International Journal of Environment and Climate Change

Volume 13, Issue 10, Page 2949-2955, 2023; Article no.IJECC.106375
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Laboratory Studies on Chromatographic Profile, Toxicity and Repellent Activity of Custard Apple (*Annona squamosa* L.) Seed Extract

Ilakkia M. [a*], Premalatha K. [b], Vellaikumar S. [c], Shanmugam P. S. [a], Harish S. [d] and Jayarajan Nelson S. [a]

[a] *Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India.*
[b] *Department of Forage Crops, Tamil Nadu Agricultural University, Coimbatore – 641 003, India.*
[c] *Department of Biotechnology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India.*
[d] *Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i102961

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/106375

Original Research Article

Received: 05/07/2023
Accepted: 07/09/2023
Published: 11/09/2023

ABSTRACT

Plant extracts have toxic substances that can kill termites and other insect pests. Many plant extracts including *Annona squamosa* have been identified for their anti-termiticidal activity. This study aims to determine the chemical profile, toxicity, and repellent activities of petroleum ether extract against *Odontotermes anamallensis* and *Microcerotermes fletcheri*. The GC-MS/MS findings of petroleum ether extract of *Annona* showed the presence of 9-octadecenoic acid (Z)- as the predominant compound followed by Octadecanoic acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester. The probit analysis revealed LC50 values of 2.38 per cent and 3.06

**Corresponding author: E-mail: ilakkiamohanram@gmail.com;*

per cent for *O. anamallensis* and *M. fletcheri*, respectively. Petroleum ether extract showed repellency with the repellency percent of 48.33 and 31.67 by *O. anamalensis* and *M. fletcheri* respectively. These findings suggest that *Annona* seed extract is a viable source of natural insecticides against termites.

Keywords: Annona squamosal; bait toxicant; petroleum ether extract; Odontotermes anamallensis Holmgren and Holmgren; Microceroteremes fletcheri Holmgren and Holmgren.

1. INTRODUCTION

Subterranean termites, a highly organized and socially sophisticated species, are renowned for their capacity to inflict substantial damage to structures and cellulose-based materials. These termites are part of the Termitidae family, representing an ecologically and economically significant group. They are among the most destructive pests globally, causing billions of dollars in damages annually. Primarily inhabiting warm and humid environments such as tropical and subtropical regions, subterranean termites have also adapted to temperate climates. Their distinct adaptations enable them to thrive underground while building intricate tunnel systems that serve as access routes to food sources and protective mechanisms against predators and environmental conditions [1].

One of the most striking features of subterranean termites is their intricate social structure. Colonies are composed of distinct castes, including workers, soldiers, and reproductive individuals, each with specific roles. Workers are responsible for foraging, tunnel construction, and feeding the colony, while soldiers defend against threats. The reproductive caste produces alates, winged individuals that partake in swarming behaviour to establish new colonies. Subterranean termites' diet primarily consists of cellulose in wood, plant debris, and other organic matter. They possess a remarkable ability to digest cellulose due to symbiotic bacteria and protozoa in their digestive systems, aiding in the breakdown of complex carbohydrates into absorbable compounds [2]. The crop dies after germination due to the termite attack. The termite attack can be avoided by deep sowing of seeds [3].

Due to their capacity to cause significant structural damage, subterranean termites demand effective control strategies. Preventive measures such as managing moisture and ensuring proper ventilation are crucial, along with chemical and physical barriers to deter termite access. Routine inspections by pest control experts are vital for early detection and mitigation. Plant extracts are potential sources of botanical insecticides to control various arthropod pests, and their use for pest management has been recorded as an alternative to synthetic chemical insecticides [4,5]. Synthetic chemicals like fipronil, and imidacloprid can be used for the management of termites but they pose a threat to the environment. Botanicals are eco-friendly and environmentally safe to use. Acetogenins of Annonacea plants (*A. squamosa* L.) have been widely reported for their high insecticidal, molluscicidal, and nematicidal properties [6]. With these views, the present study evaluated the toxicity of custard apple seed extracts against termites under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Extraction of *A. squamosa* Seeds

Custard apple (*A. squamosa*) seeds and solvent (petroleum ether) were purchased from Molychem. The sundried seeds were powdered using a mixer grinder. The extraction was done with the Soxhlet apparatus, 30g of seed powder was placed in the thimble and the round bottom flask was filled with 300 ml of solvent and the apparatus was made to run at 60˚C for 6hrs. The solvent gets boiled and evaporated and then the gets condensed and extracted from the powder in the thimble and siphon to the round bottom flask. This cycle continues for 6 hours. After 6 hrs the solvent in the round bottom flask was collected and subjected to a rotary vacuum evaporator The extract was collected after evaporating the solvents using a rotary vacuum evaporator at 45˚C for 30 minutes. The boiling point of the petroleum ether starts from 45˚c but for the evaporation of the solvent, 60 ˚C was used. Petroleum ether is used to extract the nonpolar compounds [7].

2.1.1 GC – MS/MS analysis

Gas Chromatography-Mass Spectrometry/ Mass Spectrometry (GC-MS) analysis for petroleum ether extract of *A. Squamosa* seeds were done at the Centre for Molecular Biology and Biotechnology, Department of Biotechnology, Agricultural College and Research Institute, Coimbatore, to identify the phytochemical profile of the extracts. It was carried out in QP 2020, Shimadzu GC equipped with Rxi-5 Sil MS column of length 30m and 0.25×0.25 µm thickness and dia. The column oven temperature was programmed at 70˚c and then heated to 120 ˚c with 5 ˚c/min rate, 185 ˚c with a 10˚ c/min rate, and 280˚ c with a 15 ˚c/min rate at a hold time of 2 min, 10 min, and 5min respectively. The injector temperature was 240 ˚c and helium (99.999%) was used as a carrier gas at a flow rate of 1ml/min and the split ratio was 1:10. The mass detector used in this analysis was Mass Spectral Detector (MSD). The MS was operated at 230˚ C as an ion source temperature and 280˚ C as an interface temperature. A one mL volume of sample was analyzed. The interception in GC-MS was done with the National Institute Standard and Technology (NIST) database. The spectrum of unknown compounds was compared with those of known compounds stored in the NIST library. The compound name, molecular formula, and molecular weight of the *A. squamosa* extracts were observed [7].

2.2 Laboratory Evaluation of Petroleum Ether Extract of *A. squamosa* against Subterranean Termites

2.2.1 Toxicity test

Subterranean termites *O. anamallensis* and *M. fletcheri* were collected from the field and used for laboratory studies. Compact disc food bait was prepared using 40 grams of coconut leaf powder and 0.5g of dextrose (binding agent), and then the required amount of water was added and hand-moulded. Treatments were taken as per the mortality obtained in the preliminary test. Eight treatments *viz*., 0.75%, 1%, 2%, 3%, 4%, 5%, 6%, and 7% were carried out for petroleum ether extract. The baits were impregnated with different concentrations of petroleum ether extract of custard apple seeds, and placed in a plastic container. Each concentration was replicated four times. The experimental design was a Completely Randomized Design (CRD). Each treatment was released with 20 worker termites and with water was considered as an untreated check. The treatment was maintained in BOD at 29˚ C and 85%. The observation was recorded for 24 hrs at 4 hrs intervals of termite mortality. The data was subjected to probit analysis by Finney's method [8].

2.2.2 Repellency test

Filter paper (Whatman no 1) was cut into two halves. One-half of the filter paper was treated with 1 ml of petroleum ether extract of *A. squamosa* seed at different concentrations *viz*., 1%, 2%, 3%, 4%, and 5%, and another half was maintained untreated. The treated filter paper was shade-dried and both half of the filter paper was joined using the cellophane tape and placed in the petri dish. Twenty worker termites were released in the middle of the petri dish. Then the number of termites in the treated and untreated filter paper was recorded at 1, 2, and 3 hrs intervals. Each treatment was replicated 4 times.

The percent repellent was calculated using the following formula,

$$Percent\ repellency\ (\%) = \frac{\mathrm{NC-NT}}{\mathrm{NC+NT}} \times 100$$

Where,
NC = Number of insects in the untreated half
NT = Number of insects in the treated half.

Chart 1. Based on the repellency percentage it was classified into different classes as given in the below [9]

Repellency rate (%)	Class	Interpretation
>0.01-<0.1	0	Nonrepellent
0.1-20	I	Very weakly repellent
20.1-40	II	Moderately repellent
40.1-60	III	Average repellent
60.1-80	IV	Fairly repellent
80.1-100	V	Very repellent

2.3 Statistical Analysis

The mortality of termites was recorded, and the probit analysis was done using statistical software SPSS 24.0.

3. RESULT AND DISCUSSION

3.1 Chemical Profile of Petroleum Ether Extract of *A. squamosa* Seeds Extract: GC-MS/MS Analysis

In the petroleum ether extract of *A. squamosa* seeds, fifty compounds were identified by the GC-MS/MS analysis (Table 1). Among the different compounds found, the maximum per cent area was occupied by Oleic Acid; 9-Octadecenoic acid (Z)- (52.08%), Octadecanoic acid (27.11%), n-Hexadecanoic acid (9.08%), 9-

Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (3.05%), 9-Octadecenoic acid (Z)-, oxiranyl methyl ester (0.71%), 9-Octadecenoic acid, methyl ester, (E)- (0.18%), hexadecanoic acid (0.55%), (E)-octadec-9-enoic acid (0.68%),; (Z)-pentadec-10-enoic acid (0.21%) (Fig 1). Most of these compounds were fatty acids. In general, acetogenins are fatty acids derived mainly from the plants of the Annonaceous family. These acetogenins have insecticidal, antifeedant, and antimicrobial properties. The result is on par with the results obtained [10], which reported that the compounds *viz*., Hexadecanoic acid and octadecanoic acid showed pesticidal properties.

Table 1. Phytochemical profile of petroleum ether extract of *A. squamosa* seeds

S. No	Compounds	Retention time (min)	Area	Area (%)
1	3,3-Diethoxy-1-propyne	5.068	9241373	0.37
2	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	16.860	766566	0.03
3	Benzene, (1-ethylundecyl)-	26.436	785276	0.03
4	Benzene, (1-methyldodecyl)-	27.250	5195766	0.21
5	Hexadecanoic acid, methyl ester	27.544	968479	0.04
6	n-Hexadecanoic acid	28.538	224102874	9.08
7	Acetoacetic acid-meto-TMS	28.877	4710772	0.19
8	Methyl oleate; (Z)-octadec-9-enoic acid	28.937	3417450	0.14
9	Isovalerylglycine-TMS	29.050	1800406	0.07
10	Benzene, (1-methylundecyl)-	29.265	624420	0.03
11	Palmitic acid-TMS ; hexadecanoic acid	29.847	2250012	0.09
12	Methyl linoleate; Octadeca-9,12-dienoic acid	30.715	2343728	0.09
13	9-Octadecenoic acid, methyl ester, (E)-	30.845	4337128	0.18
14	Methyl stearate ; Octadecanoic acid	31.341	1366910	0.06
15	Oleic Acid; 9-Octadecenoic acid (Z)-	32.079	1285911111	52.08
16	Octadecanoic acid	32.364	669348401	27.11
17	Glycidyl palmitate	34.740	1877134	0.08
18	2-Keto-isovaleric acid-2TMS	37.361	1254719	0.05
19	E,Z-1,3,12-Nonadecatriene	37.579	1888090	0.08
20	Oleoyl chloride	37.706	2145231	0.09
21	Methyl cis-11-icosenoate	38.282	1897372	0.08
22	2-(Dimethylamino)ethyl vaccenoate	38.397	960282	0.04
23	Methyl 3-cis,9-cis,12-cis-octadecatrienoate	38.575	5056560	0.20
24	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	38.702	17410256	0.71
25	Palmitic acid-TMS; hexadecanoic acid	39.106	13475333	0.55
26	Glycidyl palmitate	39.239	7876687	0.32
27	2-Keto-isovaleric acid-2TMS	39.397	3516087	0.14
28	Methyl cis-5,8,11,14,17-Eicosapentaenoate	39.579	2759589	0.11
29	Lauric acid-TMS; dodecanoic acid	40.426	1428037	0.06
30	2-Keto-isovaleric acid-2TMS	41.216	1773906	0.07
31	2-Hydroxyisobutyric acid-2TMS	42.004	18718061	0.76
32	Docosapentaenoic acid-TMS	42.223	8981087	0.36
33	Elaidic acid-TMS; (E)-octadec-9-enoic acid	42.328	16863607	0.68
34	Monostearin-2TMS	42.455	17872637	0.72
35	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	42.736	75388973	3.05
36	Methyl cis-10-pentadecenoate; (Z)-pentadec-10-enoic acid	43.197	5297984	0.21
37	Palmitoleic acid-TMS; hexadec-9-enoic acid	43.383	944587	0.04
38	2-Keto-isovaleric acid-2TMS	43.857	843758	0.03
39	Pyruvic acid-2TMS	44.509	1399229	0.06
40	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	44.677	1095907	0.04

S. No	Compounds	Retention time (min)	Area	Area (%)
41	Hexadecanamide	47.558	966220	0.04
42	.gamma.-Tocopherol	48.047	1316210	0.05
43	Campesterol	50.387	6007312	0.24
44	Stigmasterol	50.757	7140829	0.29
45	.gamma.-Sitosterol	51.651	15476197	0.63
46	Fucosterol	51.896	2361796	0.10
47	Oleic acid-TMS; (Z)-octadec-9-enoic acid	52.271	1700858	0.07
48	22,23-Dibromostigmasterol acetate	52.911	1904157	0.08
49	.gamma.-Sitostenone	53.790	2290627	0.09
50	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	53.954	2101603	0.09

Ramsewak *et al* [11], reported that the chemical compounds such as octadecanoic acid, decanoic acid, and hexadecenoic acids from the extract *Annona* seeds. Similarly, Babarinde et al [5] reported that oleic acids are present in the extract of the *Annona* plant*.*

Gibson *et al* [12] reported that the *Annona* extract consists of dodecanoic acid. Acda [13] reported that chemical compounds such as octadecanoic acid, hexadecenoic acid, and other fatty acids are present in the ethanolic extracts of *A. squamosa* and *A. muricata.*

3.2 Toxicity of Petroleum Ether Extract of *A. squamosa* Seeds

The probit analysis (Table 2) showed the LC50 value of 2.38 with a fiducial limit of about 2.03-2.80 and 3.06 % with a fiducial limit of about 2.58-3.64 for *O. anamallensis* and *M. fletcheri* respectively. Trisna Priadi *et al* [14] reported that the *A. glabra* extract showed insecticidal activity against wood-destroying termites *Cryptotermes cynocephalus* (dry-wood termites) and *Coptotermes curvignathus.* Sini *et al*. [15] reported that the *A. squamosa* L. extract, which contains octadecenoic acid (oleic acid), showed insecticidal activity against the insects. The toxicity of *A. squamosa* L. leaf, bark, and seed extract against *Cryptotermes brevis* was reported by Alves *et al* [16] among the leaf, bark, and seed extracts, he reported that seed extract is more effective for the wood-destroying termites *Cryptotermes brevis*.

3.3 Repellent Activity of Petroleum Ether Extract of *A. squamosa* Seeds

The petroleum ether extract showed repellency against both *O. anamallensis* and *M. fletcheri*. The highest repellency was shown at 5% concentration with the repellency percent of 48.33 and 31. 67 by *O. anamalensis* and *M. fletcheri* which were categorised under class III (Average repellent) and II (Moderately repellent) respectively (Tables 3). *O. anamalensis* showed more repellency than the *M. fletcheri*. The result was in accordance with the result reported by Acda [13]. He reported that the seed extract of *A. squamosa* showed repellency against the Asian subterranean termite *Coptotermes gestroi*. Gibson *et al*. [12] reported that the dodecanoic acid in the extract of *A. squamosa* has repellent properties. Baldin *et al*. [17] also reported the repellent activities of *Annona* extract against the soybean aphids *Aphis glycines.*

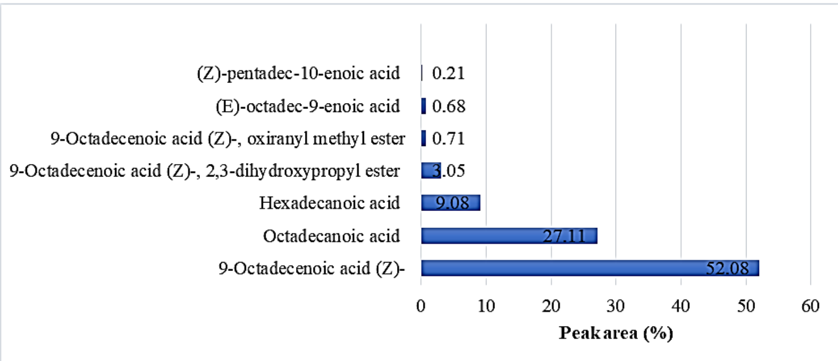

Fig. 1. Graphical representation of bioactive compounds of petroleum ether extract of *A. squamosa* seeds

Table 2. LC50 values with 95% confidence limits and probit analysis 24h of treatment.

Extracts	LC50	LL	UL	x^2 value	Equation of regression line	R^2
O. anamallensis	2.3899	2.0374	2.8035	22.823	Y=1.603X+4.4243	0.7686
M. fletcheri	3.0684	2.5828	3.6454	14.106	Y=1.368X+4.3492	0.8324

Table 3. Repellent activity of petroleum ether extract of *A. squamosa* seeds against *O. anamallensis* and *M. fletcheri*

Termites	Concentration (%)	Time intervals			Mean repellency (%)	Repellency class
		1HAT	2HAT	3HAT		
O. anamallensis	1	2.50	10.00	10.00	7.50	I
	2	15.00	22.50	25.00	20.83	II
	3	25.00	30.00	32.50	29.17	II
	4	35.00	40.00	42.50	39.17	II
	5	47.50	47.50	50.00	48.33	III
	Control	0.00	0.00	0.00	0.00	0
M. fletcheri	1	-2.50	2.50	12.50	4.17	I
	2	7.50	7.50	20.00	11.67	I
	3	15.00	17.50	25.00	19.17	I
	4	22.50	25.00	35.00	27.50	II
	5	30.00	25.00	40.00	31.67	II
	Control	0.00	0.00	0.00	0.00	0

4. CONCLUSION

The bioactive compounds of petroleum ether extract of A. squamosa seeds showed insecticidal and repellent properties against *O. anamallensis* and *M. fletcheri.* The bioactive compound 9-Octadecanoic acid is the predominant compound in the petroleum ether extract

ACKNOWLEDGEMENTS

The author acknowledged the facilities rendered by the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Peterson C. Subterranean Termites: Their prevention and control in buildings. USDA Forest Service, Wood Products Insect Research Unit; 2006
2. Breznak JA, Brune A. Role of microorganisms in the digestion of lignocellulose by termites. Annual review of Entomology. 1994 Jan;39(1):453-87.
3. Kaur R, Kaur M, Singh G. Eco-friendly Indigenous Technologies for Insect Pest Management. Indian J. Ecol. 2008;35(1) :82-6.
4. Alves KD, Bezerra AC, de Lima GB, de Oliveira Carvalho FS, de Alencar França AK, da Rocha JM, Nunes SC, de Oliveia JC, de Oliveira IV, Bezerra AC. The use *of Annona squamosa* seed, leaf and bark as an alternative in the control of *Cryptotermes brevis*. Research, Society and Development. 2021 Sep 18;10(12): e204101220307-.
5. Babarinde SA, Akinyemi AO, Odewole AF, Yisa S, Oke OO, Adeyemo AO. Phytochemical composition and insecticidal properties of mechanically extracted castor, seed oil against cowpea seed bruchid (*Callosobruchus maculatus* Fabricius) infesting Bambara groundnut. Elixir Agric. 2016 Mar 11;92:39086-92
6. Prijono D, Manuwoto S, Soemawinata RT. Insecticidal activity of sugar apple (*Annona squamosa* L.) and pond apple (*A. gl*abra L.) seed extracts against rice brown planthopper, *Nilaparvata lugens* (Stal.). In Proc. Unesco National Seminar, Depok, Indonesia 1994 (pp. 335-341).
7. Hager TE, Adam IA, Almain AA, Mohammed MM. Phytochemical screening, gc-ms analysis, antibacterial and antioxidant activity of seeds oil of *Annona squmosa* L. Sudanese medicinal plant. Journal of Pharmacy and Pharmacology. 2019;7(1):1-6.

8. Finney DJ. Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of information in medicine. 1971 Aug;10(04):237-45.
9. Appalasamy S, Diyana MA, Arumugam N, Boon JG. Evaluation of the chemical defense fluids of Macrotermes carbonarius and Globitermes sulphureus as possible household repellents and insecticides. Scientific reports. 2021 Jan 8;11(1):153.
10. Ping KY, Darah I, Chen Y, Sasidharan S. Cytotoxicity and genotoxicity assessment of *Euphorbia hirta* in MCF-7 cell line model using comet assay. Asian Pacific Journal of Tropical Biomedicine. 2013 Sep 1;3(9):692-6.
11. Ramsewak RS, Nair MG, Murugesan S, Mattson WJ, Zasada J. Insecticidal fatty acids and triglycerides from *Dirca palustris.* Journal of Agricultural and Food Chemistry. 2001 Dec 17;49(12):5852-6.
12. Gibson RW, Rice AD, Pickett JA, Smith MC, Sawicki AM. The effects of the repellents dodecanoic acid and polygodial on the acquisition of non-, semi-and persistent plant viruses by the aphid *Myzus persicae*. Annals of Applied Biology. 1982 Feb;100(1):55-9.
13. Acda MN. Chemical composition of ethanolic seed extract of *Annona squamosa* L. and *A. muricata* L. (Annonaceae) using GC-MS analysis. Philippine Agricultural Scientist. 2014 Dec 1;97(4):422-6.
14. Priadi T, Chotimah N, Ismanto A. Bioactivity Analysis of *Annona Glabra* L. Seed Extracts for Wood Protection Against Termites (*Cryptotermes Cynocephalus* Light. and Coptotermes Curvignathus Holmgren.). Indonesian Journal of Forestry Research. 2021 Oct 31;8(2):127-34.
15. Sini H, Mohanan PV, Devi KS. Studies on the insecticidal activity, cytogenecity and metabolism of fatty acid rich fraction of *Hydnocarpus laurifolia*. Toxicological & Environmental Chemistry. 2005 Jan 1;87(1):91-8.
16. Alves KD, Bezerra AC, de Lima GB, de Oliveira Carvalho FS, de Alencar França AK, da Rocha JM, Nunes SC, de Oliveia JC, de Oliveira IV, Bezerra AC. The use *of Annona squamosa* seed, leaf and bark as an alternative in the control of *Cryptotermes brevis*. Research, Society and Development. 2021 Sep 18; 10(12):e204101220307-.
17. Baldin EL, Soares MC, da Silva Santana A, Hunt TE, McMechan J, Arango AM, Louis J. Efficacy of ethanolic seed extracts of *Annona* spp. against *Aphis glycines*. Crop Protection. 2023 Aug 1;170:106268.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/106375