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ASSESSING THE EFFICACY OF *CASSIA FISTULA* AND *PONGAMIA PINNATA* EXTRACTS AGAINST *COPTOTERMES HEIMI*.

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ABSTRACT
Present study focuses on the potential use of leaf extracts from two plant species for controlling *Coptotermes heimi*. Leaves from *Pongamia pinnata* and *Cassia fistula* were extracted with ethanol solution. The results showed that *pongamia pinnata* extract was more potent as compared to *C. fistula*. GC-MS analysis of both the plants revealed several important compounds, that could be further utilizes to explore the antitermitic potential of the identified components. Our findings indicated that percentage of mortality depends on the concentration and time of exposure. Both the extracts were repellent. This research underscores the potential of plant-derived solutions for sustainable termite control, paving the way for development of environmental friendly pest management strategies.

Keywords: *C. heimi*, mortality, GC-MS, *P. pinnata*, Antitermitic.

INTRODUCTION
Termites, categorized under order Isoptera, encompass 12 families and approximately 3500 species identified to date (Davies et al., 2021; Qasim et al., 2022). These invertebrates play a significant role in humid and temperate ecosystems, contributing both beneficial and detrimental effects (Brauman et al., 2015; Qasim et al., 2022). In the Indo-Pak region, Coptotermes, Microtermes, and Odontotermes are prevalent genera known for their pestilence (Rajagopal, 2002). Coptotermes accounts for 38 out of 80 subterranean termite species (Rust and Su, 2012; Krishna et al., 2013 Qasim et al., 2022), with *Coptotermes formosanus* Shiraki and *Coptotermes gestroi* (Wasmann) being notable species within the Rhinotermitidae family (Krishna et al., 2013; Jasso-Selles et al., 2020).

Termites are classified into three main categories based on their habitat: Subterranean, damp-wood, and dry-wood termites (Baker and Marchosky, 2005; Arinana et al., 2022). Subterranean termites are closely associated with soil, where they tunnel to find water and food sources such as wood and cellulose-containing materials (Suiter et al., 2009; Arinana et al., 2022). They form networks in the ground for movement (Im and Han, 2020; Arinana et al., 2022) and are notorious for causing significant damage to wooden structures and cellulose products (Adfa et al., 2020; Peterson et al., 2006; Arinana et al., 2022).

Despite their ecological benefits, termites pose a threat to various human activities by damaging cellulose-based materials such as books, timber, buildings, and agricultural crops (Ravan et al., 2015; Ahmad et al., 2012). The economic impact of termite damage is estimated to exceed 40 billion USD annually worldwide (Rust & Su, 2012; Subekti et al., 2015; Ahmad et al., 2012), affecting agriculture directly through plant feeding and indirectly by making plants susceptible to pathogens (Paul et al., 2018; Ahmad et al., 2012).

Conventional synthetic insecticides are commonly used for termite control globally (Nyeko and Olubayo, 2005; Okari et al., 2022). However, these insecticides present various challenges including toxicity to non-target organisms, resistance development, environmental pollution, and health hazards (Taiwo, 2019; Ankit et al., 2020; Anjarwalla et al., 2016; Okari et al., 2022). Limited access to synthetic pesticides due to cost and distribution issues further complicates pest control for many farmers (Anjarwalla et al., 2016; Okari et al., 2022).

In light of the drawbacks of chemical pesticides, researchers are exploring alternative, eco-friendly methods for termite control, with plants emerging as promising candidates due to their diverse bioactive compounds (Kannaiyan, 1999; Mishra et al., 2021). Tabassum et al in 2023 find out the termiticidal activity of ethanolic leaf extracts of medicinal plants *C. gigantea* and *M. alba* against *H. indicola* and find out that both plants cause mortality and were repellent against *H. indicola* (Wasmann). Efforts are underway to identify locally available plants with insecticidal properties for termite management, as evidenced by farmers' traditional use of certain plant species to deter termites from crops (Sileshi et al., 2008; Mugerwa et al., 2014; Thomas et al., 2023). The purpose of my study was to investigate the cultural significance and economic feasibility of using plant extracts for termite control, particularly in regions where traditional knowledge of natural remedies is prevalent. Explore how local communities could benefit from utilizing plant based solutions, both in terms of cost effectiveness and preserving indigenous knowledge and practices.

MATERIALS AND METHODS

Collection of termites

C. heimi (workers and soldiers) were collected from the old trees of Populus euramericana from Lahore. Termites were maintained for few days on water soaked filter paper along with 5 grams oven dried soil in the Petri-Plates.

Collection of leaves

Leaves of the locally used plants *P. pinnata* and *C. fistula* were taken from the botanical garden of the Punjab University.

Preparation of extracts

The leaves were dried at ambient temperature for two weeks and then grinded into fine powder. Extracts were prepared by using 200 ml of Ethanol in 20 grams of powder (leaves) in the Soxhlet extractor. Further dilutions of 30%, 20% and 10% were prepared.

Gas chromatography/ GC-MS analysis

The samples of both the plants were analyzed by gas chromatography. The conditions of GC-MS include a temperature range of 50 to 250°C with a 4°C/min, along with solvent delay of 5 min. The temperature of injector was maintained at 250°C. Among noble gas Helium was used with a flow of 1.0m/L/min and volume of injected sample in split less mode was 2µL.

Anti-termitic assay

The bottom of each sterilized Petri-plate was covered with cutted circular filter paper and 0.5ml of each extract of the desired concentration (30%, 20% and 10%) was applied. Petri plates containing filter paper was then dried at ambient temperature. Hundred workers and 5 soldiers were added in each Petri plate and observations were made.

$$\%\text{age of Mortality} = \frac{\text{No. of the dead termites after test}}{\text{No. of initial termites taken for test}} \times 100$$

Repellency assay

Estimation of repellency was checked by cutting filter paper of diameter 9cm into two equal halves. One of the half of each paper was treated with 30%, 20% and 10% concentration and other half with distilled water. Then the two half of filter paper were placed in the Petri plate with a middle space between them. In the middle space 10 termites were released. Repellency was assessed by counting number of termites after every 15 min on treated (T) and untreated (UT) filter paper and experiment was conducted for a period of 2 hours. For each plant extract three replicates were made. A concentration was considered attractant if less than 21 termites out of 30 (three replicates) were present on untreated area and vice versa.

Statistical analysis

A two-way ANOVA was used to compute and assess the termite mortality percentage; statistical significance was defined as $p<0.05$. Using Probit analysis LC_{50} was determined.

RESULTS

GC-MS analysis of *P.pinnata* revealed several compounds as indicated in Table 1 and GC-MS analysis of *C. fistula* is indicated in Table 2. Components revealed in the ethanolic extract of *P. pinnata* were 4-Piperidinamine, N,1-dimethyl, (R)-(-)-14-Methyl-8-hexadecen-1-ol" U, 4-Hydroxy-N-methylpiperidine, Phytol, Cyclooctaneacetic acid,2-oxo-"H, Phthalic acid, octyl-propylpentyl ester, Sarcosine, N-(3-phenylpropionyl)-pentyl ester, 1,2- Diethl-1,2-dihydro-3,6-diphenyl—tetrazine, 2,5-cyclohexadiene-1,4-dione,2,5-bis (4-hydroxyphenyl), supraene, 1-Eicosanol, 1,1-Biphenyl, 4,4 bis (1-pyrroliding, ϱ-Tocopherol, R)-6-Methoxy-2,8-dimethyl-2-(4R,8R)- 4,8,12-trimethyltrdecyl) chroman and 1-Eicosanol. Following compounds were identified in the ethanolic extract of *C. fistula*, Rescorcinol, Neophytadiene,2-Hydroxyhexadecyl butanoate, 3-0-Methyl-d-glucose, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Bis (2- ethylhexyl) phthalate, Sulfurous acid, pentadecyl 2-propyl ester, Cis,cis,cis-7,10,13-Hexadecatrienal, -isopropyl-5-methlcyclohexyl methanol Carbonic acid, eicosyl vinyl ester, y-Tocopherol, Carbonic acid, eicosyl vinyl ester,Tetracosanol-1and Vitamin E. All compounds present in *P. pinnata* and *C. fistula* with retention time and structural formula and percent composition are shown in Table 1 and 2. The LC_{50} value for *P. pinnata* was 5.85 (Table 4) and LC_{50} value of *C. fistula* was 6.57 (Table 4). Mortality in ethanolic extract of *P. pinnata* with 30% concentration was 89% and mortality with ethanolic extract of *C. fistula* with 30% extract was 87.66%. Mortality of *P. pinnata* with 20% and 10% concentration was 78.66% and 67% respectively. Mortality of *C. fistula* at 20% and 10% concentration was 69.33% and 59.33% as shown in Figure 1. Chromatograms from GC-MS analysis of solvent-extracted leaves are shown in Figure 3 and 4.

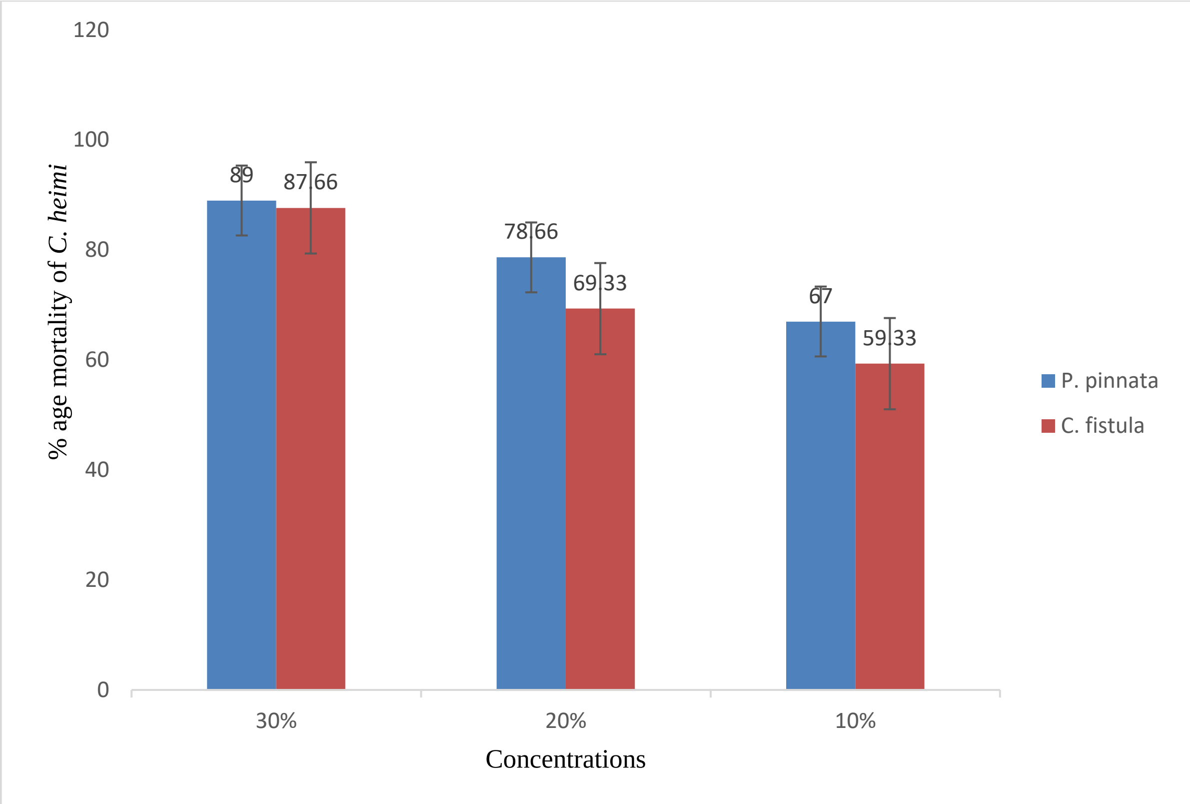


Figure 1. Percentage mortality of *C. heimi* at different leaf extract concentrations

Repellency

All concentrations of both plant extracts were repellent, as most of the termites were present on untreated filter paper. Their presence on untreated filter paper indicating repellency as shown in Figure 2.

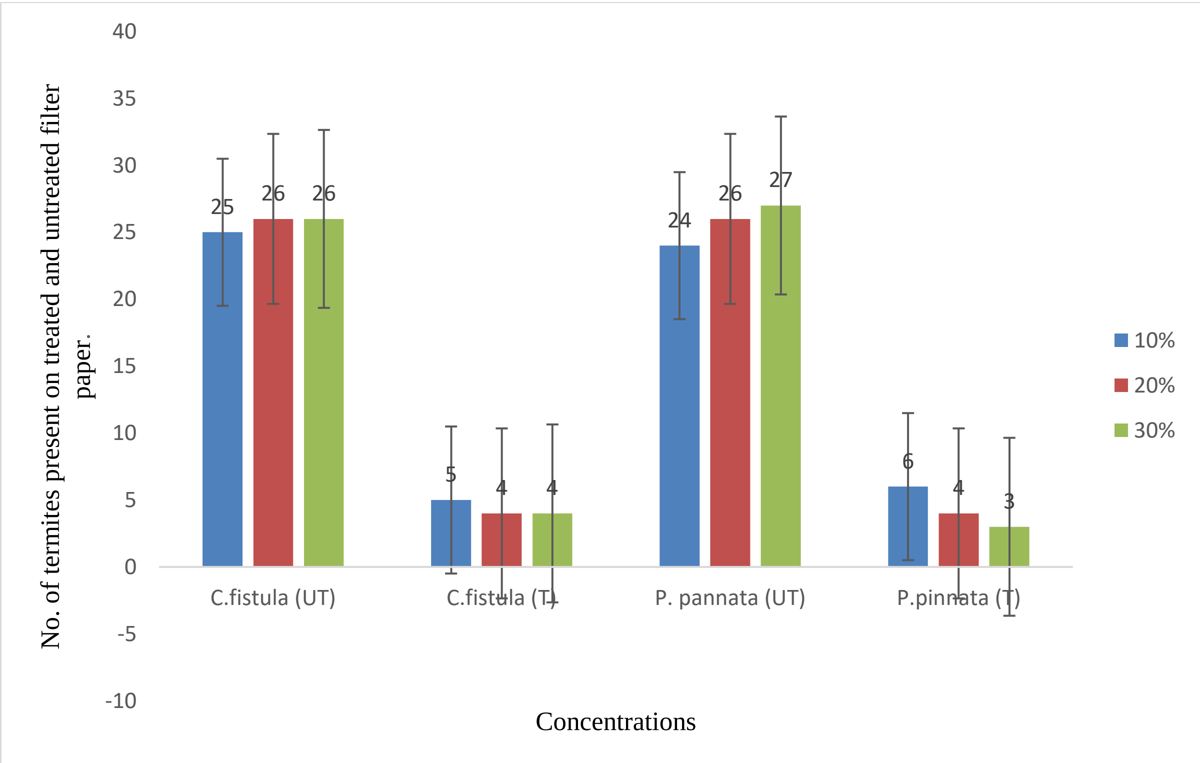


Figure 2. **Repellency test of *C. fistula* and *P. pinnata* against *C. heimi* (UT= untreated, T= treated).**

Table 1. Phytocompounds identified in ethanolic extracts of *P.pinnata*

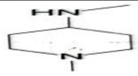
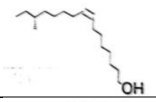
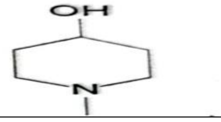
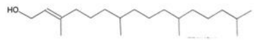
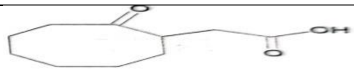
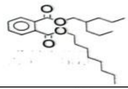
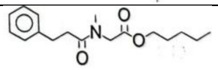
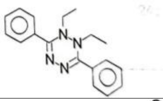
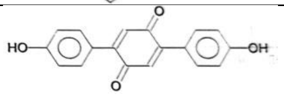
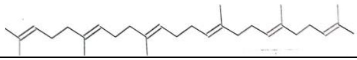
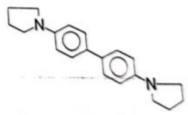
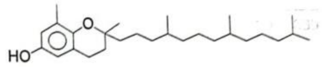
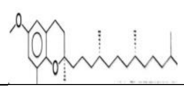
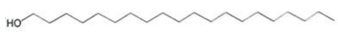
Sr #	Phytocompounds	Retention time	Relative percentage composition	Structural formula
1	4-Piperidinamine, N,1-dimethyl	13.383	10.950%	
2	(R)-(-)-14-Methyl-8-hexadecen-1-ol” U	23.782	6.679%	
3	4-Hydroxy-N-methylpiperidine	24.283	1.801%	
4	Phytol	26.713	21.989%	
5	Cyclooctaneacetic acid,2-oxo-“H	30.164	3.142%	
6	Phthalic acid, octyl-propylpentyl ester	30.455	2.367%	
7	Sarcosine, N-(3-phenylpropionyl)-pentyl ester	31.237	3.945%	
8	1,2- Diethl-1,2-dihydro-3,6-diphenyl--tetrazine	32.391	4.259%	
9	2,5-cyclohexadiene-1,4-dione,2,5-bis (4-hydroxyphenyl)	32.490	9.329%	
10	supraene	32.944	9.550%	
11	1-Eicosanol	33.201	8.190%	
12	1,1-Biphenyl, 4,4 bis (1-pyrroliding)	33.393	4.150%	
13	ǫ-Tocopherol	34.104	5.605%	
14	(R)-6-Methoxy-2,8-dimethyl-2-(4R,8R)-4,8,12-trimethyltrdecyl)chroman	34.483	3.569%	
15	1-Eicosanol	34.553	4.474%	

Table 2. Phytocompounds identified in ethanolic extracts of *C. fistula*

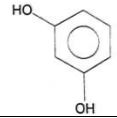
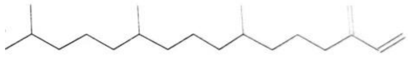
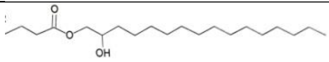
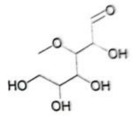
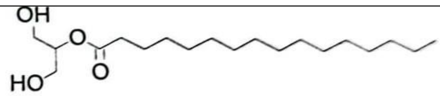
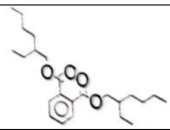
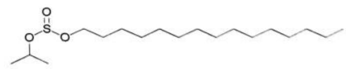
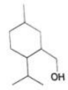
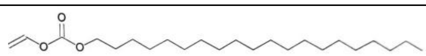
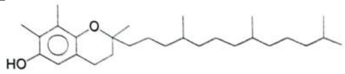
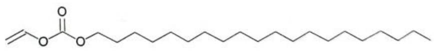
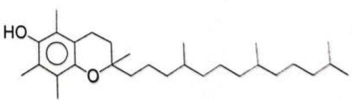
Sr #	Phytocompoundas	Retention time	Relative percentage composition	Structural formula
1	Rescorcinol	15.738	0.380%	
2	Neophytadiene	23.788	1.721%	
3	2-Hydroxyhexadecyl butanoate	24.073	0.284%	
4	3-0-Methyl-d-glucose	26.708	83.978%	
5	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	30.164	1.037%	
6	Bis (2- ethylhexyl) phthalate	30.462	0.512%	
7	Sulfurous acid, pentadecyl 2-propyl ester	31.505	0.278%	
8	Cis,cis,cis-7,10,13-Hexadecatrienal	31.616	0.240%	
9	2-isopropyl-5-methlcyclohexyl methanol	32.222	0.199%	
10	Carbonic acid, eicosyl vinyl ester	32.921	1.562%	
11	y-Tocopherol	34.227	0.362%	
12	Carbonic acid, eicosyl vinyl ester	34.437	0.549%	
13	Tetracosanol-1	34.536	5.225%	
14	Vitamin E	34.973	3.673%	

Table 3. Two-way ANOVA mortality of termites

Anova	SS	DF	MS	F (DFn, DFd)	P value
Inreraction	53.44	2	26.72	F (2, 12) = 48.10	P<0.0001
Concentration	168.1	1	956.2	F (1, 12) = 302.5	P<0.0001
Plant type	1912	2	956.2	F (2, 12) = 1721	P<0.0001
		12	0.5556		

Table 4. LC_{50}

Sr. No.	Plant name	95% Confidence limits (lower limit-upper limit)
1.	***P. pinnata***	5.85 (2.46-13.17)
2.	***C. fistula***	6.57 (1.51-23.92)

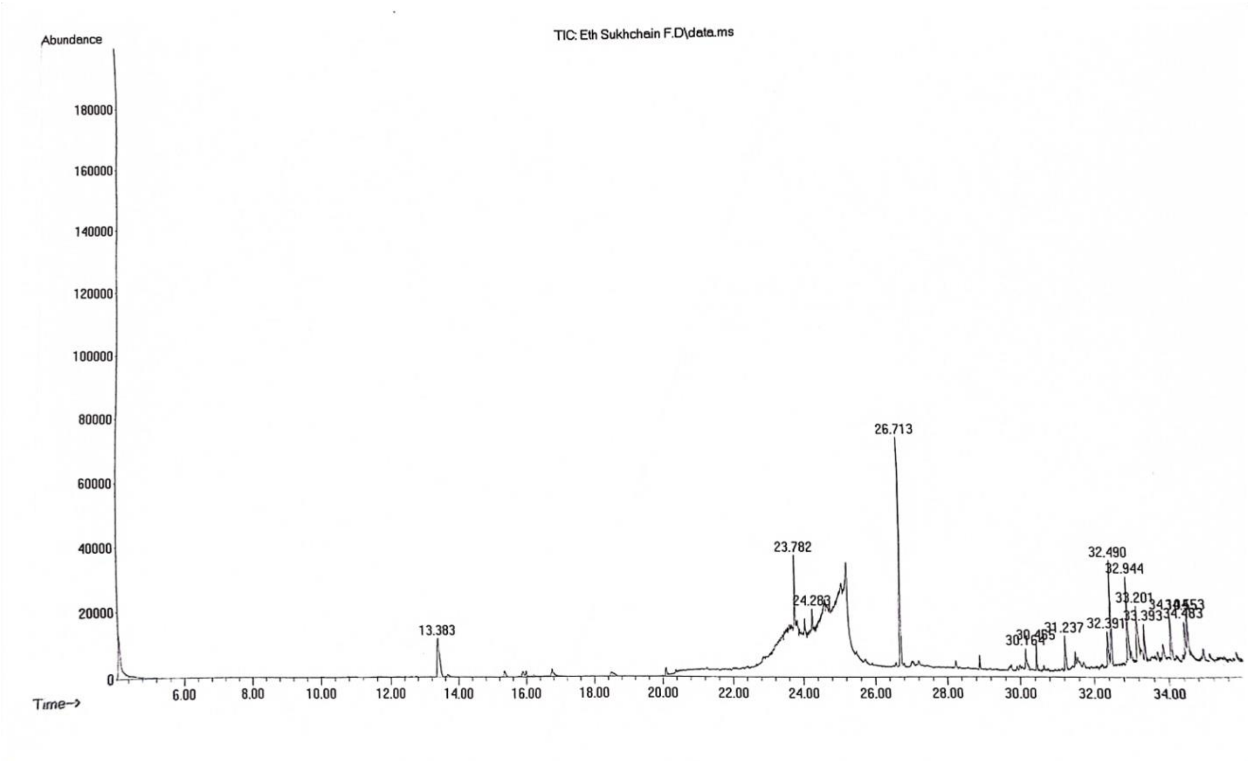


Figure 1. Compounds identified in GC-MS analysis in *P. pinnata*

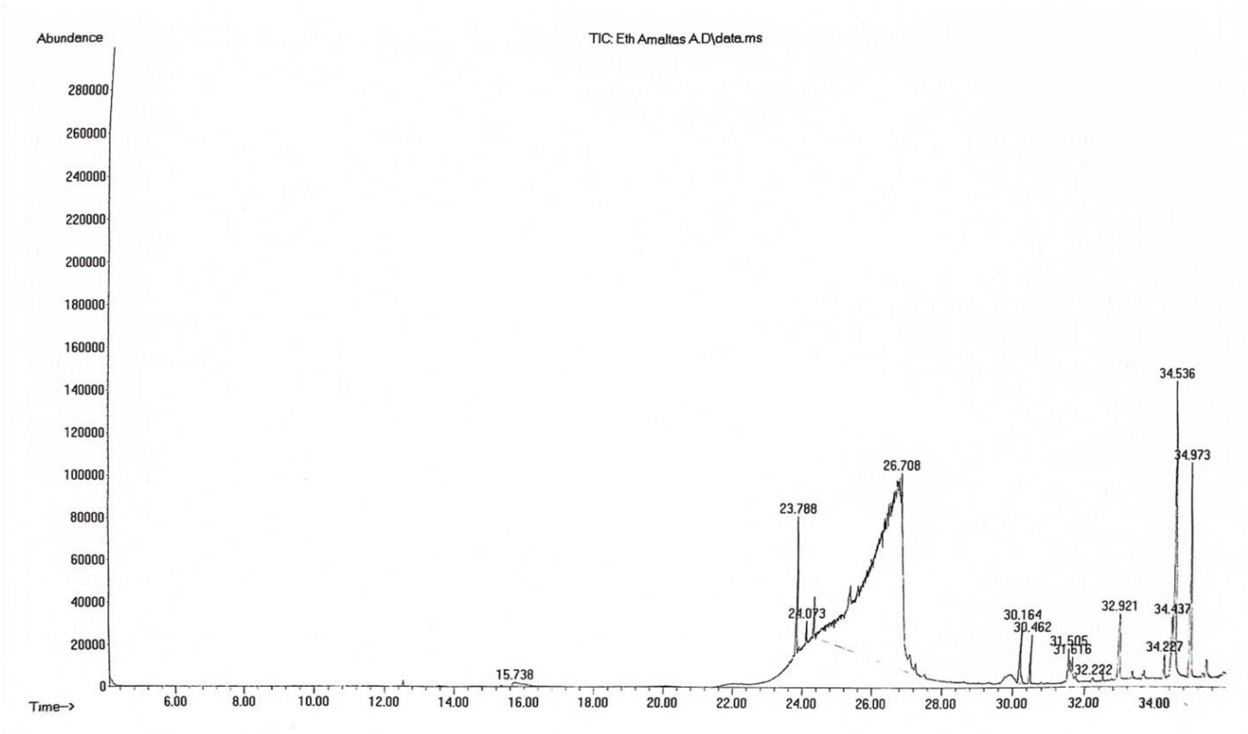


Figure 2. Compounds identified in GC-MS analysis in *C.fistula*

DISCUSSION

Highest mortality in extract of *P. pinnata* was reported in our study which is in consistent with the findings of Ahmed et al., 2022, who has also reported significant mortality of *P. pinnata* against *C. heimi* (Wasmann). He reported the termiticidal, antifeedant and repellent properties of *P. pinnata* wood against *C. heimi*. Highest mortality by using ethanolic extract of *P. pinnata*, and its toxicity can be due to the phytochemical constituents identified in its extract further Verma et al., 2011 reported that the insecticidal potential of pongamia plant extract can be due to the presence of different compounds in the plant product. Similarly Swamy et al. (2006) reported reduction of larval population by applying Pongamia oil against greater wax moth. Mortality increased by increasing the concentration similarly Ahmed et al., 2022 also reported highest mortality at highest concentration and find out that mortality was highest in organic solvent.

Leaf and seeds extracts of *P. pinnata* has already explored insecticidal especially Pongamia oil has been applied against several insects including termites (Hiremath et al. 1997, Pavela 2009, Samuel et al. 2009, Reena et al. 2012, Al-Solami et al. 2014; Ahmed et al., 2022).

In another study crude extract of *P. pinnata* caused 83.3 percent mortality after a period of two hours against *Odontotermes obesus* and all the termites died after 4 hours in Petri-plate (Sharma et al. 2011; Ahmed et al., 2022), Similarly Verma et al in 2011 reported 100% mortality against *Odontotermes obesus* by applying extract of *P. pinnata* and *J. curcas* seeds within 6 hours. The extract of *P. pinnata* is also effective against field crops, household insect's pests and stored grains as well (Kumar & Singh 2002; Ahmed et al., 2022). Leaf extracts of *P. pinnata* showed significant mortality against *Micrococcus* sp. **(**Chopade et al. 2008; Ahmed et al 2022**).** Similar findings have been reported in which leaf extracts of *P. pinnata* in petroleum ether revealed antilice and ovicidal actitivity by suppressing the nymphs **(**Samuel et al. 2009; Ahmed et al 2022**).**

Our results are in consistent with other scientist who reported significant mortality by using plant extracts against termites. Mortality in the ethanolic extract of *C. fistula is* satisfactory and it can be due to the presence of antitermitic compounds in its leaves. Effect of *C. fistula* extract against termites has already been reported. Khanikor et al., 2018 has reported the antitermitic potential of essential oil of *C. fistula* against *Odontotermes feae* Wasmann. Previously extensive study has been done on all parts of *C. fistula* and has been reported as antimicrobial and antifungal (Ali et al., 2004; Phongpaichit et al., 2004; Kiat and Chiang 2013), anti-inflammatory (Ilavarasan et al., 2005; Kiat and Chiang 2013). Zia Ullah et al 2018 have reported 100% mortality by applying extract of *C. fistula* (leaves) against *Culex quinquefasciatus* Say. So *C. fistula* extract has insecticidal properties.

C. fistula plant parts are well known for secondary metabolites (phenolic compounds) (Bahorun et al., 2005; Kiat and Chiang 2013). In the last few years' significant amount of chemical constituents has been segregated from different parts of *C. fistula* and their characters have been thoroughly studied (Rizvi et al., 2009; Kiat and Chiang 2013). The leaf extract of *C. fistula* has also reported to possess remarkable larvicidal, ovicidal and repellent activity against *Cx. quinquefasciatus* and *Anopheles stephensi* (Govindarajan et al., 2008; Govindarajan, 2009).

In the present investigation anti-termitic potential of *P. pinnata* and *C. fistula* was evaluated against *C. heimi* under Laboratory conditions. Both the plants have already been tested against termites and have been reported as anti termitic. Extract of *P. pinnata* was more potent and caused highest mortality at highest concentration and lowest mortality in low concentration. Both the extracts were repellent. Mortality of *C. fistula* extract was slightly lower than *P. pinnata*. So both the extracts are recommended for use in the future.

CONCLUSION

Our study evaluated the repellent and insecticidal properties of extracts from *P. pinnata* and *C. fistula* against termites. both plant extracts demonstrated significant repellent activity, effectively deterring termites from treated areas. Additionally, both extracts resulted in significant mortality among termite populations, indicating their potential as natural insecticides. These findings highlight the promising efficacy of plant-based alternatives for termite control. Further research into the active compounds and mechanisms of action of these plant extracts could lead to the development of sustainable and environmentally friendly pest management strategies.

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