

Laboratory evaluation of Custard Apple (*Annona squamosa* Linn.) seed extracts as bait toxicant against *Odontotermes anamallensis* Holmgren and Holmgren and *Microceroterme fletcheri* Holmgren and Holmgren (Isoptera: Termitidae)

M. Ilakkia, K. Premalatha^{1*}, S. Vellaikumar, P.S. Shanmugam,
S. Harish and K. Aravinthraju

Department of Agricultural Entomology, Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu, India.

E. Mail : premalatha.k@tnau.ac.in

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ABSTRACT

This study tested *Annona squamosa* seed bioactive compounds against two different species of termites. The seed oil was extracted mechanically and with solvents (hexane and ethyl acetate) using the Soxhlet apparatus. GC MS/MS identified the phytochemical profile of the oil and solvent extract. *A. squamosa* seed oil was found with 9-octadecenoic acid (Z)-, octadecanoic acid, n-hexadecanoic acid and oleic acid-TMS as major compounds. The predominant compound of *A. squamosa* seed extracts is found as 9- octadecanoic acid, n-hexadecanoic acid and 6-octadecanoic acid. To find the extract's LC50 value, the laboratory bioassay was carried out against *O. anamallensis* and *M. fletcheri* with *A. squamosa* seed oil and hexane and ethyl acetate extract of *A. squamosa* seed. For both species, *O. anamallensis* and *M. fletcheri* oil recorded the lowest LC₅₀ value of about 0.14 and 0.25 per cent, respectively, followed by hexane extract and ethyl acetate extract.

Keywords: *Annona squamosa*, bait toxicant, *Microceroteremes fletcheri*, seed extract, toxicant.

INTRODUCTION

Termites, also known as "eusocial roaches," belong to the order Blattodea. Despite their role in ecosystem engineering, they are highly destructive pests that damage various agricultural crops, perennial trees, forests, buildings, and wooden structures (9). *Odontotermes* species cause severe damage to the nursery and transplanted seedlings of coconut during earlier stages. More serious losses are caused when they nibble at the tender growing point. This species also damages coconut plants in the nursery by constructing mud galleries. Trunk wilting of the central shoot, stunted growth, and mud galleries are also seen on the trunk. The tender shoots of coconut seedlings have been observed to be cut off by termites, which also cause damage to the roots and shoots of seedlings, as well as on young coconut trees. The attacked plants dry gradually and finally become wilted (7).

Botanical insecticides derived from plant extracts have the potential to control a variety of arthropod pests. They are an alternative to synthetic chemical insecticides (5,6). Acetogenins from Annonaceae plants, specifically *Annona squamosa*, have been widely recognized for their potent insecticidal, molluscicidal, and nematicidal properties (8). Building upon these findings, the current study evaluated the toxicity of custard apple seed extracts against termites under laboratory conditions (Plate 1).

*Correspondence author,



Plate 1. *Annona squamosa* Linn. Plant and seeds

MATERIALS AND METHODS

The experiment was initiated in March 2023 for the procurement and extraction of oil and solvent extraction. The GC-MS/MS analysis and toxicity test were done from April to July 2023 at Tamil Nadu Agricultural University, Coimbatore (11.0122° N, 76.9354° E, 411m MSL), receiving an annual rainfall of 715 mm with a mean minimum temperature of 19 °C to 24 °C mean and maximum temperature of 30 °C to 35 °C.

Solvent extraction of *A. squamosa* Linn. seeds

Custard apple (*A. squamosa* L.) seeds and solvents (hexane and ethyl acetate) were purchased from private firms. The sundried seeds were powdered using a mixer grinder. The extraction was done with the Soxhlet apparatus, which filled 30 g of seed powder with 300 ml of solvent. The extraction was done for 6 h at 60 °C. The crude extract was collected after evaporating the solvents using a rotary vacuum evaporator at 45 °C for 30 minutes. The recovery percentage of hexane and ethyl acetate extract is 23.3 % and 20 %, respectively.

Oil extraction of *A. squamosa* Linn. Seeds

The crude oil of *A. squamosa* Sun-dried seeds were extracted using the mechanical expeller with a steamer and 10hp motor. The oil recovery percentage was 13 %.

GC-MS/MS analysis

Gas Chromatography-Mass Spectrometry/ Mass Spectrometry (GC-MS/MS) analysis for seed oil, hexane and ethyl acetate extract of *A. Squamosa* L. 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 seed oil and solvent extracts. It was carried out in QP 2020, Shimadzu GC equipped with Rxi-5 Sil MS column of length 30 m 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 analysed. The interception in GC-MS/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* Linn. extracts were observed.

Laboratory assessment of toxicity of *A. squamosa* Linn. solvent and oil extracts against different termite species

Field-collected termites *O. anamallensis* Holmgren and Holmgren and *M. fletcheri* Holmgren and Holmgren were used for the laboratory studies (Plate 2).

Odontotermes anamallensis



Microcerotermes fletcheri



Plate 2. Different species of termites used for laboratory experiments

Bait toxicant analysis

Compact disc food bait was prepared using 40 grams of coconut leaf powder and 0.5 g 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. Eleven treatments were carried out for oil and hexane extract of *Annona* seeds and eight treatments were carried out for ethyl acetate extract. The baits were impregnated with different concentrations of solvent extracts and oil 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 h at 4 h intervals of termite mortality. The data was subjected to probit analysis by Finney's method.

RESULT AND DISCUSSION

Phytochemical profile of oil and solvent extracts of *A. squamosa* Linn. seed

In general, the chemical constituents revealed the presence of monounsaturated omega 9 fatty acids (Oleic acid), saturated fatty acids (decanoic acid) polyunsaturated hydrocarbon (squalene), phytosterols (campesterol, stigmasterol, etc.), organic acid (lactic acid) and primary saturated fatty acid (palmitic acid). The major components in

Table 1. Phytochemical profile of solvent extracts of *A. squamosa* Linn. seed

S. No.	Retention time (Min.)	Area	Area (%)	Hexane extract		Ethyl acetate extract		
				Name of the compound	Retention time (Min.)	Area	Area (%)	Name of the compound
1.	26.010	3977819	0.05	Myristic acid-TMS	21.794	1155619	0.01	Lauroic acid-TMS
2.	27.564	1994395	0.02	Hexadecanoic acid, methyl ester	25.991	4581340	0.06	Myristic acid-TMS
3.	27.974	2414614	0.03	Pentadecanoic acid, TMS derivative	27.547	1231165	0.02	Hexadecanoic acid, methyl ester
4.	28.719	801216369	9.38	n-Hexadecanoic acid	27.952	1876412	0.02	Pentadecanoic acid, TMS derivative
5.	28.956	19241000	0.23	9-Octadecenal (Z)-	28.520	297886781	3.75	n-Hexadecanoic acid
6.	29.063	4830926	0.06	Hexacosane	28.770	229905	0.00	Methyl laurate
7.	29.450	6212679	0.07	Oxirane, hexadecyl-	28.873	2756140	0.03	Hexadecanoic acid, ethyl ester
8.	29.657	6659848	0.08	Oleic acid-TMS	29.583	1044574	0.01	Palmitic acid-TMS
9.	29.903	227339181	2.66	Palmitic acid-TMS	29.932	736121146	9.26	palmitic acid, TMS derivative
10.	30.257	5686354	0.07	n-Hexadecanoic acid	30.723	2401428	0.03	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
11.	30.741	3393584	0.04	8,11-Octadecadienoic acid, methyl ester	30.853	4728941	0.06	9-Octadecenoic acid (Z)-, methyl ester
12.	30.874	6360330	0.07	9-Octadecenoic acid (Z)-, methyl ester	31.124	4923592	0.06	Oleic Acid (Z)-, TMS derivative
13.	31.362	1754636	0.02	Methyl stearate	31.341	1334333	0.02	Methyl stearate
14.	32.445	5088123884	59.58	6-Octadecenoic acid	32.227	3910238295	49.19	6-Octadecenoic acid
15.	32.681	899964665	10.54	Octadecanoic acid	32.497	793422948	9.98	Octadecanoic acid
16.	33.089	382384989	4.48	Elaidic acid-TMS	33.059	690453821	8.69	Elaidic acid-TMS
17.	33.390	54617486	0.64	Stearic acid-TMS	33.433	564877600	7.11	Stearic acid-TMS
18.	33.497	120252835	1.41	Stearic acid, TMS derivative	34.768	77130664	0.97	Glycerol palmitate
19.	33.778	51836870	0.61	Palmitoyl chloride	35.130	38645150	0.49	Linoleic acid-TMS
20.	34.819	35845753	0.42	Glycerol palmitate	36.459	61394573	0.77	Eicosanoic acid
21.	36.341	16046774	0.19	Eicosanoic acid	37.157	14012443	0.18	Methyl oleate
22.	36.944	5306404	0.06	Octadecanoic acid, butyl ester	38.046	16783494	0.21	Arachidic acid, TMS derivative
23.	37.644	35186823	0.41	9,12-Octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl				
24.	37.782	81213576	0.93	Oleoyl chloride	38.303	22258569	0.28	Octadecanoic acid, 2,3-dihydroxypropyl ester
25.	38.123	6211130	0.07	Arachidic acid, TMS derivative	38.732	250771780	3.15	9-Octadecenoic acid (Z)-, octanylethyl ester
26.	38.337	1677607	0.20	Octadecanoic acid, 2,3-dihydroxypropyl ester	39.250	82052935	1.03	Glycerol palmitate
27.	38.638	52443922	0.61	9,12-Octadecadienoyl chloride (Z,Z)-	39.823	8632474	0.11	Beryl alcohol-TMS
28.	38.772	150933021	1.77	9-Octadecenoic acid (Z)-, octanylethyl ester	40.437	9709602	0.12	Oleic acid-TMS
29.	39.152	1823334	0.21	Palmitic acid-TMS	41.911	34237506	0.43	Methyl linolenate
30.	39.289	35474132	0.42	Glycerol palmitate	42.324	67673851	0.83	Elaidic acid-TMS
31.	39.838	4264584	0.05	Bis(2-ethylhexyl) phthalate	42.943	15805865	0.20	Glycerol palmitate
32.	42.387	46810576	0.55	Palmitoleic acid-TMS, hexadec-9-enoic acid	43.344	29669193	0.37	1,3-Dihydroxypropan-2-yl octadec-9-enoate, 2TMS
33.	42.497	45447270	0.53	3-(Hexadecyloxy) propan-1-ol, TMS	43.830	4270789	0.05	2,3-Dihydroxypropyl eicosanoate, 2TMS derivative
34.	42.821	91444738	1.07	9,12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	48.960	3712272	0.05	Triacetone
35.	43.452	330111965	0.39	1,3-Dihydroxypropan-2-yl octadec-9-enoate, 2TMS	50.410	6775283	0.09	Campesterol
36.	45.057	3248107	0.04	Oleic acid-TMS, (Z)-octadec-9-enoic acid	50.783	5468879	0.07	Stigmasterol
37.	45.236	15486886	0.18	rac-1-Oleoyl-2-palmitoylglycerol, TMS derivative	51.680	11421142	0.14	beta-Stigmasterol
38.	47.581	2918318	0.03	Hexadecanamide				
39.	48.992	2390417	0.03	Methyl oleate, (Z)-octadec-9-enoic acid				
40.	50.432	7981056	0.09	Campesterol				
41.	50.804	9277152	0.11	Stigmasterol				
42.	51.706	19756982	0.23	beta-Stigmasterol				
43.	54.290	3308896	0.04	9-Octadecene, 1,1'-[1,2-ethanediylbis(oxy)]bis-(Z,Z)-				

A. squamosa seed oil and solvent extracts are saturated and unsaturated fatty acids such as n-hexadecanoic acid, 9-octadecenoic acid and octadecenoic acid (Table 1, 2&3). According to Adesanwo *et al.* (2), 9-octadecanoic acid was found to be the most abundant compound in the *A. squamosa* seed extracts. Several studies have identified major compounds in the extracts of *A. squamosa* and *A. muricata*. Deewatthanawong *et al.* (4) found that hexadecanoic is the dominant compound in both *A. squamosa* and *A. muricata* extract. Babaiwa *et al.* (3) reported that hexadecenoic acid is the most prevalent compound in *A. muricata* extract, while Zahid *et al.* (13) identified n-hexadecanoic acid, octadecanoic acid and 9-octadecanoic acid as the predominant compounds in the solvent extraction of *A. squamosa* seed extract along with palmitic acid, oleic acid and stearic acid. Similarly, Hagr *et al.* (5) also reported hexadecanoic acid and 9-octadecanoic acid as the major compounds of *A. squamosa* seed extract. Various compounds have been found in *A. squamosa* seeds including palmitic acid, oleic acid, steric acid, campesterol and stigmasterol. Furthermore, both *A. squamosa* and *A. muricata* seed extracts contain palmitic acid and oleic acid reported by Acda (1) (Fig. 1, 2 & 3 and Annexure 1).

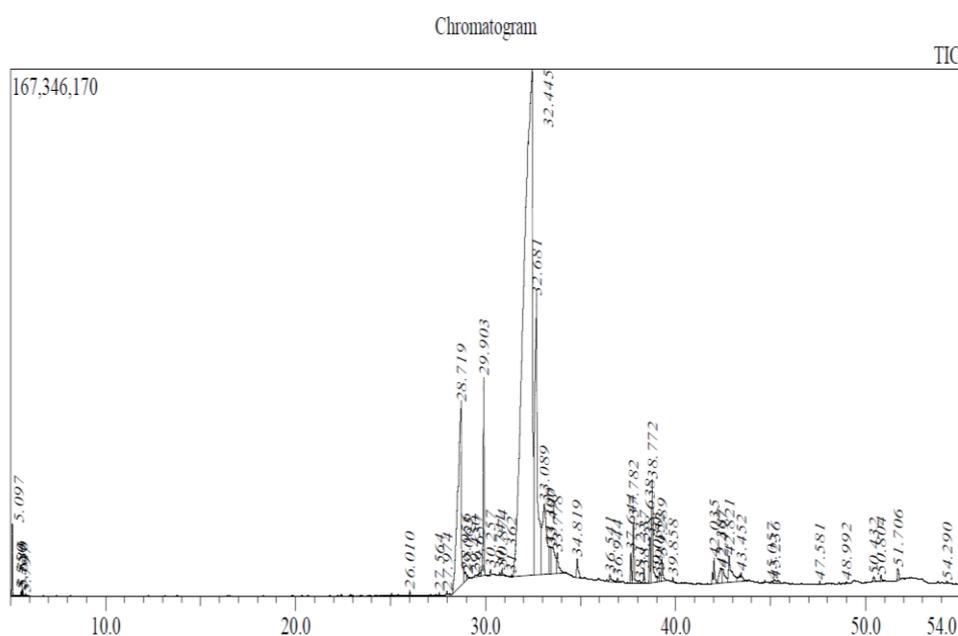


Figure 1. Phytochemical profile of *A. squamosa* L. seeds (Hexane extract)

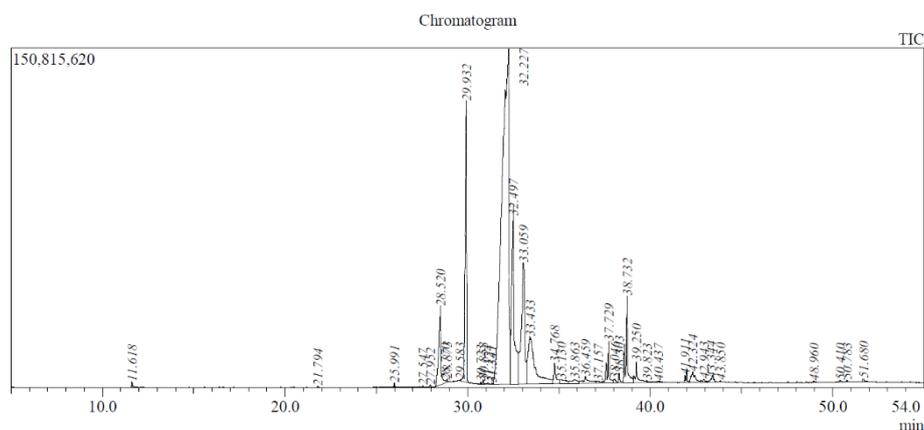
Table 2. Phytochemical profile of *A. squamosa* Linn. Seed oil

S. No.	Phytochemical profile of hexane extract of <i>Annona squamosa</i> Linn. seeds			
	Retention time (Min.)	Area	Area (%)	Name of the compound
1.	29.821	1767808	0.43	Palmitic acid-TMS; hexadecanoic acid
2.	31.678	104945284	25.36	Oleic Acid; 9-Octadecenoic acid (Z)-
3.	32.057	32590320	7.88	Octadecanoic acid
4.	38.673	3365002	0.81	9-Octadecenoic acid (Z)-, oxiranyl methyl ester
5.	39.063	1670015	0.40	Palmitic acid-TMS; hexadecanoic acid
6.	39.217	1192049	3.29	Oleic acid-TMS; (Z)-octadec-9-enoic acid
7.	39.357	1072238	0.26	2-Keto-isovaleric acid-2TMS
8.	43.833	1124113	0.27	2-Methylhippuric acid-2TMS
9.	44.491	1494488	0.36	Lactic acid-13C3-2TMS
10.	44.640	1843360	0.45	Squalene
11.	45.991	844107	0.20	Ethylmalonic acid-2TMS
12.	50.370	10441127	2.52	Campesterol
13.	50.736	10621623	2.57	Stigmasterol
14.	51.629	21437824	5.18	.gamma.-Sitosterol

Table 3. LC₅₀ values with 95% confidence limits and probit analysis parameters for workers of *Odontotermes anamallensis* Holmgren and Holmgren and *Microcerotermes fletcheri* Holmgren and Holmgren after 24 h of treatment.

Extracts	<i>Odontotermes anamallensis</i>						<i>Microcerotermes fletcheri</i>					
	LC ₅₀	LL	UL	χ^2 value	Equation of regression line	R ²	LC ₅₀	LL	UL	χ^2 value	Equation of regression line	R ²
Oil	0.1471	0.1188	0.1823	4.3887	Y= 1.3077X+6.0819	0.9764	0.2528	0.201	0.318	4.6449	Y= 0.9984X+5.5969	0.9794
Hexane	0.2867	0.2306	0.3565	3.8812	Y= 1.0254X+5.5587	0.9808	0.4710	0.3605	0.6156	5.3885	Y= 0.7829X+5.2559	0.9654
Ethyl acetate	1.8760	1.6265	2.1638	13.659	Y= 2.0661X+4.4605	0.8582	2.4608	2.0458	2.960	12.985	Y= 1.3112X+4.5028	0.8167

*Mean of four replication

Figure 2. Phytochemical profile of *A. squamosa* L. seeds (Ethyl acetate extract)

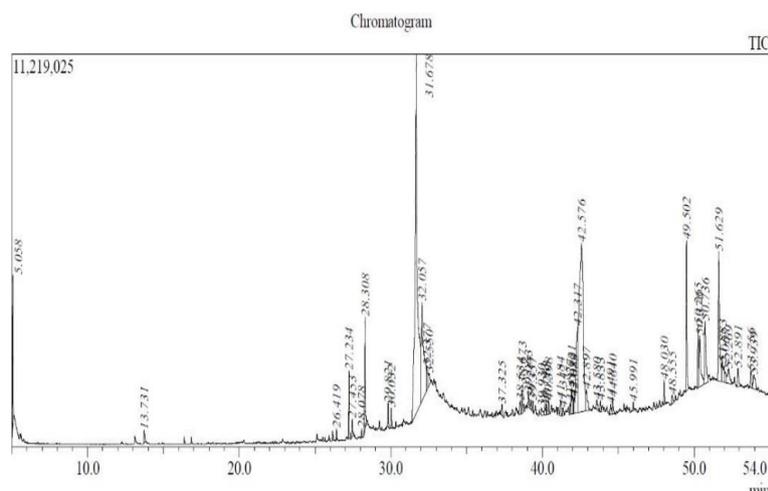


Figure 3. Phytochemical profile of *A. squamosa* L. seeds (seed oil)

Toxicity of *Annona squamosa* seed extracts

The probit analysis showed the LC 50 of 0.14, 0.28 and 1.87 for oil, hexane and ethyl acetate, respectively, to *O. anamallensis* Holmgren and Holmgren. The LC50 (Table 4) value of oil is less, followed by hexane extract and ethyl acetate extract of *A. squamosa* Linn. seeds which may be due to the toxic compounds in the extract. Among *O. anamallensis* Holmgren and Holmgren and *M. fletcheri* Holmgren and Holmgren the *O. anamallensis* Holmgren and Holmgren is more susceptible to the *A. squamosa* extract than the *M. fletcheri* Holmgren and Holmgren. The LC 50 value for oil, hexane and ethyl acetate against *M. fletcheri* Holmgren and Holmgren was 0.25, 0.47 and 0.46, respectively. Among the solvent extract, hexane showed the lowest LC 50 (0.28 and 0.47 respectively) against *O. anamallensis* Holmgren and Holmgren and *M. fletcheri* Holmgren and Holmgren, which may be because the predominant compounds 6-Octadecenoic acid, Octadecenoic acid, and Hexadecanoic acid is more in the hexane extract (Fig. 4). Sini *et al.* (10) reported that the *A. squamosa* Linn. extract, which contains octadecenoic acid (oleic acid), showed insecticidal activity against the *Leptocorisa acuta*. Similarly, Vetal and Pardeshi (12) reported that among the ethanol and hexane extract of *A. squamosa* Linn. the hexane extract showed more insecticidal activity against *Spodoptera litura* than the ethanolic extract. Trisna Priadi *et al.* (11) reported that the *A. glabra* extract showed insecticidal activity against wood destroying termites *Cryptotermes cynocephalus* (dry-wood termites) and *Coptotermes curvignathus*.

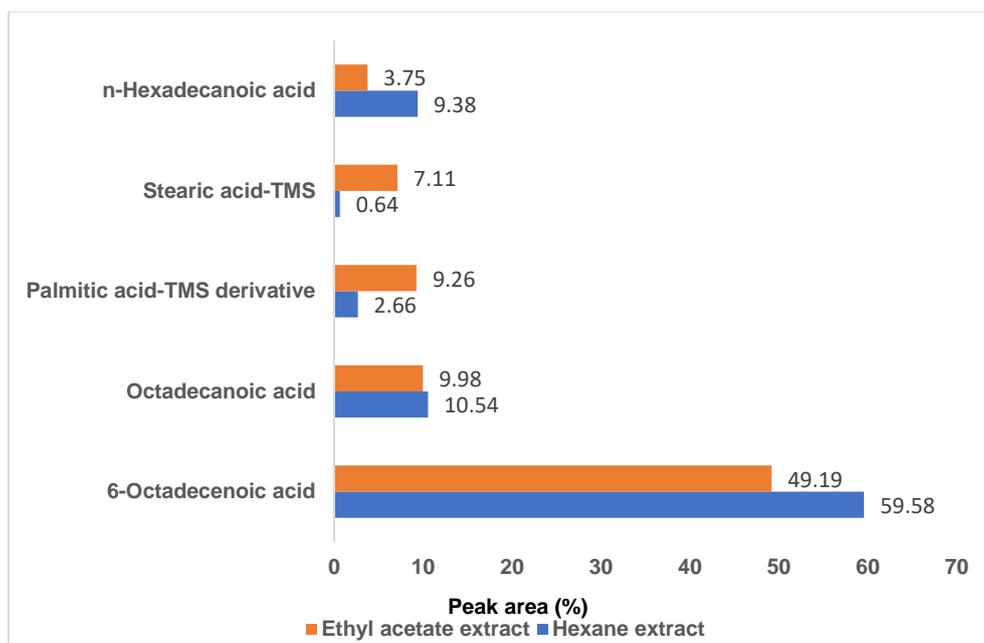


Figure 4. Comparison of phytochemical profile of *A. squamosa* L. seed extracts

CONCLUSIONS

Forty-three, 37 and 14 phytochemicals were identified in hexane and ethyl acetate extracts and seed oil of *A. squamosa*. 6-Octadecenoic acid was predominant in the extracts and 9-Octadecenoic acid was predominant in the seed oil. A comparison of toxicity revealed that the oil extract was more toxic than hexane and ethyl acetate extracts. *O. anamallensis* Holmgren and Holmgren were more susceptible to *A. squamosa* L. seed oil and solvent extract than *M. fletcheri* Holmgren and Holmgren.

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DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We have not excluded any author that substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

REFERENCES

1. Acda, M.N. (2014). Chemical composition of ethanolic seed extract of *Annona squamosa* L. and *A. muricata* L. (Annonaceae) using GC-MS analysis. *Philippine Agricultural Scientist* **97** (4): 422-426.
2. Adesanwo, J.K., Akinloye, A.A., Otemuyiwa, I.O. and Akinpelu, D.A. (2020). Chemical Characteristics and Biological Activities of *Annona squamosa* Fruit Pod and Seed Extracts. *Journal of Exploratory Research in Pharmacology* **6** (1): 5-15.
3. Babaiwa, U.F., Okonkwo, T.J., Erharuyi, O., Eraga, S.O. and Akerele, J.O. (2022). Studies on the antimicrobial properties and GC-MS analysis of ethyl acetate extract of *Annona muricata* seeds. *Journal of Phytomedicine and Therapeutics* **21** (2): 812-824.
4. Deewatthanawong, R, P., Kongchinda, P., Deewatthanawong, J.P. and Insung, A. (2019). GC-MS Analysis and Biopesticide Properties of Different Crude Extracts of *Annona squamosa* and *Annona muricata*. *International Journal of Agricultural Technology* **15** (6): 859-868.
5. Hagr, T. E., Adam, I. A., Almain, A. A. and Mohammed, M.M. (2019). Phytochemical screening, GC-MS analysis, antibacterial and antioxidant activity of seeds oil of *Annona squamosa* L. Sudanese medicinal plant. *Journal of Pharmacy and Pharmacology* **7**(1): 1-6.
6. Kevyn Danuway Oliveira Alves. (2021). The use of *Annona squamosa* seed, leaf and bark as an alternative in the control of *Cryptotermes brevis*. *Research, Society and Development* **10** (12): 1-6.
7. Logan, J.W., Cowie, R.H. and Wood, T.G. (1990). Termite (Isoptera) control in agriculture and forestry by non-chemical methods: a review. *Bulletin of Entomological Research* **80** (3): 309-330.
8. Prijono, D., Manuwoto, S. and Soemawinata, R.T. (1994). Insecticidal activity of sugar apple (*Annona squamosa* L.) and pond apple (*A. glabra* L.) seed extracts against rice brown planthopper, *Nilaparvata lugens* (Stal.). In *Proc. UNESCO National Seminar, Depok, Indonesia* pp. 335-341.
9. Rasib, K.Z. and Wright, D.J. (2018). Comparative efficacy of three bait toxicants against the subterranean termite *Reticulitermes Santonensis* (Isoptera/Blattoidea: Rhinotermitidae). *Biomedical Journal of Scientific & Technical Research* **11** (3): 8536-8559.
10. Sini, H., Mohanan, P.V. and Devi, K.S. (2005). Studies on the insecticidal activity, cytogenecity and metabolism of fatty acid rich fraction of *Hydnocarpus laurifolia*. *Toxicological & Environmental Chemistry* **87**(1): 91-98.
11. Trisna Priadi, Nurul Chotimah and Agus Ismanto. (2021). 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* **8**(2): 127-134
12. Vetal, D. S. and Pardeshi, A. B. (2019). Insecticidal potential of ethanol and hexane solvent seed extract of *Annona squamosa* against *Spodoptera litura* Fab. *Journal of Pharmacognosy and Phytochemistry* **8**(3): 842-845.
13. Zahid, M., Arif, M., Rahman, M.A., Singh, K. and Mujahid, M. (2018). Solvent extraction and Gas Chromatography-Mass Spectrometry analysis of *Annona squamosa* L. seeds for determination of bioactives, fatty acid/fatty oil composition and antioxidant activity. *Journal of Dietary Supplements* **15**(5): 613-623.

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