and%20in%20vitro%20environments)

antifungal potential of extracts was evaluated in terms of the fungal growth inhibition zone (Table 1).

Pathogenic fungi cause infection in humans or other organisms. Approximately 300 fungi are known to be pathogenic to humans (Viegas *et al.*, 2019). Fungal plant pathogen species are primarily in the phyla Ascomycota and Basidiomycota (Doehlemann *et al.*, 2017). Pathogenic fungi, particularly yeasts, have the ability to gain access to the bloodstream, reaching the internal organs of patients and causing systemic or disseminated fungal infections (Barros *et al.*, 2022). Earlier, it was studied that *Candida albicans* can cause heart, brain, eyes and blood infections; *Cryptococcus neoformans* causes infection in the immune systems; *Aspergillus fumigates* damages lungs; *Coccidioides immitis* occurs by breathing into the lungs; *Histoplasma capsulatum* causes histoplasmosis with symptoms like mild flu; *Blastomyces dermatitidis* causes infection in lungs through inhalation and *Pneumocystis jirovecii* causes damage in lungs without causing symptoms, individuals with weakened immune systems, such as people with HIV/AIDS, cancer patients, and people with inflammatory or autoimmune diseases are at greatest risk (Martin-Loeches *et al.*, 2022). Methanolic extract of *L. camara* was used against *C. albicans* and *A. niger* (Zare, 2021).

In our studies, the antifungal activity of the ethanolic extract of *L. camara* was examined against *Aspergillus niger* causes cutaneous infections and pulmonary disease (Dai *et al.*, 2016). *P. sinensis* is not a pathogenic fungus. It enhances soil fertility. In the present work, *L. camara* has not shown any antifungal activity against *P. sinensis.* Such types of extract that are supported for beneficial microbes can be suggested to apply as an insecticide in agricultural land. Their particular activities against pathogenic microbes can support agricultural crop systems. It proves that *L. camara* can be used in soil because it does not cause any damage to *P. sinensis*.

Our findings are consistent with earlier research that has established ethanol as the most effective solvent for recovering more extractable chemicals from a variety of medicinal plants. Formerly, ethanolic extract was used less by researchers for their studies than methanolic extract (Mansoori *et al.*, 2020). However, a review of the literature reveals that, as of now, there are very few publications on the comparative yield analysis derived from ethanolic solvent (Swamy *et al.*, 2015).

GC-MS Analysis

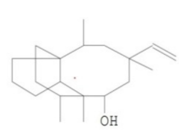
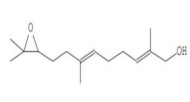
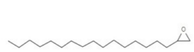
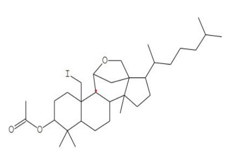
GC-MS is an efficient method for the identification of compounds present in the extract. Here only the best result: 10% ethanolic extract of *L. camara* was sent to JNU, Delhi. In this extract, the GC-MS chromatogram showed the presence of 27 compounds. According to our study, these 27 compounds can be categorized into two different classes (Table 2) and (Table 3). The names of different compounds identified have been mentioned in both Tables 2 and 3 with their Rt (retention time) and with different percent areas and structures. Out of 27, 23 compounds (Table 2) are mentioned in the literature for their characteristics and economic importance. We can state the remaining four compounds out of 27 as novel compounds, as they are not described in the literature for their characteristics (Table3).

Most valuable medicines are made up of phytochemicals, which are very essential to health. Several chemicals which are derived in this GC-MS report act as a drug that is currently used in more countries in the world. The various phytocomponents have been identified from the GC-MS chromatogram (Fig. 7) and compared with the NIST and WILEY libraries.

Methanolic extract of *L.camara* revealed 65 peaks in previous studies based on separation, retention time and percent area. The mass spectra of all identified compounds were matched with WILEY8.LIB and NIST08.LIB and the function of only 19 components was predicted, functions of other components could not be identified due to a shortage of library data equivalent to compounds. Most of the components identified and reported belong to different biological activities like antioxidants, anticancer, antifungal, antibacterial, antiviral, anti-inflammatory, etc. (Mansoori *et al*., 2020).

In our report peak of 27 components are present, among which application of 23 components were found and 4 are novel compounds. Out of 27, 3 compounds come under the functional group of benzoic acid, i.e., diethyl phthalate, dibutyl phthalate and di-n-octyl phthalate, which is used in making insecticides, plastics and beauty goods like nail polishes. 1 compound i.e., 2-amino-3-cyano-6-methyl-4,5,6,7-tetra hydropyrido(3,4-b) thiophene comes under the carboxy group, which is used in the making primary bile acid and white crystalline substances. 2 compounds, i.e., 2-Pentadecanone,6,10,14-trimethyl; 8-Octadecanone comes under *Carbonyl group* which is used by several species of insect as a pheromone. *6 compounds, i.e.,* (S,E)-6-Hydroxy-6-methyl-2-((2S,5R)-5-methyl-5-vinyltetr; (S,E)-6-Hydroxy-6-methyl-2-((2S,5R)-5-methyl-5-vinyltetr; Ledol; 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-; (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]d/ Humulene epoxide; Cedran-diol,(8S,14)- *comes under Hydroxyl group* + alcohol group which is having some biological activities like antioxidant, anti-metastatic, anticancer, anti-inflammatory, antimicrobial, anti-inflammatory and antitumor. Also, ledol can cause cramps, paralysis, and delirium. One chemical, namely LilacalcoholD, is categorized as an oxolane and is utilized in the production of household goods, soaps, pesticides, fragrances, and food additives. 6 compounds i.e., Pregn-4-ene-3,20-dione/ beta.-progesterone; Neophytadiene; Phytol, acetate; Geranyl linalool isomer b; Undec-10-ynoicacid,tridec-2-yn-1-ylester; Phytol $$ 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- comes under methyl group which is having some biological activities like anti-inflammatory, antimicrobial, antioxidant, antinociceptive, antimicrobial and cytotoxic properties. Also can be used in the treatment of cancer and post-translational modification. 1 compound, i.e., 1H-purine-2,6-dione, 3,7-dihydro-1,3,7-trimeth/ coffein, comes under the alkaloid group, which is used in making insecticides. 1 compound, i.e., Octadecane, 1-isocyanate/ Isocyanic acid, and octadecyl ester comes under alkane hydrocarbons, which is used in making wool fabric softeners and intermediates for synthetic auxiliaries. 1 compound i.e., 5Beta.-guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-/isocurcumenol, (+)-comes under monocarboxylic acid, which is used for treating wounds, cancer and antiaging. 1 compound, i.e., squalene, comes under triterpenoids which contain properties like anticancer, antioxidant, drug carrier, detoxifier and skin hydrating. While 4 were novel compounds i.e., 2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradeca –; 9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-o -; Oxirane,hexadecyl-; Lanostan-3.beta.-ol,11.beta.,18-epoxy-19-iodo-,acetate –.

Table 3: Novel compounds found in GCMS analysis

S. No.	*Name of compound*	*Chemical structure*	*RT*	*Area%*
1.	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)] tetradeca – $C_{20}H_{34}O$		15.007	0.67
2.	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-o – $C_{15}H_{26}O_2$		15.775	1.59
3.	Oxirane,hexadecyl- $C_{18}H_{36}O$		17.012	0.21
4.	Lanostan-3. beta. -ol,11. beta.,18-epoxy-19-iodo-, acetate – $C_{32}H_{53}IO_3$		39.424	5.88

Insecticidal activity of ethanolic extract of *L.camara*

Insecticidal results revealed that 10% ethanolic extract of *L. camara* gave better results on the plant *Calotrope gigantea*

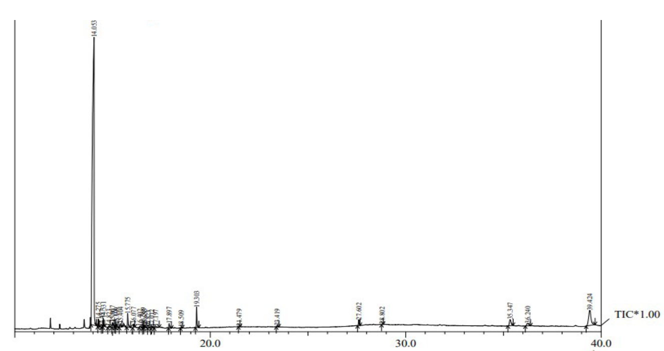


Fig. 7: Chromatogram of ethanolic extract of leaf of *L.camara*

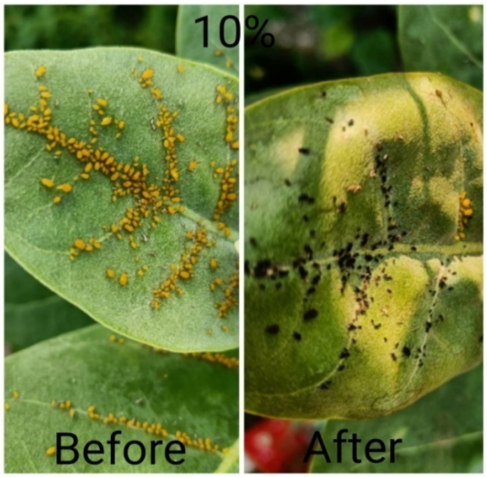


Fig. 8: Plant- C*alotrope giganea* Insect**-** *Oleander aphid* concentration- 10%

Fig. 9: Plant- C*alotrope giganea* Insect**-** *Oleander aphid* concentration- 5%

against the insect *Oleander aphid* (Figs 8 and 9) and *Hibiscus rosa-sinensis* beside the bug *Aphis fabae* (Fig. 10). Ethanolic extract of *L. camara* was sprayed on the leaves of infected plants where insects and larvae were present. Observations were made after every third day of spray. Yellow dots were present on *C. gigantea* diseased plants, indicating the presence of *O. aphid* on the leaves. After 3rd day of spraying of *L. camara* extract on *C. gigantea* leaves, yellow spots disappeared. The absence of yellow spots on *C. gigantea* leaves is an indication of the eradication of disease from them. The fresh, green and healthy appearance of *C. gigantea* leaves, even after 15 days, is confirmation that it is totally eliminated from its disease host. Therefore, yellow spots were absent on the plant.

Similar results were observed in diseased plants of *H. sinensis* had the presence of bright black spots, which indicate the presence of *A.fabae* on the leaves. After 3rd day of spraying of *L. camara* extract on *H.sinensis* leaves, black spots became deadly, which totally disappeared after 6 days of observation. The absence of black spots on *H.sinensis* leaves is an indication of the extermination of disease from them. *H.sinensis* leaves were undried, green and in good condition even after one month of

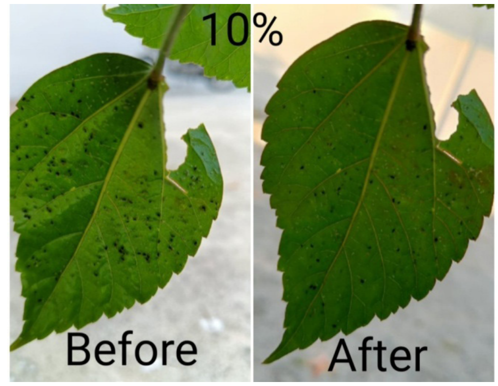


Fig. 10: Plant- *Hibiscus rosa-sinensis* Insect**-** *Aphis fabae* Concentration- 10%

spray, which was the confirmation that the disease host had completely removed the plant. Therefore, black spots were absent on the plant. On the basis of above observations, it is confirmed that 10% ethanolic extract of *L.camara* is referred as anti-insecticidal activity.

One of the biggest issues that reduces plant output is biotic stress brought on by insect pests. Synthetic pesticides still play a significant role in crop protection (Tlak Gajger & Dar, 2021). Biopesticides being eco-friendly, have significant advantages over conventional pesticides. They are also less prone to pest resistance and less toxic to non-target organisms. Biopesticides have diverse modes of action, making them versatile (Khursheed *et al*., 2022). So there is a need of herbal insecticide, so there is a replacement of herbal extract of *L. camara*. In the past, maize weevils were managed using ethanolic and methanolic extracts of *L. camara* against *Sitophilus zeamais* (Ayalew, 2020).

The ethanolic extract of *L. camara* was tested in our research for its anti-insecticidal properties against the *Oleander aphid*, which causes discoloration, curled leaves, yellowing, and slowed plant growth on *Calotrope gigantea* (Singh & Singh, 2021) and *Hibiscus rosa-sinensis* against the insect *Aphis fabae* which causes several damages through sucking sap, injuring leaves, the excretion of honeydew - encouraging the growth of sooty mold and the spread of plant viruses (Bennour *et al*., 2021).

Conclusion

The present investigation shows that the ethanolic leaf extract of *L. camara*, has a wide spectrum of bioactive compounds, which can be utilized as a medicinal source in the development of drugs. The identification of the composition and structure of biomolecules with potential therapeutic applications was detected by GC-MS. According to a preliminary screening of the ethanolic extract of *L. camara* leaf, the extract has antioxidant, anti-metastatic, anticancer, anti-inflammatory, antimicrobial and antitumor properties. The present study focuses on the antimicrobial properties of some more pathogens along with the insecticidal activity against plant-damaging insects. Reports detected from prior and present studies might result in the conception of medicinal formulations to save plants against insects and microbes. In our studies, on the basis of the qualities of the ethanolic extract of *L. camara,* an herbal insecticide (Lantocide) was prepared. Presently world is facing the problem of the dreadful effects of chemical pesticides. Therefore, we are here with a solution of herbal insecticide. And so this extract may be further researched for commercial use as an improved herbal insecticide.

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Author's Contribution

Conceptualization Dr. Mamta Gokhale, Data curation Kashish Agrawal, Formal Analysis Dr. Rumana Faraz, Investigation Kashish Agrawal, Methodology Dr. Mamta Gokhale, Project administration Dr. Rumana Faraz, Supervision Dr. Mamta Gokhale and Dr. Rumana Faraz, Validation Dr. Mamta Gokhale, Writing Kashish Agrawal, Writing-review and editing Kashish Agrawal, Dr. Mamta Gokhale and Dr. Rumana Faraz.

Conflict Of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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