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RESEARCH ARTICLE

Exploring termiticidal properties and GC/MS-based chemical profile of *Lantana camara* (L.)

Devindra Gundakanal[1], K Premalatha[1]*, M Murugan[1], PS Shanmugam[1], S Harish[2] & D Uma[3]

[1]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[2]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[3]Department of Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural University, Coimbatore 641 003, India

*Email: premalatha.k@tnau.ac.in

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Abstract

India accounts for almost 10% of the worlds' termite species. *Odontotermes horni* is one of the most important termites, posing significant threats to agricultural and horticultural ecosystems. The persistent nature of synthetic termiticides necessitates the development of alternative control strategies. The present study investigates the phytochemical composition and potential termiticidal and acetylcholinesterase (AChE) inhibitory properties of *Lantana camara* (L.) leaf against *O. horni.* Methanolic, ethyl acetate, and aqueous extracts of *L. camara* (L.) leaves were used to assess their toxicity. The methanolic extract exhibited the highest potency, with an LC_{50} of 0.98%, followed by ethyl acetate (1.40%) and water extract (8.81%). The per cent mortality recorded at 2.5% concentration for methanol, ethyl acetate, and aqueous extract was 96.20, 78.75 and 21.25, respectively. By fumigant action, the *L. camara* (L.) leaf extract at a 5% concentration of methanol, ethyl acetate and aqueous extract resulted in 68.75, 42.50 and 26.25 percent mortality, respectively. The methanolic extract showed the highest AChE inhibition (52.39%), followed by the ethyl acetate (47.00%) and the aqueous (43.53%) extracts after 24 hours of exposure. GC-MS analysis of plant volatiles of the *L. camara* (L.) revealed 196 compounds, including terpenoids, known for termiticidal activity.

Keywords

acetylcholinesterase inhibition; *Lantana camara* (L.); *Odontotermes horni*; termiticidal activity

Introduction

Termites are social insects that live in large colonies and feed on cellulose-based materials. A termite colony typically comprises reproductives (King and Queen), soldiers for defence, and workers tasked with foraging, grooming, nest maintenance, and caring for the young (1). Globally, termites are classified by their habits and habitats into wood and ground inhabitants, including mound-builders, subterranean termites, and carton-nest builders (1). In the genus *Odontotermes*, species such as *O. obesus*, *O. redemanni*, and *O. wallonensis* are known for building mounds, while *O. horni* is classified as a subterranean species. In India and Sri Lanka, *O. horni* is entirely subterranean, whereas in Cambodia, earthen mounds have been observed to be constructed up to one meter high (2).

Termites seriously threaten agriculture, particularly in water-stressed

regions, affecting many crops, including sugarcane, cotton, cereals, vegetables, fruits, legumes, oilseeds, and ornamental plants (3, 4). Successful control of termites has been a persistent challenge, mainly due to their cryptic feeding behaviour (Feeding in concealed or hidden locations makes them challenging to detect and target) (1). Applying slow-acting, non-repellent pesticides to the soil is suggested to control termites, particularly against subterranean species (5). Termites can be controlled through synthetic termiticides, which remain the primary method to prevent termite attacks on wooden structures and crops (6). However, the extensive use of synthetic insecticides has led to several challenges, including phytotoxicity, mammalian toxicity, pesticide resistance, secondary pest outbreaks, and environmental risks (5, 7). These problems highlight the necessity for alternative control methods (7).

Botanicals offer a potential alternative to highly hazardous synthetic pesticides for termite management. Various plant extracts and essential oils exhibit termiticidal activity against different termite species, comparable to synthetic termiticides (6, 8). So, potential plant-based termiticides must be evaluated and optimized to ensure practical feasibility and economic viability. One of the advantages of botanical termiticides is their diverse modes of action, including toxicity, antifeedant, repellent, and oviposition deterrent effects (9, 10). The plant the *L. camara* (L.) is rich in triterpenoids and other biologically active secondary metabolites and has been traditionally used in Indian medicine for its antimicrobial, fungicidal, insecticidal, and nematicidal properties (11). Previous studies have demonstrated that extracts from its aerial parts exhibit insecticidal activity against various pests (12, 13). Hence, the present study evaluated the toxicity of *L. camara* (L.) against *O. horni* and to investigate its phytochemical composition.

Materials and Methods

Laboratory studies were carried out at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore (Latitude: 11.01 N; Longitude: 76.92 E) to investigate the insecticidal property of *L. camara* (L.) leaf extract against subterranean termites (*O. horni*).

Collection and identification of termites

Termite soldiers and workers were collected from the field and placed in plastic containers. In the laboratory, termites were carefully separated from debris using forceps and a camel brush. For taxonomic identification, soldiers were preserved in vials containing 70% ethyl alcohol and stored at room temperature. Workers intended for molecular characterization were preserved in absolute alcohol vials and stored at -20°C. Soldiers were examined under a stereo zoom binocular microscope (LEICA M205C), and morphometric measurements were recorded for five soldiers per sample using Leica Image Analyzer software (version 4.12.0). Identification of the soldiers was based on standard keys and descriptions (2, 14, 15).

Plant extraction

The leaf samples of *L. camara* (L.) collected from Chinmaya Nagar, Coimbatore, Tamil Nadu (Latitude: 11.02; Longitude: 76.89) were shade-dried for 7-10 days (Fig. 1.). Then, the shade-dried leaves were powdered using a mixer grinder. One kilogram of *L. camara* (L.) leaves yielded 292 grams of powder. Different solvents, including methanol, ethyl acetate, and water, were extracted using a Soxhlet apparatus. A total of 30 g of *L. camara* (L.) leaf powder was placed in the thimble. The round-bottom flask was filled with 300 millilitres of solvent, and the apparatus was maintained at temperatures of 65–75°C for methanol and ethyl acetate and 100°C for water for six hours. The crude extract was obtained by evaporating the solvents using a rotary vacuum evaporator at temperatures below 45°C for 30 min. After the evaporation of solvents from *L. camara* (L.) leaf extracts, the recovery percentage of each solvent extract was calculated. The recovery percentages of methanol, ethyl acetate, and water extracts were 13.33, 11.86 and 16.83%, respectively.

No-choice bioassays for evaluating toxic potential

No choice bioassay was conducted using the standard filter paper method (16). The filter papers were immersed in extracts (methanol, ethyl acetate, aqueous) at concentrations of 0.5%, 1%, 1.5%, 2%, and 2.5% for durations ranging from 10 to 30 sec, followed by air drying at room temperature for approximately 10 to 15 min. Distilled water-soaked filter papers were used as the control. Subsequently, each dried filter paper was positioned at the base of a 5 cm diameter and 1 cm height Petri dish. Twenty workers were introduced to each Petri dish and exposed to the treatments. Each treatment was replicated four times. The Petri dishes were incubated in a growth chamber at 25 ± 2° C temperature, with 85% relative humidity, and in com-

Fig. 1. *L. camara* (L.) leaf sample for extraction.

plete darkness. Mortality rates were monitored and recorded after 24 hours. Deceased and inactive termites were gathered using a camel hairbrush, and the percentage mortality rate was calculated. Based on per cent mortality, the test was serially conducted with different ranges of concentrations.

Fumigant toxicity bioassay

The fumigant toxicity test was carried out using standard procedures with slight modifications (17). Filter paper discs with a diameter of 4.25 cm were impregnated with methanol, ethyl acetate, and aqueous extracts of *L. camara* (L.) leaf at varying concentrations (1%, 2%, 3%, 4%, and 5%). Subsequently, these treated filter papers were positioned at the top of mud pots (4.5 cm in diameter and 8.6 cm in height). Within each mud pot, a diet cup measuring 3.8 cm in diameter and 4 cm in height, housing 20 workers, was placed and covered with a 60 mesh cloth. The mud pots were sealed with lids, subjecting the insects to the treated filter papers for 3 days. Each treatment was replicated four times, and the mortality count was documented at 24 hour intervals. (Fig. 2.).

ACh esterase inhibitory activity

The enzyme activity of termite workers' acetylcholinesterase (AChE) was evaluated after exposure to LC_{50} concentrations from no-choice bioassays of solvent extracts of *L. camara* (L.) leaf by following standard methodology

Fig. 2. Experimental set-up for fumigant study of *L. camara* (L.) leaf extracts against *O. horni*.

(18). Twenty termites were sampled from each control and treatment group. Subsequently, their cephalic regions were excised to facilitate the determination of AChE activity. The heads were washed with ice-cold phosphate buffer (0.1 M, pH 7.5) and homogenized in 1 mL of ice-cold phosphate buffer (0.1 M, pH 7.5) containing 10 mL L^{-1} Triton X-100 using a Teflon pestle. The homogenate was centrifuged at 20000 rpm for 15 min at 4°C. The resulting supernatant was re-centrifuged at 20000 rpm for 15 min at 4 °C and utilized as the enzyme source. Enzyme aliquots (50 µL) and DTNB (100 µL, 0.01 M) were added to 2.8 ml of 0.1 M phosphate buffer (pH 8.0). This mixture added 20 µL of dimethyl sulfoxide (DMSO), and the solution was incubated at 37°C for 15 min. The reaction was initiated by adding 30 µL of ATChI, followed by an additional 10 min incubation at 37°C. Absorbance was measured at 412 nm using a Unico 2802 UV spectrophotometer. The percentage inhibition was determined using the following formula in Eqn. 1.

Collection of volatiles using a volatile collection unit

A Volatile Collection Unit was employed to extract volatiles from *L. camara* (L.) plant samples. Initially, the chamber was pressurized with air to create a vacuum. The plant samples were then hermetically sealed within a glass jar,

$$\% \text{ Inhibition} = \frac{(\text{Absorbance Control} - \text{Absorbance Test})}{\text{Absorbance Control}} \text{X100} \quad \text{.....(Eqn. 1)}$$

and air was introduced through activated charcoal at a consistent rate of 100 millilitres per min. The volatiles were adsorbed onto Porapak Q tubes and subsequently eluted using 10 mL of HPLC-grade hexane. The eluted plant volatiles were subsequently concentrated to 1 mL by a turbo vacuum evaporator (30 bar). The resulting crude extract was introduced into a GC-MS to identify volatile compounds.

GC-MS analysis of volatiles from L. camara (L.)

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Shimadzu QP-2010 Plus instrument at the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore (19). The system was equipped with a split injector and an ion-trap mass spectrometer, with the detector temperature maintained at 220°C. A fused silica capillary column (RXI-1MS) with a thickness of 0.25 µm, a length of 30 m, and an inner diameter of 0.25 mm was utilized, operating over a temperature range of 60°C to 260°C. The temperature of the column was programmed to rise from 60°C to 260°C at a rate of 3.0°C per min. The mass range for the injector and detector was set to 500 m/z, with injector and detector temperatures maintained at 220°C and 200°C, respectively. Helium was the carrier gas, flowing at 0.95 mL per min. The chromatogram generated by the GC-MS was analyzed via mass spectrometry, and compound identification was achieved by comparing the spectra of unknown compounds to those in the National Institute of Standards and Technology (NIST) database.

Results and Discussion

The termites used in the study were *O. horni* (Wasmann) (Termitidae: Isoptera), which was confirmed by morphometric methods (2, 14, 15) and molecular methods (NCBI Accession number: PQ146896).

O. horni has a straw yellow to reddish brown head capsule with pale yellow to brown antennae and purplish brown mandibles (Fig. 3.). Mandibles are sabre-shaped, with the left featuring a prominent tooth. The post mentum is sub-rectangular, and the pronotum is saddle-shaped.

Toxicity of L. camara (L.) *leaf extracts*

The result for the no-choice test was different concentrations of methanol, ethyl acetate, and aqueous extract

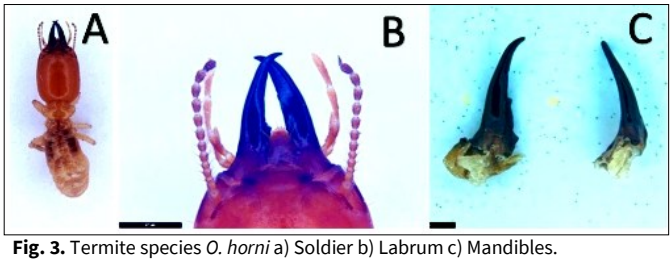

Fig. 3. Termite species *O. horni* a) Soldier b) Labrum c) Mandibles.

of *L. camara* (L.) leaf against *O. horni* was given in Table 1. The methanolic extract showed the highest efficacy, with termite mortality increasing significantly with concentration and time. The methanolic extract had an LC_{50} value of 0.98%, indicating strong effectiveness in inducing mortality. Similarly, the ethyl acetate extract showed an LC_{50} value of 1.40%, indicating significant efficacy against the termites.

In contrast, the aqueous extract had a much higher LC_{50} value of 8.81%, indicating lower efficacy than the other extracts. These results suggest that the methanolic and ethyl acetate extracts of *L. camara* (L.) are significantly more effective against *O. horni* than the aqueous extract, based on their respective LC_{50} values (Table 1). The percent mortality of *O. horni was* also substantially different from that of various solvent extracts. After 24 hours, the highest percent mortality was observed in 2.5% of a methanolic extract with 96.20%. Meanwhile, the lowest percent mortality (22.80%) was observed in 0.5% of methanolic extract.

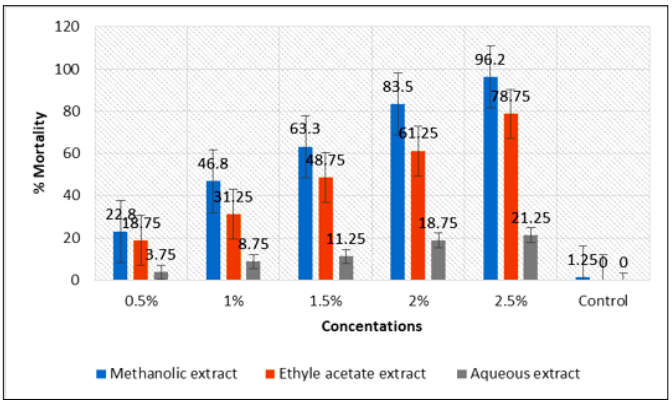

Fig. 4. Studies on bio efficacy of solvent extracts of *L. camara* (L.) leaf against *O. horni*: No choice test.

considering the mortality percentage of *O. horni* in different treatments of solvent extracts. At 74 hours after treatment (HAT), 5 % of the methanolic extract of *L. camara* (L.) showed the highest mortality percentage, 68.75%.

Similarly, the ethyl acetate extract also demonstrated increasing mortality, with a higher mortality percentage

Table 1 Probit analysis studies on bio efficacy of different solvent extracts of *L. camara* against *O. horni* (LC_{50})

Extracts	LC_{50} (%)	95 % Fiducial limit of LC_{50} (%)		χ2 value	Equation of regression line	R^2
		LL	UL			
Methanol	0.97	0.77	1.23	2.2539	y = 3.3618x + 5.067	0.9173
Ethyl acetate	1.40	1.08	1.82	1.1448	y = 2.3046x + 4.6707	0.9386
Aqueous	8.81	12.78	60.74	0.0909	y = 1.4134x + 3.6307	0.9802

Table 2 Probit analysis studies on fumigant toxicity of different solvent extracts of *L. camara* leaf against *O. horni* (LC_{50})

Extracts	LC_{50} (%)	95 % Fiducial limit of LC_{50} (%)		χ2 value	Equation of regression line	R^2
		LL	UL			
Methanol	3.36	2.42	4.68	0.2757	y = 1.8743x + 4.0139	0.9780
Ethyl acetate	6.24	4.13	9.44	0.4859	y = 2.8088x + 2.6432	0.9634
Aqueous	9.51	3.99	22.67	1.0071	y = 2.1566x + 2.5863	0.8912

Similarly, at 2.5% of ethyl acetate extract, the percent mortality was 78.75, whereas 0.5% ethyl acetate extract resulted in only 18.75% mortality at 24 HAT. The aqueous extract showed the least efficacy, with a mortality percentage of 21.25% at a 2.5% concentration. The untreated check in all the experiments resulted in zero mortality (Fig. 4.). The researchers assessed the efficacy of various plant species against *O. wallonensis*. They found that the aqueous extract of *L. camara* demonstrated superior effectiveness to extracts from other plant species. The present study revealed the exceptional effect of methanolic extract, consistent with prior studies suggesting that aqueous extracts generally require higher doses or longer exposure times to achieve similar efficacy to organic solvents (20, 21).

Fumigant toxicity

The methanolic extract was the most toxic to termites, with an LC_{50} value of 3.36%. The ethyl acetate extract had an LC_{50} value of 6.24%, while the aqueous extract had the highest LC_{50} value of 9.51%, indicating it was the least effective. (Table 2). A similar trend was observed while of 42.50% at 5%. In contrast, the aqueous extract exhibited the lowest toxicity across all concentrations, and 26.25% mortality was recorded at 5% (Fig. 5.). The fumigant action of *L. camara* is primarily due to its bioactive compounds like Caryophyllene oxide, Coumaran and Other volatile compounds in the extracts may also enhance its efficacy against stored grain pests, making *L. camara* a promising alternative to chemical fumigants (22). This is well documented in storage pest management (23).

AChE inhibition activity

The methanolic extract exhibited the highest percent inhibition, followed by the ethyl acetate extract and the aqueous extract. At 24 hours post-treatment, the methanolic extract inhibited AChE activity by 52.39%. The ethyl acetate extract achieved 47.00% inhibition, and the aqueous extract 43.53% by 24 hours (Table 3). This effect aligns with the mechanisms of anticholinesterase insecticides, which interfere with nerve-muscle coordination by preventing ACh breakdown (24). Studies have shown that terpenoids can effectively replace synthetic insecticides in controlling pests on stored products. Several reports indicate that terpenoids and various plant volatiles induce insect mortality by inhibiting the enzyme acetylcholinesterase (AChE)

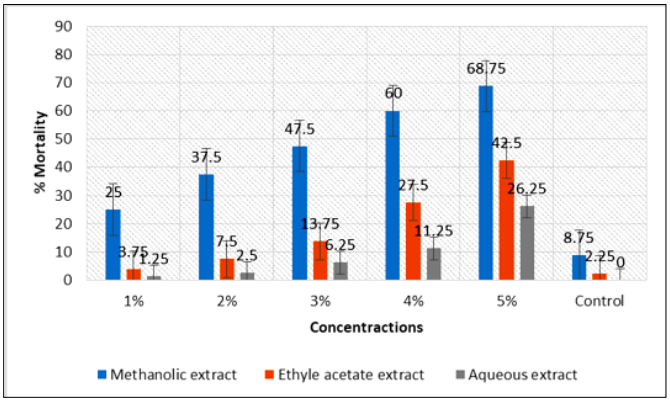

Fig. 5. Studies on fumigant toxicity of solvent extracts of *L. camara* (L.) against *O. horni*: Per cent mortality

(25). A known terpenoid, 1,8-cineole, has been described as a potent AChE inhibitor (26). It is well documented that *L. camara* (L.) contains a high concentration of terpenoids, including 1,8-cineole. Our study identified several terpenoids, including 1,8-cineole, α-terpineol, caryophyllene oxide, caryophyllene, and α-pinene, using GC-MS analysis. These findings further validate the presence of AChE inhibitory compounds in *L. camara* (L.).

Characterization of bioactive compounds in L. camara (L.)

The diverse bioactive compounds present in *L. camara* (L.) contributes significantly to its effectiveness in pest control. Understanding the phytochemical profile of plant volatile is crucial for identifying its potential applications in managing termite populations, especially given the growing demand for eco-friendly alternatives to synthetic pesticides. In this study, the plant volatiles from the leaves of *L. camara* (L.) were characterized by GC-MS analysis (Fig. 6.). Various compounds were identified that may play pivotal roles in their insecticidal properties. The study identified 196 compounds belonging to various classes of phytochemicals (Supplementary Table 1). The analysis identified several compounds with termiticidal properties. In the organoboron group, ethyl boronic acid comprised 0.517% of the total area. Among the sesquiterpenes, caryophyllene accounted for 0.440%, while the element isomer contributed 0.028%. Caryophyllene oxide, a sesquiterpene oxide, was present at 0.029%. These results are consistent with prior studies indicating the critical role of terpenoids and their derivatives in pest control (27, 28). Among the monoterpenes, thujone accounted for 0.064%, and α-pinene contributed 0.010%. The analysis also identified linalool, a terpene alcohol, at 0.040% and α-terpineol, another monoterpenoid alcohol, which accounted for 0.019%. In addition, eucalyptol (1,8-cineole) was found at 0.024% and (R)-(+)-α-terpineol, a monoterpenoid alcohol, at 0.006%. Previous studies have shown that these compounds can affect insect behaviour, further supporting the potential use of *L. camara* extracts in pest control (29-31). These compounds contribute to the termiticidal properties of the *L. camara* (L.).

Conclusion

The increasing threat of termites to agricultural productivity underscores the pressing need for pest control strategies that are both effective and environmentally sustaina-

Table 3 Studies on Ach esterase inhibition activity of solvent extracts of *L. camara* leaf against *O. horni*

Hours	Methanolic extracts of *L. camara* (0.97%)		Ethyl acetate extracts of *L. camara* (1.40 %)		Aqueous extracts of *L. camara* (8.81 %)	
	Enzyme activity*	% Inhibition	Enzyme activity*	% Inhibition	Enzyme activity*	% Inhibition
2	107.38 ± 0.88	5.10	109.29 ± 3.34	4.26	110.00 ± 0.67	3.95
4	93.88 ± 0.57	10.83	97.76 ± 3.12	9.01	101.67 ± 3.32	7.21
6	78.60 ± 0.91	18.41	82.32 ± 0.05	16.50	89.45 ± 0.73	12.96
12	47.00 ± 1.34	36.9	49.94 ± 0.91	34.96	55.71 ± 1.40	31.31
24	26.76 ± 0.60	52.39	33.16 ±1.01	47.0	37.65 ± 0.69	43.53

*enzyme activity in micromol min^{-1} mg $protein^{-1}$ values are followed by ± to indicate standard deviation

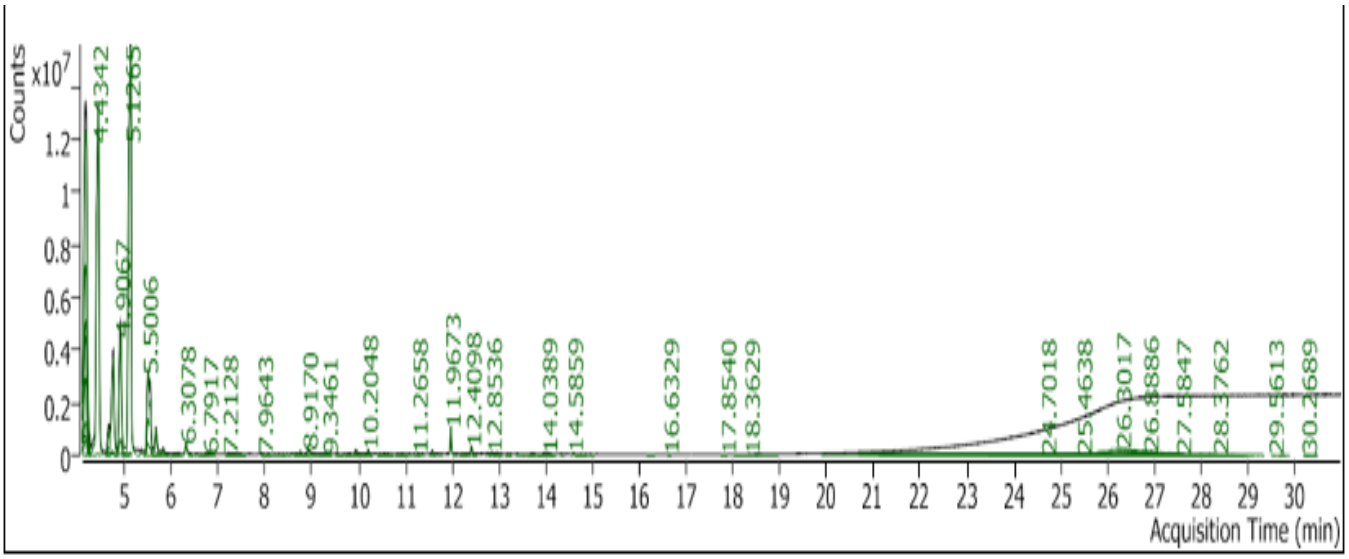

Fig. 6. Chemical Profile of the plant volatiles of *L. camara* (L.) leaf.

ble. The present study highlights the strong termiticidal properties of *L. camara* (L.) leaf extracts. Since *L. camara* (L.) is a known invasive species, its use for termiticidal purposes could also help mitigate its proliferation. Future research should focus on exploring the full potential of *L. camara* (L.) for pest control, considering both the environmental benefits and challenges associated with its widespread use.

Additionally, the upscaling of lab findings to field applications is crucial. Developing practical, cost-effective methods for large-scale extraction and application of its bioactive compounds could open up opportunities for commercial ventures. Young entrepreneurs could be encouraged to explore this avenue, focusing on sustainable pest management solutions that align with ecological and economic goals.

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Authors' contributions

KP conceived and designed the study, supervised the research, and contributed to manuscript writing and editing. DG conducted the laboratory experiments, collected the data, and performed the data analysis. MM and PSS assisted in the experimental design, data interpretation, and statistical analysis. SH, DU, and MM contributed to the review of the relevant literature and manuscript preparation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Supplementary data

Supplementary Table. 1. Studies on the phytochemical profile of *L. camara* leaf volatile: GC-MS analysis.

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