

Evaluation of plant-based extracts as a sustainable alternatives for termites control

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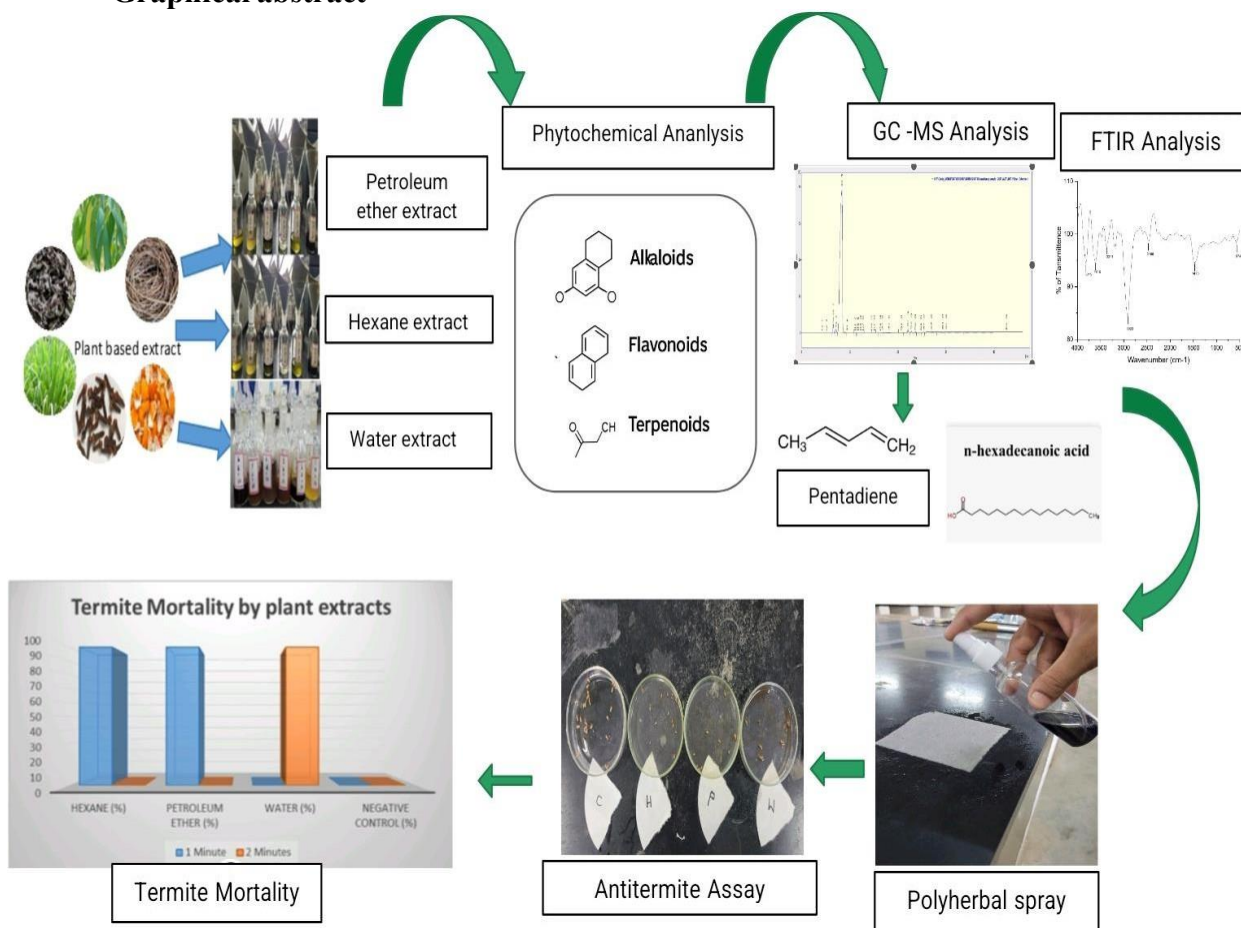
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ABSTRACT

Termites, highly structured social insects of the order Isoptera, are major biological threats to wooden infrastructure, agricultural products, plantations, and stored materials. Although chemical insecticides are widely used, their long-term application has led to increasing termite resistance, environmental degradation, and toxicity to non-target species. These drawbacks emphasize the growing need for eco-conscious, biodegradable alternatives. Plant-based biopesticides, rich in bioactive metabolites, offer a sustainable and safer approach to termite management. This study explores the insecticidal potential of six botanicals—*Eucalyptus globulus*, *Cymbopogon citratus*, *Syzygium aromaticum*, *Artemisia absinthium*, *Citrus sinensis*, and *Chrysopogon zizanioides*—using petroleum ether, hexane, and distilled water as extraction media. Phytochemical analysis confirmed the presence of key secondary metabolites, including alkaloids, saponins, flavonoids, steroids, carbohydrates, terpenoids, phytosterols, and quinones, while polyphenols, anthraquinones, and cardiac glycosides were absent. Advanced GC-MS profiling identified major constituents such as pentadiene and hexadecanoic acid, and FTIR spectroscopy revealed functional groups like hydroxyl (O–H), alkene (C=C), halogenated chains (C–C), amines (N–H), and alkyl aryl ethers. A distinctive aspect of this research is the comparative solvent-based assessment showing extremely rapid anti-termite activity. Hexane and petroleum ether extracts achieved 100% termite mortality within one minute, while aqueous extracts reached full lethality in two minutes. These findings establish the remarkable efficacy of solvent-optimized plant formulations as natural, fast-acting, and environmentally sustainable alternatives to conventional synthetic termiticides paving the way for safer pest control strategies in agricultural and structural settings.

Keywords: Termite Control, Plant-Based Extracts, Phytochemical Analysis, GC-MS Analysis, Sustainable Pest Management.

Graphical abstract



SUSTAINABLE GREEN ALTERNATIVES FOR TERMITE CONTROL

1. Introduction

Termites are highly destructive polyphagous pests that cause extensive damage to plants, finished products, household goods, and agricultural crops such as rice, sugarcane, millet and barley. They also affect fodder crops and create tunnels in tropical and subtropical soils. Despite their destructive nature, termites play a vital role as primary invertebrate decomposers, breaking down dead organic matter and contributing to key ecological processes like soil enrichment, plant decay, nitrogen and carbon cycling, and microbial activity. These insects are generally small, ranging in size from 4 to 15 millimeters (0.16 to 0.59 inches) in length (Bignell and Eggleton *et al.*, 2000).

Termites are social insects that live in organized groups known as colonies. A colony of termites consists of various castes, including workers, soldiers, nymphs, queens, and kings, each performing distinct roles and physical tasks. These insects belong to the order Blattodea, formerly classified as Isoptera, and are categorized into three primary groups: damp wood termites, dry wood termites, and subterranean termites (Paul and Rueben *et al.*, 2005). Termites, constructing the nests that can be underground, above ground, or in trees. Subterranean termites inhabit the soil and wood in contact with it, causing extensive destruction (Su and Scheffrahn *et al.*, 1988). Dry-wood termites, on the other hand, live exclusively within wood, where they nest and feed (Myles *et al.*, 2007). Damp-wood termites infest wood with varying degrees of moisture and decay, such as logs, stumps, dead trees, and structures exposed to wet soil, damp tree trunks, or environments with high humidity.

Termites play a critical role in agriculture but are also responsible for considerable economic losses due to their capacity to damage crops, trees, and structures, earning them the nickname "silent destroyers" (Sileshi *et al.*, 2010). While synthetic chemical termiticides such as cyclodiene, hydroquinone, indoxacarb and baiting system have been used for years, they have proven ineffective in significantly reducing termite populations and have harmful impacts on ecosystems (Rosemary *et al.*, 2023). Therefore, there is a need to explore alternative solutions, such as natural or plant-based pesticides with anti-termite properties is both timely and highly relevant (Badshah *et al.*, 2004)

India's rich biodiversity, including around 700 medicinal plant species, has been utilized for their

bioactive enzymes in traditional and folk medicine. Plant-derived natural compounds such as lignans, coumarins, flavonoids, carbohydrates, fatty acids, aromatic polyketides, terpenoids,

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steroids, and alkaloids are crucial in managing termites and pests (Haouas *et al.*, 2011). Although more than 1,000 plants have been studied for their insecticidal phytochemicals, only a few have been developed for commercial pest control applications. Among these, alkaloids represent the largest group of plant-based chemical toxins (Badshah *et al.*, 2004). These plants produce secondary metabolites as part of their natural defense mechanisms. They are essential in protecting the plants from other plants, fungi, insects, and germs etc.,

Researchers have investigated the toxicity, attraction, and repellent properties of plant extracts on various termite and insect species. Natural anti-termite compounds are valuable due to their diverse modes of action against different termite species (Haouas *et al.*, 2011). Despite this, there is still a pressing need for effective botanical formulations to mitigate termite-related losses (Amoabeng *et al.*, 2013). Liu *et al.*, 2024 formulated a nanoemulsion containing *E. globulus* essential oil and nutmeg, demonstrating enhanced termiticidal activity, midgut damage, and enzyme inhibition in *Odontotermes formosanus* compared to bulk oil. Pramod *et al.*, 2023 studied on *Syzygium aromaticum* stem and leaf essential oil noted high fumigant toxicity against stored-product pests, with over 96% mortality at 100 μ L after 5 days, attributable to eugenol and β -caryophyllene. In another study Muhammad Zeeshan *et al.*, 2020 evaluated comparative bioefficacy against *Odontotermes obesus* (termites) and found that the essential oil of *Cymbopogon citratus* was among the most effective termite inhibitors. We have hypothesised that this is one region to investigate the hopes of finding such termite control agents. This research reports on the anti-termite properties of poly herbal extracts like *Chrysopogon zizanioides*, *Artemisia absinthium*, *Syzygium aromaticum*, *Citrus sinensis*, *Eucalyptus globulus*, and *Cymbopogon citratus*.

2. Materials and methods

2.1. Plant collection

Six herbal plants were collected from various locations in and around Chennai, including medicinal herb stores. The plants gathered were *Eucalyptus globulus*, Lemongrass (*Cymbopogon citratus*), Clove (*Syzygium aromaticum*), *Artemisia absinthium*, *Citrus sinensis*, and *Chrysopogon zizanioides*. Leaf, Fruit, Root and Flower buds part was used for extraction (Table 1).



Fig.1. a) *Eucalyptus globulus*

b) *Cymbopogon Citratus*

c) *Syzygium aromaticum*

d) *Artemisia absinthium*

e) *Citrus sinensis*

f) *Chrysopogon zizanioides*

Table 1. Selected plants and its parts used for crude extracts preparation

S.No	Botanical Name	Family	Common Name	Plant Part used for the study
1.	<i>Eucalyptus globulus (a)</i>	Myrtaceae	Blue Gum	Leaves
2.	<i>Cymbopogon citratus (b)</i>	Poaceae (Gramineae)	Lemongrass	Leaves
3.	<i>Syzygium aromaticum (c)</i>	Myrtaceae	Clove	Flower Buds
4.	<i>Artemisia absinthium (d)</i>	Asteraceae	Worm wood	Leaves
5.	<i>Citrus sinensis (e)</i>	Rutaceae	Sweet orange	Fruit peel
6.	<i>Chrysopogon zizanioides (f)</i>	Poaceae	Vetiver	Root

2.2. Plant sample preparation

Plants like *Eucalyptus globulus*, *Cymbopogon citratus*, *Syzygium aromatic*, *Artemisia absinthium*, *Citrus sinensis*, and *Chrysopogon zizanioids* (Fig1a to 1f) were shade-dried at room temperature. The dried plant sample was ground into a fine powder, sieved, and stored in clean container for further use.

2.3. Sample extraction

All the six powders were extracted using three solvents such as–Petroleum ether (P), n Hexane (H), distilled water (W) to capture a wide spectrum of bio-active compounds. Hexane, petroleum

ether, and distilled water were employed due to their distinct solvation capacities, enabling the recovery of a broad spectrum of phytoconstituents. Hexane, a strongly lipophilic solvent, efficiently isolates low-polarity metabolites such as terpenoids, volatile oils, and aliphatic compounds with known insect-repelling properties. Petroleum ether, slightly more versatile in its non-polar nature, facilitates the extraction of neutral compounds including phytosterols, simple alkaloids, and lipid-soluble secondary metabolites. In contrast, distilled water, a highly hydrophilic medium, solubilizes polar phytochemicals such as polyphenolics, glycosides, and biosaponins. This strategic solvent selection based on differential polarity enhances the chemical diversity of the extracts and supports a more comprehensive assessment of their termiticidal efficacy.

10gms of each herbal plants powdered material were separately extracted with 50ml of each solvent. Extraction was done in an orbital shaker agitator at room temperature ($25 \pm 2^\circ\text{C}$) at 150 rpm after a period of 72 hours. The flasks were made lightproof by covering them with aluminum foil and parafilm so as to avoid degradation of sensitive phytoconstituents by light. The incubated extracts were filtered using Whatman No. 1 filter paper. Filtrate was collected, named accordingly and stored in sterile amber glass vials at 4°C until the time they were required to conduct other experiments. (Fig-2).

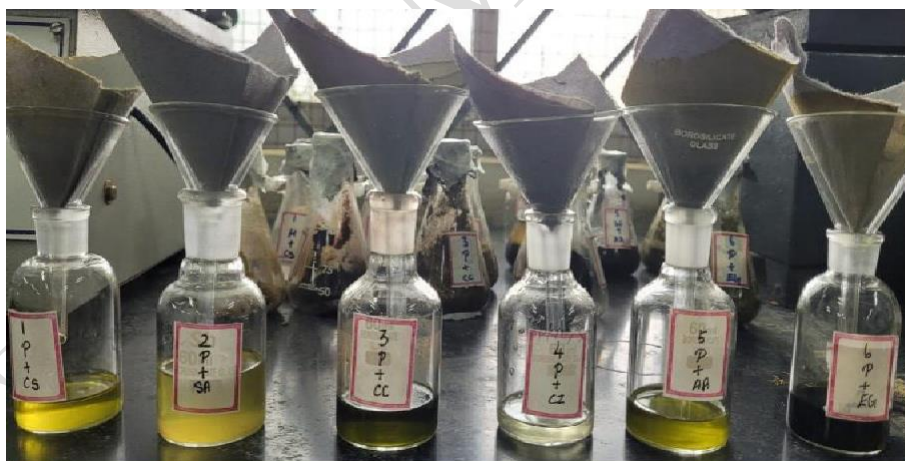


Fig.2.Solvent extraction of plant samples

2.4. Qualitative Tests

The plant extract was taken in order to perform a qualitative analysis and ascertain the presence of various phytochemicals. For the same, depending on visual observations such as change in solution appearance (colour change, precipitation formation, ring formation), at the end of each test, the presence of phytoconstituents such as carbohydrates, oils, proteins, alkaloids, diterpenes,

flavonoids, phenols, phytosterols, saponins, tannins, and terpenoids was qualitatively confirmed to know the absence (-) or presence (+) methods described by Peiris, *et al.*, (2023).

2.5. Poly herbal Spray Formulation

The plant extracts were filtered and then concentrated by removing any remaining solvents through evaporation. Every extract was utilized to analyse the qualitative phytochemicals. Each 1 ml of the petroleum ether extracts from each of the six samples was used to generate the polyherbal formulation solution, which was then combined in a reagent bottle. In a similar manner, two additional reagent bottles were labelled for future use, and the formulation for the other two solvent extracts—hexane and water—was completed.

I - *Citrus sinensis* (C.s) + Hexane solvent / Petroleum ether / water

II - *Syzygium aromaticum* (S.a) + Hexane solvent / Petroleum ether / water

III - *Cymbopogon citratus* (C.c) + Hexane solvent / Petroleum ether / water

IV - *Chrysopogon zizanioides* (C.z) + Hexane solvent / Petroleum ether / water

V - *Artemisia absinthium* (A.b) + Hexane solvent / Petroleum ether / water

VI - *Eucalyptus globulus* (E.g) + Hexane solvent / Petroleum ether / water

2.6. Gas Chromatography-Mass Spectroscopy (GC-MS)

Gas Chromatography-Mass Spectroscopy (GC-MS) was used in this work to detect the phytochemical components in medicinal plants. The extract was introduced into a Shimadzu GCMS-QP2010 Ultra after being filtered, suspended, and purified. Mass spectra were obtained at 40-600 m/z and 70 eV scan intervals. By comparing each component's average peak area to the total area, the relative percentage of each component was determined. By contrasting unknown components with recognized chemicals in the National Institute Standard and Technique database, the identity was established. While GC-MS is a robust technique for profiling volatile and semi-volatile compounds, it does have limitations. Non-volatile, thermally unstable, or high-molecular-weight compounds may not be detected accurately.

2.7. FTIR Analysis

Fourier Transform Infrared spectrophotometer (FT-IR) (FTS- 8000, Japan) was used to analyze functional groups present in the plant extract by the standard KBr method, with spectral resolution set at 4 cm⁻¹ and the scanning range from 600 to 3600 cm⁻¹. Translucent sample discs were prepared by encasing 10 ml of poly herbal (hexane, petroleum ether, and water) extracts within 100 mg of KBr pellet. The samples were ground with KBr in the ratio of 1 mg⁻¹⁰ mg in a mortar

and pestle, and 1 mg of homogenous mixture placed in sample discs pressed using a hydraulic press and mounted into the FT-IR machine for analysis. While FTIR is a reliable and rapid technique for identifying characteristic functional groups, it has some limitations. Compounds present in very low concentrations may not produce detectable absorption bands, and overlapping peaks can make it difficult to distinguish between similar functional groups.

2.8. Site selection for Repellency test

Experiments were carried out in our college campus near Paiyanoor, where various plants, herbs, and shrubs commonly consumed by termites were abundant. Termite colonies were observed both on the ground's surface and in shaded areas, often near water sources. These termites primarily fed on dead plants, herbs, and shrubs. The dense vegetation provided shade, shielding the termites from direct sunlight. During the hot summer climate, we tested plant extracts on dried wood species (*Kalotermitidae*) to evaluate termite feeding preferences, resistance, and anti-termite properties.

2.9. Antitermitic Activity

Antitermite bioassay was performed as per the methodologies proposed by the researchers (Ranjith *et al.*, 2017). The petri dishes were sterilized in an oven for two hours, and Whatman filter paper was used as a control. Three different solvent extracts were applied to the filter paper, with solvent alone serving as a positive control and blank paper as a negative control. The filter papers, treated with plant extracts, were air-dried and placed in petri dishes. Ten adult worker termites from family *Kalotermitidae* (*Cryptotermes brevis*) were randomly selected using a blind draw method and gently transferred into sterilized petri dishes containing treated filter papers. Random placement was ensured by mixing the termite pool before each selection to avoid positional or behavioral bias. Each treatment hexane, petroleum ether, and aqueous extracts was conducted in triplicate ($n = 3$) to account for biological variation and enhance statistical accuracy. Both positive (solvent only) and negative (untreated filter paper) controls were included. All trials were performed under consistent environmental conditions ($25 \pm 2^\circ\text{C}$, 65% RH, ambient light). The dead termites were then counted to calculate the mortality percentage. The mortality rates for the test and control groups were determined using a specific formula.

$$\text{Mortality(\%)} = \frac{\text{Number of dead termites}}{\text{Number of initial termites in the test}} \times 100$$

3. Results and discussion

3.1. Phytochemical Analysis of plant extracts

This study demonstrated the biocontrol potential of six plant extracts i.e *E.globulus*, *C.citratus*, *S.aromaticum*, *A.absinthium*, *C.zizaniodes* and *C.sinesis*. The chemical nature of solvent affects solubility, ultimately produce diversity in both number and quantity of isolated phytochemicals. Three solvents Hexane, Petroleum ether and water were selected on the basis of polarity indices which showed a diverse pattern in the composition. The presence of various phytochemicals was determined by use of standard qualitative tests (Peiris,2023). The intensity of observed color or precipitate was semi-quantitatively determined as follows: "-" indicates the absence of the compound, "+" indicates moderate presence of the compound, and "++" as a strong presence of the compound. Table 2 shows qualitative phtyochemicals composition of all three extracts of plants.

Table 2 : Qualitative phytochemical Analysis of plant extracts

Plant	Solvent	Terpenoids	Carbohydrates	Phytosterol	Alkaloids	Quinones	Saponins	Steroids	Flavonoids	Cardiac Glycosides	Anthraquinones	Polyphenols
<i>Citrus sinensis</i>	Hexane	+	+	+	+	+	+	+	+	+	+	-
	Petroleum ether	-	+	+	-	+	+	+	+	+	+	+
	Water	++	-	-	+	+	-	-	+	+	-	-
<i>Syzygium aromaticum</i>	Hexane	++	+	++	+	++	+	+	-	+	+	-
	Petroleum ether	++	++	+	-	++	+	++	+	++	-	-
	Water	++	+	++	+	++	-	+	-	-	+	-
<i>Cymbopogon citratus</i>	Hexane	+	+	+	-	+	-	+	+	+	-	-
	Petroleum ether	+	-	-	+	+	+	-	+	+	+	-
	Water	++	+	-	+	+	+	-	+	+	+	-
<i>Chrysopogon zizaniodes</i>	Hexane	+	+	++	-	+	-	+	+	+	-	-
	Petroleum ether	+	+	++	-	+	+	+	-	++	-	+
	Water	+	-	-	-	+	+	+	++	++	-	-
<i>Artemisia absinthium</i>	Hexane	+	-	++	-	++	-	+	+	+	-	-
	Petroleum ether	+	+	++	+	-	+	+	++	-	-	-
	Water	+	+	+	+	++	+	+	+	+	-	-
<i>Eucalyptus globulus</i>	Hexane	++	-	-	+	++	+	+	+	+	-	-
	Petroleum ether	-	+	-	+	-	-	+	++	+	-	-

	Water	++	+	+	+	+	+	-	-	++	-	-
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{- Absent, + Present (low intensity), ++ Strongly present (high intensity)}

The hexane extract of plants, after being subjected to phytochemical screening, was found that it contains a variety of bioactive chemicals, including Terpenoids, Carbohydrates, Phytosterol, Alkaloids, Quinone, Saponins, Flavonoids and Cardiac Glycosides, but it was devoid of Glycosides, Anthraquinone and Polyphenols. This active compounds have potential antitermites property. From previous studies the methanolic extracts of *Eucalyptus globulus* plant show the presence of phytochemicals such as Flavonoids and phenols (Raho *et al.*,2012, Silva *et al.*,2003 ,Batish *et al.*,2008). Similar research study reported by Mazhar abbas, the ethanolic extract were used for the evaluation of phytochemical analysis showed the presence of active constituents like flavonoids,Tannins, Alkaloids,Terpenoids etc.,(Mazhar Abbas *et al.*,2013).

A detailed phytochemical screening of the chloroform ,ethanolic and methanolic extracts of *Lantana camara* leaves was performed, which confirmed the presence of various bioactive compounds including alkaloids, tannins, steroids, glycosides, carbohydrates, and flavonoids, consistent with findings from earlier research (Sharma *et al.*,2022). From previous study report observed that *Cymbopogon citratus* showed the presence of alkaloids, flavonoids,phenolics,tannins, saponins,steroids and citrals shows good termite control (Rosemary Anietie *et al.*,2023) . Our study result in accordance with previous study report, that the phytochemicals found in *Garcinia kola* aqueous extract are: Carbohydrates; Alkaloids; Glycosides; Phenolic Compounds, Tannins, Phytosterols, Triterpenoids and Saponins (Adesuyi *et al.*, 2012).

3.2. Gas Chromatography & Mass Spectrometer (GC-MS) Analysis

3.2.1. Gas Chromatography & Mass Spectrometer (GC-MS) Analysis of Hexane extracts

The gas chromatogram obtained from the Petroleum ether, Hexane, Water extract of plant sample showed several peaks representing different bioactive compounds. The identification of compounds was based on their Area, Retention time, Height and Compound name and Molecular formula.

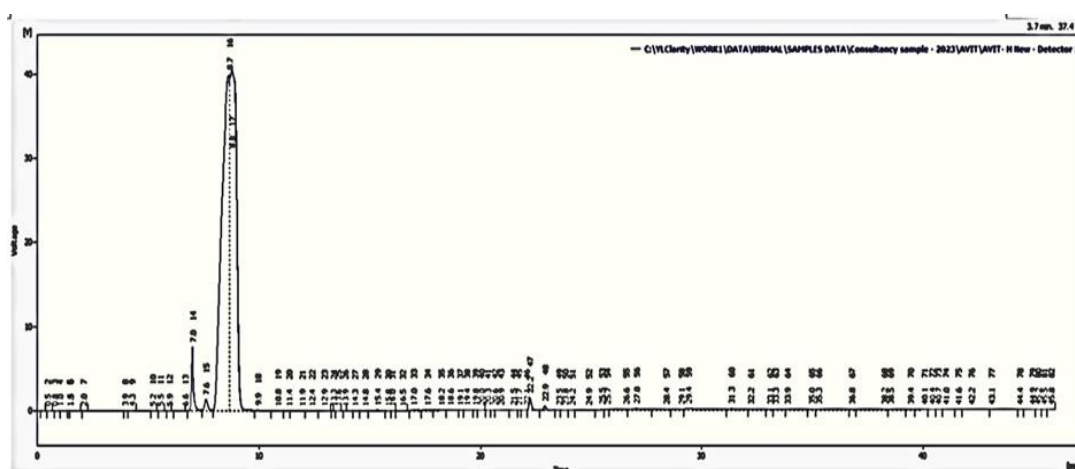


Fig: 3- GC-MS- Spectrum of Hexane extract of plants

Gas chromatography and mass spectroscopy analysis was carried out in Hexane extract of plants as shown in Table-3. Chromatogram GC-MS analysis of the Hexane extracts of plants showed the presence of many major peaks (Fig:3) and the components corresponding to the peaks were determined as follows.

Table -3- Phytochemical constituents identified in Hexane extract of plants by using GC-MS analysis

Some secondary metabolites identified using GC–MS with the help of a NIST 17 spectral data base.

S.NO	Retention Time [Min]	Area [Mv.s]	Height [Mv]	Compound Name
1	8.797	833290.70	40532.407	Octadecan
2	8.663	93515.971	40015.150	Pyridine
3	6.990	54203.287	7555.301	Pentadiene
4	7.603	13240.285	1328.334	Trimethyl cyclohexanone
4	22.240	11682.421	1500.644	Benzofurandiol
5	26.583	3613.673	75.387	Hexadecanoic acid
6	29.403	12786.791	196.286	Dimethoxybenzylone
7	38.243	3448.948	39.236	Diphenyl methane
8	31.347	4335.366	198.714	Decane
9	59.302	1333.873	3396.019	Octadecenamide
10.	35.280	4398.957	54.368	Octadecanenitrile

GC-MS run on hexane extract disclosed several phytochemicals worth recording, with the most prolific being octadecan (RT: 8.797 min) known to have antibacterial and insect-repellent properties, with the peak area and height being very large. Others that were significant included hexadecanoic acid (RT: 26.583 min) a fatty acid that has been shown to possess antioxidant and antibacterial activities and pyridine (RT: 8.663 min) which might have added to the neurotoxic and pesticidal activity. In addition to this, the chemicals found in herbal research to possess anti-inflammatory and antibacterial effect like dimethoxy benzynone, benzofurandiol, diphenyl methane and trimethyl cyclohexanone were identified. Although in lesser entities, the presence of minor chemicals such as methyl dodecanamide, cyclo-5-pentenone and n-amyl benzene could have added up to the total bioactivity of the extract. In a recent study observed that GC-MS profiling of the hexane extract of *E.globulus* revealed the presence of aromadendrene(24.83%), globulol(10.33),heneicosane(15.56%)and hexadecanoic acid, methyl ester(7.59%) (Fikadu Kifle *et al.*,2025).The previous research study reported by Rasib, the methanolic extracts were used for the GC-MS analysis showed the presence of n-Hexadecanoic acid, 9,12-Octadecadienoic acid,16-Methyloxacyclohexadeca-3,5-dien-2-one etc., (Rasib *et al.*,2017). The compounds detected in GC-MS analysis of hexane extract shows the hexadecanoic acid and octanoic acid are prominent for their established insect-repelling and toxicological effects. Hexadecanoic acid is reported to disrupt cellular lipid bilayers and interfere with metabolic pathways in insects, while octanoic acid exhibits contact lethality and efficiently penetrates the insect cuticle. These bioactive molecules are likely responsible for the rapid knockdown effect observed in termites, possibly through a synergistic mechanism of action.

3.2.2. Gas Chromatography & Mass Spectrometer (GC-MS) Analysis of Petroleum ether extracts of plants

Plant extract in petroleum ether was subjected to gas chromatography and mass spectroscopy examination, as Table -4. Numerous main peaks were detected in the petroleum ether extracts of plants by GC-MS analysis (Fig.4), and the active compounds that corresponded to the peaks were identified as follows.

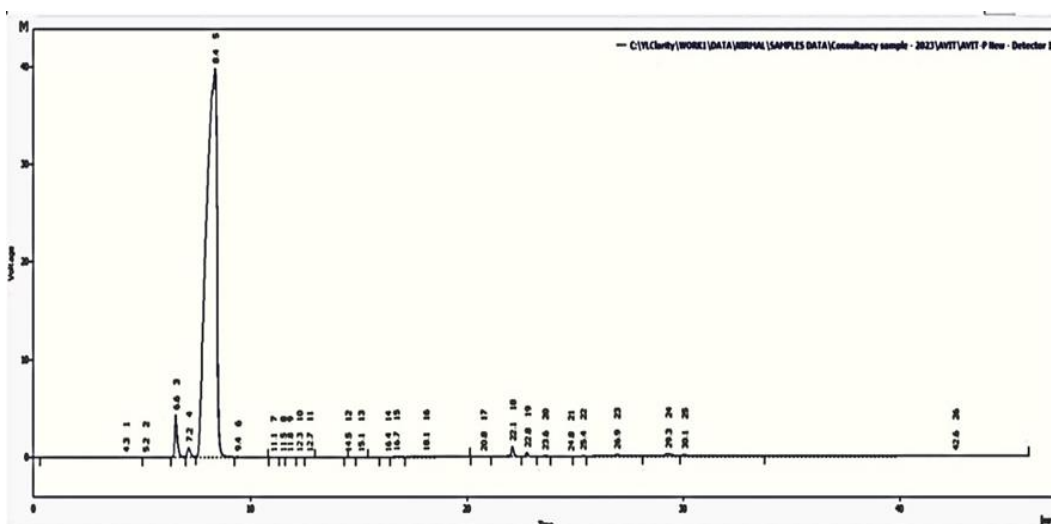


Fig: 4.GC-MS- Spectrum of Petroleum ether extract of plants

Table - 4 - Phytochemical constituents identified in Petroleum ether extract of plants by using GC-MS analysis

S.NO	Retenti on Time [Min]	Area [Mv.s]	Height [Mv]	Compound Name
1	4.267	1498.744	9.144	2,4-Methyl phenyl cyclopentane
2	5.160	32530.189	4269.442	n-Tetradecane
3	7.163	9229.149	965.145	Pentadecane
4	8.367	1330552.399	39770.684	Hexadecane
5	18.110	1706.854	33.905	9-Octadecenamide
6	22.113	9558.082	1087.109	1-Dodecane
7	22.783	3447.391	400.117	Diphenylmethane
8	23.623	1887.485	103.341	2,4-Diphenyl-1-butene
9	24.783	2723.970	56.812	Hexadecanoic acid
10	25.373	2131.537	67.936	Oleanitrile

Based on peak area, the most abundant bioactive chemicals that were detected in GC-MS analysis of petroleum ether extract included Hexadecane, pentadecane, and 9-Octadecenamide. Long-chain alkanes, namely the hexadecane and pentadecane, are well known to have antibacterial and anti-inflammatory effects. Many fatty acid amides, such as 9-Octadecenamide (oleamide) are connected with neuroactive and anticancer effects. Antioxidant activity may also arise when the aromatic hydrocarbons such as 2,4-Diphenyl-1-butene and diphenylmethane are present. The presence of all these substances testifies to the existence of pharmacologically active substances in the extract. The research study reported by Mohamed Z. M. Salem, the distilled water extract

were used for the GC-MS analysis of *L. latifolia* extract. The main constituents, were linalool, lavandulol, terpinyl acetate, camphor, linalyl acetate, fenchol, isobornyl acetate etc., (Mohamed, *et al.*, 2020). Another study reported that petroleum ether extract from *E. tithymaloides* gave a prominent compounds 1-heptadecene and n-hexadecanoic acid (Suleiman *et al.*, 2023). Among the predominate phytoconstituents identified in the petroleum ether extract, hexadecanoic acid and n-tetradecane are notable for their insecticidal efficacy. Hexadecanoic acid compromises the structural integrity of cellular membranes and disrupts metabolic functions in insects, leading to physiological failure. In contrast, n-tetradecane functions as a volatile hydrocarbon with repellent properties, likely interfering with chemosensory signaling and behavioral responses in termites. These compounds may act in concert, contributing to the observed termiticidal activity through multifaceted disruption of vital biological pathways.

3.2.3. Gas Chromatography & Mass Spectrometer (GC-MS) Analysis of Water extracts of plant

Fig- 5. Gas Chromatography & Mass Spectrometer (GC-MS) Analysis of Water extracts of plant

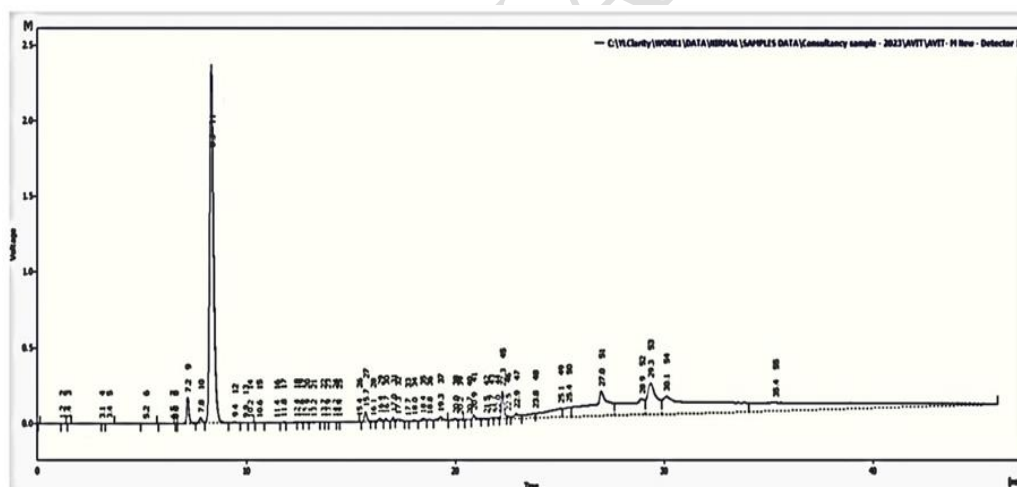


Fig: 5.GC-MS- Spectrum of Water extract of plants

Water extract was subjected to gas chromatography and mass spectroscopy examination, as shown in Table- 5. Numerous prominent peaks were detected in the aqueous extracts of plants by chromatogram GC-MS analysis (Fig.5), and the compounds that corresponded to the peaks were identified as follows.

Table:5- Phytochemical constituents identified in Water extract of plants by using GC-MS analysis

S.NO	Retention Time [Min]	Area [Mv.s]	Height [Mv]	Compound Name
1	7.180	1243.249	173.174	Trimethyl hexadecine
2	8.327	28892.495	2369.441	Pentadecane
3	22.267	1368.381	183.357	Heptadecanitrile
4	25.053	3149.003	55.257	n-Amyl benzene
5	25.437	1488.134	63.482	Benzene-1,3-Propanediyl
6	26.997	10204.241	163.761	Hexadecanoic acid
7	28.930	7306.762	103.671	Oleanitrilic acid
8	29.340	6502.181	205.101	Octadecanenitrile
9	30.097	17848.419	115.564	Methly sterate
10	35.350	20584.419	3899.827	2,3Triphenyl-1-hexane

Gas Chromatography and Mass Spectrometer (GC-MS) screening of the water extract of the plants revealed several bioactive substances that can be used in medicine. Notably, hexadecanoic acid (palmitic acid) and methyl stearate were significant components recognized to have anti-inflammatory, anti-cancer, and antioxidant properties. Octadecanenitrile and oleanitrilic acid are long-chain nitrile derivatives that also are cytotoxic and antibacterial. Anticancer and antioxidant aromatic compounds are 2,3-triphenyl-1-hexane and 1,3-benzenediol. Also, although less reactive, pentadecane as well as trimethyl hexadecine can also serve as hydrocarbon biomarkers contribute to membrane contact. The identification of compounds was based on their peak area (which represents the percentage of that compound), Retention time and height. Additionally, a previous study on the aqueous extract of *C. sinensis* identified 19 compounds through GC-MS analysis, including 1-butanol, 3-methyl acetate, trifluoroacetic acid, vitamin E, oleic acid, along with 15 other phytochemical constituents (Usman Adamu *et al.*, 2022). From the previous research study reported by Mohamed Salem, the distilled water extracts were used for the GC-MS analysis of *S. aromaticum* extract. The main component was eugenol (99.16%) were identified (Mohamed *et al.*, 2020). The water extract contains, hexadecanoic acid and n-tetradecane emerged as key bioactive compounds with potential termiticidal roles. Hexadecanoic acid compromises cellular stability and impairs essential metabolic processes, ultimately causing insect mortality and n-tetradecane acts as a volatile hydrocarbon with repellent and fumigant

properties. Together, these constituents may act synergistically, contributing to the potent termite mortality observed in the aqueous extract.

3.3. FTIR ANALYSIS OF PLANT EXTRACT

3.3.1. FTIR spectrum of Hexane extracts

FTIR characterization studies are used to identify the functional molecules of the phytochemicals present in the plant extracts. The FTIR spectrum of Hexane extract of plants reveals the peak values and the probable functional groups present in it.

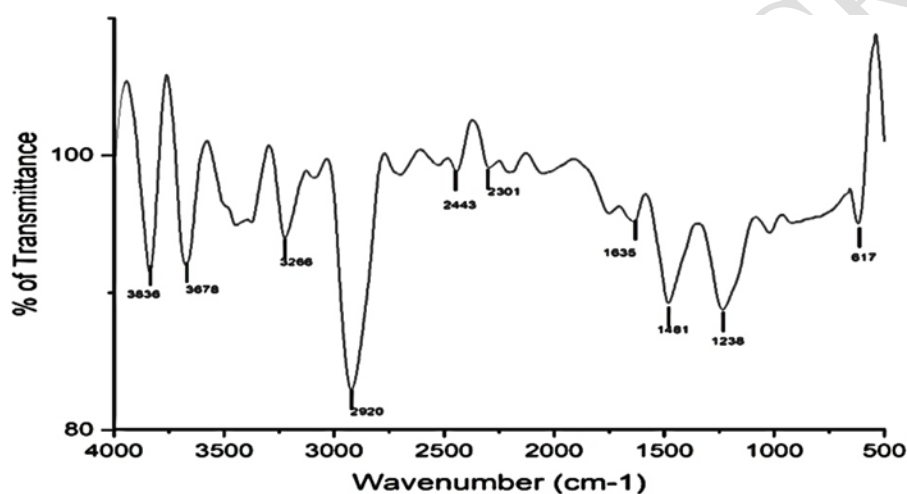


Fig 6. FTIR spectrum of hexane extracts

TABLE: 6. FT-IR Intepretation of hexane extracts

SIN O	WAVENUMBER	FUNCTIONAL GROUP	INTENSITY	IR FREQUENCY RANGE cm ⁻¹	TYPE OF BOND
1.	3266	O-H Alcohol	Strong	3550-3200	Stretching
2.	2301	O=C=O Carbon dioxide	Strong	2275-2400	Stretching
3.	1635	C=C Alkene	Medium	1662-1626	Stretching
4.	1238	C-O Alkyl aryl ether	Strong	1275-1200	Stretching

5.	617	C-I Halo	Strong	600-500	Stretching
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The FT-IR spectra for Hexane extract of plants showed the peak at 3678cm^{-1} which indicates the O-H alcohol group stretching; the peak at 2920cm^{-1} which indicates the amine group; the peak at 2310cm^{-1} which indicates the Carbon dioxide group; the peak at 1635cm^{-1} which indicates the Alkene compound; the peak at 1238cm^{-1} which indicates the Alkyl aryl ether compound; the peak at 617cm^{-1} which indicates the Halo compound. The inference drawn from this study is that the Hexane extract of plants contains functional groups that are active, such as alkene, amines, and alcohol (Fig-6, Table-6). From the previous research study the FTIR analysis is done for the methonolic extract shows the presence of functional group such as amine, alcohol, alkene, ether etc., (Amuthavalli *et al.*,2020). Bore *et al.* (2022) conducted qualitative screening of *Mitracarpus hirtus* leaf extracts against *Spodoptera frugiperda* pest using FTIR spectroscopy and reported key functional groups such as alcohols, phenols, alkanes, carboxylic acids, aldehydes, and these molecular signatures phenolic OH, alkene C=C, alkane C-H that are frequently associated with insecticidal, repellent, or antimicrobial properties.

3.3.2. FTIR spectrum of petroleum ether extracts

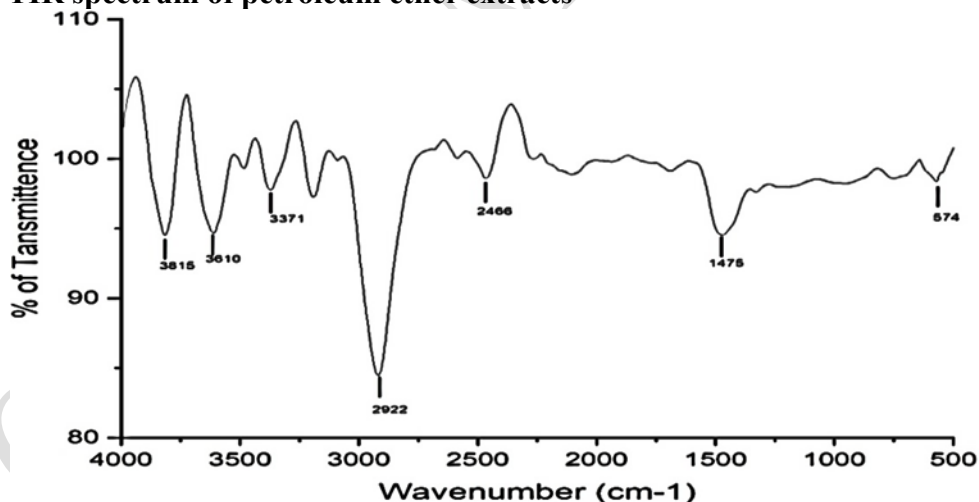


Fig -7: FTIR Spectrum of Petroleum ether extracts

TABLE-7 -FT-IR Intepretation of petroleum ether extracts

S.NO	WAVENUMBER	FUNCTIONAL GROUP	INTENSITY	IR FREQUENCY RANGE cm^{-1}	TYPE OF BOND
1	3610	O-H Alcohol	Medium	3700-3584	Stretching
2	3371	N-H Amine	Medium	3400-3300	Stretching
3	2922	C-H Alkane	Medium	2840-3000	Stretching
5	574	C-Br Halo	Strong	690-515	Stretching

The FT-IR spectra of Petroleum ether extract of plants showed the peak at 3610cm^{-1} which indicates the O-H alcohol group stretching; the peak at 3371cm^{-1} which indicates the amine group; the peak at 2922cm^{-1} which indicates the alkane group; the peak at 574cm^{-1} which indicates the halo compound (Fig-7, Table-7). Observed that the Petroleum ether extract of plants contains functional groups that are active, such as alkane, amines, and alcohol. From the previous study the FTIR analysis is done for the Ethanolic extract of *Aerva lanata* (L.) shows the presence of functional group such as C-CO-C, C=O, alkane, ether etc., (Ragavendan *et al.*,2011). Mondal *et al.*, (2020) performed FTIR studies on *E. globulus* and *C.citratus* and identified terpenoid constituents (1,8-cineole, limonene) typical FTIR-associated groups like C-H alkanes and C=C alkenes are present in its essential oil . These compounds are well-documented for fumigant toxicity and neurotoxic effects in insects. The methanolic extract from *S. foetida* seed was subjected to FT-IR analysis to identify the active functional groups namely amine, alkenes, aldehyde, ketones, esters, amides, alcohols, phenols, halogen and arenes , it shows good antitermites and antibacterial activity (Amuthavalli *et al.*,2020)

3.3.3. FTIR spectrum of water extracts

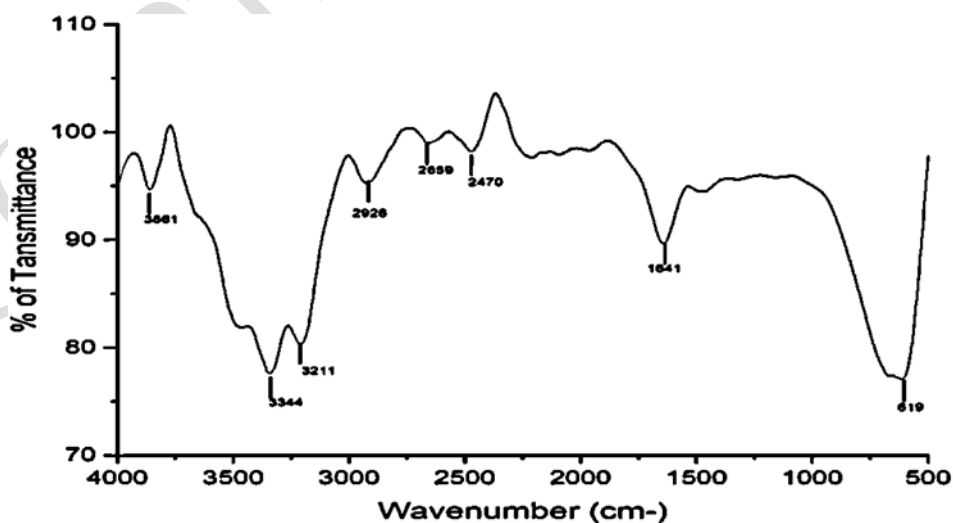


TABLE -8-FT-IR Interpretation of water extracts

S.NO	WAVENUMBER	FUNCTIONAL GROUP	INTENSITY	IR FREQUENCY RANGE cm ⁻¹	TYPE OF BOND
1	3344	N-H Amine	Medium	3300-3350	Stretching
2	3211	O-H Carboxylic	Strong	2500-3300	Stretching
3	2926	O-H Alcohol	Weak	3200-2700	Stretching
4	1641	C=C Alkene	Strong	1648-168	Stretching
5	619	C-Br Halo	Strong	690-515	Stretching

The FT-IR spectra for water extract of plants showed the peak at 3341cm⁻¹ which indicates the Amine group stretching; the peak at 3211cm⁻¹ which indicates the Carboxylic group; the peak at 1641cm⁻¹ which indicates the alkene group; the peak at 619 cm⁻¹ which indicates the halo compound (Fig-8, Table-8). The conclusion drawn from this study is that the Water extract of plants contains functional groups that are active, such as alkene, amines, and alcohol, Carboxylic. The previous study FTIR analysis is done for the Aqueous extract of *Aerva lanata* (L.,) shows the presence of functional group such as Alkane, C=O etc., (Ragavendan *et al.*, 2011).

3.4. Repellency Test

In order to study the termites' feeding preferences, resistance, and anti-termite activity, we treated wood species that had been buried under the soil with control wood stick for a period of week and left undisturbed. When treated with the plant extract, the wood sample that was removed after a week showed resistance to termites.

3.5. Anti-termites activity assay

Three extracts of plants were subjected to termite toxicity trials to ascertain whether they can be used as insecticides. The percentage of mortality calculated out of 10 termites was evaluated. The antitermites activity results shows the highest mortality rate was observed for the Hexane and petroleum extract of plant sample for the exposure period 1-minutes when compared water extracts (2 min). But all three extracts, shows 100% mortality. It indicates the strong anti-termites property (Fig-9, Fig-10).

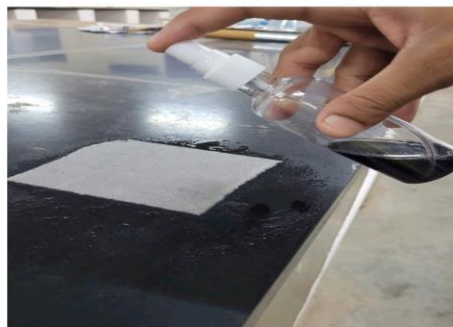


Fig: 9. Polyherbal spray -sprayed over the filter paper sheet (6 cm × 6 cm)

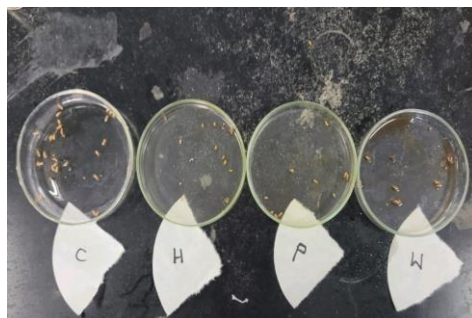


Fig: 10. Antitermites activity in sterilized petri dishes (9 cm diameter)

C- Control, **H-** Hexane – Polyherbal spray, **P-** Petroleum ether – Poly herbal spray **W-**Water-Poly herbal spray

The bioactive compounds in the plant extracts exhibit significant anti-termite properties. A similar outcome was reported by Abdullah *et al.*, 2015, where ethanolic and petroleum ether extracts of *A. galanga* at a minimum concentration of 1% proved effective as anti-termite agents, causing 90% mortality after 2 hours of exposure. Extract concentrations ranging from 2% to 5% resulted in mortality rates of 95-100% at various exposure times. Mortality was evaluated based on the death of 10 termites. Rosemary *et al.*, 2023 reported that *C.citratus* aqueous extract exhibited the highest termiticidal activity even at low concentration (2g) against *Macrotermes sp* .In previous research study reported by Lo Pinto Mirella *et al.*,2023 after 24 hours treatment with *Syzygium aromaticum* the lowest lethal dose DL50 (1.28mul/l) exposed good efficacy on termite mortality.The results of the anti-termites activity showed the highest mortality rate for the hexane and petroleum extracts after a 1-minute exposure period, compared to the negative control. Nasser R *et al.* (2024) demonstrated that nanoemulsified *Eucalyptus globulus* essential oil caused significant mortality and midgut tissue disruption in *Odontotermes formosanus* a result also observed with our hexane and petroleum ether extracts . In another study, Majeed, *et al.* (2020) reported that *Cymbopogon citratus* essential oil exhibited potent toxicity and repellency against *O. obesus*, supporting our finding of 100% mortality within minutes . Additionally, Essien *et al.* (2023) found that *C. citratus* leaf extract killed *Macrotermes bellicosus* termites at concentrations as low as 2 g per dish, consistent with the effectiveness of our poyherbal aqueous extract. While the results are promising in a controlled lab setting, field implementation poses challenges. Factors like extract formulation stability, application method (e.g., spray, coating, fumigation), and dosage standardization must be optimized. The efficacy of botanical termiticides may vary with external

environmental factors such as temperature, humidity, and light exposure. Future studies should include field trials under diverse ecological conditions to evaluate the robustness of the formulations. Compared to synthetic termiticides like fipronil, chlorpyrifos, and imidacloprid botanical extracts may require more frequent application, their biodegradability, lower ecotoxicity, and lower risk of resistance offer major advantages. With proper formulation, botanical termiticides could become part of integrated pest management (IPM) programs. Together, these comparisons underscore this present study's validation of known botanical efficacy and highlight its novelty in achieving exceptionally rapid and complete termite mortality using diverse, solvent-based formulations.

4. Conclusions

From the present study it was concluded that *herbal plants* contain many active phytochemicals. Qualitative phytochemical analysis showed the presence of alkaloids, saponins, flavonoids, carbohydrate, steroids, quinone, phytosterol and terpenoids. The GC-MS analysis of plant extract shows the presence of various active compounds. The active functional groups was observed and confirmed by FT-IR. The FT-IR spectra for plant extract shows the functional group such as alcohol, alkene, ether, amine, carboxylic, halo compounds. Among the three tested extracts hexane and petroleum ether extracts exhibited the highest termite mortality within one minute of exposure, whereas the aqueous extract was effective within two minutes. These findings highlight the potential of these plant extracts as eco-friendly termite control agents. Thus, the results obtained from the present experiments are encouraging and support the effectiveness of the *plant sample* in control of termites. In future work the stability of phytochemical compounds over time and their efficacy under field conditions remain to be evaluated. Additionally, the potential impact of these extracts on non-target organisms, including beneficial soil invertebrates or pollinators, was not assessed. Further research is needed to isolate and characterize the specific bioactive constituents responsible for termite control, optimize formulations for long-term use, and evaluate their environmental safety and selectivity.

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Declaration of competing interest

The authors declare no relevant competing interests.

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