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Evaluation of the Repellent Activity and Biopesticide Properties of *Annona squamosa* (Linn.) Leaf Extracts against the Stored Grain Pest *Callosobruchus maculatus* (Fab.)

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A B S T R A C T

The use of a natural pesticide obtained from plant-based substances has proven to be an effective alternative to conventional pesticides. *Annona squamosa* Linn. (Custard apple), a potential medicinal plant, is grown throughout tropical and subtropical regions of the world, including India. In the phytochemical studies, it was found that these extracts contain significant secondary metabolites that contribute to their insecticidal effects. *Annona squamosa* leaf powders were extracted with various solvents and their phytochemical components were determined qualitatively and quantitatively using GC-MS analysis. Petroleum benzene and ethanol extracts contained the highest concentrations of terpenoids, fatty acids, phenolic compounds, and steroids. These extracts also contained biopesticides such as geranylacetone, dihydroactinidiolide, phytol, linoleic acid, palmitic acid, caryophyllene, and ethyl-4-hydroxyphenylacetate. A study was conducted on the biopesticide chemicals extracted from powdered *Annona squamosa* leaves using different solvents and their effectiveness against cowpea beetles, *Callosobruchus maculatus*. A higher repellency (48±1.26%) was achieved at a higher concentration (50 mg/ml) of petroleum benzene extract of *Annona squamosa* after 360 minutes of treatment followed by ethanol extract (47.2±1.49%), water extract (37.6±0.97%), benzene (42.4±0.97%) and chloroform (41.6±0.97%). We measured the levels of toxicity after 24 hours, 48 hours, and 72 hours of exposure. After 72 hours, Petroleum benzene caused the highest level of toxicity (LD50 = 16.22 mg/ml) of *Callosobruchus maculatus*, followed by ethanol (LD50=23.98 mg/ml), water (LD50 = 44.66 mg/ml), Benzene (LD50 = 89.13 mg/ml), and chloroform (LD50 = 102.32 mg/ml. Our results suggested that *Annona squamosa* phytocompounds are valuable ways of protecting stored grains against *Callosobruchus maculatus.*

***Key words*:** Biopesticide, *Callosobruchus maculatus, Annona squamosa,* Repellent activity, Toxicity activity

One of the most important aspects of agriculture is pest management. Eco-friendly methods of controlling microbes and pathogens have become increasingly popular in agriculture for researchers who prioritize safety for the environment and non-target species. In general, synthetic chemicals used as pesticides have negative after-effects. Discovering plant-derived compounds and using them as pesticides is undoubtedly an efficient way to control pests without disrupting the ecological balance [1]. In recent years, various plant-based pesticides having active components have been developed, including nicotine (from tobacco), rotenone (from *Derrissps.*, *Tephrosiasps*), pyrethrum (from *Chrysanthemumsps*), azadirachtin (from *neem*) and other comparable chemicals. One of those botanicals that deserve more attention is *Annonaceae*, one of those potent plant families. This is a large family of plants with approximately 130 genera and 2300 species. Members are primarily confined to tropical zones, and the family has a well-developed old and new world distribution. *Anonaceae* have been the subject of a lot of research since the 1980s, due to their presence of acetogenins, a class of long-chain fatty acid derivatives with a variety of biological activities. Among the many uses for *Annona squamosa*, commonly known as 'Seethapazhzm' in Tamil and 'Custard Apple' or 'Sugar Apple' in English, its insecticidal activity is among the most promising [2].

Study was conducted on the effect of the *A. squamosa* leaf extract on skin repellent activity against three mosquito species, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus.* The highest concentration provided protection against *Anopheles stephensi* for over 126.2 minutes. Low concentrations provided protection against *Aedes aegypti* and *Culex quinquefasciatus* for 52.4 and 50.4 minutes, respectively. A dose-dependent repellent effect was observed by the authors [3]. An analysis of hexane, chloroform, ethyl acetate, acetone, and methanol dried leaf and bark extracts of *Annona squamosa* L., *Chrysanthemum indicum* L., and *Tridax*

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procumbens L. against fourth instar larvae of malaria vectors Anopheles subpictus Grassi and Japanese encephalitis vectors Culex tritaeniorhynchus Giles (Diptera: Culicidae) was conducted by Kamaraj *et al*. [4]. The pure compound annotemoyin-1 isolated from chloroform extract of *Annona squamosa* Linn seeds was evaluated by Shahnaj Parvin *et al*. [5] for its pesticidal activity against *Tribolium castaneum* (Herbst), which is a significant pest of grains stored in laboratories [6]. The LD50 values of the pure compound were determined for adults and different larval stages. As a result of the above facts, we evaluated the repellent effect and biopesticide properties of *Annona squamosa* (Linn.) leaf extracts against the stored grain pest *Callosobruchus maculatus* (Fab.).

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *Annona squamosa* were collected in Kasilingapuram village, in the Thoothukudi district, Tamil Nadu, India, during the months of October and November (2019). Plant specimens have been identified and authenticated by Dr. C. Babu, Head and Associate Professor of Botany at Pioneer Kumaraswamy College, Nagercoil. After thoroughly rinsing the leaves under running water, they were shade-dried for 7-8 days at room temperature to remove dust. A fine powder was then made from plant leaves and stored in an airtight container for future use.

Extract preparation

In a Soxhlet extractor, 50 grams of dry leaves were dissolved in 250 ml of petroleum benzene (40-60°C), benzene, chloroform, ethanol, and water. Solvent is poured into the Soxhlet loop, where extraction occurs until the solvent is colorless [7]. At room temperature, the extracts were made into a concentrated state and stored in airtight containers to allow the solvent to evaporate. For further use, the solution was frozen at 4°C [8].

Phytochemical analysis

The leaves of *Annona squamosa* were used to test for phytochemicals using a previously described method [9]. In order to establish the chemical composition profile of the extracts, numerous qualitative chemical tests were conducted on the individual extracts. Following standard procedures, the crude powder is extracted with different solvents and tested to determine what phytoconstituents are present in each one. Generally, tests are performed to determine if Terpenoids, Steroids, Fatty Acids, Phenolic Compounds, Alkaloids, Saponins, and Flavonoids are present.

Gas Chromatography Mass Spectrum (GC-MS) analysis

We used GCMS analysis of *Annona squamosa* plant extracts from Heber Analytical Instrumentation Facility (HAIF), Bishop Heber College, Trichy-620 017 to investigate its phytochemistry. The analyses were performed with GC-MS equipment (GC MS QP2020; SHIMADZU), which consists of an autosampler, a sample injector, a gas chromatograph (GC-2010) and a mass spectrometer. GC-MS system was composed of SHRxi-5Sil-MS capillary standard non-polar column (Dimension: 30.0 m, Diameter: 0.25mm, Film thickness: 0.25µm which is 100% Dimethyl poly siloxane The electron ionization energy system used had an ionization energy of 70eV. It was carried out with helium gas (99.99%) at a rate of 1.20ml/min and an injection volume of 5µl (split ratio: 10). The oven temperature was programmed from 50°C (isothermal for 2 min.), increasing to 280°C for 10 min. Mass spectra were taken at 70eV at a scan interval of 0.3 seconds with scan range of 50 - 500 m/z. A total of 21 minutes were spent running the GC. We calculated the percentage of each component based on its average peak area divided by the total peak area. To analyze mass spectra and chromatograms, we used Shimadzu's GC-MS real-time software package.

Identification of components

The interpretation of GC-MS mass spectra was done using data from the National Institute Standard and Technique (NIST14) [10] and WILEY8 [11] having more patterns. We compared the spectrum of the unknown component to the spectrum of the known components from the NIST14 and WILEY8 libraries. Molecular formula, Name, Molecular weight, and Structure were identified for each component of the test material.

Insect collection and rearing

Callosobruchus maculates Fab. was collected from a farmer in Kasilingapuram village. All experiments were conducted at the PG and Research Department of Zoology PMT College, Melaneelithanallur. In the beginning, 50 pairs of adults aged 1–2 days were placed in jars containing cowpea seeds, sealed, and allowed a maximum of 7 days for mating and oviposition, the pest reared on cowpea grains in the laboratory at 28 ± 1°C and 65 ± 5% relative humidity (RH). The parents were removed and cowpea seeds containing eggs were transferred into new cowpea seeds in breeding jars, which were covered with cloth fastened with rubber bands to prevent contamination and insect escape. All experiments were carried out using progenies of the pest.

Repellency tests

The repellent effects of the plant extract and some of their individual components against *Callosobruchus maculates* were tested using the area partiality method [12]. The solutions of plant extract were prepared in ethanol, and in all cases, a volume of 1 mL was applied to a half-filter paper disk, as uniformly as possible, to obtain the desired plant extract volume per unit area of 10, 20, 30, 40, and 50 mg/ml, using ethanol as solvent. The other half of the filter paper was treated with an equal volume of ethanol as a vehicle control. Test areas consisted of 9 cm Whatman No. 1 filter paper cut in half (31.8 cm^2). The treated and control half disks were air-dried for 10 min to evaporate the solvent. Treated in addition untreated halves were reattached with adhesive tape and placed in 90 mm glass Petri dishes. Thirty adults of *Callosobruchus maculates* of both sexes were released at the center of each filter paper disk. Dishes were covered and placed in darkness at 22-2°C and 75±10% RH. The numbers of *Callosobruchus maculates* present on the treated and untreated portions of the experimental paper halves were recorded for each dish after 60, 120, 180, 240, 300, and 360 sec of exposure. Percentage repellency (PR) for a given exposure time was computed as follows: PR = [(Nc -Nt)/(Nc-Nt)] x100, where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively. Five replicates were used for each tested concentration Nerio *et al*. [13].

Toxicity assay

In toxicity assay, the *Annona squamosa* extracts were used against adults of *Callosobruchus maculates* at 28 ± 1°C and 65 ± 5% RH. Newly developed adults (1–15 days old) were used in these studies. *Cassia auriculata* leaf extracts were tested against *Callosobruchus maculates* adults by using glass jars (1L) as chambers (replicates) and filter paper pieces (3 x 3 cm)

connected to the underside of the screw caps. Each jar contained 30 insects and the plant extracts were applied by 10, 20, 30, 40, and 50 mg/ml to filter paper pieces. The treatment and control were repeated five times. Filter paper pieces were treated with solvent alone as a control. An insect mortality percentage was observed and recorded after 24, 48, and 72 hours for each concentration, and the lethal concentration causing 50% mortality (LC50) were calculated from log-concentration mortality regression lines. The insects were considered dead if they did not move their legs or antennae [14-15].

Statistical analysis

One-way analysis of variance (ANOVA) and the least significant difference (LSD) multiple range test were performed on the data to determine significant ($P < 0.05$) differences among variable concentrations. It was hope that Finney's Probit analysis [16] would help estimate lethal concentration (LC) and LC50 values were considered significantly different when their respective 95% fiducial limits did not overlap.

RESULTS AND DISCUSSION

The present study was carried out on the plant of *Annona squamosa* leaves to identify the presence of biopesticide components. Phytochemical tests, being economical and fast, are recommended for the quality control of insecticidal secondary metabolism. In the present study, phytochemicals were confirmed to be present in different solvent extracts of *Annona squamosa*.

Qualitative phytochemical analysis A.squamosa leaves extracts

Plants have a toxicity value due to some chemical substances that possess a strong physiological effect on insects. The most important of these compounds are alkaloids, terpenoids, steroids, fatty acids, and phenols. The qualitative phytochemical analysis of various solvent extracts of *Annona squamosa* leaves was showed in (Table 1). The phytochemical analysis results revealed that the presence of alkaloids, terpenoid, steroid, fatty acid and phenolic compounds. There was high intensity of terpenoids in petroleum benzene extract and low intensity in chloroform and ethanol extracts [17]. Fatty acids were detected in high intensity in petroleum benzene, chloroform, benzene, and ethanol extracts. The presence of steroids was found in high intensity in ethanol and water extracts. The alkaloids were found to be in very low intensity in ethanol extracts. The phenolic compound was present in low intensity in petroleum benzene and benzene extracts. Saponin was found in very small amounts in ethanol and water extracts, while flavonoids were found in very small amounts in benzene, chloroform, and ethanol extracts [18-19].

Table 1 Preliminary phytochemical screening of extract of powdered leaves of *Cassia auriculata*

Phytochemicals	Solvents				
	Petroleum benzene	Benzene	Chloroform	Ethanol	Water
Terpenoids	+++	+	+	++	+
Steroids	-	-	-	+++	++
Fatty acids	+++	+++	+	++	+
Phenolic compounds	+	++	-	+++	++
Alkaloids	-	-	-	++	-
Saponin	-	-	-	++	+
Flavonoids	+	-	+	++	-

+ → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; - → absent

GC-MS analysis of Annona squamosa leaves extract

The most effective way to determine the functional groups that make up bioactive constituents of Terpenoids, Steroids, Fatty Acids, Phenolic Compounds, Alkaloids, Saponins, and Flavonoids is through GC-MS. In this study, we analyze the results of Gas Chromatography - Mass Spectroscopy on the various solvent extracts of *Annona squamosa*, as shown in (Table 2, Fig 1). Among forty-five compounds identified in the petroleum benzene extract, eight showed to be toxic in nature. The GC-MS analysis of petroleum benzene extract of *Annona squamosa* revealed the presence of toxic compounds like Geranyl acetate (0.39), Dihydroactinidiolide (0.79), Neophytadiene (1.21), Phytol (1.59), Ascorbic Acid (6.36), Linoleic acid (1.31), Octadecanedioic acid (3.56) and squalene (8.48). Benzene extracts, twenty compounds were identified and four of those compounds appeared toxic. The toxic compounds in benzene extracts, such as beta-Caryophyllene (1.16), Stearic acid (1.42), Nonadecane (0.61) and Dioctyl phthalate (58.18). Among the 25 compounds identified in the chloroform extracts, four were toxic. A toxic compound such as Valencene (1.18), Tetradecan-1-ol (0.82), Palmitic acid (10.43) and Dioctyl phthalate (25.54). The ethanol extracts identified thirty compounds out of which six were toxic. Toxic compound such as Ethyl 4-hydroxyphenylacetate (5.6), D-Phenylalaninol (1.67), Palmitic acid (3.69), Ethyl stearate (3.25), Oxandrolone (1.24) and Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (3.09). The water extracts showed that 10 compounds were identified and two of these compounds were toxic, such as Cholecalciferol (1.67) and 4-Dodecylphenol (1.28).

Terpenoids are widely used in the fragrance and food industries, as well as in a wide range of pharmacological applications. Terpenoids are the largest class of natural products derived from plants. They include essential oils, flavours, fragrances, lipid-soluble pigments and toxic compounds [20]. A toxin that causes paralysis and mortality led to the development of the most successful commercial pesticides [21-22]. Phenolic compounds possess a hydroxyl (—OH) group, attached to the benzene ring or to another aromatic ring structure, such as catechol, resorcinol, hydroquinone, pyrogallol, etc. In various studies [23], phenolic compounds found in oak have been found to have a negative effect on the growth of gypsy moths. Various studies have shown that plant phenolic compounds act as one of the primary defences against insects [24-25]. Insecticides using fatty acids are natural. Its relatively low toxicity to vertebrates, ease of soil decomposition, and lack of resistance by target insects make it an excellent natural insecticide [26]. Lauric acid is a saturated fatty acid with a 12-carbon atom chain (medium chain fatty acid) that acts both as a physical and chemical insecticide [27]. Cholecalciferol is an acute (single-feeding) or chronic (multiple-feeding) rodenticide toxicant with unique activity for controlling commensal rodents as well as anticoagulant-resistant rats [28]. An association between the monoterpene, sesquiterpene, and oxygenated monoterpene content of *H. opposita* and its insecticidal activity against *Callosobruchus maculatus* [29-30]. In recent years, GC-MS has

become one of the most recommended tools for monitoring and tracking organic pollutants in the environment. It is the tool used to test for prohibited performance enhancing drugs such as anabolic steroids in athletes' urine samples. It is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes, etc. GC-MS is an extremely powerful technology that provides a rare opportunity to characterization and identification of new compounds synthesized or derivatized [31].

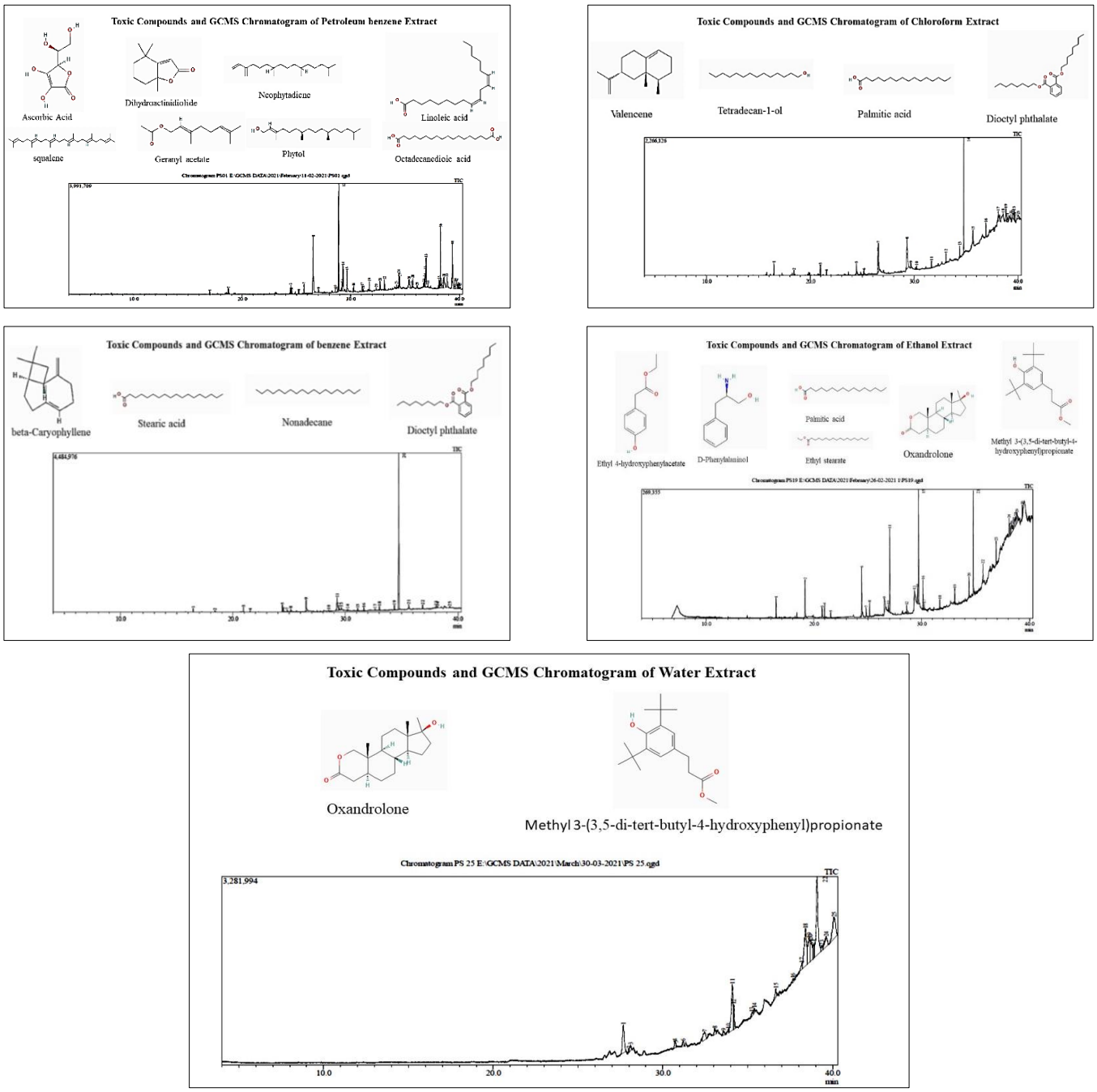

Fig 1 GC-MS Chromatogram and toxic compounds of *Annona squamosa* leaves different solvent extract

Repellence activity

(Table 3) were showed that repellence activity of methanol, ethyl acetate and hexane extracts of *Annona squamosa*. Highest repellency (48±1.26%) about was achieved at higher concentration (50 mg/ml) of petroleum benzene extract of *Annona squamosa* after 360 minutes of treatment, followed by ethanol extract (47.2±1.49%), water extract (37.6±0.97%), benzene (42.4±0.97%) and chloroform (41.6±0.97%). The highest individual repellency activity was achieved at petroleum benzene extract of *Annona squamosa* against stored grain insect pests *C. maculatus*. The individual replicate with mean value was showed that highest repellence activity in petroleum benzene extract at 1 h interval. The repeated measure analysis of *Annona squamosa* against *C. maculatus* between various doses of 10, 20, 30, 40 and 50 mg/ml after 1, 2, 3,4, 5and 6 h respectively were significant at $p < 0.05$ level.

Some monoterpenes such as α--pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents of a number of EO described in the literature, as presenting mosquito repellent activity [32-35]. Among sesquiterpenes, β-caryophyllene is most cited as a strong repellent against *A. aegypti* [36]. Although repellent properties of several EO regularly appear to be associated with the presence of monoterpenoids and sesquiterpenes [37-38], other authors [39] have found that phytol, a linear diterpene alcohol, has high repellent activity against Anopheles gambiae. Moreover, the oxygenated compounds phenylethyl alcohol, β-citronellol, cinnamyl alcohol, geraniol, and α-pinene, isolated from the essential oil of *Dianthus caryophyllum*, showed strong

repellent activities against ticks (*I. ricinus*) [40]. In our study, petroleum benzene leaf extract from Annona squamosa was more effective at repelling insects than other extracts such as ethanol, water, chloroform, and benzene. The petroleum benzene extract of Annona squamosa was found to contain eight toxic compounds, including Geranyl acetate, Dihydroactinidiolide, Neophytadiene, Phytol, Ascorbic Acid, Linoleic acid, Octadecanedioic acid and squalene. These compounds act as repellent activity against stored grain pests *Callosobruchus maculatus.*

Table 2 Phytochemical analysis of *Cassia auriculata* leaves different solvent extract

Solvent	Retention time (min)	Peak area	Molecular weight	Molecular formula	Name of the compound	Name of the phytochemical	Toxicity
Petroleum benzene	16.984	0.39	194	$C_{12}H_{20}O_2$	Geranyl acetate	monoterpenoid	Skin, eye, and respiratory irritations health hazards
	18.707	0.79	180	$C_{11}H_{16}O_2$	Dihydroactinidiolide	Lactone	Acute toxicity, oral
	24.454	1.21	278	$C_{20}H_{38}$	Neophytadiene	diterpene	Health hazards
	25.188	1.59	296	$C_{20}H_{40}O$	Phytol	diterpenoid	Skin, eye, and respiratory irritations health hazards
	26.544	6.36	652	$C_{38}H_{68}O_8$	Ascorbic Acid	Vitamins	Fungicides
	29.183	1.31	280	$C_{18}H_{32}O_2$	Linoleic acid	omega-6 fatty acid	Skin, eye, and respiratory irritations
	29.658	3.56	284	$C_{18}H_{34}O_4$	Octadecanedioic acid	alpha,omega-dicarboxylic acid	Skin, eye, and respiratory irritations
	38.280	8.48	410	$C_{30}H_{50}$	squalene	triterpene	Health hazards
Benzene	16.453	1.16	204	$C_{15}H_{24}$	beta-Caryophyllene	sesquiterpene	Skin, eye, and respiratory irritations health hazards
	29.630	1.42	284	$C_{18}H_{36}O_2$	Stearic acid	long-chain fatty acid	Skin, eye, and respiratory irritations
	30.195	0.61	268	$C_{19}H_{40}$	Nonadecane	Hydrocarbon	Skin, eye, and respiratory irritations health hazards
	37.750	58.18	390	$C_{24}H_{38}O_4$	Dioctyl phthalate	Phthalic Acids	Skin, eye, and respiratory irritations health hazards
Chloroform	18.418	1.18	204	$C_{15}H_{24}$	Valencene	Sesquiterpenes	Skin, eye, and respiratory irritations health hazards
	21.555	0.82	214	$C_{14}H_{30}O$	Tetradecan-1-ol	Fatty Alcohols	Health hazards
	26.539	10.43	256	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acids	Skin, eye, and respiratory irritations health hazards
	37.770	25.54	390	$C_{24}H_{38}O_4$	Dioctyl phthalate	Phthalic Acids	Skin, eye, and respiratory irritations health hazards
Ethanol	19.168	5.6	180	$C_{10}H_{12}O_3$	Ethyl 4-hydroxyphenylacetate	ester	Skin, eye, and respiratory irritations health hazards
	20.744	1.67	151	$C_9H_{13}NO$	D-Phenylalaninol	aminoacids	Health hazards
	26.532	3.69	256	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acids	Skin, eye, and respiratory irritations health hazards
	30.115	3.25	312	$C_{20}H_{40}O_2$	Ethyl stearate	Fatty aster	Skin, eye, and respiratory irritations
	38.560	1.24	306	$C_{19}H_{30}O_3$	Oxandrolone	oxa-steroid	Skin, eye, and respiratory irritations health hazards
	38.560	3.09	306	$C_{18}H_{28}O_3$	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	Ester	Skin, eye, and respiratory irritations health hazards
Water	30.640	1.67	454	$C_{27}H_{44}O$	Cholecalciferol	steroid	Rodenticides, acute toxicity, oral
	35.388	1.28	262	$C_{18}H_{30}O$	4-Dodecylphenol	Phenolic compounds	Skin, eye, and respiratory irritations health hazards

Table 3 Repellent Activity-Annona Squamosa

Solvent	Concentration (mg/ml)	60	120	180	240	300	360	Average	P value	Significance
Benzene	10	3.2±0.8	6.4±0.97	12±1.26	16.8±0.8	20.8±1.49	24±1.26	13.86	<0.005	***
	20	6.4±0.967	12±1.26	16.8±1.49	20.8±0.8	26.4±0.97	31.2±0.8	18.93	<0.005	***
	30	8.8±1.49	12.8±1.49	20.8±0.8	25.6±0.97	31.2±0.8	34.4±0.97	22.26	<0.005	***
	40	12.8±1.49	17.6±0.97	24±1.26	28±1.26	33.6±0.97	39.2±0.8	25.86	<0.005	***
	50	16.8±1.49	20.8±1.49	27.2±0.8	34.4±0.97	36.8±0.8	42.4±0.97	29.73	<0.005	***
Chloroform	10	2.4±0.97	4.8±0.8	11.2±0.8	16±1.26	20±1.26	23.2±0.8	12.93	<0.005	***
	20	5.6±0.97	11.2±0.8	16±1.26	20±1.26	24.8±1.49	29.6±0.97	17.87	<0.005	***
	30	8±1.26	11.2±0.8	20±1.26	24±1.26	30.4±0.97	33.6±0.97	21.2	<0.005	***
	40	12±1.26	16.8±0.8	23.2±0.8	26.4±0.97	32±1.26	38.4±0.97	24.8	<0.005	***
	50	15.2±1.49	20±1.26	26.4±0.97	32.8±1.49	35.2±0.8	41.6±0.97	28.53	<0.005	***
Ethanol	10	4±0.8	7.2±0.8	13.6±0.97	20.8±1.49	24±1.26	26.4±0.97	16.00	<0.005	***
	20	7.2±0.8	13.6±0.97	19.2±0.8	23.2±0.8	28±1.26	34.4±1.6	20.94	<0.005	***
	30	10.4±0.97	14.4±1.97	23.2±0.8	28±1.26	33.6±0.97	37.6±0.97	24.54	<0.005	***
	40	13.6±0.97	19.2±0.8	26.4±0.97	31.2±1.49	36.8±1.49	42.4±0.97	28.27	<0.005	***
	50	18.4±0.97	23.2±0.8	29.6±0.97	38.4±0.97	43.2±0.97	47.2±1.49	33.33	<0.005	***
Petroleum benzene	10	7.2±0.8	8.8±1.49	16±1.26	22.4±1.49	28±1.78	26.4±0.97	18.13	<0.005	***
	20	8.8±0.8	13.6±0.97	20±1.26	24.8±0.8	30.4±0.97	35.2±1.49	22.13	<0.005	***

	30	11.2±0.8	15.2±0.8	24±1.26	28.8±24	34.4±0.97	39.2±1.49	25.47	<0.005	***
	40	15.2±0.8	20±1.26	28±1.26	32±1.78	37.6±1.6	45.6±0.97	29.74	<0.005	***
	50	20±1.26	25.6±0.97	31.2±0.8	38.4±0.97	43.2±1.49	48±1.26	34.40	<0.005	***
Water	10	1.6±0.97	3.2±0.8	9.6±0.97	12.8±1.49	16±1.26	20.8±0.8	10.66	<0.005	***
	20	2.4±0.97	8±1.26	11.2±0.8	15.2±1.49	20±1.26	25.6±1.6	13.73	<0.005	***
	30	5.6±0.97	7.2±0.8	13.6±0.97	19.2±1.49	22.4±0.97	28.8±1.49	16.13	<0.005	***
	40	8.8±1.49	14.4±0.97	19.2±1.49	21.6±0.97	28.8±1.49	33.6±0.97	21.06	<0.005	***
	50	11.2±1.49	17.6±0.97	21.6±0.97	24.8±0.8	32±1.26	37.6±0.97	24.13	<0.005	***

Each datum represents for five replicates (Mean ± SE, %), adults (n = 30)

Table 4 Toxicity and profit analysis of different extract to cowpea beetle, *Callosobruchus maculates*

Plant material	Hours	Concentration (mg/ml) 10	20	30	40	50	Mean	P value	Significant	LC_{50} (mg/ml)
Petroleum	24	12.8±1.49	24±1.26	42.4±0.97	55.2±0.8	60.8±1.49	39.04	0.001	***	38.01
Benzene	48	20.8±1.49	38.4±0.97	48.8±1.497	60.8±0.8	72±1.26	48.16	0.0018	***	28.18
	72	31.2±1.49	48±1.26	61.6±0.97	73.6±0.97	87.2±0.8	60.32	0.003	***	16.22
Benzene	24	2.4±0.97	4.8±0.8	8.8±1.49	14.4±0.97	20.8±1.49	10.24	0.001	***	162.18
	48	5.6±0.97	11.2±0.8	16±1.26	20.8±1.49	28±1.26	16.32	0.0012	***	154.88
	72	8.8±1.49	18.4±0.97	27.2±0.8	28.8±1.49	37.6±0.97	24.16	0.0005	***	89.13
Chloroform	24	3.2±0.8	8±1.26	10.4±0.97	16.8±0.8	23.2±1.49	12.32	0.0030	***	165.95
	48	6.4±0.97	9.6±1.6	18.4±0.97	24±1.26	32.8±1.49	18.24	0.0035	***	109.62
	72	12.8±0.8	16.8±1.49	25.6±1.6	32±1.26	38.4±0.97	25.12	0.0039	***	102.32
Ethanol	24	12±1.26	20.8±1.49	36.8±0.8	45.6±1.6	56±1.26	34.24	0.0015	***	50.11
	48	16.8±1.49	32.8±0.8	45.6±1.6	52±1.26	66.4±0.97	42.72	0.0008	***	33.88
	72	25.6±0.97	40.8±0.8	54.4±1.6	64.8±1.49	76.8±0.8	52.48	0.002	***	23.98
Water	24	6.4±0.97	12.8±1.49	23.2±0.8	29.6±1.6	34.4±0.97	21.28	0.000003	***	85.11
	48	8.8±1.49	16±1.26	23.2±0.8	32.8±1.49	41.6±0.97	24.48	0.003	***	77.62
	72	20.8±1.49	33.6±0.97	39.2±0.8	48±1.26	52.8±1.49	38.88	0.0009	***	44.66

***Highly significant

Toxicity assay

Petroleum benzene, benzene, chloroform, ethanol, and water extracts of *Annona squamosa* showed excellent fumigant activity against *Callosobruchus maculatus*, and the time needed to cause 50% (LC50) mortality dropped with increased concentration (Table 4). Plant extracts from the leaves were fumigated against the pest with various concentrations of 10, 20, 30, 40, and 50 mg/ml and exposure times of 24, 48, and 72 hours, respectively. The mean mortality activity of petroleum benzene extract is observed 39.04, 48.16and 60.32in 24, 48 and 72 hours respectively (Fig 2). The benzene extract mean observed mortality percentage is 10.24 (24hr), 16.32 (48hr) and 24.16 (72hr). Chloroform extract showed the mean value of observed mortality is 12.32, 18.24 and 25.12 in 24, 48 and 72 hours respectively. The mean mortality activity of ethanol extracts observed 34.24, 42.72, and 52.48 in 24, 48 and 72 hours respectively. The water extract mean observed mortality percentage is 21.28 (24hr), 24.48 (48hr) and 38.88 (72hr). The highest toxicity (87.2±0.8% and LC50 value 16.22 mg/ml) of *Callosobruchus maculatus* was caused by Petroleum benzene, followed by ethanol 76.8±0.8±1.26% and LC50 value 23.98 mg/ml), water (52.8±1.49% and 44.66 mg/ml) Benzene (37.6±0.97% and 89.13 mg/ml) and chloroform (38.4±0.97% and 102.32 mg/ml) after 72 hours.

The essential oil of *Vernonia arborea* can be used to manage *Callosobruchus maculatus* and other insect pests in stored products. It acts as an insecticide, reducing the rate of female oviposition, population growth, and development [41]. Novel botanical insecticides, especially their hexane fraction, have a similar or higher biological activity than the most popular botanical insecticides like *Azadirachta indica* against stored grain pests like *Callosobruchus maculatus* [42]. Considering its high levels of toxicity against cowpea weevils in storage, *Hapalosiphon welwitschii* leaves powder extracts should be used as postharvest insecticides of plant origin for stored cowpea management [43].

Analogues of secondary metabolites have the possibility of interfering with various vital components of the cellular signaling system, or interfering with vital enzymes and signals in the nervous system (such as neurotrans- mitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction), or blocking metabolic pathway functions [44]. Toxic effects of essential oils or their constituents in insects and other arthropods point to a neurotoxic mode of action; most prominent symptoms are hyperactivity and hyperexcitation leading to rapid knockdown and immobilization [45].

The results showed that petroleum benzene extract exhibited the highest fumigant toxicity. This was because petroleum benzene extract contained the highest concentration of terpenoids, fatty acids, and toxic substances such as thymol Mohammad *et al.* [46], Mohaddeseh *et al.* [47]. Furthermore, ethanol extract showed a more toxic effect against *Callosobruchus maculatus*. Ethanol extract exhibits fatty acids, terpenoids, glycosides, acrylate, and cholecalciferol. Water extracts are found in steroids and organometallic compounds and show moderate activity. Cowpea beetle, *Callosobruchus maculatus* responded to benzene and chloroform extracts with the least toxicity. A toxin causes disturbances of the nervous system, which can lead to paralysis and death, giving rise to the most successful commercial pesticides of all time [48-49]. The results of our study concluded that *Cassia auriculata* phytocompounds are highly effective at preventing the growth of *Callosobruchus maculatus* on grains stored in storage.

CONCLUSION

As a result of the present study, botanical compounds may be used commercially as insect control agents. The powdered leaves of Annona squamosa exhibit a high level of toxic and inhibitory activity against *Callosobruchus maculatus*. In the various solvent plant extract tests, petroleum benzene and

ethanol proved to be most effective against high toxic compounds. Traditional plant products have shown great success in controlling the stored grain pest, *Callosobruchus maculatus*, by reducing the severity of the damage caused by insect pests cowpea seeds can be stored efficiently and effectively with Annona squamosa extracts, which are inexpensive and easy to adapt, thus increasing its availability to farmers. Although these results are promising, further testing is needed, especially to isolate and identify the active principal component of the product, as well as to evaluate both the product's cost-benefit ratio and its capability to control insect infestations in grain stores.

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