

## Original Research Article

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

Comment [DA1]: What studies????

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### ABSTRACT

Plant extracts have toxicants that can kill termites and other insect pests. Many plant extracts including *Annona squamosa* have been identified for their anti-termicidal activity. This study aims to determine the chemical profile, toxicity, and repellent activities of petroleum ether extract against *Odontotermes anamallensis* and *Microcerotermes fletcheri*. Extraction was done using the Soxhlet apparatus, and its compounds were analysed using the GC-MS/MS. The toxicity test was done by using the bait and impregnating it with the extract and the repellency test was done using filter paper. The GC-MS/MS findings of petroleum ether extract 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% and 3.06% for *O. anamallensis* and *M. fletcheri*, respectively. Petroleum ether extract showed repellency with the repellency percent of 48.33 and 31.67 by *O. anamallensis* and *M. fletcheri* respectively. These findings suggest that *Annona* seed extract is a viable source of natural insecticides against termites.

Comment [DA3]: Eeds Major Revision very poorly written

Keywords: *Annona squamosa*, bait toxicant, petroleum ether extract, *Odontotermes anamallensis*, and *Microcerotermes fletcheri*

Comment [DA4]: Sp name must be completed with authority (botanical)

### 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.

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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 (5). The crop dies after germination due to the termite attack. The termite attack can be avoided by deep sowing of seeds (6).

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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 (2,3). Acetogenins of Annonacea plants (*A. squamosa*L.) have been widely reported for their high insecticidal, molluscicidal, and nematocidal properties (10). 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 private firms. The sundried seeds were powdered using a mixer grinder. The extraction was done with the Soxhlet apparatus, which filled 30g of seed powder with 300 ml of solvent. The extraction was done for 6 hrs at 60° C. The crude extract was collected after evaporating the solvents using a rotary vacuum evaporator at 45°C for 30 minutes.

**Comment [DA7]:** Must be explained in details

**Comment [DA8]:** Specification like boiling point 40-60 or 60-80

**Comment [DA9]:** Name of the firm

**Comment [DA10]:** Wrong method pet ether starts boiling at 40 degree ..explain why at 60 degree

**Comment [DA11]:** reference

**Comment [DA12]:** reference to use GCMS for crude extract

#### 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 0.25x0.25µm 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.

### 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.

**Comment [DA13]:** Dear Authors: pet Ether Extract are insoluble in water. How you prepared the various concentrations? Explain in the extraction procedure why u have chosen only pet ether extract ?????

**Comment [DA14]:** what is the concentration ? it must be written in mg/mL

**Comment [DA15]:** reference

#### 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,

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

**Comment [DA16]:** elaborate it

Chart 1 :Based on the repellency percentage it was classified into different classes as given in the below

**Comment [DA17]:** Method reference

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

### 3. STATISTICAL ANALYSIS

The mortality of termites was recorded, and the probit analysis was done using statistical software SPSS 21.0

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### 4. RESULT AND DISCUSSION

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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
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

#### 4.1 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 (Fig 2). 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 (9), which reported that the compounds viz., Hexadecanoic acid and octadecanoic acid showed pesticidal properties.

Figure 1: Chromatographic profile of petroleum ether extract of *A. squamosa* seed

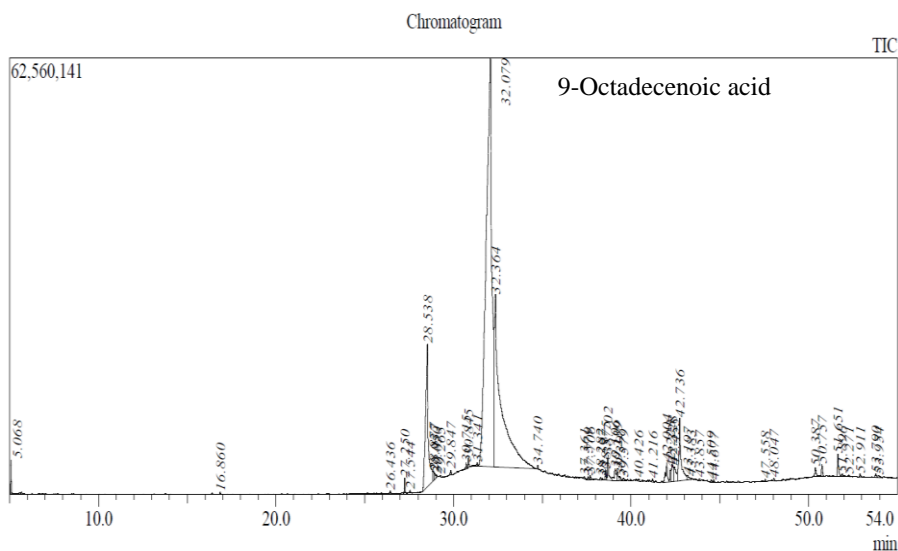
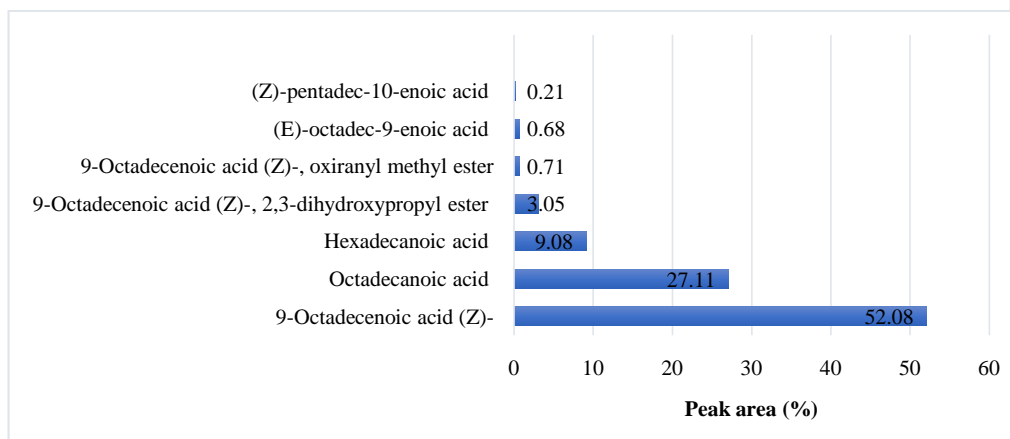


Figure 2: Graphical representation of bioactive compounds of petroleum ether extract of *A. squamosa* seeds

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Ramsewak *et al*(11), reported that the chemical compounds such as octadecanoic acid, decanoic acid, and hexadecanoic acids from the extract *Annona* seeds. Similarly, Babarinde et al (3) reported that oleic acids are present in the extract of the *Annona* plant.

Gibson *et al* (6) reported that the *Annona* extract consists of dodecanoic acid. Acda (1) reported that chemical compounds such as octadecanoic acid, hexadecanoic acid, and other fatty acids are present in the ethanolic extracts of *A. squamosa* and *A. muricata*.

#### 4.2 Toxicity of petroleum ether extract of *A. squamosa* seeds

The probit analysis (Table 2) showed the LC<sub>50</sub> 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*(13) reported that the *A. glabra* extract showed insecticidal activity against wood-destroying termites *Cryptotermes cynocephalus* (dry-wood termites) and *Coptotermes curvignathus*. Sini *et al.* (12) 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*(2). Among the leaf, bark, and seed extracts, he reported that seed extract is more effective for the wood-destroying termites *Cryptotermes brevis*.

Table 2: LC<sub>50</sub> values with 95% confidence limits and probit analysis 24h of treatment.

Extracts	LC <sub>50</sub>	LL	UL	$\chi^2$ value	Equation of regression line	R <sup>2</sup>
<i>O. anamallensis</i>	2.3899	2.0374	2.8035	22.823	Y=1.603X+4.4243	0.7686
<i>M. fletcheri</i>	3.0684	2.5828	3.6454	14.106	Y=1.368X+4.3492	0.8324

#### 4.3 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. anamallensis* and *M. fletcheri* which were categorised under class III (Average repellent) and II (Moderately repellent) respectively (Tables 3). *O. anamallensis* showed more repellency than the *M. fletcheri*. The result was in accordance with the result reported by Acda (1). He reported that the seed extract of *A. squamosa* showed repellency against the Asian subterranean termite *Coptotermes gestroi*. Gibson *et al.* (6) reported that the dodecanoic acid in the extract of *A. squamosa* has repellent properties. Baldin *et al.* (4) also reported the repellent activities of *Annona* extract against the soybean aphids *Aphis glycines*.

**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		
<i>O. anamallensis</i>	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
<i>M. fletcheri</i>	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

## 5. 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

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