

Research Article

GC-MS profiling and efficacy of *Crotalaria ramosissima* Roxb. leaf extracts in controlling termite, *Odontotermes obesus*

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ABSTRACT: *Crotalaria ramosissima* Roxb. is a Fabaceae family genus that prolifically grows as a weed in southern regions of India and is known for insect-repellent activity. The purpose of the current study is to assess the efficacy of anti-termite activity of the different solvents extracts and formulations of *C. ramosissima* against *Odontotermes obesus* which is prevalent in the south dry zone that destroys 10% of crops and wood on an average by feeding. The extracts showed anti-termite efficacy by No-choice bioassay method where the ethyl acetate extract showed highest termite mortality rates of 24.23±1.51%, 43.93±1.51%, 74.23±1.51% at 12, 24, 36h time intervals and methanol extract showed 16.66±1.51%, 30.29±4.00%, 54.54±2.62% at 12, 24, 36 h time intervals. The TLC analysis of ethyl acetate extract resulted in three bands which were separated through column chromatography and named Fraction-1, Fraction-2, and Fraction-3 and upon further investigation Fraction-3 showed maximum mortality rate of 81.81±2.62% at 36 hrs of time interval with a lethal concentration (LC-50) 248.44mg/ml. The GC-MS profiling of fraction-3 led to the identification of compounds viz. 1,2-benzenedicarboxylic acid, 1,2,4-oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl], phenethylamine-2-acetoxy-n,n-bis[2-(benzimid-2-yl)ethyl], and 9-octadecenoic acid (z)-2-hydroxyethyl ester and phenol, p-(1-ethyl-1-methylbutyl). These results may aid in the synthesis of novel effective bioactive compounds that needs to be explored for their toxicity against insects, as was enlightened in the current study.

KEYWORDS: *Crotalaria ramosissima*, GC-MS analysis, herbal-pesticide, termites

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INTRODUCTION

Some aromatic plants and their extracts have been used to control pests since ancient times and from historical sources it is evident that, in Europe and other countries aromatic plants are used particularly in grain storage rooms during post-harvest time (Pavela, 2016). Plant extracts are used for many beneficial activities and pest control is one of them. Plants and insects co-evolved with different survival strategies, resulting in the development of an elegant defence mechanism in which active metabolites are released in response to herbivores from specific structures which are toxic, repellent, or anti-nutritional to some animals and humans (Rani & Jyothsna 2010). Plant volatile compounds are used specifically for insecticidal purposes (Hare, 2011). Plant metabolites such as alkaloids, phenolics, terpenoids, tannins, saponins, proteinase inhibitors and oxidative enzymes, affect growth and have synergistic effects in the digestion and metabolism of insects (Hanley *et al.*, 2007). Phytohormones such as jasmonic acid, salicylic acid, and ethylene are known to induce disease resistance in plants by

blocking neurotransmitter channels in insects and retard their growth (Duffey & Stout, 1996).

Around 93 species of the *Crotalaria* genus are distributed in India (Subramaniam & Pandey 2014), which is primarily used for hemp, fibre, green manure, animal feed (forage) and also as ethnomedicine for skin diseases (Tirkey, 2006). Pyrrolizidine alkaloids (PAs) found in the *Crotalaria* genus plant roots, leaves, flowers, fruits, and seed have been identified as a source of contaminants in food and beverages causing hepatotoxicity in animals and central nervous system problems in humans (Pearson, 2001). *C. ramosissima* is found only in the Indian Peninsula region and distributed in open, dry deciduous forests; it does not occur in the Western Ghats (Subramaniam & Pandey, 2014; Subramaniam *et al.*, 2015; Thomas *et al.*, 2012). Village people use it for foraging and as pest control in grain storage rooms. *C. ramosissima* flowers are already reported for flavanone, crotamosmin, trimethoxychalcone, and essential oil major component like sesquiterpenes. However, their biological activities are not known (Khalilullah *et al.*, 1992; Rao & Narukulla, 2007).

The white ants and termites are eusocial, polyphagous insects, which act as pests (Isoptera:Termitidae) and are found all over the world. They originated in South Africa and primarily consist of seven families among which, four families of termites are known for their destructive activities, namely Rhinotermitidae, Kalotermitidae, Hodotermitidae, and Termitidae. Their largest family includes more than 2100 species (UNEP/FAO/Global IPM Facility Expert Group on Termite Biology and Management members, 2000). Termites are renowned for surviving in harsh conditions and are frequently resistant to toxic chemical pesticides (Upadhyay *et al.*, 2010). Termites are also responsible for the destruction of household materials, severely destroying wood and its products, forest trees, buildings and altering soil profiles. Agriculture has suffered greatly as a result of termites, which have destroyed crops worldwide, including rice, maize, sugarcane, cotton, legumes, sunflowers and groundnuts (Mishra *et al.*, 2021; Elango *et al.*, 2012; Ahmad *et al.*, 2021). Three billion dollars worth of wood structures are damaged by termites annually in the United States (Verma *et al.*, 2018) and surprisingly 217 million dollars is being used for pest control especially to control the termites in America (Rust & Su, 2012). Each year, a total of 15–20% of the maize crop is lost to termite activity, with around 1478 million rupees in losses reported in India (Joshi *et al.*, 2005). Every year, India loses 35.12 million dollars worth of crops due to termites. Chemical pesticides and biological agents are commonly used to control termite infestation, however, chemical pesticides used in the control of termites known to pollute 75% of urban surfaces, which are persistent and dangerous to humans, linked to food chains, and affecting ecological balances (Pandey *et al.*, 2012). Bifenthrin, Chlorfenapyr, Cypermethrin, Fipronil, Imidacloprid and Permethrin are the most commonly used termite pesticides worldwide (Elango *et al.*, 2012). However, disposal of these chemicals is a problem because they accumulate and cause harm to other beneficial insects and the environment.

The phytobased products are easily available and can be adopted to control termites. Plant roots, leaves, stems, fruit, seed and bark have different modes of action and also they attract termites and act as temitoxicants as reported by Mishra *et al.*, (2021). The repellents, feeding deterrents, toxicants, growth retarders, chemo sterilants and wisely essential oils of *Dipterocarpous*, *Cinnamomum* and *Cymbopogon*, etc., are mostly used against termites (Verma *et al.*, 2018). Phytochemicals are components which kill termites by exerting anti-feedant action. The majority of termite-toxic chemical structures isolated and elucidated from plant extract are 2-bromocompounds, 2-chloro analogues, halo acid methyl esters, (Karr *et al.*, 2004; King *et al.*, 2005) shorter sugar chain and noviflumuron is highly potent which is depend on a number of fluorine molecules present for

termicidal activity (Ohara *et al.*, 1991). *Crotalaria burhia* root extract reported anti-termite activity against *O. obesus* showed 73.33% mortality after 24 h treatment (Ranjith *et al.*, 2017). The prenylated chalcone named crotarorixin was isolated from *C. orixensis* (Narender *et al.*, 2005). *In vitro* anti-malarial activities of crotaorixin as well as a few prenylated chalcones isolated from *C. medicagenia* and *C. ramosissima* were evaluated to exhibit 100% inhibition against *Plasmodium falciparum*. Chalcones are reported as anti-feedant and deterrent against insects compound reported from *Crotalaria* genus (Díaz-Tielas *et al.*, 2016) and *C. ramosissima* known to have 1,2,4-oxadiazole-3(1,3-benzodioxol-5-yl)-5-[2(4-methoxyphenyl)ethyl] heterocyclic aromatic chemical compound having a broad spectrum applications in insecticidal activities (Lanchana & Garampalli, 2024).

The *C. ramosissima* is an herb with silky hairs that is highly aromatic, sticky, and completely covered with oily glands. It is unfit for consumption due to the unpleasant odour and grows as a weed in between agricultural fields. Locally it is known as an insect repellent and used to test it for its anti-termite activity. *Odontotermes obesus* is found in southern dry regions and is predominant in the districts of Mysore, Mandya, Tumkur, Chitradurga, Hassan, Coorg, Shimoga, Kolar and Bangalore, Karnataka (Basavarajappa *et al.*, 2002). They are not encountered in lateritic soils and in heavy rainfall areas. However, they have been reported to infest various crops in Karnataka. Published report on phytochemicals impact on termite activity is meagre. Therefore, in the present study, phytochemical analysis of *C. ramosissima* leaf extracts, GC-MS profiling and anti-termite activity was carried out in a pursuit to get a novel compounds from plants against termites.

MATERIALS AND METHODS

Plant collection and extraction

Healthy plants were collected from Pavagada, Tumkuru district (14°10'13.8"N 77°06'18.2"E) identified and authenticated with the help of local floras. Plant herbarium has been deposited at the Department of Studies Botany, University of Mysore, Manasagangothri. Dried plant leaves were ground into powder; 25g of powder sample was used successively for extraction with 250 ml of acetone, ethyl acetate, ethanol and methanol using Soxhlet apparatus for 8h. The collected extract was concentrated in vacuum evaporator pressure fixed at 22-26 mm Hg with 40°C and stored in 4°C (Harborne, 1998).

Antitermitic activity

Odontotermes obesus species termite species were collected from Mysuru and identified and authenticated at



Figure 1. Graphical representation of anti-termite activity of *Crotalaria ramosissima*.

the Department of Studies in Zoology, University of Mysore. Only worker and soldier termites were collected from the termite mound without harming the main colonies and were stored in the laboratory by maintaining $28 \pm 2^\circ\text{C}$ temperature and $>85\%$ Relative Humidity (RH) at dark condition.

No-choice bioassay method

The no-choice bioassay method (Kang *et al.*, 1990) was used to evaluate the termiticidal activity. *C. ramosissima* leaf powder and Soxhlet extracts of acetone, ethyl acetate, ethanol and methanol were utilised. 500mg/ml of each extract dissolved in 10ml of acetone used as a stock solution, 2ml of stock solution was sprayed onto a sterile Whatman filter paper with a diameter of 9cm which was then put in

Petri dishes and allowed to dry for an hour under laminar air being utilised. Twenty healthy workers and two soldier termites were put on filter paper, with 2ml of acetone and distilled water sprayed on filter paper used as a control. The termites were then incubated at $28^\circ\text{C} \pm 2$ and $>85\%$ RH. To maintain humidity, a few drops of water were sprayed around the edges of the petri dishes. Termite mortality was observed every 12 h and the experiment continued up to 36 h. The percentage of mortality was calculated using a formula given by Kang *et al.* (1990).

$$(\%) \text{Mortality} = \frac{\text{Number of dead termites}}{\text{Number of termites kept for test}} \times 100$$

Isolation of bioactive compound

Among all extracts, ethyl acetate extract showed the most significant anti-termite activity hence it was subjected to further separation using Thin Layer-Chromatography (TLC) and Column Chromatography to isolate bioactive chemical constituents. 50 μL of ethyl acetate extract was spotted on a TLC silica gel plate (TLC silica gel 60 F254, Merk, Germany) and air-dried. The spotted TLC plate was dipped in a solvent system of Hexane: Ethyl acetate (1:1). The TLC plate dried for clear bands appearance its Retention Factor (R_f) values were calculated. Aluminium oxide was also used for Column Chromatography to separate the natural products, especially alkaloids and terpenoids as per the protocol of Feng *et al.* (2011). The column (20 mm x 280 mm) was columnisation by neutral aluminium oxide (150g aluminium oxide of $\text{pH} 7 \pm 5$ applying wet stow with 100% methanol) washed with 100% of methanol, 2g of ethyl acetate extract added and solvent system, Hexane: Ethyl acetate (1:1) introduced to wash extract with a fixed flow rate of 2 ml/min to elute. Collected fractions were completely vaporized to remove solvent traces and then stored for further analyses.

GC-MS analysis of ethyl acetate fractions

The GC-MS analysis of *C. ramosissima* ethyl acetate extract column fractions was carried out on a Shimadzu GC-MS chromatogram of Model -QP2010S of a capillary column of ELITE-5MS of 30 m length, 0.25 mm inner diameter and 0.25 μm thickness. GC-MS Solutions software was used to identify compounds and compare them to mass spectral data libraries from NIST 11 (National Institute of Standards and Technology, Washington, DC, USA) and Wiley 8.

Statistical analysis

Termites' mortality rates were measured in triplicates for each test and statistical analysis one-way Analysis of Variance (ANOVA) with significant effects was determined by F values ($p > 0.05$). All the data analysis was performed by using the software SPSS 16.0.

Table 1. Termite mortality percentage at different solvent leaf extracts of *C. ramosissima*

Extract	Concentration of sample mg mL ⁻¹	Mortality % 12 h	Mortality % 24 h	Mortality % 36 h
Ethyl acetate extract	500	24.23±1.51 ^a	43.93±1.51 ^a	74.23±1.51 ^a
Methanol extract	500	16.66±1.51 ^b	30.29±4.00 ^b	54.54±2.62 ^b
Ethanol extract	500	06.05±0.00 ^c	13.33±2.60 ^c	24.23±3.60 ^c
Acetone extract	500	04.00±1.51 ^c	10.51±1.42 ^c	20.00±1.51 ^c
Water plant powder	500	NA	NA	NA
Acetone	0	NA	NA	NA
Water	0	NA	NA	NA

Each value is the mean for three replicates n=3) and ± indicates standard errors. Means denoted by the same alphabet letter(s) within the same column are not significantly different (p<0.05) according to (ANOVA with significant treatment according to Tukey's HSD;p<0.05). Note: NA- No Activity.

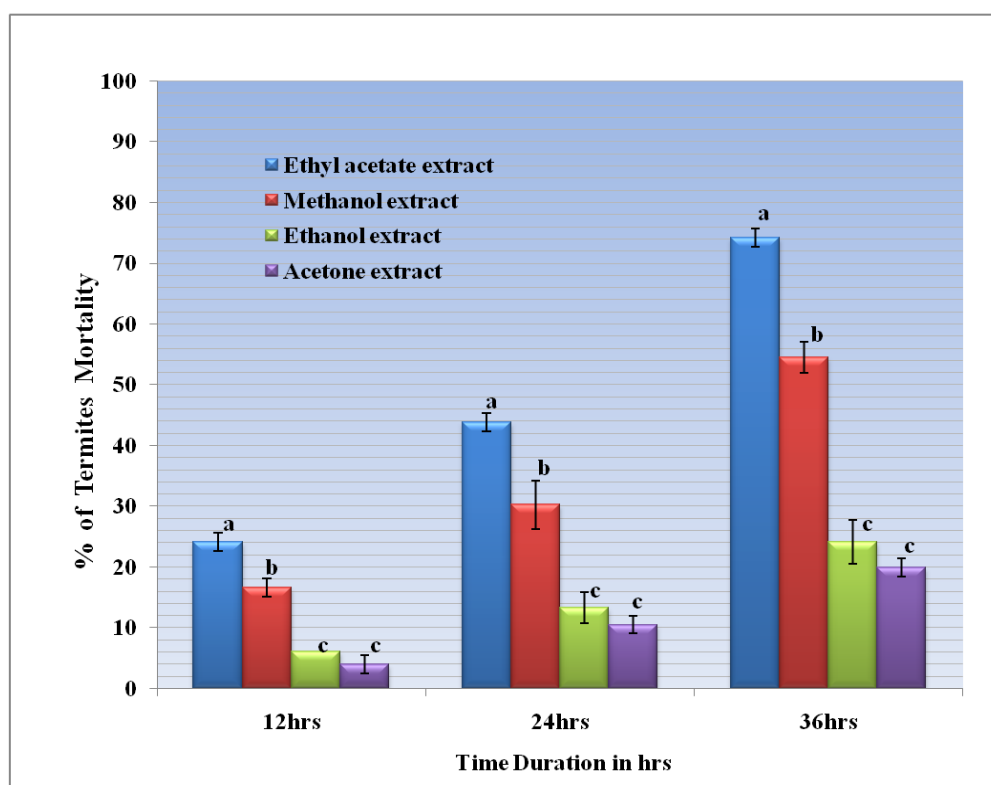


Figure 2. Graphical representation of termite's mortality percentage of different solvent leaf extracts of *C. ramosissima* followed by bars sharing the same letters are not significantly different (p<0.05) according to Tukeys HSD vertical bars are standard errors.

RESULTS

Terminicidal activityof *C. ramosissima* leaf extracts

Plant extracts showed promising results against *O. obesus* after 12, 24 and 36 h of incubation time (Figure 1). A high mortality rate was observed in ethyl acetate and methanol extracts, though there was a slight difference between them (Table 1). The mortality rate was found to be 24.23±1.51%, 43.93±1.51%, 74.23±1.51% for the ethyl acetate extract and 16.66±1.51%, 30.29±4.00%, 54.54±2.62% for the

methanol extract at 12, 24 and 36, respectively at the extract concentration of 500 mg/ml, while ethanol extract, acetone extract, crude plant powder were found ineffective against the termite (Figure 2).

Terminicidal activity of *C. ramosissima* column extracts

TLC analysis of ethyl acetate extract showed majorly three major visible bands (Figure 3) at 365nm and Rf values were determined as 0.35, 0.5, and 0.55. Ethyl acetate extract was used for column chromatography to separate bioactive

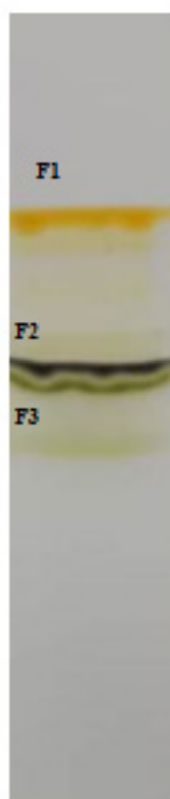


Figure 3. TLC analysis of ethyl acetate extract.

compounds, and a total of 9 fraction mixtures were pooled into three fractions. Fraction-1 and Fractions-2 showed moderate results in anti-termite activity (Table 2), while Fraction-3 showed highest mortality rate of $39.38 \pm 1.51\%$, $60.60 \pm 3.03\%$, $81.81 \pm 2.62\%$ at 12, 24 and 36h of time interval, respectively with lethal concentration (LC-50) of 672.26 mg/ml at 12h, 375.74 mg/ml at 24 h, 248.44 mg/ml at 36 (Table 3).

GC-MS analysis of column fraction

GC-MS analysis of fraction-3 let to the identification of compounds viz. 1,2-benzenedicarboxylic acid,

Table 3. Termite mortality in terms of lethal concentration (LC-50) from *C. ramosissima* column fraction-3

Time duration in hours	Concentration mg mL ⁻¹	Regression equation	Regression coefficient (R ²)
24	375.74	$y=0.101x+12.05$	0.973
36	248.44	$y=0.135x+16.46$	0.984

1,2,4-oxadiazole, and 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl], phenethylamine-2-acetoxy-n,n-bis[2-(benzimid-2-yl)ethyl], and 9-octadecenoic acid (z)-2-hydroxyethyl ester phenol, p-(1-ethyl-1-methylbutyl)and along with some other volatile oils, terpenoids, unsaturated and saturated fatty acids (Table4) (Figure 4).

DISCUSSION

Available literature suggests that few plant extracts are more potent in the field of anti-termite activities. India has diversified climate zones where termites also exhibit diversity in population and density. Botanical extracts are the most promising bio-pesticide which reduces termite population within a short period of exposure (Mahapatro *et al.*, 2017). Seed treatments with plant extracts having anti-termite property is also believed to help (Jimma, 2014). *Crotalaria ramosissima* has leaves with glandular trichomes which contain poisonous alkaloids, terpenoids and flavonoids that repel insects (War *et al.*, 2012). Results of present study reports the anti-termite activity of *C. ramosissima* and best results were obtained in ethyl acetate extract fractions of column chromatography. The compounds primarily contained phthalic acids, alkaloids, terpenoids and fatty acids of which 1, 2-benzenedicarboxylic acid which has insecticidal and phytotoxic activity against insects. Similar results were observed in *C. burhia* root aqueous extracts which showed 73.63%-76.67% mortality of *O. obesus* at 24 h of incubation, which was mediated by mucin, gum and secondary metabolites (Rajith *et al.*, 2017). *Capparis deciduas* and its combinatorial mixture anti-termite activity

Table 2. Termite mortality percentage of column fractions of *C. ramosissima* leaf extracts.

Extract	Concentration of sample mg mL ⁻¹	Mortality % 12 h	Mortality % 24 h	Mortality % 36 h
Fraction-1	500	14.72 ± 1.72^a	17.96 ± 2.80^a	17.96 ± 2.80^a
Fraction-2	500	16.45 ± 1.72^b	19.48 ± 3.24^b	37.87 ± 1.51^b
Fraction-3	500	39.38 ± 1.51^b	60.60 ± 3.03^b	81.81 ± 2.62^c
Acetone	0	NA	NA	NA
Water	0	NA	NA	NA

Each value is the mean for three replicates (n=3) and $\pm$ indicates standard errors. Means followed by the same letter(s) within the same column are not significantly different ($p < 0.05$) according to Tukey's HSD. Note: NA- No Activity

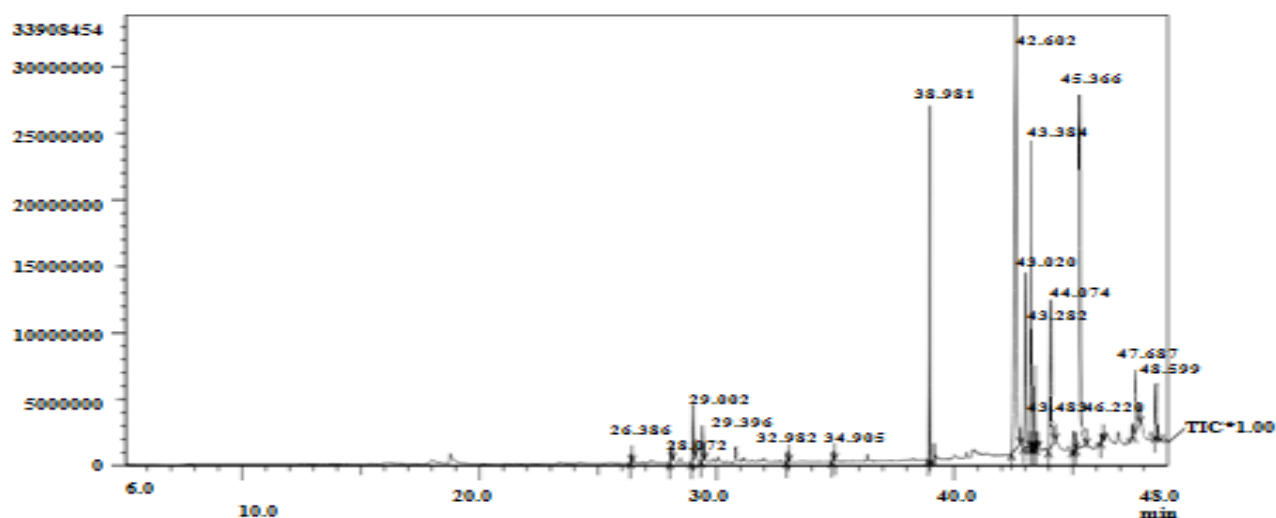


Figure 4. GC-MS Chromatogram of ethyl acetate column fraction peaks with retention time.

Table 4. GC-MS analysis of *C. ramosissima* Column Fraction-3 and probable bioactive compounds activities

Peak No.	RT	Area of %	Compound name	Molecular formula	Biological activities
1	26.386	0.22	Neophytadiene	C ₂₀ H ₃₈	Insect-repellent activities (Caceres <i>et al.</i> , 2015; Laosinwattana <i>et al.</i> , 2018)
2	28.072	0.43	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C ₁₆ H ₂₂ O	Insecticidal, Mosquito repellent (Huang <i>et al.</i> , 2021)
3	29.002	1.85	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	
4	29.396	1.20	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	C ₂₀ H ₃₀ O ₄	
5	32.982	0.26	Hexadecanoic acid, butyl ester	C ₂₀ H ₄₀ O	Insect repellent (de Paula, 2003)
6	34.905	0.31	Oxiraneoctanoic acid, 3-octyl-, methyl ester, trans-	C ₁₉ H ₃₆ O ₃	
7	38.981	6.05	1,2-Benzenedicarboxylic acid	C ₈ H ₆ O	
8	42.602	24.77	9-Octadecenoic acid (z)-, 2-hydroxyethyl ester	C ₂₀ H ₃₈ O	Insect repellent in grain storage, Cytotoxic activities (Awojide <i>et al.</i> , 2023)
9	43.020	7.38	1,2,4-oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl]-	C ₁₈ H ₁₆ N ₂ O ₄	larvicidal activity, nematicidal, insecticidal pesticide, and herbicidal (Luo <i>et al.</i> , 2022; Zhu <i>et al.</i> , 2020)
10	43.282	3.91	9-octadecenoic acid (z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	C ₂₅ H ₄₆ O ₆	Insect repellent, antimalarial (Okokon <i>et al.</i> , 2017)
11	43.384	9.42	9-octadecenoic acid (z)-, 2,3-bis(acetyloxy)propyl ester	C ₂₅ H ₄₄ O	
12	43.483	0.20	1-linolenic acid, 2,3-diacylglycerol	C ₃₉ H ₇₂ O ₅	Cosmetic preparation
13	44.074	7.61	2,4-hexadien-1-one, 1,3,5-triphenyl-	C ₂₄ H ₂₀ O	Antihelmintic and Insecticidal agents (Gupta & Kant, 2013)
14	45.044	1.03	elaidic acid, benzyltrimethylsilyl ester	C ₂₀ H ₃₈ O ₂	Larvicidal, insect repellent (Ramsewak <i>et al.</i> , 2001)
15	45.366	27.64	phenethylamine, 2-acetoxy-N,N-bis[2-(benzimidazol-2-yl)ethyl]-	C ₂₉ H ₂₅ N ₃ O ₆	Unknown
16	46.220	0.28	2h-cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-	C ₁₅ H ₂₂ O	Insecticidal potent reported (Ayaz <i>et al.</i> , 2016)
17	47.687	4.39	phenol, p-(1-ethyl-1-methylbutyl)-	C ₁₃ H ₂₀ O	Insecticidal (Pavela, 2011; Stasiuk & Kozubek 2010; Bagle <i>et al.</i> , 2013)
18	48.599	3.03	phenol, p-(1-ethyl-1-methylbutyl)-	C ₁₃ H ₂₀ O	

has been reported by Upadhyay *et al.*, (2010), where a very low dosage of plant extract was able to exhibit mortality against *O. obesus* through synergistic action. Extracted Oil from *Murraya koenigii* and *Callistemon citrinus* showed anti-termite activity with 100% mortality with LC50-535.805ppm, LC90-4743.531 ppm and LC50-177.891ppm LC90-749.502 ppm respectively and the GC-MS report revealed that phenols are the main constituents which are responsible for termicidal activity (Mishra *et al.*, 2021).

The compound 1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl] obtained from GC-MS separation in the present study is known to be synthesized in plants from chalcones, which have cytotoxic and nematocidal activities which are used as low-risk chemical pesticides to control a variety of agricultural diseases (Zhu *et al.*, 2020; Biernacki *et al.*, 2020). 9-Octadecenoic acid (z)-2-hydroxyethyl ester which is an oleic acid is known to have the anti-termicidal property generally used to control pests in grain storage rooms. Along with other fatty acids, volatile essential oils are reported as insect repellents and feeding deterrents (Scheffrahn & Rust, 1983; Awojide *et al.*, 2019). Other compounds reported from present study are terpenoids, which can block the Na⁺ channel of insects, disrupt larval growth and also induce the production of phytoecdysone and kill insects (Mithofer & Boland, 2012). Phenols exhibit cytotoxic effects and direct toxicity, which disrupts endocrine activity of the insect (Gajger & Dar, 2021). Corroborating the past results of other plant extracts with the present study, it may be concluded that *C. ramosissima* plant extracts have promising insecticidal properties and may be useful in termite control.

CONCLUSION

Biopesticides are the world's future, and increasing awareness of organic pesticides may increase their use and marketing. *Crotalaria ramosissima* leaf extracts were evaluated for efficacy of anti-termite activity; crude plant powder showed no activity, ethyl acetate and methanol extract showed significant activity; and ethyl acetate extract of column fraction resulted in a high percentage mortality of termites due to the presence of potent bioactive compounds. Majorly, 1,2-benzenedicarboxylic acid, 1,2,4-oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl], phenmethylamine-2-acetoxy-n,n-bis[2-(benzoimid-2-yl)ethyl], 9-octadecenoic acid (z)-2-hydroxyethyl ester and phenol, p-(1-ethyl-1-methylbutyl) combination showed promising effect against termite, *O. obesus*.

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REFERENCES

- Ahmad, F., Fouad, H., Liang, S. Y., Hu, Y., and Mo, J. C., 2021. Termites and Chinese agricultural system: Applications and advances in integrated termite management and chemical control. *Int Sci*, **28**(1): 2-20. <https://doi.org/10.1111/1744-7917.12726> PMID:31529680 PMCid:PMC7818233
- Awojide, S. H., Anifowose, A. J., Aderogba, A. A., and Tayo, A. S., 2019. Chemical composition and bioactivity of essential oil from *Monodora myristica* against grain storage insects. *Int J Sci Res Innov*, **6**(6): 181-186.
- Awojide, H. S., Fadunmade, E. O., Oyewole, A. K., Adeyemo, G. A., Ogunniran, T. D., Ademikanlu, D. E., and Adebayo, E. A. 2023. *Monodora myristica* essential oils bioactivity against two grain storage insects. *Adeleke Univ J Eng Tech*, **6**(1): 123-129.
- Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Ovais, M., Ahmad, W., and Zeb, A. 2016. Chemical profiling, antimicrobial and insecticidal evaluations of *Polygonum hydropiper* L. *BMC Complement Altern Med*, **16**, 1-14. <https://doi.org/10.1186/s12906-016-1491-4> PMID:27919287 PMCid:PMC5139080
- Bagle, A. V., Jadhav, R. S., Gite, V. V., Hundiware, D. G., and Mahulikar, P. P. 2013. Controlled release study of phenol formaldehyde microcapsules containing neem oil as an insecticide. *Int J Polym Mater Polym Biomater*, **62**(8): 421-425. <https://doi.org/10.1080/00914037.2012.719142>
- Basavarajappa, S., Naveed, A., and Hosetti, B. B. 2002. Zones in Karnataka. *Persp Ani Ecol Reprod*, 1-12.
- Biernacki, K., Daško, M., Ciupak, O., Kubiński, K., Rachon, J., and Demkowicz, S. 2020. Novel 1, 2, 4-oxadiazole derivatives in drug discovery. *Pharma*, **13**(6): Article 111. <https://doi.org/10.3390/ph13060111> PMID:32485996 PMCid:PMC7345688
- Caceres, L. A., McGarvey, B. D., Briens, C., Berruti, F., Yeung, K. K. C., and Scott, I. M. 2015. Insecticidal properties of pyrolysis bio-oil from greenhouse tomato residue biomass. *Journal of Analytical and Applied Pyrolysis*, **112**: 333-340. <https://doi.org/10.1016/j.jaap.2015.01.003>
- Díaz-Tielas, C., Graña, E., Reigosa, M. J., and Sánchez-Moreiras, A. M. 2016. Biological activities and novel applications of chalcones. *Planta Daninha*, **34**: 607-616. <https://doi.org/10.1590/S0100-83582016340300022>

- de Paula, J. P., Gomes-Carneiro, M. R., and Paumgarten, F. J. 2003. Chemical composition, toxicity and mosquito repellency of *Ocimum selloi* oil. *J Ethnopharmacol*, **88**(2-3), 253-260. [https://doi.org/10.1016/S0378-8741\(03\)00233-2](https://doi.org/10.1016/S0378-8741(03)00233-2)
- Duffey, S. S., and Stout, M. J. 1996. Antinutritive and toxic components of plant defense against insects. *Arch Insect Biochem Physiol*, **32**(1): 3-37. [https://doi.org/10.1002/\(SICI\)1520-6327\(1996\)32:1%3C3::AID-ARCH2%3E3.0.CO;2-1](https://doi.org/10.1002/(SICI)1520-6327(1996)32:1%3C3::AID-ARCH2%3E3.0.CO;2-1)
- Elango, G., Rahuman, A. A., Kamaraj, C., Bagavan, A., Zahir, A. A., Santhoshkumar, T., and Rajakumar, G. 2012. Efficacy of medicinal plant extracts against Formosan subterranean termite, *Coptotermes formosanus*. *Ind Crop Prods*, **36**(1): 524-530. <https://doi.org/10.1016/j.indcrop.2011.10.032>
- Feng, D., Li, L., Yang, F., Tan, W., Zhao, G., Zou, H., and Zhang, Y. 2011. Separation of ionic liquid [Mmim] [DMP] and glucose from enzymatic hydrolysis mixture of cellulose using alumina column chromatography. *Appl Microbiol Biotechnol*, **91**: 399-405. <https://doi.org/10.1007/s00253-011-3263-x> PMID:21617929
- Gajger, T. I., and Dar, S. A. 2021. Plant allelochemicals as sources of insecticides. *Insects*, **12**(3): Article 189. <https://doi.org/10.3390/insects12030189> PMID:33668349 PMCID:PMC7996276
- Gupta, V., and Kant, V. 2013. A review on biological activity of imidazole and thiazole moieties and their derivatives. *Sci Int*, **1**(7): 253-260. <https://doi.org/10.17311/sciintl.2013.253.260>
- Hanley, M. E., Lamont, B. B., Fairbanks, M. M., and Rafferty, C. M., 2007. Plant structural traits and their role in anti-herbivore defence. *Perspect Plant Ecol Evol Syst*, **8**(4): 157-178. <https://doi.org/10.1016/j.ppees.2007.01.001>
- Harborne, A. J. 1998. Phytochemical methods a guide to modern techniques of plant analysis. Springer Dordrecht.
- Hare, J. D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annu Rev Entomol*, **56**: 161-180. <https://doi.org/10.1146/annurev-ento-120709-144753> PMID:21133760
- Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., and Shao, H. 2021. Phthalic acid esters: Natural sources and biological activities. *Toxins*, **13**(7): Article 495. <https://doi.org/10.3390/toxins13070495> PMID:34357967 PMCID:PMC8310026
- Jimma, E. 2014. Efficacy of botanical extracts against termites, *Macrotermes* spp. (Isoptera: Termitidae) under laboratory conditions. *Int J Agri Res*, **9**(2): 60-73. <https://doi.org/10.3923/ijar.2014.60.73>
- Joshi, P. K., Singh, N. P., Singh, N. N., Gerpacio, R. V., and Pingali, P. L. 2005. Maize in India: Production systems, constraints, and research priorities. CIMMYT, Mexico.
- Kang, H. Y., Matsushima, N., Sameshima, K., and Takamura, N., 1990. Termite resistance tests of hardwoods of Kochi growth. I. The strong termiticidal activity of kagonoki (*Litsea coreana*). *Mokuzai Gakkaishi. J Wood Sci*, **36**(1): 78-84.
- Karr, L. L., Sheets, J. J., King, J. E., and Dripps, J. E., 2004. Laboratory performance and pharmacokinetics of the *benzoylphenylurea noviflumuron* in eastern subterranean termites (Isoptera: Rhinotermitidae). *J Econ Entomol*, **97**(2): 593-600. <https://doi.org/10.1093/jee/97.2.593> PMID:15154487
- Khalilullah, M. D., Sharma, V. M., Rao, P. S. and Raju, K. R. 1992. Crotaramosmin, a new prenylated flavanone from *Crotalaria ramosissima*. *J Nat Prod*, **55**(2): 229-231 <https://doi.org/10.1021/np50080a013>
- King, J. E., DeMark, J. J., and Griffin, A. J. 2005. Comparative laboratory efficacy of noviflumuron and diflubenzuron on *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiol*, **45**(3): 779-785.
- Lanchana H. A., and Garampalli, R. H. 2024. Analysis of phytochemical constituents, antibacterial, antioxidant and GC-MS profiling of *Crotalaria ramosissima* leaf extracts. *Int J Pharm Sci Drug Res*, **16**(3): 426-434. <https://doi.org/10.25004/IJPSDR.2024.160314>
- Laosinwattana, C., Wichittrakarn, P., and Teerarak, M. 2018. Chemical composition and herbicidal action of essential oil from *Tagetes erecta* L. leaves. *Ind Crops Prod*, **126**: 129-134. <https://doi.org/10.1016/j.indcrop.2018.10.013>
- Luo, L., Liu, D., Lan, S., and Gan, X. 2022. Design, synthesis, and biological activity of novel chalcone derivatives containing an 1, 2, 4-Oxadiazole Moiety. *Front Chem*, **10**: Article 943062. <https://doi.org/10.3389/fchem.2022.943062> PMID:35936084 PMCID:PMC9354253
- Mahapatro, G. K., Debajyoti, C., and Gautam, R. D. 2017. Indian Indigenous Traditional Knowledge (ITK) on termites: Eco-friendly approaches to sustainable management. *Ind J Trad Know*, **16**(2): 333-340.

- Mishra, P., Verma, M., Saket, J., Arpita T., Anand, P., Anupam D., and Satyawati S. 2021. Biological approaches of termite management: A review. *Curr Bot*, **12**: 121-131. <https://doi.org/10.25081/cb.2021.v12.7021>
- Mithofer, A., and Boland, W. 2012. Plant defense against herbivores: Chemical Aspects. *Annu Rev Plant Biol*, **63**: 431-450. <https://doi.org/10.1146/annurev-arplant-042110-103854> PMID:22404468
- Ohara, S., Kato, A., Hayashi, Y., and Itou, Y. 1991. Chemical structure and biological activity of saponins. *Baiomasu Henkan Keikaku Kenkyu Hokoku*, **27**: 54-73.
- Okokon, J. E., Augustine, N. B., and Mohanakrishnan, D. 2017. Antimalarial, antiplasmodial and analgesic activities of root extract of *Alchornea laxiflora*. *Pharm Biol*, **55**(1): 1022-1031. <https://doi.org/10.1080/13880209.2017.1285947> PMID:28183236 PMCID:PMC6130711
- Pandey, A., Chattopadhyay, P., Banerjee, S., Pakshirajan, K., and Singh, L. 2012. Antitermitic activity of plant essential oils and their major constituents against termite *Odontotermes assamensis* Holmgren (Isoptera: Termitidae) of North East India. *Int Biodeterior Biodegrad*, **75**: 63-67. <https://doi.org/10.1016/j.ibiod.2012.09.004>
- Pavela, R. 2011. Insecticidal properties of phenols on *Culex quinquefasciatus* say and *Musca domestica* L. *Parasitol Res*, **109**: 1547-1553. <https://doi.org/10.1007/s00436-011-2395-3>
- Pavela, R. 2016. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects – A review. *Plant Prot Sci*, **52**(4): 229-241. https://doi.org/10.1300/J133v03n01_06
- Pearson, W. 2001. Pyrrolizidine Alkaloids in higher plants: Hepatic veno-occlusive disease associated with chronic consumption. *J Nutraceuticals Funct Med Foods*, **3**(1): 87-96. https://doi.org/10.1300/J133v03n01_06
- Ramsewak, R. S., Nair, M. G., Murugesan, S., Mattson, W. J., and Zasada, J. 2001. Insecticidal fatty acids and triglycerides from *Dirca palustris*. *J Agric Food Chem*, **49**(12): 5852-5856. <https://doi.org/10.1021/jf010806y> PMID:11743774
- Rani, P. U., and Jyothsna Y. 2010. Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiol Plant*, **32**: 695-701. <https://doi.org/10.1007/s11738-009-0449-2>
- Ranjith, M., Deotale, V., Bajya, D. R., Manoharan, T., and Gajalakshmi, M., 2017. Evaluation of termiticidal activity and phytochemical analysis of *Crotalaria burhia* (Buch-Ham) and *Anacardium occidentale* (L.). *J Pharmaco Phyto*, **6**(2): 172-176.
- Rao, M. S., and Narukulla, R., 2007. A new trimethoxychalcone from *Crotalaria ramosissima*. *Fitoterapia*, **78**(6): 446-447. <http://dx.doi.org/10.1016/j.fitote.2007.03.011> PMID:17616261
- Rust, M. K., and Su, N. Y. 2012. Managing social insects of urban importance. *Annu Rev Entomol*, **57**: 355-375. <https://doi.org/10.1146/annurev-ento-120710-100634> PMID:21942844
- Scheffrahn, R. H., and Rust, M. K. 1983. Drywood termite feeding deterrents in sugar pine and antitermitic activity of related compounds. *J Chem Ecol*, **9**: 39-55. <https://doi.org/10.1007/BF00987769> PMID:24408618
- Stasiuk, M., and Kozubek, A. 2010. Biological activity of phenolic lipids. *Cell Mol Life Sci*, **67**: 841-860. <https://doi.org/10.1007/s00018-009-0193-1> PMID:20213924 PMCID:PMC11115636
- Subramaniam, S., Pandey, A. K., and Rather, S. A., 2015. A revised circumscription of the species in Bracteatae complex (section Calycinae) in the genus *Crotalaria* L.: Evidence from nuclear and chloroplast markers. *Plant Syst Evol*, **301**(9): 2261-2290. <https://doi.org/10.1007/s00606-015-1228-8>
- Subramaniam, S. and Pandey, A. K. 2014. Reinstatement of *Crotalaria pellita* (Leguminosae, Papilionoideae) and a new combination for its variety. *Phytotaxa*, **183**(1): 051-055. <http://dx.doi.org/10.11646/phytotaxa.183.1.6>
- Thomas, B., Kumar, K. M. P., George, S., Rajendran, A., and Balachandran, I. 2012. A new variety of *Crotalaria ramosissima* (Fabaceae) from Tamil Nadu, India. *Asian Pac J Trop Biomed*, **2**(3), S1412-S1414. [https://doi.org/10.1016/S2221-1691\(12\)60427-4](https://doi.org/10.1016/S2221-1691(12)60427-4)
- Tirkey, A. 2006. Some ethnomedicinal plants of family-Fabaceae of Chhattisgarh state. *Indian J Tradit Knowl*, **5**(4): 551-553.
- UNEP/FAO/Global IPM Facility Expert Group on Termite Biology and Management members. 2000. Finding alternatives to Persistent Organic Pollutants (POPs) for termite management. https://nature.berkeley.edu/upmc/documents/UN_termite.pdf

- Upadhyay, R., Jaiswal, G., and Ahmad, S., 2010. Anti-termite efficacy of *Capparis decidua* and its combinatorial mixtures for the control of Indian white termite *Odontotermes obesus* (Isoptera: Odontotermitidae) in Indian soil. *J Appl Sci Environ Manag*, **14**(3). <https://doi.org/10.4314/jasem.v14i3.61475>
- Verma, M., Verma, S., and Sharma, S., 2018. Eco-friendly termite management in tropical conditions. *Termites Sustainable Management*. pp. 137-164. https://doi.org/10.1007/978-3-319-68726-1_6 PMCID:PMC6768136
- War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., and Sharma, H. C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signal Behav*, **7**(10), 1306-1320. <https://doi.org/10.4161/psb.21663> PMID:22895106 PMCID:PMC3493419
- Zhu, L., Zeng, H., Liu, D., Fu, Y., Wu, Q., Song, B., and Gan, X. 2020. Design, synthesis, and biological activity of novel 1, 2, 4-oxadiazole derivatives. *BMC Chem*, **14**: 1-12. <https://doi.org/10.1186/s13065-020-00722-1> PMID:33292412 PMCID:PMC7680602