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Original Research Article

Insecticidal Efficacy and Chemical Composition of hexane Oil extracts From the Leaves of *Euphorbia milii* and *Cassia occidentalis*

Abstract

The efficacy of [illegible] of essential oil[illegible] [illegible]*ntalis* and *Euphorbia milii* leaves on selected i[illegible] [illegible]e essent[illegible] [illegible]soxhlet apparatus with hexane, and test inse[illegible] [illegible]at 3 dose [illegible].6g) for 24 hours. The results showed co[illegible] [illegible]tivity of *E. mi*[illegible] [illegible]d LD_{90}s as 0.583 and 1.108 for ~~*P. americana*~~, 0.681 and 1.215 for ~~*T.virridisima*~~ and 0.488 and 0.893 for *Anopheles gambaie,* respectively. The *C. occidentalis* oils showed insecticidal activity of 0.889 and 1.689 for *P. americana*, 1.013 and 1.973 for *T. virrdissima*, and 0.722 and 1.847 for *Anopheles gambaiae,* respectively. The corresponding values for the conventional insecticide, SWAN were 0.417 and 1.017 for *P. americana,* 0.607 and 1.111 for *T. virridissima* and 0.40 and 0.743 for *A. gambaiae,* respectively.The GC-MS analysis of the essential oils revealed the presence of hexadecanoic acid,2 hydroxy-1-(hydroxymethyl) ethyl ester , 6, 9,12,15-docosatetraenic acid methyl ester for *E.milii* and Phthalic acid isobutyl octyl ester, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester , 9-octadecenoic acid (z)-methyl ester, methyl stearate, phthalic acid,2-ethylhexyl isohexyl ester, decane, oleic acid, 4,7,-methano-1h-indene,3a,4,7,7a-tetrahydro and n-hexadecanoic acid for *C. occidentalis.* Overall, data from this study provides strong evidence that these oils possess bioactive secondary metabolites with commendable degrees of insecticidal efficacy.

Keywords: Insecticidal, Efficacy, Chemical, Composition, hexane, extracts

Author Contributions: Conceptualization, Obioma Ohaeri; Data curation, Chibuzor Okonkwo; Formal analysis, Chibuzor Okonkwo; Investigation, Chibuzor Okonkwo; Methodology, Chibuzor Okonkwo; Resources, Chibuzor Okonkwo; Supervision, Obioma Ohaeri; Validation, Obioma Ohaeri; Writing – original draft, Chibuzor Okonkwo; Writing – review & editing, Obioma Ohaeri

Introduction

Insects are chief competitors for food and fibre resources throughout the world. Insecticides are essential tools for preventing/minimizing insect damage to and significantly increasing the quality and quantity of

UNDER PEER REVIEW

crops, and for improving the quality of life for humans and livestock. A public call for sustainability in agriculture has resulted in numerous government initiatives to develop environmentally friendly agricultural practices [1]. Many secondary plant metabolites are known for their insecticidal properties, and in many cases plants have a history of use as home remedies to kill or repel insects [2].Plants play pivotal roles in ecological systems as control agents because they constitute a rich source of bioactive chemicals [3]. The discovery of plant secondary metabolites which are either toxins or repellents to herbivores that feed on them opened the vista for their assessment as insecticides. These secondary compounds represent a large reservoir of chemical structures with biological activity [4]. The term "natural" conveys a sense of wholesomeness or safety. Natural products, including natural insecticides can generally be described as chemical, mineral or biological compounds or substances produced by living organisms. These products vary in their toxicity to non- target organisms as well as in their effectiveness in controlling specific insect pests. Essential oils are among the best known natural substances tested against insects.

A large percentage of sprayed insecticides and herbicides are known to reach a destination other than their target species, simply because they are sprayed across an entire agricultural field or household [5]. Again**,** flowing water can carry pesticides into aquatic environments while wind can carry them to other fields potentially affecting other species. Other avenues of non-target contact emerge from poor production, transport and storage practices [6].Since the synthetic pesticides used in the control of insect pests such as organophosphate pesticides and organochlorine insecticides have been associated with various forms of cancer, neurological disorders and lung irritations in humans [7], there is an urgent need to develop safe alternatives that are cost effective, convenient to use and environmentally friendly [8].

Over time, repeated application of a particular insecticide, increases pest resistance and can facilitate the pest's resurgence [9].There is therefore need for a potent but safer and cost-effective alternative to the existing conventional pesticides, particularly in the developing countries where there are inadequate occupational safety standards,insufficient enforcement, poor labelling of pesticides, illiteracy and insufficient knowledge of pesticide hazards [10].

Euphorbia milii and *Cassia occidentalis* are candidate plants whose empirical observation/evidence strongly suggests that they possess chemical compounds possibly oils, with insecticidal properties. Whereas*E.mi* shrub with very thorny grooved stems and branches; *C.occiden* ows 5-8m high and is found in many tropical areas. It is also known *Akidiogbara* in Igbo, *Dora rai* in Hausa and *Aboorere* in Yoruba [11]; it is claimed to scare away insects and reptiles from its environs by indigenes of Akwa Ibom State in Nigeria, thus making it a popular domestic plant in that part of the world.

[1]. It is claimed to repel insects

UNDER PEER REVIEW

Another plant of interest in the current study is *Euphorbia milii*, not much has been reported about its insecticidal activity despite the empirical evidence of its insect repellent activity. On close and repeated observation, the leaves of *Euphorbia milii* are usually not affected by insect pest attacks even when other plants around it suffer serious attacks from these pests. The plant is known by the common names; crown of thorns, Christ plant and/or red shrub and is not indigenous to Nigeria, but is believed to have been imported to Nigeria from India [12].There are claims by inhabitants of Okwuta in Ibeku Umuahia Abia State Nigeria that the plant may be linked with insecticidal tendencies. No study however has investigated the insecticidal activity or the compounds responsible for the observed effects. This underscores the need for the current investigation.

The American cockroach (*Periplanetaamericana*), is one of the largest species of the common cockroaches, and often considered a pest. They were i[illegible] the United States from Africa as early as 1625 [13]. They are reddish brown and ha[illegible] the body region behind the head. The American cockroach, which is one o[illegible]g insects [14] has a pair of large compound eyes, and is a very active night insect that shuns light. The odorous secretions produced by it can alter the flavour of food [15]. They can also pick up disease-causing bacteria, such as *Salmonella*, on their legs and later deposit them on foods and cause food infections or poisoning. House dust containing cockroach faeces and body parts can trigger allergic reactions and asthma in certain individuals [16].

nocturnal

Mosquitoes are small, midge-like flies which belong to the family *culicidae.* The females of most species are ecto-parasites, whose tube-like mouth parts (called a probocis) pierce the host's skin to suck blood. They are holometabolous insects and therefore grow through an egg, larva, pupae to adult stage. The larvae and pupae are aquatic, whereas the adults are free flying [17]. Mosquitoes thrive in environments with poor drainage system especially during rainy seasons, for example; fish ponds, irrigation ditches and lowland rice fields. They are the most indisputable medically significant arthropoddisease vectors. The vector-borne diseases caused by mosquitoes pose a major health problem in most countries. It affects the socio economic status of many nations and is an important pest against human; it transmits parasites and pathogens which continue to have disadvantageous impact on human beings. Diseases like filariasis, dengue fever, yellow fever, malaria, Japanese encephalitis and *chikungunya* are some of the deadly diseases spread by mosquitoes [18].

Bush cricket are herbivorous orthopterous insects with hind legs adapted for leaping and having chewing mouth parts. They are insects of the order *orthoptera*, sub-order *caelitera,* they are sometimes referred to as short-horned grasshoppers to distinguish them from the katydid (bush crickets) which have much longer antennae. They are typically ground-dwelling insects with powerful hind legs which enable them to escape from threats by leaping vigorously. They do not undergo complete metamorphosis, so

UNDER PEER REVIEW

they hatch from egg into a nymph or hopper which undergoes five molts to become an adult [19]. Grasshoppers are plant eaters, sometimes becoming serious pests of cereals, vegetables and pastures, especially when they swarm in their millions as locusts and destroy crops over wide areas. They eat large quantities of foliage both as adults and during their development. They protect themselves from predators by camouflage; when detected, many species attempt to startle the predator with a brilliantly-coloured wing-flash while jumping and if adult, launching into the air, usually flying for only a short distance [20].

This research was aimed at determining the insecticidal actions of oil extracts from the leaves of *Euphorbia milii* and *Cassia occidentalis* using the insect pests described above, in order to ascertain their level of effectiveness/potency as compared to the commonly used synthetic ones, as well as investigate their chemical composition in order to determine the chemical compounds likely responsible for its insecticidal actions.

2. Materials and Methods

The major instruments used in this research include; Soxhlet extractor Manufactured by B.BRAN Scientific and Instrument Company England, Thermo Scientific Rotary evaporator Model R-300 USA, Gas chromatography-Mass spectrometry analyser (GCMS-QP2010 PLUSSHIMADZU, JAPAN), Electric blender, AKAI TOKYO JAPAN, Model No: BDOO11DA-1033M, made in PRC. Echotherm Chilling/Heating dry bath. ~~(Towey Pines Scientific, Gas chromatography-Mass spectrometry analyser (GCMS-QP2010 PLUSSHIMADZU, JAPAN).~~ Weighing balance (Symmetry Colle-Parmer Instrument Co, USA.

plant names need to be italicised

2.1. Collection and Identification of Plant Samples

The leaves of five plants including; Euphorbia milii, Cassia occidentalis, Acanthus montanus, Erythrophleumafricana and Ipomeaasarifolia were initially screened for insecticidal activities. *Euphorbia milii*, *Cassia occidentalis* and *Acanthus montanus* were harvested from GPS mobile location Latitude=4.961538, Longitude= 8.349273, No 4 Edim Otop close, off victory way, Satellite town Calabar, Cross Rivers State, Nigeria on the 18th of October 2015 while *Erythrophleum africana* and *Ipomea asarifolia* were harvested at Latitude; 5.522745, Longitude; 7.527940, Okwuta village, off Ehimmiri Housing Estate [illegible] ia State on the 16th of October 2015 [illegible] eared healthy, leaves bright green in col[illegible] vers intact at the time of the harvest. T[illegible] and fifty grams (250g) of each plant was used for the screening.Out of the five plants, E.milli and *C.occidentalis* showed the highest insecticidal efficacy and were thus used for further studies. Prior to screening, the plants

were

italics

UNDER PEER REVIEW

were identified by a botanist in the Department of Biological Sciences (Botany), College of Natural Sciences, Michael Okpara University of Agriculture Umudike Abia State.

2.2. Test Insects

Test insects were identified by a Zoologist in the the Department of Zoology and Environmental Biology, Faculty of sciences, University of Calabar. All insects used were of adult stage (except for mosquito), this is because they exhibit the greatest destructive and infectious tendencies at this stage. Insects were kept very active as at the time of the experiment, no symptoms of any disease or weakness was observed. Their response to environmental factors and stimuli, movement and general behaviour indicated that they were physiologically sound at the time of the procedure. The adult cockroach is recognised by its reddish brown colour with a pale brown or yellowish band around the edge of the pronotum, they also have a pair of slender jointed cerci at the tip of the abdomen. *T.virridissima* was distinguished by its very long and thin antennae which can reach up to three (3) times the length of the body differentiating it from grasshoppers which carry short antennae. Mosquito larvae are green while Imago are pale green to brown with a thin brown longitudinal stripe on their back, wings are well developed [21].

2.3. Treatment of Test Insects

Periplaneta Americana (American Cockroach) was trapped from a domestic sewage pit in a plastic container perforated at the base and baited with some food particles.*Tettigoniaviridissima*, (Green Bush Cricket) was caught from the grass fields in the University of Calabar staff quaters, and*Anopheles gambaiae* (African Malaria Mosquito) were trapped in plastic basins containing stagnant water in the University of Calabar, the insects were provided with commercial and domestic household food and green grass for *T.virridissima*throughout the period of the experiment. SWAN all-purpose commercial insecticide was purchased in Calabar, Cross Rivers State.

2.4. *Preparation of plant oil*

The leaves of *E.milii* and *Cassia occidentalis* harvested from open field in Etta-Agbor, Calabar were carefully selected, washed with clean water and air-dried for a period of three weeks. The dried leaves were pulverized using an electric blender, put in an air-tight container and used subsequently for soxhlet oil extraction. Oils from the leaves of *Euphorbia milii* and *Cassia occidentalis* were extracted by continuous extraction in soxhlet apparatus for sixteen hours using n-hexane as solvent according to the method described by A.O.A.C [22]. Fifty grammes (50g) of the ground plant samples were weighed separately and loaded into a thimble placed inside the soxhlet extractor, about 600mls of n-hexane was added to a round bottom flask which was attached to the soxhlet extractor and condenser. The side arm

UNDER PEER REVIEW

was lagged with glass wool. The solvent was heated using an isomantle 70 C and began to evaporate moving through the apparatus to the condenser. The condensate was collected in the reservoir containing the thimble. Once the level of solvent reached the siphon it poured back into the flask and the cycle continued lasting for about 16hours. After many cycles the desired compound was concentrated in the distillation flask and solvent was removed using a rotary evaporator. The non-soluble portion of the extracted solid remained in the thimble, and was discarded. [illegible]d oil was weighed an[illegible] weight used to calculate the percentage oil yield. For *E.milii* w[illegible]ur grams (4g) of oil [illegible] 3.4 from fifty grams (50g) of gr[illegible]esulting to 8% oil yield, and for *C.occident*[illegible] realized five grams (5g) of oil [illegible]fty grams (50g) of powder amounting to 10% oil yield. This procedure was done twice.

2.5. Gas Chromatography-Mass spectrometry (GC-MS) analysis of Soxhlet extracted oils.

The extracted oils were analysed separately, one after another by subjecting them to chemical profiling via GC-MS technique as described by Sahilk *et al.* [23]. The analysis was performed using a QP2010 PLUS GC-MS SHIMADZU JAPAN equipped with GC-2010 capillary column with Viscosity Comp Time: 0.2 sec, Pumping time: 5sec, Injection. Port Dwell Time:0.3 sec, Washing volume:8uL,Column oven temperature:80.0°C, Injection Temp: 250.00°C, Flow control Mode: Linear velocity, Pressure:108.0KPa, Total flow:6.2mL/min, Column flow;1.58mL/min, Linear velocity:46.3cm/sec, Purge Flow:3.0mL/min, Split Ratio:1.0.The oven temperature was set between 80.0°C to 280.0°C, Hold time between 1.00-5.00 min at a rate of 10.00.The equilibrium time was 3.0 min, Ion source temperature:230.00°C, Interface temp:250.00°C, Solvent Cut Time: 250 mins,Threshold:1000, Start time:3.00 min, End time:28.00 min, Event Time:0.50 sec, Scan speed:1250,Sample Inlet Unit: GC. The chemical compounds in the oils were identified based on GC retention time on GC-2010 capillary column matched with EI MS library of the NIST/EPA/NIH Mass Spectral Library 2005 [24] as the reference library. The analysis was carried out at the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State.

2.6. Contact insecticidal activity test on insects

Cotton lint soaked with 500mg (0.5g) of each of the oil extracts separately was placed inside a cylindrical, perforated empty tin; the test insects (10 adults in each tin) were placed in the tins and covered with a net to prevent escape of insects and to avoid death by suffocation. The insects were monitored for 24 hours to observe mortality. The control passed through similar treatment but with distilled water only [24]. For the mosquito, the larval stage which is active in water was used. The larvae were trapped in a bowl of stagnant water and the treatment was administered into their habitat and

UNDER PEER REVIEW

monitored for 24 hours to observe mortality. The control test was performed without the oil or SWAN treatment [26]. All experiments were done in triplicates. LD_{50} and LD_{90} of the oils were estimated using regression analysis [27].

2.7. Statistical analysis

The LD_{50} values for the insects were determined using 'Probit Analysis" developed by D.J Finney [28]. The data from bioassays (mortality proportions and corresponding doses) gave an S-shape curve which was made linear by transforming the proportions to probits and doses to log 10. The LD_{50} values were estimated using regression analysis [27].

3. Result

Table 1: Insecticidal effects (mean mortality rate) of plant extracts on test insects.

Treatment	**Mean Mortality Rate (%)**		
Concentration (g)	***P.americana***	***T.viridissima***	***A. gambaiae***
Euphorbia milii (0.4)	76.67 ± 5.77^{e}	66.67 ± 5.77^{d}	70.0 ± 10^{d}
Cassia occidentalis (0.4)	60.00 ± 10^{d}	56.67 ± 5.77^{d}	53.33 ± 5.77^{c}
Acanthus montanus (0.4)	36.67 ± 5.77^{c}	43.33 ± 5.77^{c}	43.33 ± 5.77^{c}
Ipomeasarifolia (0.4)	[illegible]	[illegible]33 $\pm 5.77^{b}$	23.33 ± 5.77^{b}
Erythropleum Africana ([illegible]	[illegible]	[illegible]5.77^{b}	26.67 ± 5.77^{b}
Control (no treatment)	[illegible]	[illegible].77^{a}	3.33 ± 5.77^{a}

std error of the mean? OR std deviation

Values are mean ±SEM of three determinations. Values with the same superscript letters are not statistically significant at 95% confidence level (P<0.05).

There was a significant (P<0.05) increase in the mean mortality rate of *P.americana, T.virridissima* and *A.gambaiae*when treated with *E.milli, C.occidentalis, A.montanus, I.sarifolia* and *E.africana* as compared to their control groups. However *E.milii*and *C.occidentalis* showed the highest significant (P<0.05) increase in mean mortality rate when compared to the control groups.

Table 2: Percentage yield of soxhlet extracted oil from leaves

Plant	**pH**	**Weight of plant powder us**	**Weight equivalent rac oil obtained**	**% yield of oil**
E.milii	3.4	50	4.0	8
C.occidentalis	3.9	50	5.0	10

UNDER PEER REVIEW

For each fifty grammes (50g) of *E.milii* plant powder used for soxhlet extraction, 4.0 grammes (g) equivalent of oil extract was obtained which translated to 8% yield. For *C.occidentalis* plant powder out of fifty grammes used, 5.0 grammes (g) equivalent of oil extract was obtained which translated to 10% oil yield.

Table 3: Chemical composition of bioactive primary metabolites obtained from soxhlet extracted *E.milii* oil as revealed by GC-MS analysis

S/N	Name of compound	Retention time(RT)	Peak area concentrati
1.	Phenol	3.91	1.52
2.	Phenol,2 methyl	5.12	1.49
3.	n-Nonaldehyde	5.55	0.85
4.	Phenol,2,3,dimethyl	6.41	1.07
5.	Azulene	6.82	0.94
6.	2-Prop 2-enoyloxyl tetradecane	7.78	0.88
7.	1-Undecene,8-methyl	8.82	0.78
8.	1-Dodecene	9.50	1.13
9.	Phthalic acid, di-(1-hexen-5yl) ester	12.02	4.85
10.	1-Pentadecene	14.42	0.75
11.	2-Undecanone,6,10,-dimethyl	15.22	1.15
12.	Pentadecanoic acid,14-methyl-,methylester	16.68	5.77
13.	n-Nonadecanoic acid	17.44	1.63
14.	1-Heptadecanol	19.55	0.92
15.	11 Octadecanic acid, methyl ester	19.81	2.24
16.	Octadecanoicacid,methyl ester	20.17	1.28
17.	1,propene-1,2,3-tricarboxylic acid,tributyl ester	20.62	1.15
18.	Tributylacetylcitrare	21.88	3.84
19.	Palmitin,1,2-di-,2-aminoethyl hydrogen phospha	22.30	14.60
20.	4, 8, 12, 16-Tetramethylheptadecan-4-olide.	22.94	2.09
21.	Hexadecanoic acid,2-hydroxy-1-(hydroxymethy ester	24.46	29.46
22.	6,9,12,15-Docosatetraenoic acid, methyl ester	24.61	14.58
23.	Hexadecanoic acid,2-(acetyloxy)-1-[(acetylox l]ethyl ester	25.32	3.76
24.	Hexadecanoic acid,2,3-dihydroxypropyl ester	26.33	3.29

The GC-MS analysis of *E.milii* soxhlet extracted oil revealed twenty-four (24) peaks showing that the oil contained twenty-four (24) compounds. These compounds range from; phenols, acyclic olefins, esters, ketones, carboxylic acids and alcohols. The compounds with the highest concentrations in *E.milii* oil extract include; hexadecanoic acid, 2 hydroxy-1-(hydroxymethyl) ethyl ester**,** 6,9,12,15-Docosatetraenic acid, methyl ester**,** palmitin 1,2-di-2,aminoethyl hydrogen phosphate. Others include; phthalic acid, di-(1-hexen-5y) ester and pentadecanoic acid, 14 methyl-, methyl ester.

UNDER PEER REVIEW

Table 4: Chemical composition of bioactive primary metabolites obtained from soxhlet extracted *C.occidentalis*oil as revealed by GC-MS analysis.

S/N	Name of Compound	Retention time(RT)	Peak area% (concentration).
1.	Ethylbenzene	4.54	1.87
2.	o-Xylene	4.66	5.87
3.	1,3,5,7-Cyclooctatetraene	5.01	6.70
4.	Benzene,1-ethyl-2-methyl	6.03	7.95
5.	Benzene 1,2,4-trimethyl	6.16	2.45
6.	Decane	6.56	15.10
7.	4,7-Methano-1H-indene,3a,4,7,7a-tetrahydro	7.11	13.36
8.	1-Propyne,3-Phenyl	7.41	6.71
9.	Cyclobutane,1,2-bis(1,3-butadienyl).	8.04	1.97
10.	Undecane	8.10	1.82
11.	Bicyclo[2,2,1]hept-2-ene,1-methyl	8.30	1.15
12.	5-Phenylbicyclo[2,2,1]hept-2-ene	12.58	2.74
13.	Z-4-Nonadecen-1-ol acetate	19.42	1.34
14.	Pentadecanoic acid,14-methyl,methyl ester	20.45	0.98
15.	n-Hexadecanoic acid	21.02	11.63
16.	Oleic acid	22.77	14.66
17	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	23.57	2.58
18.	Palmitin1,2-di-,2-aminoethyl hydrogen phosphate	23.94	[illegible]

For *C.occidentalis* oil extract, a total of eighteen (18) peaks [illegible] observed. Among these chemical compounds the ones wi[illegible] Oleic acid**,** 4,7-methano-1H-indene,3a,4,7,7a-tetrahydro [illegible] found in moderate concentrations include; O-xylene**,** 1, 3, [illegible] methyl and 1-propyne, 3 phenyl.

I would have preferred to see this data represented as graphs, especially since these were used to determine the LD50 and LD90.

Table 5: Contact Insecticidal activity of SWAN, *C. oc*[illegible] and *E.mi*[illegible]

E.milii								
P.americana			***T. virridissima***			***A.gambaiae***		
Conc (mg)	Mortality	%Mortality	Conc (mg)	Mortality	%Mortality	Conc (mg)	Mortality	%Mortality
0.2	3	30	0.2	1	10	0.2	3	30
0.2	2	20	0.2	2	20	0.2	3	30
0.2	2	20	0.2	2	20	0.2	4	40
0.4	3	30	0.4	2	20	0.4	4	40
0.4	3	30	0.4	2	20	0.4	5	50
0.4	4	40	0.4	3	30	0.4	5	50
0.6	5	50	0.6	4	40	0.6	6	60
0.6	5	50	0.6	5	50	0.6	7	70
0.6	6	60	0.6	5	50	0.6	7	70
1.24	10	100	1.348	10	100	1.013	10	100

UNDER PEER REVIEW

C.occidentalis								
0.2	2	20	0.2	2	20	0.2	3	30
0.2	2	20	0.2	2	20	0.2	3	30
0.2	1	10	0.2	2	20	0.2	2	20
0.4	2	20	0.4	2	20	0.4	4	40
0.4	2	20	0.4	1	10	0.4	4	40
0.4	3	30	0.4	2	20	0.4	4	40
0.6	4	40	0.6	3	30	0.6	5	50
0.6	4	40	0.6	4	40	0.6	6	60
0.6	3	30	0.6	4	40	0.6	4	40
1.889	10	100	2.21	10	100	1.448	10	100
SWAN								
0.2	4	40	0.2	3	30	0.2	3	30
0.2	3	30	0.2	2	20	0.2	3	30
0.2	4	40	0.2	2	20	0.2	3	30
0.4	5	50	0.4	3	30	0.4	4	40
0.4	5	50	0.4	3	30	0.4	4	40
0.4	4	40	0.4	4	40	0.4	5	50
0.6	6	60	0.6	5	50	0.6	7	70
0.6	6	60	0.6	5	50	0.6	8	80
0.6	7	70	0.6	6	60	0.6	8	80
1.167	10	100		10	100	0.829	10	100
Control								
0.2	0	0	0.2	0	0	0.2	0	0
0.2	0	0	0.2	0	0	0.2	2	20
0.2	1	10	0.2	0	0	0.2	1	10
0.4	0	0	0.4	0	0	0.4	0	0
0.4	0	0	0.4	1	10	0.4	1	10
0.4	0	0	0.4	0	0	0.4	1	10
0.6	0	0	0.6	0	0	0.6	1	10
0.6	0	0	0.6	1	10	0.6	1	10
0.6	1	10	0.6	0	0	0.6	0	0
	10	100		10	100		10	100

Table 6: Lethal effect derived from exposing test insects to 0.2, 0.4 and 0.6g of *E. milii*, *C. occidentalis* and SWAN treatments.

Treatments (0.2,0.4, 0.6g)	***P.americana***		***T.viridissima***		***A.gambaie***	
Lethality	**LD50**	**LD90**	**LD50**	**LD90**	**LD50**	**LD90**
E.milii	0.583	1.108	0.681	1.215	0.488	0.893
C.occidentalis	0.889	1.689	1.013	1.973	0.722	1.847
SWAN	0.417	1.017	0.607	1.111	0.400	0.743

UNDER PEER REVIEW

The result of the contact insecticidal activity of both plant extracts and SWAN showed that both extracts possess commendable insecticidal effects. Unlike SWAN whose active ingredients have been isolated and formulated into an industrially and commercially active form, *E.milii and C.occidentalis* may perform as well or even better if their active components are isolated and formulated into an industrially pure and active form.

4. Discussion

The insecticidal screening test of the selected plants showed that *Euphorbia milii* leaf extract caused a mean mortality rate of 76.67% ±5.77 for *P.americana,* 66.67% ±5.77 and 70.0 % ± 10 for *T.virridissima*and *A.gambaiae*respectively. *Cassia occidentalis* leaf extract caused a mean mortality rate of 60.00% ±10, 56.67% ±5.77 and 53.33% ±5.77 for*P.americana, T.virridisssima* and *A.gambaiae* respectively. These two plants exhibited the strongest insecticidal effects among screened plants. This means that *Euphorbia milii* may be a promising potent insecticidal agent. *C.occidentalis* also showed promising insecticidal potentials as can be seen from the result (Tables 1) followed by *Acanthus montanus* which caused mean mortality rates of 36.67% ±5.77, 43.33%±5.77 and 43.33% ±5.77*for P.americana, T.virridissima and A.gambaiae* respectively. *Ipomeasarifolia* and *Erythropleuafricana had average mean mortality rates of 23.33 %*±5.77 *and 25.56*±10 on the test insects and *thus* did not exhibit much insecticidal properties compared to the other plants. The control recorded an average mean mortality rate of; 5.56±5.77 on test insects.

On this basis, *E.milii* and *C.occidentalis* were used for the rest of the study. Even though there is scarcely any literature on the insecticidal potential of *E.milii*, which provoked this study in the first place, crude leaf extract of *C.occidentalis* has been reported to show growth regulating and adulticidal potential in *Anopheles stephensi* [29].*C.occidentalis*has also been reported to suppress wood damage by termites causing mortality of worker termites within the shortest duration of application [30]. There are also reports that *C.occidentalis*extracts possess significant larvicidal potential against the malaria vector *Anopheles stephensi* [30]. *C.occidentalis* was reported to be the probable cause of hepatomyo-encephalopathy in children in western Uttar Pradesh [32]. No study has however investigated or reported the active principles li

Need to cite literature to back the claim that these compounds have insecticidal properties

The GC-MS analysis of of twenty-four (24) compounds ranging ds and alcohol. The major active compo sible for its insecticidal properties include; hexadecanoic acid, 2 hydroxy-1-(hydroxymethyl) ethyl ester which had a concentration of 29.46%, followed by; 6,9,12,15-Docosatetraenic acid, methyl ester with a concentration of 14.58% and palmitin 1,2-di-2,aminoethyl hydrogen phosphate with a concentration of

UNDER PEER REVIEW

14.60%. Others include; phthalic acid, di-(1-hexen-5y) ester, pentadecanoic acid and 14 methyl-methyl ester which had concentrations of 4.85% and 5.77% respectively. All the compounds listed above are esters derived from fatty acids. The first three compounds which are all derivatives of saturated fatty acid may hold promising potentials as insecticidal agents.

For *C.occidentalis,* the oil extract revealed eighteen (18) chemical compounds. The ones with the highest concentrations include; Decane, Oleic acid, 4,7-methano-1H-indene,3a,4,7,7a-tetrahydro and n-Hexadecanoic acid, which had concentrations of

Need to cite literature to back the claim that these compounds have insecticidal properties

Other compounds found in moderate c

cyclooctatetraene (6.70%), benzene, 1-ethyl-2

The insecticidal activities observed with*E.mi*

of the following predominant components; hexadecanoic acid 2 hydroxy-1-(hydroxymethyl) ethyl ester**,**palmitin 1,2-di-2 aminoethyl hydrogen phosphate and pthalic acid. Pthalic acid and 6,9,12,15-Docosatetraenic acid, methyl ester were absent in *C.occidentalis* oil extract but present in *E.milii* oil extract, thus the oils may exert their insecticidal activities through different unique routes. Also, the mechanism of action of *E.milii* oil extract may be largely different from that of *C.occidentalis*.According to Dalia [33] hexadecanoic acid an

et al.

yl ester was instrumental to the insecticidal activity exhibited by *Casimiroa edulis La Llav*

(Rutaceae) leaf extract and its fractions against *Spodopteralitteralis* larvae. Oladipupo*et al* [34] also observed that the presence of hexadecanoic acid and n-hexadecanoic acid methyl ester in significant concentrations in the leaf extracts of *Chromolaenaodorata* was accountable for the insecticidal action of the leaf towards adults of *S.zeamays*. Hexadecanoc acid and its dervatives was observed to be abundantly present in both plant extracts, this may account for some similarities in their mechanism of action.

Pthalic acid (PA); another major constituent of *E.milii* oil extract and its esters (1, 2-benzene dicarboxylic acid) may also be partly responsible for the insecticidal efficacy of the plant. P.A has been reported to exhibit insecticidal activity against *G.mellonella* in a dose-dependent manner. Also, one hundred percent (100%) insect mortality was observed at 108hrs after injection of 1M Pthalic acid [35] Since pthalic acid and 6,9,12,15-Docosatetraenic acid, methyl ester are major chemical components contained in *E.milii*oil extract but not in *C.occidentalis* oil extract, the oils may carry out their insecticidal effects through different mechanisms. It also indicates that the oil extracts may belong to different classes of insecticides. For *C.occidentalis* oil its insecticidal efficacy is likely due to the presence of the following predominant components: Decane**,** Oleic acid**,** 4, 7-methano-1H-indene, 3a, 4, 7, 7a-tetrahydro and n-hexadecanoic acid. Apart from n-hexadecanoic acid which was also present in *E.milii* oil extract, the other major components were absent in *E.milii* oil.

UNDER PEER REVIEW

Decane, is an alkane hydrocarbon with the chemical formula $C_{10}H_{22}$. Like other alkanes, it is a non-polar solvent, and is readily combustible [36]. Decane has been classified as an organo-chlorine pesticidal agent contained in Mirex (an organochlorine pesticide)[37], this means that *C.occidentalis*oil may belong to the organochlorine class of insecticides.Another compound discovered in *C.occidentalis* oil extract but not in *E.milii*oil extract is 4,7,-methano-1H-indene 3a,4,7,7a-trahydro which is known by the commercial name" chordene" and also used in the manufacture of insecticides [38]. Chordene is also an organo-chlorine pesticide whose acute toxic action is on the CNS [39].This is the second organochlorine compound found in *C.occidentalis* oil extract. This compound is also used as an acaricide, acarifuge and as an abortifacient [40].The compound; 4,7,-methano-1H-indene 3a,4,7,7a-trahydro is an antagonist of acetyl choline; it inhibits acetyl cholinesterase which is the main target of most neuro-toxic insecticides. Thus the oil extract may also double as a neuro-toxic insecticide. The presence of a wide vaiety of biological compounds in natural plants is one of the advantages they possess over the synthetic insecticides which posses only one or two compounds. The complexity and functionality of the various biological compounds in natural plants when combined together is likely to make them more effective as insecticides and less likely to yield to pest resistance. This compound; 4,7,-methano-1H-indene 3a,4,7,7a-trahydro is also an inhibitor of 5-Alpha-Reductase which converts testosterone into the more potent dihydrotestosterone (DHT). Its inhibition results in decreased conversion of testosterone to DHT, leading to increased testosterone and estradiol [41]. This may affect fertility and reproduction negatively in mammals.

Oleic acid, another major component found in *C.occidentalis*oil extract is classified as a monounsaturated omega-9 fatty acid[42].It is used as an acidifier, acidulant, inhibits arachidonic acid and uric acid production, and increases aromatic amino acid decarboxylase activity [43]. Tare and Sharma [44] compared the larvicidal properties of different fatty acid constituents against *Aedesaegypti* and found that oleic acid was the most effective. In another study by Oladipupo*et al* [34], leaf extract of *Chromolaenaodorata* which contained oleic acid methyl ester and hexadecanoic acid methyl ester exhibited insecticidal action towards adults of *S.zeamais*after 96hrs. Interestingly, Oleic acid is emitted by the decaying corpses of a number of insects, including bees and *Pogonomyrmex* ants, and triggers the instincts of living workers to remove the dead bodies from the hive. The oleic acid smell also may indicate danger to living insects, prompting them to avoid others who have succumbed to disease or places where predators lurk [45].Interestingly, n-hexadecanoic acid and its derivatives were found in both plants analysed. This arouses special interest on this compound as a potential organic insecticidal agent. N-hexadecanoic acid has been reported to possess hemolytic pesticide properties and as an inhibitor of 5-alpha-reductase [46]. Its ester; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl

UNDER PEER REVIEW

ester has been reported as haemolytic, pesticidal, nematicidal, and as an inhibitor of 5-alpha reductase by Akpuaka*et al* [47]. Note that hexadecanoic acid is the IUPAC nomenclature for palmitic acid [48].

The values of the LD_{50}s and LD_{90}s obtained for SWAN (the positive control) and *E.milii* oil extracts on test insects were quite close. This shows that the oil extract from *E.milii* could compete effectively with SWAN given the same conditions. The LD_{50} and LD_{90} values obtained for *C.occidentalis* oil extract was slightly higher than the other two treatments (SWAN and *E.milii*). Thus even though *C.occidentalis* oil extract have shown evidence of strong insecticidal activity, it may not be as potent as *E.milii* oil extract and SWAN. This indicates that the plants especially *E.milii* may hold meaningful potentials as insecticidal agents, being able to compete reasonably with SWAN even in the crude, unrefined and impure form unlike SWAN whose base is a pure isolated compound formulated into an industrially active form. The result is also indicative of the fact that hexadecanoic acid, 2 hydroxy-1-(hydroxymethyl) ethyl ester**,** 6,9,12,15-Docosatetraenic acid, methyl ester and palmitin 1, 2-di-2, amino-ethyl hydrogen phosphate as combined naturally in *E.milii* oil extract makes for a more potent insecticidal combination than; decane**,** oleic acid4**,** 7-methano-1H-indene,3a,4,7,7a-tetrahydro and n-hexadecanoic acid as combined naturally in *C.occidentalis* oil extract. It is also very likely that a combination of both plant extracts will make for an even stro[illegible]nt possibly stronger than the positive control (SWAN) [illegible]

In conclusion, *E.milii* oil extract holds promis[illegible] and natural insecticides currently in use. *C.*[illegible] agent, though not as potent as *E.milii* oil ext[illegible] these oils are carefully isolated, purified and [illegible] available form, we may have in our hands an[illegible] developed quickly in insects exposed freque[illegible]tance to these natural oils is not likely to occur very easily given the complexity of natural chemicals that combine together to make these oils effective. They may therefore serve as good alternatives to synthetic insecticides.

This is only true if you use the extracts as a whole and not as a purified commercially optimised single compound. This is in contrast to your statement in the previous sentence where you state the compds should be purified

The study has shown that *E.milii* and *C.occidentalis* plant extracts could be exploited and utilized as potent insecticidal agents, since their activities favourably compare with the positive control (SWAN) a synthetic insecticide. *A. montanus* was also shown to possess some insecticidal properties however the activity was not strong enough compared to *E. milii* and *C.occidentalis*. The active principles found in the two plant oil extracts especially those in high concentrations, are thought to be highly responsible for the insecticidal effects exhibited by the plants. The hexadecanoic acid present in both plant oils in appreciable quantity is of particular interest in this study, as it has been previously shown to be a potent-

UNDER PEER REVIEW

insecticidal agent. Also Phthalic acid and Octadecanoic acid and their derivatives tend to possess strong insecticidal actions.

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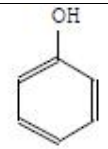
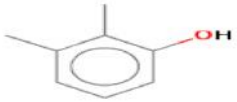
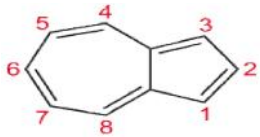
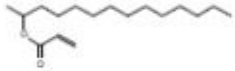
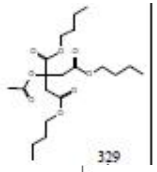
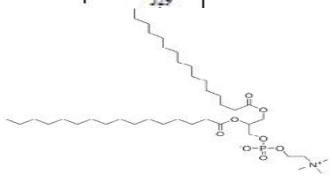
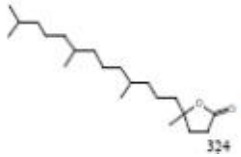
Table 4.3.3: Chemical composition of soxhlet extracted *E.milii* oil using hexane

S/N	Name of Compound	Retention time(RT)	Mol Formular	Mol weight	Peak area% (concentration).	Compound nature	Chemical structure of compound
1.	Phenol	3.91	C_6H_6O	94	1.52	Phenolic compound	
2.	Phenol,2 methyl	5.12	$C_7H_{18}O$	108	1.49	Phenol	
3.	n-Nonaldehyde	5.55	$C_9H_{18}O$	142	0.85	Alkyl aldehyde	
4.	Phenol,2,3,dimethyl	6.41	$C_8H_{10}O$	122	1.07	Phenol	
5.	Azulene	6.82	$C_{10}H_8$	128	0.94	Polycyclic aromatic comp	
6.	2-Prop 2-enoyloxyl tetradecane	7.78	$C_{17}H_{32}O_2$	268	0.88	Saturated hydrocarbon	
7.	1-Undecene,8-methyl	8.82	C_2H_{24}	168	0.78	Acyclic olefins	

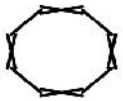
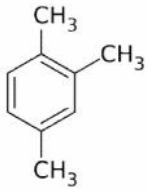
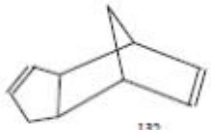
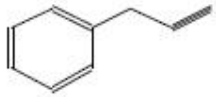
8.	1-Dodecene	9.50	$C_{12}H_{24}$	168	1.13	Acyclic olefins	
9.	Phthalic acid, di-(1-hexen-5yl) ester	12.02	$C_{20}H_{26}O_4$	330	4.85	Ester	
10.	1-Pentadecene	14.42	$C_{15}H_{30}$	210	0.75	Acyclic olefins	
11.	2-Undecanone,6,10,-dimethyl	15.22	$C_{13}H_{26}O$	198	1.15	Ketone	
12.	Pentadecanoic acid,14-methyl-,methyl ester	16.68	$C_{17}H_{34}0_2$	270	5.77	Ester	
13.	n-Nonadecanoic acid	17.44	$C_{19}H_{38}O_2$	298	1.63	Carboxylic acid	
14.	1-Heptadecanol	19.55	$C_{17}H_{36}O$	256	0.92	Alcohol	
15.	11 Octadecanic acid, methyl ester	19.81	$C_{19}H_{36}O_2$	296	2.24	Ester	
16.	Octadecanoic acid,methyl ester	20.17	$C_{19}H_{38}O_2$	298	1.28	Ester	
17.	1,propene-1,2,3-tricarboxylic	20.62	$C_{18}H^{30}O_6$	342	1.15	Ester	

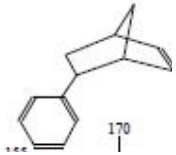
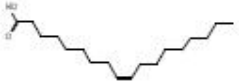
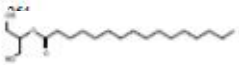
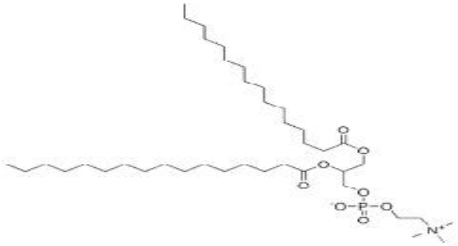
	acid,tributyl ester						
18.	Tributyl acetylcitrare	21.88	$C_{20}H_{34}O_8$	402	3.84	Ester	
19.	Palmitin,1,2-di-,2-aminoethyl hydrogen phosphate	22.30	$C_{37}H_{74}NO_8P$	691	14.60	Ester	
20.	4, 8, 12, 16-Tetramethylheptadecan-4-olide.	22.94	$C_{21}H_{40}O_2$	324	2.09	Furanone/ester	
21.	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	24.46	$C_{19}H_{38}O_4$	330	29.46	Ester	
22.	6,9,12,15-Docosatetraenoic acid,	24.61	$C_{23}H_{38}O_2$	346	14.58	Ester	

	methyl ester						
23.	Hexadecanoic acid,2-(acetyloxy)-1-[(acetyloxy) methyl]ethyl ester	25.32	$C_{23}H_{42}O_6$	414	3.76	Ester	
24.	Hexadecanoic acid,2,3-dihydroxypropyl ester	26.33	$C_{19}H_{38}O_4$		3.29	Ester	

Table: 4.3.4 Chemical composition of soxhlet extracted *C.occidentalis* oil using hexane

S/N	Name of Compound	Retention time(RT)	Mol Formular	Mol weight	Peak area% (concentration).	Compound nature	Chemical Structure of Compound
1.	Ethylbenzene	4.54	C_8H_{10}	106	1.87	Benzene	
2.	o-Xylene	4.66	C_8H_{10}	106	5.87	Benzene	

3.	1,3,5,7-Cyclooctatetraene	5.01	C_8h_8	104	6.70	Monocyclic,unsaturated hydrocarbom	
4.	Benzene,1-ethyl-2-methyl	6.03	C_9H_{12}	120	7.95	Benzene	
5.	Benzene 1,2,4-trimethyl	6.16	C_9H_{12}	120	2.45	Benzene	
6.	Decane	6.56	$C_{10}H_{22}$	142	15.10	Alkane hydrocarbon	
7.	4,7-Methano-1H-indene,3a,4,7,7a-tetrahydro	7.11	$C_{10}H_{12}$	132	13.36	Aromatic comp	
8.	1-Propyne,3-Phenyl	7.41	C_9H_8	116	6.71	Benzene	
9.	Cyclobutane, 1,2-bis(1,3-butadienyl).	8.04	$C_{12}H_{16}$	160	1.97	Aromatic benzene	
10.	Undecane	8.10	$C_{11}H_{24}$	156	1.82	Alkane	

						hhydrocarbon	
11.	Bicyclo[2,2,1]hept-2-ene,1-methyl	8.30	C_8H_{12}	108	1.15	Aromatic hydrocarbon	
12.	5-Phenylbicyclo[2,2,1]hept-2-ene	12.58	$C_{13}H_{14}$	170	2.74	Ester	
13.	Z-4-Nonadecen-1-ol acetate	19.42	$C_{21}H_{40}O_2$	324	1.34	Ester	
14.	Pentadecanoic acid,14-methyl,methyl ester	20.45	$C_{17}H_{34}0_2$	270	0.98	Ester	
15.	n-Hexadecanoic acid	21.02	$C_{16}H_{32}0_2$	256	11.63	Carboxylic acid	
16.	Oleic acid	22.77	$C_{18}H_{34}0_2$	282	14.66	Fatty acid	
17.	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl	23.57	$C_{19}H_{38}O_4$	330	2.58	Ester	

	ester						
18.	Palmitin1,2-di-,2-aminoethyl hydrogen phosphate	23.94	$C_{37}H_{74}NO_8P$	691	1.13	Ester