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EXTRACTION, ISOLATION, CHARACTERIZATION AND PHYTOCHEMICAL SCREENING OF *EUPHORBIA BALSAMIFERA* LEAF EXTRACT FOR PESTICIDAL ACTIVITY AGAINST *CALLOSOBRUCHUS MACULATUS*

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ABSTRACT

Pest infestations of stored agricultural products cause substantial global losses, highlighting the need for ecofriendly alternatives to synthetic pesticides. This study explores the insecticidal potential of *Euphorbia balsamifera* leaf extracts against *Callosobruchus maculatus*, a major storage pest. Ethanol was used to obtain the crude extracts which were then fractionated into n-hexane, chloroform, ethyl acetate, methanol, and aqueous fractions. Phytochemical analysis confirmed the presence of bioactive compounds including alkaloids, flavonoids, saponins, and terpenoids. The bioassay was conducted at varying concentrations ranging from 0.5 to 2.5 mg/µL, with each treatment performed in triplicate, and mortality rates were recorded over 72 hours. The *n*-hexane fraction exhibited the highest insecticidal activity, achieving 87% mortality at the highest concentration of 2.5 mg/µL. Further analysis using FT-IR and GC-MS identified key functional groups and bioactive compounds, including azelaic acid and 1-hexyl-2-nitrocyclohexane, known for their pesticidal properties. These findings suggest that *Euphorbia balsamifera* leaf extract could serve as natural, biodegradable alternatives to synthetic pesticides for storage pest control, contributing to sustainable pest management in agricultural.

Keywords: *Euphorbia Balsamifera, Callosobruchus maculatus,* Storage pests, Phytochemical Screening, Bioactive compounds, *n*-hexane fraction, FT-IR, GC-MS, Sustainable pest control

INTRODUCTION

Insects' pests inflict severe damage to stored food grains in many parts of the world. Though effective and reliable, several discouraging aspects of synthetics pesticides such as high cost, non-biodegradability, and the harmful effects on humans and the environment have urged agriculturalist to look for an alternative approach that is powerful, eco-friendly and economically viable. Plants volatile organic compounds are known to possess insecticidal properties. They maybe advocated for alternatives to synthetic insecticides (Kabrambam, *et al.,* 2021).

The commercially used synthetic pesticides have been proven to be toxic not only to humans and other animals, but also to non-target plant, the surrounding organisms around the plant, and the environment. There are also increased concerns regarding the development of pest resistance towards these synthetic pesticides. As such, bio pesticides, which are defined as the certain kinds of pesticides derived from natural sources such as plants, bacteria, fungi, animals and some minerals, are potential alternative pesticides and are gaining increasing attention (Aadil *et al.,* 2022). The used of agrochemicals causes a number of environmental issues, including harm to human health, contamination of ground water and other aquatic habitats, and oil degradation and lowers soil quality and turns many formally productive agricultural lands into unproductive ones (Hassan *et al.,* 2025). Traditional pest control strategies have relied heavily on synthetic chemical pesticides. However, these methods have raised concerns due to their environmental impact, health risks, and the increasing problem of pest resistance (Sharma *et al.,* 2020).

In order to improve efficiency of crop production and reduce food crisis in a sustainable manner, while preserving consumer's health, plant-derived pesticides may be a green alternative to synthetic ones. They are cheap, biodegradable, ecofriendly and act by several mechanisms of action in a more specific way, suggesting that they are less of hazard to humans and the environment. Natural plant products with bioactivity toward insects include several classes of molecules, for examples: terpenes, flavonoids, alkaloids, polyphenols, cyanogenic, glucosides, quinones, amides aldehydes, thiophenes, amino acids, saccharide and polyketides. Those compounds have important ecological activities in nature, such as antifeedant, attractant, nematicide, fungicide, repellent, insecticide, insect growth regulator and allelopathic agents acting as promising source for novel pest control agents or biopesticides (Augusto *et al.,* 2021).

Euphorbia Balsamifera is a dioecious succulent dendroid shrub, popularly known as "sweet tabaiba" or "tabaiba dulce" (the plant is also known in Africa as "ifernane", "fernán", "yaro", "aliyara", (Pablo & Recarda, 2023). It is distributed along the dry coastal areas of all the Canary Islands where it is a common element of the thermophilous vegetation. The species is less common along the coast of southern Morocco and northern Western Sahara where it is found forming scattered populations (Riina *et al*., 2021).

Figure 1: *Euphorbia Balsamifera* Leaves

Callosobruchus maculatus is a primary pest of stored cowpea seeds, causing significant damage that leads to weight loss and reduced seed viability. The larvae develop inside the seeds, feeding on the cotyledons, which results in both quantitative and qualitative losses. In severe infestations, losses can be as high as 100%, rendering the seeds unsuitable for consumption or planting (Ashamo *et al*., 2022).

Figure 2: Cowpea Weevils *Callosobruchus maculatus*

Murugesan *et al*., (2021) carried out the investigation of crude leaf extract of *Solanum torvum* (Sw.), their preliminary phytochemical screening and ability to protect the stored green from *Callosobruchus maculatus* (F.) adult infestation. The *Solanum torvum* (Sw.) ethyl acetate leaf extract was exhibited strong contact toxicity and repellent activity against *Callosobruchus maculatus* (F.) adult. The toxicity was significantly improved while extended treatment times and concentrations of *Solanum torvum* (Sw.). The study shows that, the mortality was reached over the ethyl acetate leaf extract nearly 98% at the dose of 900 µg/cm^2 after 72 h, followed by methanol (70%) and hexane (48%) leaf extract. Contact toxicity value of *Solanum torvum* (Sw.) leaf extract LC_{50} at 72 h interval was observed at 393.271 µg/mL, 632.338 µg/mL and 894.333 µg/mL for ethyl acetate, methanolic and hexane extract respectively against *Callosobruchus maculatus* (F.) adult.

Oladejo *et al.,* (2023) investigated the effects of *Euphorbia balsamifera* Leaf and stem powders and the combination on the adult's bruchids establishment, mortality, oviposition, and subsequent emergence. The results revealed that the bruchids were able to establish but after 24hours, adult mortality commenced and increased significantly as the dosage and duration of the experiment increases, except in the control (0.0g) treatment. The mortality effects of these powders on insects may depend on chemical composition of the treated powders which may suggest a role in its pesticidal, anti-feedant and repellent potencies against C. maculatus. The highest mean mortality (88.33%) was recorded on the combination (20.0g). It was concluded from this study that combinations of both powders at 20.0g could be used as alternative pesticides against bruchids infestations in stored cowpea grains.

MATERIALS AND METHODS

Solvents, Reagents: Methanol (CH_3OH), ethanol (CH_3CH_2OH), ethyl acetate $(CH_3)_2CH_2CO_2$, n-hexane, chloroform ($CHCl_3$), dimethyl sulfoxide $(CH_3)_2S{=}O$, Sodium hydroxide (NaOH), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), Acetic anhydride $(CH_3CO)_2O$, ferric chloride ($FeCl_3$), silica gel and distilled water.

Apparatus & Equipment: Beaker, TLC plate, capillary tube, glass wool, column, mortar and pestle, rotary evaporator, water bath, conical flask, syringe, cotton swab, sterile borer, reagent bottle, spatula, sample bottle, Whatman filter paper, muslin cloth, aluminium foil paper, petri dish, measuring cylinder, weighing balance, test tube, separating funnel and buchner funnel.

Sample Collection, Identification and Pre-treatment

Fresh leaf of *Euphorbia Balsamifera* was collected at Faskari Local Government, Katsina State. The plant was identified

and authenticated at the Biology Department, Umaru Musa Yar'adua University Katsina. The sample was rinsed thoroughly with distilled water to remove all dirt and dust, and then rinsed again with distilled water. The leaf was dried under shade at room temperature, and then grinded into powder and stored in an air tight container until extraction.

Preparation of Crude Extracts

150g of the powdered plant material was immersed in a stoppered container containing 525mL of ethanol giving a solid-to-solvent ratio of 1:3.5 (w/v) and keep at room temperature with occasional shaking for two weeks. The solutions was filtered using muslin cloth followed by Whatman filter paper, the filtrate was concentrated using rotary evaporator and stored in a container prior to analysis as reported by (Shafeeqa *et al.,* 2022).

Fractionation of crude extracts

The ethanolic extract obtained was fractionated subsequently using solvents of increasing polarities which are; N-hexane, Chloroform, Ethyl acetate, Methanol and Water. A fraction of the extract was partitioned between water and chloroform mixture (50:50). This was shaken for about one hour and allowed to settle for 24 hours in a separating funnel. The water, chloroform and interface fractions were separated in glass beakers and labeled respectively. These fractions were again concentrated using a vacuum rotary evaporator, weighed, labeled and stored in a refrigerator at 40C respectively.

Similarly, a fraction of each of the chloroform soluble extract was partitioned in a mixture of absolute methanol and n-hexane (50:50). Again, the methanol and n-hexane fractions were concentrated using vacuum rotary evaporator, weighed, labeled and stored as above. Finally, each of the water soluble fractions was partitioned between water and ethyl acetate (50:50). The water and ethyl acetate fractions were concentrated using a vacuum rotary evaporator, weighed, labeled and stored as above (Fatope *et al.,* 1993 and Adoum *et al.,* 1997).

Figure 3: Fractionation of the crude extract

Bioassay test for Anti pest Activity of the fractions

The Bioassay assessment of the fractions was carried out at Biology Department, Umaru Musa Yar'adua University Katsina, Nigeria using standard methods as reported by Ahmed *et al.,* (2020).

Incubation of Cowpea weevils (*Callosobruchus maculatus*), Preparation of Extract Stock Concentration and Anti Pest Activity Test

Cowpea weevils (*Callosobruchus maculatus*) were used as the test insects. The insects were cultured in the laboratory at ambient laboratory conditions (28 ± 5°C), feeding on cowpea seeds. Only adult weevils, 3-5 days old were used for the bioassay (Ahmed *et al.,* 2020).

The insecticidal activity of each fraction was evaluated using five different concentrations: 0.5, 1.0, 1.5, 2.0, and 2.5 mg/µL of water. The concentrations were prepared by dissolving the respective fraction in water to the required amount; each concentration was applied to 20g of cowpea seeds placed in separate Petri dishes. The seeds was thoroughly mixed with the solution and allowed to air-dry, after drying, 10 adult cowpea weevils were introduced into each Petri dish. Each treatment including control were performed in triplicate, along with a control where the seeds were treated with water only, the dishes were covered and maintained under laboratory conditions at (28 ± 5°C and 75 ± 10% RH). Mortality of the weevils was recorded after 24, 48, and 72 hours of exposure (Ahmed *et al.,* 2020).

Figure 4: Biological Assay of the Fractions

Isolation of Bioactive Compounds

Thin Layer Chromatography:

Thin layer chromatography studies of the plant extracts was carried out for qualitative determination of compounds in the extracts and the best solvent system for column chromatography. The spotted plate was placed in the appropriate solvents using a gradient elution method running from non-polar to polar solvent (mobile phase) using silica gel as adsorbent (stationary phase) and the R_f values was determine from the formula as reported by (Gowthama *et al.,* 2020);

$$\text{Retention factor } (R_f) = \frac{\text{distance travelled by the sample (cm)}}{\text{solvent front (cm)}}$$

Compound with the highest R_f value gets eluted first in the column.

Column Chromatography

In column chromatography, the column was packed by adding the slurry containing 25g of fresh silica gel dissolved in the appropriate solvents. The excess solvent that flows through the plugged-in cotton was collected. The silica was allowed to settle by closing the tap and transferring the extract to the solvent layer once, the solvent settles above the silica gel. The column was filed with the solvent until the colored compounds gets eluted through the column. Each fraction was collected separately and label consecutively for further analysis on thin layer chromatography.

Thin Layer Chromatography

The collected fractions were placed as a spot on TLC plates to visualize compounds contained in each fraction, fractions that contain similar profile of compounds were combined, excess solvent was removed and the bioactivity of the fractions was tested. Fraction with the highest bioactivity was purified using column chromatography.

Characterization of Pure Compounds

Fourier Transform infrared spectroscopy (FT-IR): The IR spectroscopy was used to confirm any functional group present in the isolated compounds.

Gas chromatography- Mass spectroscopy (GC-MS): GC-MS was employed to determine the molecular formula of the compounds and their full identity.

Phytochemical Screening

Phytochemical Constituents of *Euphorbia balsamifera* Leaf

Table 1 showed the phytochemical screening of *Euphorbia balsamifera* leaf powders. It revealed the presence of a wide range of phytochemical constituents including steroids, flavonoids, alkaloids, saponins, and anthraquinones. This could be the reason for their wide range of biological activities.

Table 1: Phytochemical composition of *Euphorbia balsamifera* leaf extract

Phytochemical Composition	***Euphorbia Balsamifera***
Anthraquinone	+
Alkaloids	+
Balsams	+
Cardiac glycosides	_
Flavonoids	+
Saponins	+
Steroids	+
Tannin	+
Terpernoids	_
Volatile Oil	_

Key: + Present; - Absent

Bioassay for *Euphorbia Balsamifera* Leave Fractions

Euphorbia Balsamifera

Table 2: Ethanolic Fraction (F001) Percentage Mortality and Probit Value

Conc(mg/µL)	Log_{10}(concentration)	%mortality	Probit
0.5	-0.301029996	27	4.39
1.0	0	37	4.67
1.5	0.176091259	50	5.00
2.0	0.301029996	60	5.25
2.5	0.397940009	70	5.52

LC50 = 1.37mg/µL ; LC90 = 8.70mg/µL

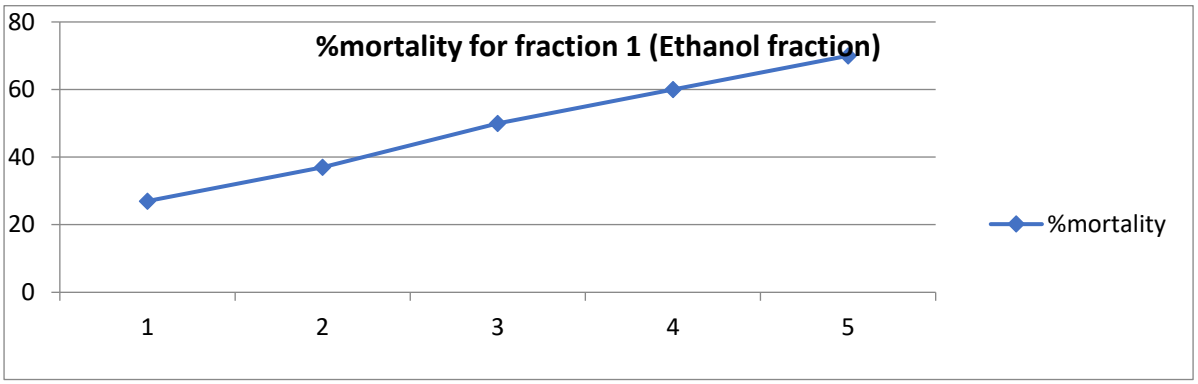

Figure 5: Plot of %mortality against concentration for Ethanol fraction

Table 3: Chloroform Fraction (F002) %Mortality and Probit Value

Conc(mg/µL)	Log_{10}(concentration)	%mortality	Probit
0.5	-0.301029996	15	3.96
1.0	0	27	4.39
1.5	0.176091259	47	4.92
2.0	0.301029996	47	4.92
2.5	0.397940009	50	5.25

LC50 = 1.93 mg/µL LC90 = 9.80 mg/µL

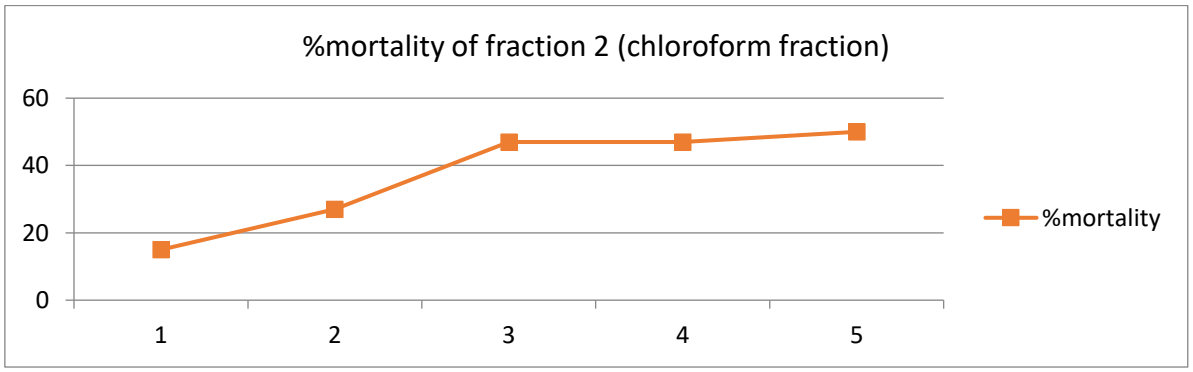

Figure 6: Plot of %mortality against concentration for chloroform fraction

Table 4: Aqueous Fraction (F003) %Mortality and Probit Value

Conc (mg/µL)	Log_{10}(concentration)	%mortality	Probit
0.5	-0.301029996	5	3.36
1.0	0	10	3.72
1.5	0.176091259	20	4.16
2.0	0.301029996	20	4.16
2.5	0.397940009	30	4.48

LC50 = 5.93 mg/µL LC90 = 39.50 mg/µL

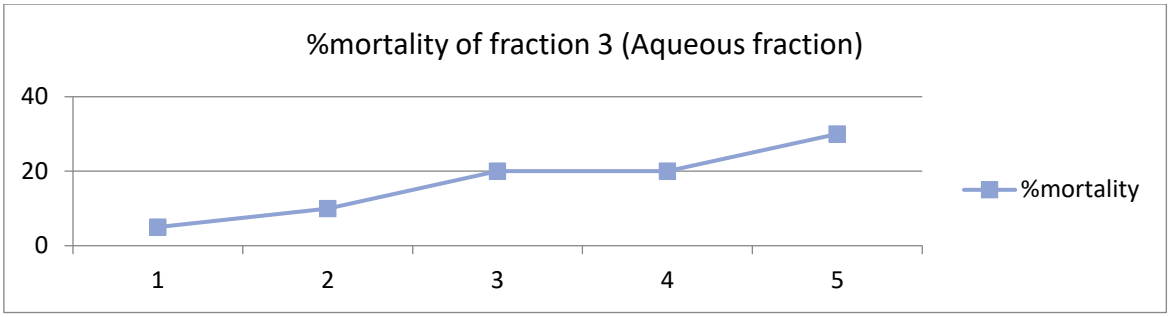

Figure 7: Plot of %mortality against concentration for aqueous fraction

Table 5 Ethyl Acetate Fraction (F004) %Mortality and Probit Value

Conc (mg/µL)	Log_{10}(concentration)	%mortality	Probit
0.5	-0.301029996	10	3.72
1.0	0	17	4.05
1.5	0.176091259	27	4.39
2.0	0.301029996	30	3.48
2.5	0.397940009	40	4.75

LC50 = 17.30 mg/µL LC90 = 627.24mg/µL

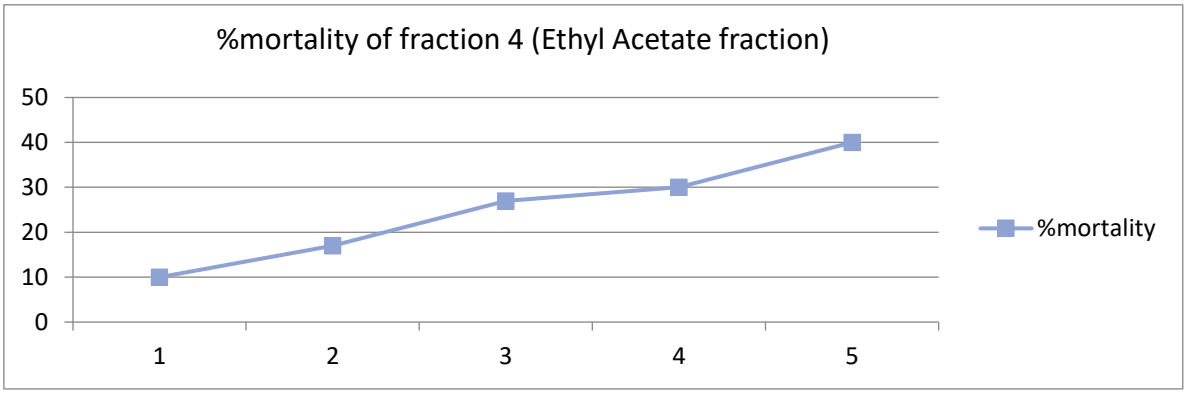

Figure 8: Plot of %mortality against concentration for Ethylacetate fraction

Table 6: Methanol Fraction (F005) %Mortality and Probit Value

Conc(mg/µL)	Log_{10}(concentration)	%mortality	Probit
0.5	-0.301029996	20	4.16
1.0	0	30	4.48
1.5	0.176091259	47	4.92
2.0	0.301029996	60	5.25
2.5	0.397940009	67	5.44

LC50 = 1.56 mg/µL LC90 = 7.45 mg/µL

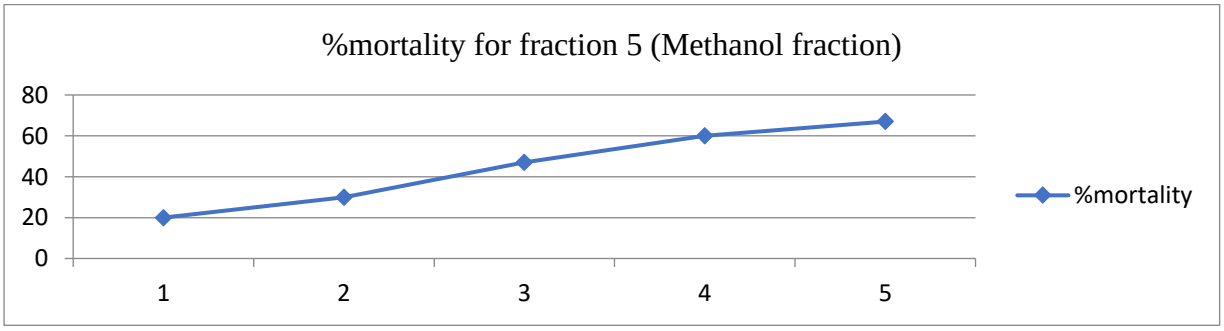

Figure 9: Plot of %mortality against concentration for methanol fraction

Table 7: N-Hexane Fraction (F006) %Mortality and Probit Value

Conc(mg/µL)	Log_{10}(concentration)	%mortality	Probit
0.5	-0.301029996	40	4.75
1.0	0	53	5.08
1.5	0.176091259	70	5.52
2.0	0.301029996	80	5.84
2.5	0.397940009	87	6.13

LC50 = 0.76 mg/µL LC90 = 3.37 mg/µL

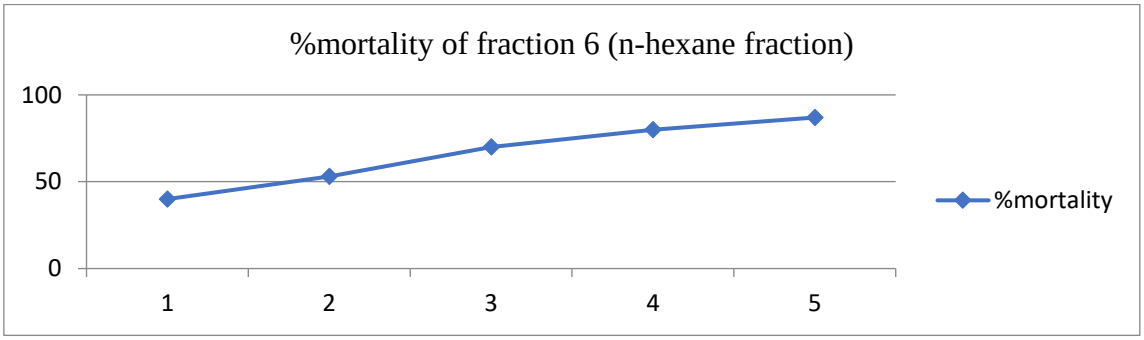

Figure 10: Plot of %mortality against concentration for n-hexane fraction

Thin Layer Chromatography Crude Extract

The most active fraction was subjected to thin layer chromatography (TLC). TLC was performed using n-hexane: ethyl acetate (9:1) as mobile phase. The result showed a spot at R_f of 0.42

Figure 11: The T.L.C profile of n-hexane under UV lamp

Furrier Transformation Infrared Spectroscopy (FT-IR) Result

FT-IR analysis was performed on *Euphorbia Balsamifera* leaf extract to identify the functional groups responsible for its potential bioactivity. Understanding these functional groups provides insight into the chemical composition of the extract, which is crucial for evaluating its pesticidal properties. The results of this analysis serve as a foundation for further phytochemical and biological studies.

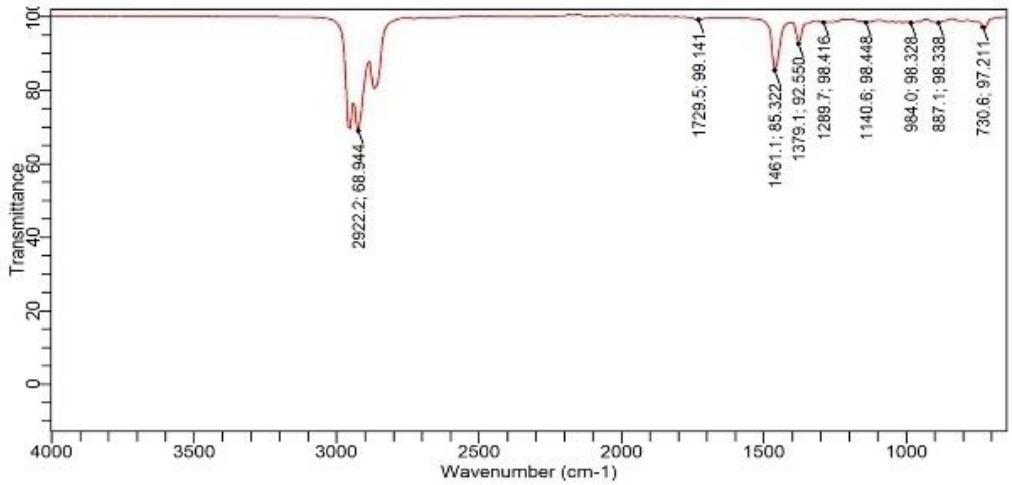

Figure 12: FT-IR Spectra of the n-hexane fraction of *Euphorbia Balsamifera* showing key functional groups

Table 8: FT-IR Absorption peaks and functional Groups Assignments for the N-Hexane Fraction of *Euphorbia Balsamifera*

S/N	Frequency (cm^{-1})	Bond type	Functional group
	2922.23286	C-H stretch	Aliphatic compounds (alkanes)
	1729.48476	C=O stretch	Carbonyl groups (ketones, aldehydes, carboxylic acids, esters)
	1461.12	CH_2 bending	Alkanes
	1379.12	C-H bending	Alkanes, methyl groups
	1289.66	C-N stretch	Amine, amides
	1140.57	-C-O stretch	Alcohols, ethers or esters
	984.02	-C=C bending	Alkenes
	887.11	C-H bending	Alkenes, aromatic compounds
	730.56	C-H bending	Aromatic compounds, alkenes derivatives

FT-IR Analysis of *Euphorbia Balsamifera* Leaf Extract Revealed the Presence of Various Bioactive Functional Groups, as Shown In Table 8

Fourier-transform infrared (FT-IR) spectroscopy was employed to identify the functional groups present in the n-hexane fraction crude extract of *Euphorbia Balsamifera* leaves. The results revealed the presence of various bioactive compounds based on the observed absorption frequencies and their corresponding bond assignments as shown in figure 12

The presence of a strong absorption band at 2922.23 cm^{-1}, attributed to C-H stretching vibrations, confirms the presence of aliphatic compounds, likely long-chain hydrocarbons, fatty acids, or terpenoids. These compounds are known to contribute to plant defense mechanisms, providing protective barriers against pests and microbial attacks. In addition, CH_2 bending (1461.12 cm^{-1}) and CH_3 bending (1379.12 cm^{-1}) further support the presence of alkane structures which are commonly found in plant-derived oils and waxes.

A significant absorption peak at 1729.48 cm^{-1}, corresponding to C=O stretching vibrations, suggests the presence of carbonyl-containing compounds, such as ketones, aldehydes, esters, and carboxylic acids. These functional groups are commonly found in bioactive secondary metabolites with antimicrobial and insecticidal properties.

The peak observed at 1140.57 cm^{-1}, associated with C-O stretching, indicates the presence of alcohols, ethers, or esters, which are structural components of flavonoids, tannins, and glycosides. These classes of compounds have been widely reported for their antioxidant, antimicrobial, and insecticidal activities.

The identification of a C-N stretching band at 1289.66 cm^{-1} suggests the presence of amines and amides, which are commonly found in alkaloids. Alkaloids are nitrogen-containing organic compounds known for their potent biological activities, including toxicity to insects.

The detection of C=C bending vibrations at 984.02 cm^{-1} indicates the presence of alkenes, which are characteristic of terpenoids and essential oils. Additionally, the peaks at 887.11 cm^{-1} and 730.56 cm^{-1}, associated with C-H bending vibrations, confirm the presence of aromatic compounds and substituted alkenes. These findings suggest that *Euphorbia Balsamifera* extract of n-hexane fraction contains phenolic compounds, flavonoids, and tannins, which have been widely studied for their pesticidal and antimicrobial properties.

Overall, the FT-IR analysis of *Euphorbia Balsamifera* leaf extract provides strong evidence of the presence of multiple bioactive functional groups, including alkanes, carbonyls, alcohols, ethers, amines, and aromatic compounds. The combination of these chemical constituents suggests that the plant extract possesses potential insecticidal and pesticidal properties, supporting its use in sustainable pest control strategies. These findings align with previous studies that highlight the efficacy of plant-derived secondary metabolites in pest management. The n-hexane fraction of *euphorbia balsamifera* exhibited an LC_{50} of 0.76mg/µL against *Callosobruchus maculatus*, indicating substantial activity. In comparison, Murugesan *et al.,* (2021) reported and LC_{50} of 0.000393mg/µL for the ethyl acetate leaf extract *solanum torvum* against the same pest. This comparison underscores the potential of *E. balsamifera* as a mutual pesticide, while also highlighting the variability in the efficacy among different plant extracts.

GC-MS Analysis

GC-MS analysis of isolated n-hexane fraction of leave of *Euphorbia balsamifera* plant was carried out using (G89ON Modern). Peak identification was carried out by comparism of the mass spectral obtained with mass spectral data available on NIST version shown in Table 9 below.

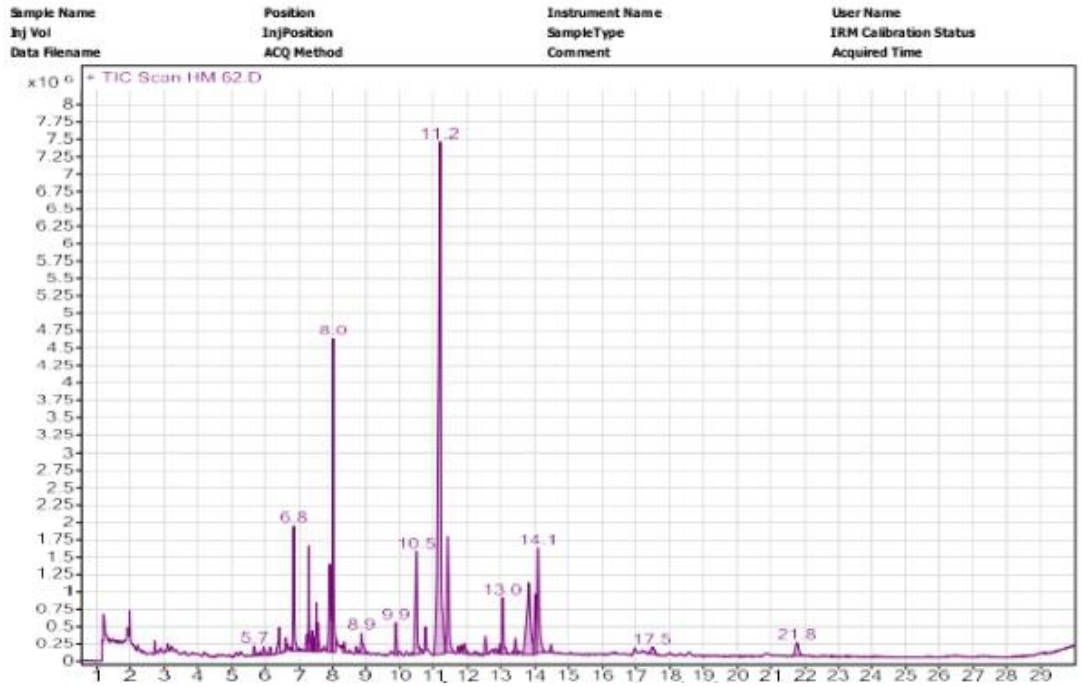

Figure 13: Phytocompounds Identified From GC-MS Result

Table 9: GC-MS Analysis Results

peak	R. Time	Area%	Base peak	Molecular weight	Compound
1	5.7	1.15	150	192	4-Acetoxy-3-methoxystyrene
2	5.9	1.47	55	150	DL-Arabinose
3	6.1	1.05	131	170	(R)-(+)-Citronellic acid
4	6.4	3.09	151	205	DL-α-Lipoamide
5	6.6	1.11	55	230	Bis(1-hydroxycyclohexyl) peroxide
6	6.8	11.45	108	216	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-
7	7.2	2.32	55	172	Cyclooctane, (methoxymethoxy)
8	7.3	7.3	143	230	Diethyl suberate
9	7.4	1.52	55	78	2-Mercaptoethanol
10	7.4	1.32	55	192.5	10-Chloro-1-decanol
11	7.5	4.28	55	156	(Z)-4-Decen-1-ol
12	7.6	2.71	107	205	DL-α-Lipoamide
13	7.9	12.95	152	188	Azelaic acid
14	8	25.2	152	188	Azelaic acid
15	8.3	1.31	55	213	1-Hexyl-2-nitrocyclohexane
16	8.7	1.15	55	192.5	10-Chloro-1-decanol
17	8.9	3.51	55	192.5	10-Chloro-1-decanol
18	9.9	3.99	149	278	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
19	10.5	16.2	149	334	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
20	10.7	2.42	149	306	Phthalic acid, hex-3-yl isobutyl ester
21	11.2	100	149	278	Dibutyl phthalate
22	11.4	18.93	149	362	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester
23	11.7	1.04	149	150	DL-Arabinose
24	11.9	2	149	192.5	10-Chloro-1-decanol
25	12.2	1.04	149	238	7-Hexadecenal, (Z)
26	12.5	2.68	149	304	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester
27	12.8	1.11	149	192.5	10-Chloro-1-decanol
28	13	1.08	55	236	cis,cis-7,10,-Hexadecadienal
29	13	7.74	55	213	1-Hexyl-2-nitrocyclohexane

30	13.4	1.88	74	256	Tetradecanoic acid, 12-methyl-, methyl ester
31	13.8	20.65	55	213	1-Hexyl-2-nitrocyclohexane
32	14	8.68	67	236	cis,cis-7,10,-Hexadecadienal
33	14.1	18.07	55	213	1-Hexyl-2-nitrocyclohexane
34	14.5	1.03	88	238	7-Hexadecenal, (Z)
35	17.5	1.97	55	213	1-Hexyl-2-nitrocyclohexane
36	21.8	3.5	149	390	Diisooctyl phthalate

Gas chromatography-mass spectrometry (GC-MS) analysis was used to perform additional characterization. According to the GC-MS analysis results, *E. balsamifera* is composed of 36 different components. The primary compound is Azelaic acid, which has a retention time of 8 minutes and a percent area of 25.2%. The secondary compound is 1-Hexyl-2-nitrocyclohexane, which has a retention time of 13.8 minutes and a percent area of 20.65%

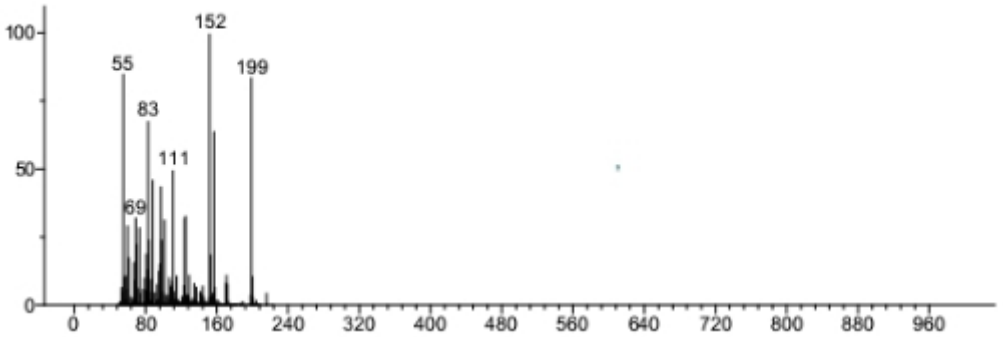

Figure 14: GC-MS spectrum of Azelaic acid

The mass spectral analysis of Azelaic acid (C9H16O4) reveals distinct fragment ions at m/z 55, 69, 83, 111, 152, and 199, corresponding to specific cleavage pathways. Below is the stepwise fragmentation process for this compound.

The molecular ion peak (M^+) is observed at m/z 188, confirming the molecular weight of Azelaic acid, the peak at 152 m/z results from the loss of CO_2 (-44 Da) due to decarboxylation, a typical fragmentation pattern of dicarboxylic acids, the peak at 111 m/z suggests the removal of the second -CO_2 group, forming a stable hydrocarbon ion, the peaks at 83, 69, and 55 m/z correspond to the stepwise breakdown of the alkyl chain, forming smaller hydrocarbon fragments. The GC-MS fragmentation pattern of Azelaic acid exhibits characteristic peaks at m/z 188, 152, 111, 83, 69, and 55, which arise from sequential decarboxylation and alkyl cleavage. The loss of two carboxyl groups (-CO_2) at m/z 152 and 111 is a key marker of dicarboxylic acids, confirming the structural identity of azelaic acid.

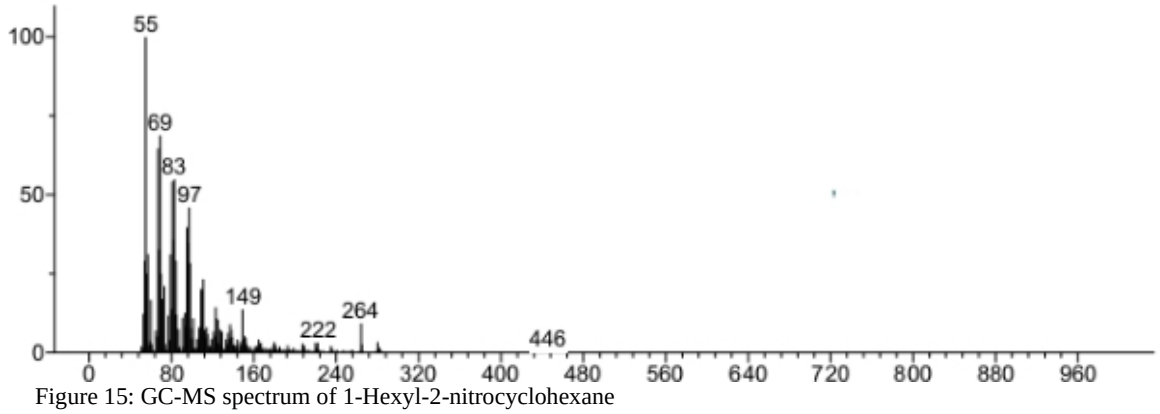

Figure 15: GC-MS spectrum of 1-Hexyl-2-nitrocyclohexane

The mass spectral analysis of 1-Hexyl-2-Nitrocyclohexane (C12H23NO2) reveals distinct fragment ions at m/z 55, 69, 83, 97, 149, 222, 264, which correspond to various cleavage pathways. Below is the stepwise fragmentation process for this compound.

The molecular ion peak (M^+) is observed at m/z 225, confirming the molecular weight of 1-Hexyl-2-Nitrocyclohexane, the peak at 179 m/z indicates the loss of the NO_2 functional group, a characteristic feature of nitroalkanes, the peak at 149 m/z results from the fragmentation of the cyclohexane ring, leading to a stable ion, the peaks at 97, 83, 69, and 55 m/z represent progressive cleavage of alkyl chains, forming stable hydrocarbon fragments. The fragmentation pattern of 1-Hexyl-2-Nitrocyclohexane in GC-MS confirms its structural stability through key fragment ions at m/z 225, 179, 149, 97, 83, 69, and 55. These peaks arise from stepwise loss of the nitro group (-NO_2), cyclohexyl cleavage, and alkyl chain breakdown, leading to a distinct mass spectral profile.

CONCLUSION

The results of this study confirm that *Euphorbia balsamifera* leaf extracts possess strong insecticidal activity against *Callosobruchus maculatus*. The presence of diverse phytochemicals, along with the identification of potent compounds like azelaic acid, supports the plant's traditional use in pest control and its potential as an eco-friendly biopesticide.
The n-hexane fraction exhibited the highest efficacy, suggesting that non-polar compounds play a key role in its pesticidal action. This discovery also provides a scientific foundation for developing plant-based pest control solutions that could reduce reliance on harmful synthetic chemicals.

RECOMMENDATIONS

To build on these findings, the following steps are recommended:

i. Further Isolation and Characterization: Future studies should isolate and structurally elucidate additional bioactive compounds from the most effective fractions (n-hexane and methanol) using advanced techniques like HPLC and 2D NMR.
ii. Formulation Development: Research should focus on developing stable and practical formulations (e.g., sprays or powders) for real-world storage applications, ensuring ease of use and long-term effectiveness.
iii. Toxicity and Safety Studies: Before large scale use, the extracts should be evaluated for toxicity on non-target organisms, including humans, beneficial insects, and the environment.
iv. Field Testing: Large scale: trials under different storage conditions (temperature, humidity) are necessary to assess the extracts' effectiveness in actual agricultural settings.
v. Comparative Studies: The efficacy of *Euphorbia balsamifera* extracts should be compared with conventional synthetic pesticides to determine their competitiveness in terms of cost, efficiency, and environmental impact.
vi. Synergistic Combinations: Exploring mixtures with other plant-derived pesticides could enhance potency and broaden their pest control spectrum, potentially reducing the required dosage and delaying pest resistance.

By pursuing these recommendations, *Euphorbia balsamifera* could be developed into a viable, sustainable pest management solution, benefiting farmers and reducing the ecological footprint of grain storage practices.

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