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Bioactivity of Ethanolic Leaf Extracts of *Acacia nilotica* and *Eucalyptus camaldulensis*, against *Coptotermes heimi*

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ABSTRACT: *Termite infestation poses a significant threat to structural integrity and agricultural productivity worldwide. This study was conducted to investigate the efficacy of ethanolic extracts of Acacia nilotica and Eucalyptus camaldulensis against Coptotermes heimi, focusing on mortality and repellency. The ethanoloic leaf extract of E. camaldulensis showed mortality of 79.33% at 30% concentration while A. nilotica at 30% of concentration revealed a mortality of 74.66%. The LC50 value of E. camaldulensis was 7.70% while that of A. nilotica was 13.03%. Both plants were found repellent against C. heimi, but A. nilotica was non-repellent at 10% concentration. Additionally, an analysis of Gas-Chromatography-Mass Spectrometry (GC/MS) was performed to identify and quantify the chemical constituents present in the plant extracts. The GC/MS analysis of E. camaldulensis revealed an important compound Eucalyptol, which has the highest percentage composition of 57.81 % while GC/MS of A. nilotica contains a high concentration of γ-Sitosterol along with Vitamin E and Behenic-alcohol. This study underscores the potential of A. nilotica and E. camaldulensis leaf extracts as ecofriendly alternatives for termite control.*

Keywords: *C. heimi*, GC/MS, Termite control, *A. nilotica*, *E. camaldulensis*

INTRODUCTION

One of the most common eusocial Isoptera insects are termites, which have intricate work divisions within each colony (Padwal et al 2023). Globally, there are currently 282 genera and nearly 3000 termite species, nearly 1000 reported from Africa (Van Huis, 2017; Ajayi et al., 2020). In Pakistan, there exist 53 distinct termite species, with 13 of them recognized as pests damaging forestry, buildings, and agriculture (Hassan et al., 2018; Ahmed et al., 2020). Termites exhibit diverse feeding patterns, falling into categories such as wood feeders, soil feeders, grass feeders, or fungus growers (Brauman et al., 2015; Ahmad et al., 2021).

An enormous amount of money, estimated to be several hundred million rupees annually, is lost because of termites, as their entire yield loss affects between 10 and 25 percent of the nation's crops (Ranjith et al., 2021; Padwal et al., 2023). Termite creates a major problem in both localities (urban and rural) across the world because they cause significant damage to wood structures, plants, and crops in addition to causing financial losses Khanum (Uzunovic et al., 2008; Khanum and Javed, 2020).

Globally, they indiscriminately target wood, with their attacks most prevalent in warmer climates (Aihetasham et al., 2017). Depending on the invasion species, managing termite colonies successfully requires a variety of specialized abilities. Identifying damage and finding management measures can be made easier with an understanding of termite ecology (Khan et al., 2016; Khanum and Javed, 2020).

Historically, the primary method for combating termites was the use of synthetic insecticides (Hertel, 2000; Venkateswara et al., 2005; Sattar et al., 2014; Aihetasham et al., 2015). Unfortunately, the risk is not only posed to mammals but also to birds, were carcinogenic, and contributed to environmental pollution. With the increasing prevalence of termite infestations, the need of finding treatments that are both safe for humans and the environment has become more pronounced (Meepagala et al., 2006; Aihetasham et al., 2015).

The development of botanical pesticides to manage various insect pests is being influenced by the negative consequences of synthetic pesticides. Powders and extracts derived from several bioactive plants that have insecticidal, repulsive, and anti-feeding qualities (Isman, 2006; Kassie, 2019). Since of their inherent biodegradability, plant-based pesticides are favored for the management of insect pests since they are less hazardous to non-target organisms (Prabakar & Jebanesan, 2004; Kassie, 2019). In addition to being toxic to a wide range of insect pests, many plant extracts also modify the behavior of the pests they are intended to harm (Abbas et al., 2013; Aihetasham et al., 2017). When it comes to controlling termites, plants with both insecticidal and repellent qualities are thought to work best. It is safe to utilize plant bioactive components (Aihetasham et al., 2017).

The goal of the current research was to assess the potential toxicity of *E. camaldulensis* (sufaida) and *A. nilotica* (kikar) against *C. heimi*. Our objectives were to prepare Ethanolic extracts of selected plants by using the Soxhlet extractor, to evaluate the toxicity of these extracts against *C. heimi* by calculating LC_{50} along with Repellency test, and the structural characterization of the leaves extract by GC/MS.

MATERIALS AND METHODS

Termite Collection

Workers and soldiers of *C. heimi* were taken from the old trees of *Populus euramericana* from Canal Bank, University of the Punjab, Quaid-e-Azam campus, Lahore. Termites were maintained in the laboratory at 26±2 ºC for few days, on filter paper soaked with water along with five grams dried soil into the Petri-Plates.

Collection of Leaves

Leaves of the locally available medicinal plant *E. camaldulensis* (sufaida) and *A. nilotica* (kikar) were taken from the botanical garden at the University of the Punjab.

Preparation of Extracts

The leaves were air 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 (Aihetasham et al., 2017). Rotary evaporator was used to obtain dried residues and stored in refrigerator for making stock solution. Further dilutions of 30%, 20% and 10% were prepared.

Gas chromatography/ GC/MS analysis

The samples of both the plants were analysed by gas chromatography (Trace GC-TSQ Evo 9000 mass spectrometer (Thermo Scientific, Austin, TX, USA) with a TC-SMS direct capillary column). The temperature of the protocol ranged from 80 to 280℃, increasing at a rate of 10℃/min, and remained constant for 10 minutes at 280℃. The injector maintained a temperature of 280℃ throughout the analysis. The carrier gas (helium) was used with a flow of 0.8 ml per minute. The compounds present in extracts were identified based on their retention time and structural formulas. Cross-referencing of mass spectral data with known authentic compounds, information obtained from relevant literature sources, were followed for verification of each compound.

Anti-termitic Assay

Anti-termitic assay was performed by following the methodology of Smith (1979). The base of each sterilized Petri-plate was surfaced with filter paper and 0.5ml of each extract of the desired concentration (30%, 20% and 10%) was applied. Petri plates along with filter paper were then dried at ambient temperature. A Hundred workers and 5 soldiers were placed in each Petri-plate and findings were made after 24 hours.

$$\text{Mortality \%} = \frac{\text{Number of dead insects after treatment}}{\text{Number of initial insects taken for treatment}} \times 100$$

Test for Repellency

Repellency test was conducted by cutting filter paper of 9cm diameter into 2 equal parts. One half of each filter paper was treated with 30%, 20% and 10% concentration of extracts and the other half with distilled water. Then the two halves of filter paper were placed in the Petri plate with a cut place between them. A total of 10 termites were released in the middle space. Repellency was assessed by counting number of termites after every 15 min on treated (T) and untreated (UT) filter paper and test was performed for a period of 2 hours. For each concentration of plant extracts three replicates were made. A treatment concentration was considered repellent when 21 (sum of three replicates) of 30 termites were present on untreated filter paper for five consecutive readings (Aihetasham, et al., 2017)

Statistical Analysis

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

RESULTS

The ethanolic leaf extracts of *A. nilotica* and *E. camaldulensis* were used to determine their efficacy against *C. heimi.* Greater mortality was observed at 30% concentration of both plant extracts while decreased at 20% and low in 10% concentration (Fig. 1, Table 3) The LC_{50} values of *A. nilotica* and *E.camaldulensis ethanolic leaf extracts against C. hemi were* 13.029 and 7.70, respectively (Table 4).

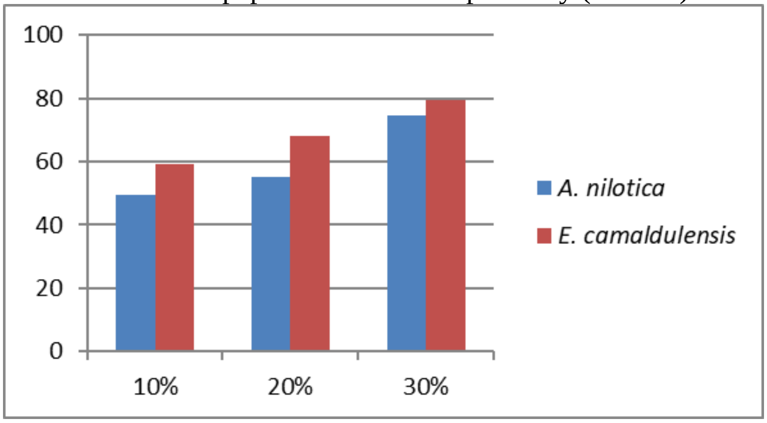


Fig. 1. Percentage mortality of *C. heimi* at different ethanolic leaf extract concentrations (10%, 20%, 30%) of *A. nilotica* and *E. camaldulensis*

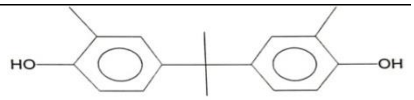
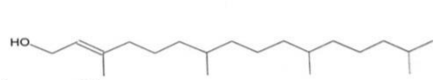
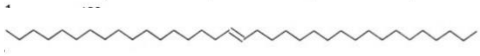
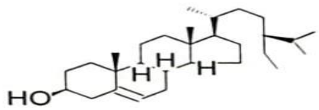
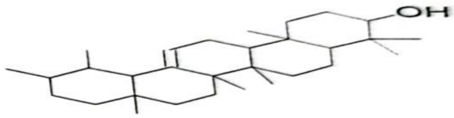
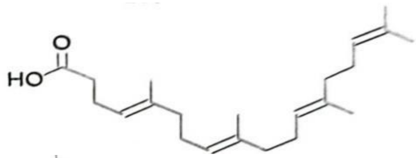
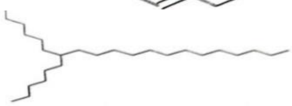
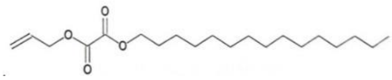
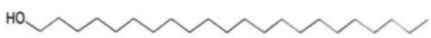
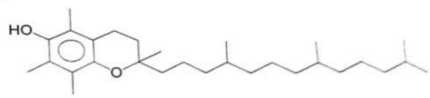
Sr #	Phytocompounds	Retention time (RT)	Relative % composition	Structural formulas
1	Bisphenol C	17.283	3.373%	
2	Neophytadiene	23.782	9.274%	
3	Phytol	26.702	2.848%	
4	17-Pentatriacontene	28.690	1.836%	
5	γ -Sitosterol	30.030	28.262%	
6	α – Amyrin	32.000	4.733%	
7	5,9,13,17-Tetramethyl 4,8,12,16-octadecatetraec acid	32.490	2.528%	
8	Eicosane,7-hexyl	32.915	2.491%	
9	Oxalic acid,allyl pentadecyl ester	34.419	1.696%	
10	Behenic-alcohol	34.495	17.464%	
11	Vitamin E	34.950	23.868%	
12	1-Eicosanol	35.416	1.628%	

Table I. Phytocompounds analysed in *A. nilotica* (ethanolic extract)

The GC/MS analysis of plant indicated several important components. The GC/MS analysis of *A. nilotica* (Table 1, Fig. 2) revealed following compounds: Bisphenol C, Neophytadiene, Phytol, 17-Pentatriacontene, γ – Sitosterol, α – Amyrin, 5,9,13,17-Tetramethyl 4,8,12,16-octadecatetraenoic acid, Eicosane, 7-hexyl, Oxalic acid, allyl pentadecyl ester, Behenic-alcohol, Vitamin E, 1-Eicosano (Table 1 and Fig. 3). The GC/MS analysis of *E. camaldulensis* revealed several compounds: α – Pinene, Eucalyptol, Eucalyptol, Terpinen-4-ol, α – Terpineol, 1H-Cycloprop[e]azulene,1a,2,3,4a,5,6,7b-octahydro-1,1,4,7-tetrametyl-,[1aR, Aromandendrene, (1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene, Alloaromadendrene,1H-Cycloprop[e]azulene,1a,2,3,5,6,7,7a,7b-0ctahydro-1,1,4,7-tetramethyl-,[1Ar], Naphthalene, 12,3,5,6,8a-hexdydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis), (-)-Globulol, 1H-Cycloprop[e]azulen-4-0l, decahydro-1,1,4,7-tetramethyl-,[1aR-(1aα,4β,4aβ,7),1H-Indene,1-ethylideneoctahydro-7a-methyl-,(1Z,3aα,7aβ), Phytol, γ– Sitosterol, Olean-12-en-3-ol, acetate, (3β), Lupeol (Table 2).

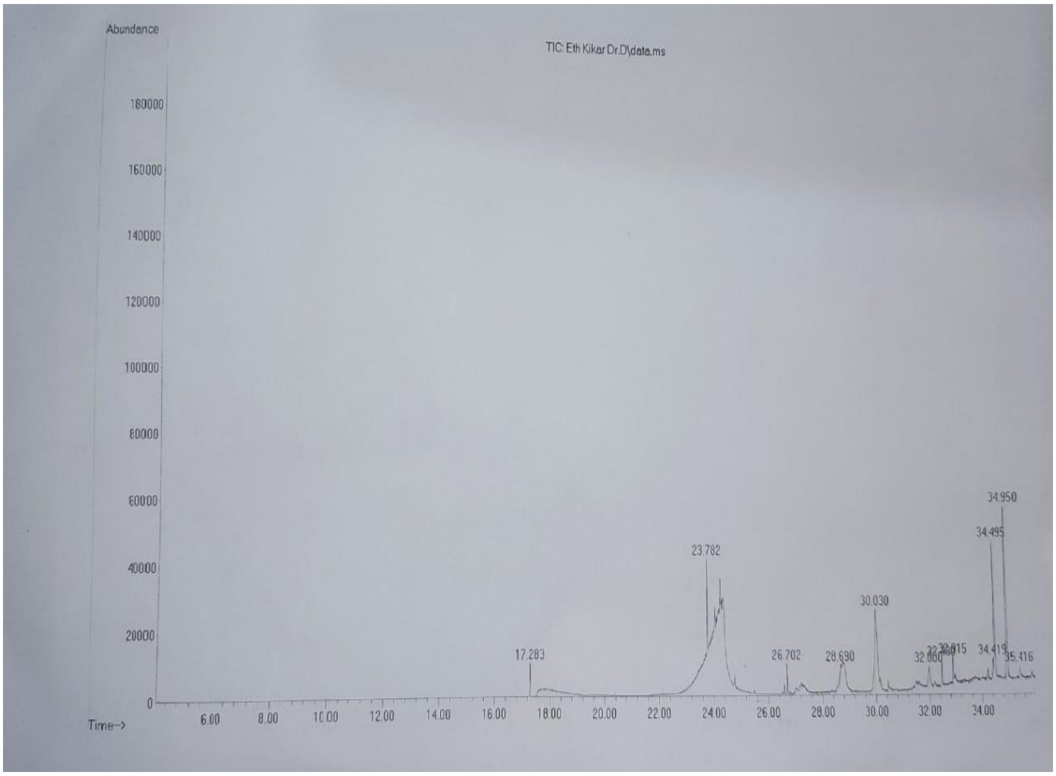
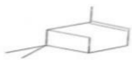
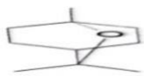
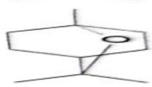
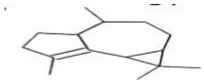
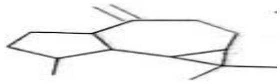
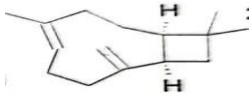
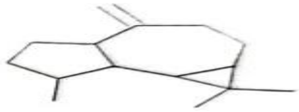
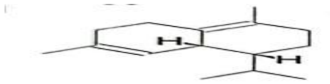
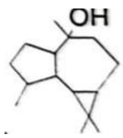
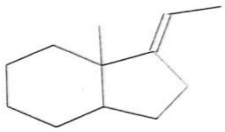
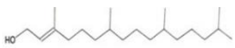
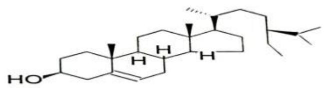
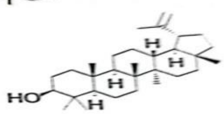


Fig. 2. Compounds identified in GC-MS analysis in *A. nilotica*

Table 2. Phytocompounds analysed in ethanolic extracts of *E. camaldulensis*

Sr #	Phytocompounds	Retention time	Relative percentage composition	Structural formula
1	α – Pinene	5.806	0.691%	
2	Eucalyptol	8.289	7.737%	
3	Eucalyptol	8.493	57.811%	
4	Terpinen-4-ol	12.189	1.358%	
5	α – Terpineol	12.538	1.749%	

6	1H-Cycloprop[e]azulene,1a,2,3, 4a,5,6,7b-octahydro-1,1,4,7-tetrametyl-, [1aR	15.989	0.751%	
7	Aromandendrene	17.231	1.193%	
8	(1R,9R, E)-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0]undec-4-ene	17.837	8.062%	
9	Alloaromadendrene	18.227	2.299%	
10	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-0ctahydro-1,1,4,7-tetramethyl-,[1Ar]	18.734	0.960%	
11	Naphthalene,12,3,5,6,8a-hexdydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)	18.845	1.412%	
12	(-)-Globulol	19.311	0.667%	
13	1H-Cycloprop[e]azulen-4-0l, decahydro-1,1,4,7-tetramethyl-,[1aR-(1aα,4β,4aβ,7)	20.483	4.810%	
14	1H-Indene,1-ethylideneoctahydro-7a-methyl-,(1Z,3aα,7aβ)	20.605	1.718%	
15	Phytol	21.066	0.895%	
16	ץ – Sitosterol	26.708	0.908%	
17	Olean-12-en-3-ol, acetate, (3β)	30.030	1.283%	
18	Lupeol	31.033	1.771%	

All the concentrations of both the plants were repellent, except 10% concentration of *A. nilotica* (Fig. 4). An observed concentration was notified repellent as twenty-one or more tested insects were found on untreated (UT) filter paper.

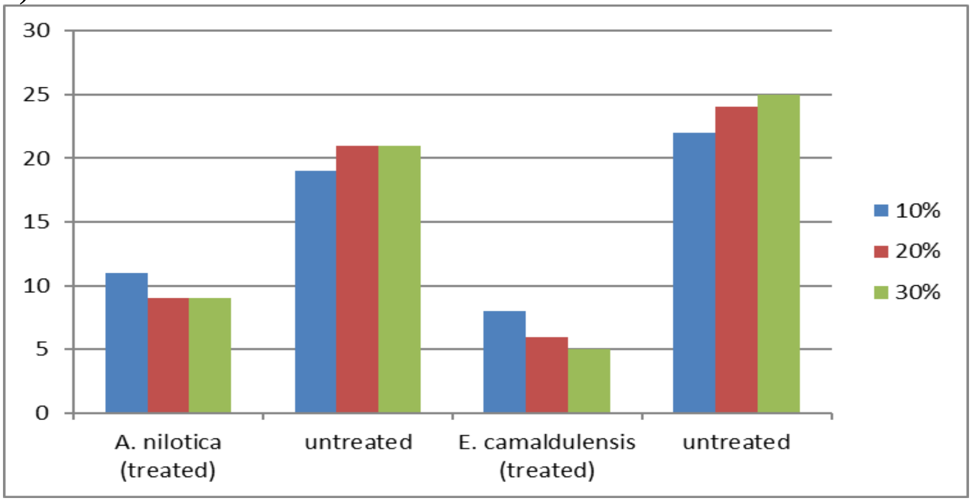


Fig. 4. Repellency test of *A. niolotica* and *E. camaldulensis* against *C. heimi*

Table 3. Two-way ANOVA for mortality of *C. heimi* (workers) after being exposed to *A. nilotica* and *E. Camaldulensis* ethanolic leaf extracts

ANOVA Table	SS (Type III)	Df	MS	F (DFn, DFd)	P value	Significance
Interaction	49.00	2	24.50	F (2,12) =33.92	P<0.0001	Yes
Plants	364.5	1	364.5	F (1,12) = 504.7	P<0.0001	Yes
Concentrations	1625	2	812.7	F (2,12) = 1125	P<0.0001	Yes
Residual	8.667	12	0.7222			

Table4. LC_{50} of *A. nilotica* and *E. camaldulensis* ethanolic extracts against *C. heimi.*

Sr. No.	Plant name	LC_{50}
1.	***A. nilotica***	13.029
2.	***E. camaldulensis***	7.70

DISCUSSION

The plants used in this study to control termites are *A. nilotica* and *E. camaldulensis* which contained biological active compounds. *E. camaldulensis* extracts were more repellent and toxic to *C. heimi* as caused 79.33% mortality at 30% concentration.

No prior research has been documented by using *A. nilotica* extract against termites, although its pesticidal properties are known. Numerous active components found in various parts of *A. nilotica* have been mentioned in diverse literature sources. Despite the absence of specific studies on the termiticidal effects of *A. nilotica* extracts, the promising results warrant further investigation at both laboratory and field levels. It is essential to identify and evaluate the specific anti-termitic toxicant compounds within the potent extract. Initial findings from this research indicate that *A. nilotica* ethanolic extract exhibits remarkable anti-termitic properties against *C. heimi*.

Pande et al. (1981) reported that the *A. nilotica* (leaves and seeds) carry various components, including crude proteins, phosphorous, tannins, galactose, sulfides, pentosan, arabinose, catechol, galacton, calcium, saponins, and silica. The observed effect of *A. nilotica* extract against termites can be linked to Edriss et al. (2012), who noted its larvicidal effects, on the *Anopheles arabiensis*. Additionally, Vivekanandhan et al. in 2018, documented significant larvicidal activity from the seed pod solvent extract and essential seed oil of *A. nilotica* against 3 mosquito species: *Anopheles stephensi* with 5.239 LC_{50}, *Aedes aegypti* having 3.174 LC_{50} and *Culex quinquefasciatus* having 4.112 LC_{50}.

An extra significant finding is of Edriss et al. (2012), which identified alkaloids, saponins, flavones, tannins, triterpenes, and sterols in the petroleum ether and ethanolic extracts of *A. nilotica*. The tannins in *A. nilotica* are known for their potent biocidal properties (Fagg and Greaves, 1990; Edriss et al., 2012). The current findings align with the research by Baeshen and Baz (2003), which tested *A. nilotica* leaf extract against the *Aedes aegypti* and *Culex pipiens*, observing a remarkable reduction of larvae nearly about 89.8 percent in less than 24 hours and maintaining stability for twelve days. The current findings are consistent with the phytochemical analysis conducted by Solomon & Shittu (2009), which identified various compounds in the ethanolic leaf extract of *A. nilotica*. These compounds include phenols, flavonoids, tannins, alkaloids, triterpenoids, volatile oils, hydrolysable tannins, and saponin glycosides."

The ethanolic extract of *E. camaldulensis* demonstrated notable efficacy against *C. heimi*, with mortality rates increasing in correlation with higher extract concentrations. Jembere et al. (2005) similarly noted that mortality rates rose with increased extract concentration, observing minimal mortality at 10% and maximal mortality at 30%. Qureshi et al. (2016) supported these findings by reporting that the benzene-ethanol extract from the sapwood, heartwood and bark of *E. camaldulensis* exhibited high toxicity towards flagellates in termite species, including *C. heimi*, resulting in significant population reduction ($p < 0.05$). The population of flagellates in *C. heimi*, showed a reduction of 86.66% (908±82.7), 90.6% (681±214) and 100% (0.0) in the bark, sapwood and heartwood. Furthermore, Alavijeh et al. (2014) corroborated the significant mortality of *E. camaldulensis* extract against pests like *Microcerotermes diversus*, highlighting its efficacy through contact, digestive, and fumigation toxicity.

Additionally, our findings are supported by Siramon et al. (2009), who demonstrated the potent antitermitic properties of *E. camaldulensis* leaf essential oil. They reported significant effectiveness against *C. formosanus* through both contact and fumigation methods, with LC_{50} values ranging from 12.68 to 17.5 mg/g and 12.65 to 17.5 mg/petri dish (100 cm^3), respectively. The extract also exhibited inhibition of acetylcholinesterase activity, impacting the nervous system. Furthermore, Mouna et al. (2021) corroborated these insecticidal properties, noting a 93% mortality rate with a 50% concentration of *E. camaldulensis* extract against *Aphis fabae*.

Among various compounds present in Eucalyptus essential oil, the 1, 8-cineol is the most essential and showed a notable role in insecticidal activity (Duke, 2004). Principally, Eucalyptol was found as an eminent constituent, showing retention time of 8.493

with relative percentage composition of 57.811%. Eucalyptol, the principal active component, present within the leaf extract may be responsible for toxicity against *C. heimi*.

The toxic and repellent effects of these plants are likely influenced by their chemical composition and the insects' susceptibility. To support our findings, we conducted a phytochemical screening, which confirmed the presence of active molecular families, including tannins and saponins.

CONCLUSION

Laboratory bioassay with *A. nilotica* and *E. camaldulensis* indicated their potential to be used as termiticides. Specifically, E. camaldulensis ethanolic extract was strongly repellent and toxic causing highest mortality of *C. heimi* at 30% concentration. The result of the present findings showed that botanical extracts are both economical and effective when used to control termites.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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