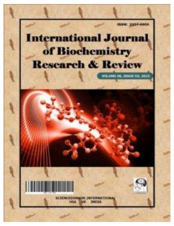

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Insecticidal Efficacy and Chemical Composition of Hexane Oil Extracts from the Leaves of *Euphorbia milii* and *Cassia occidentalis*

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Authors' contributions

This work was carried out in collaboration between both authors. Author OCO designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author COO managed the analyses of the study. Author COO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Insecticidal efficacy of oils from the leaves of *Cassia occidentalis* and *Euphorbia milii* on selected insect pests was studied. The oils were extracted via Soxhlet apparatus with hexane, and test insects exposed to the oils at 3 dose levels (0.2 mg, 0.4 mg and 0.6 mg) for 24 hours. The results showed contact insecticidal activity of *E. milii* oil at LD_{50}s and LD_{90}s (mg/kg) as 0.583 and 1.108 for *Periplaneta americana*, 0.681 and 1.215 for *Tettigonia viridissima* 0.488 and 0.893 for *Anopheles gambaie, C. occidentalis* oil gave LD_{50}s and LD_{90}s (mg/kg) of 0.889 and 1.689 for *P. americana*, 1.013 and 1.973 for *T. virdissima*, and 0.722 and 1.847 for *Anopheles gambaie*. The corresponding values for the conventional insecticide; SWAN were 0.417 and 1.017 for *P. americana,* 0.607 and 1.111 for *T. viridissima* and 0.40 and 0.743 for *A. gambaie,* respectively. The GC-MS analysis of

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plant oils revealed for *E.milii*, the presence of hexadecanoic acid, 2 hydroxy-1-(hydroxymethyl) ethyl ester; 6, 9,12, 15-docosatetraenic acid methyl ester. For *C. occidentalis,* the presence of: 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 were revealed. Overall, data from this study provides strong evidence that these oils possess bioactive metabolites with commendable degrees of insecticidal efficacy.

Keywords: Insecticidal; efficacy; chemical composition; Euphorbia milii; Cassia occidentalis.

1. INTRODUCTION

Insects are major enemies of human, livestock and agricultural crop yield all over the world. Insecticides are important tools used for the control and reduction of insects and the damages they cause in order to increase agricultural crop yield and improve the quality of life for humans, domestic animals and livestock. There are presently many mechanisms by which various insecticides control insect pests [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]. Natural products, including natural insecticides can generally be described as substances produced by living organisms which vary in their toxicity to non-target organisms as well as in their effectiveness in controlling specific insect pests.

Since the synthetic pesticides used in the control of insect pests such as organophosphate pesticides and organo-chlorine insecticides have been associated with various forms of cancer, neurological disorders and lung irritations in humans [5], there is an urgent need to develop safe alternatives that are cost effective, convenient to use and environmentally friendly [6]. Also, repeated application of a particular insecticide, increases pest resistance and can facilitate the pest's resurgence [7]. There is need for a potent, safer and cheaper alternative to the existing conventional pesticides, particularly in the developing countries where there are inadequate occupational safety standards, insufficient enforcement, poor labeling of pesticides, illiteracy, poverty and insufficient knowledge of pesticide hazards [8].

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. milii* is a low-growing evergreen shrub with very thorny grooved stems and branches; 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 *E. 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 [9]. 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.

C. occidentalis or Fedegoso is a small tree that grows 5-8 m high and is found in many tropical areas. It is also known as coffee senna in English, *Akidi ogbara* in Igbo, *Dora rai* in Hausa and *Aboorere* in Yoruba all in Nigeria [10]. It is claimed to repel 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.

The American cockroach (*Periplaneta americana*), is one of the largest species of the common cockroaches, and often considered a pest. They were introduced to the United States from Africa as early as 1625 [11]. They are reddish brown and have a yellowish margin on the body region behind the head. The

American cockroach, which is one of the fastest running insects [12] has a pair of large compound eyes, and is a very active nocturnal insect that shuns light. The odorous secretions produced by it can alter the flavour of food [13]. 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 [14].

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 [15]. 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 arthropod disease 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 [16].

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 they hatch from egg into a nymph or hopper which undergoes five molts to become an adult [17]. 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 [18].

This research was aimed at determining the insecticidal efficacy of oil extracts from the leaves of *E. milii* and *C. occidentalis*, on selected insect pests 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 their insecticidal actions.

2. MATERIALS AND METHODS

The major instruments used in this research include; Soxhlet extractor (B. Bran scientific and instrument company, England), Rotary evaporator (Thermo scientific model R-300 USA), Gas chromatography-mass spectrometry analyzer (GCMS-QP2010 plus Shimadzu, Japan), Electric blender (Akai Tokyo Japan, model No: BDOO11DA-1033M, made in PRC), chilling/heating dry bath (Echotherm, weighing balance (Symmetry Colle-Parmer Instrument Co, USA).

2.1 Collection and Identification of Plant Samples

The leaves of five plants including; *Euphorbia milii, Cassia occidentalis, Acanthus montanus, Erythrophleum africana* and *Ipomea asarifolia* were initially screened for insecticidal activities. *E. milii*, *C. occidentalis* and *A. 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 *E. africana* and *I. asarifolia* were harvested at Latitude; 5.522745, Longitude; 7.527940, Okwuta village, off Ehimmiri Housing Estate Umuahia, Abia State on the 16th of October 2015. All plant appeared healthy, leaves bright green in colour and flowers intact at the time of the harvest. Two hundred and fifty grams (250 g) of each plant was used for the screening. Of the five plants screened, *E. milli* and *C. occidentalis* showed the highest insecticidal efficacy and were used for further studies. Prior to screening, the plants 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), because they exhibit the greatest destructive and infectious tendencies at this stage. Insects were healthy and very active as at the time of the experiment, no symptoms of any diseases or weaknesses were observed. Their response to environmental factors and stimuli, movement and general behaviour indicated that they were physiologically sound at the time of the procedure [19].

2.3 Treatment of Test Insects

P. americana (American cockroach) was trapped from a domestic sewage pit in a plastic container perforated at the base and baited with some food particles. *T. viridissima*, [great green bush cricket] was caught from the grass fields in the University of Calabar staff quarters, and *A. gambaie* (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 while green grass was provided for *T. virridissima* throughout the period of the experiment. SWAN all-purpose commercial insecticide was purchased from Whatt market in Calabar, Cross Rivers State.

2.4 Preparation of Plants and Extraction of Oil

The leaves of *E.milii* and *C.occidentalis* 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 *s* were extracted by continuous extraction in Soxhlet apparatus for sixteen hours using n-hexane as solvent according to the method described by association of official agricultural chemist (A.O.A.C) [20]. Fifty grams of each ground sample was weighed separately and loaded into a thimble placed inside the Soxhlet extractor, thereafter, 600 mls of n-hexane was added to a round bottom flask which was attached to the Soxhlet extractor and condenser. The side arm was lagged with glass wool. The solvent was heated using an isomantle set at 70°C. 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 for 16 hours. 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.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Soxhlet Extracted Oils

The extracted oils were analyzed separately, one after another by subjecting them to chemical profiling via GC-MS technique as described by Sahilk et al. [21]. 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: 5 sec, injection. port dwell time:0.3 sec, washing volume:8 uL, column oven temperature: 80.0°C, injection temp: 250.00°C, flow control mode: linear velocity, pressure:108.0KPa, total flow:6.2 mL/min, column flow;1.58 mL/min, linear velocity:46.3 cm/sec, purge flow:3.0 mL/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°C/min. 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 [22] 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 lints soaked with 500 mg (0.5 g) of each of the oils were 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. Mortality was observed 24 hours post treatment. The control passed through similar treatment but with distilled water only [23]. 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, mortality was observed 24 hours post exposure. The control test was performed without the oil or SWAN treatment [24]. All experiments were done in triplicates. LD_{50} and LD_{90} of the oils were estimated using regression analysis [25].

2.7 Statistical Analysis

The LD_{50} values for the insects were determined using 'Probit Analysis" developed by D.J Finney [26]. 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 [25].

3. RESULTS

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 1).

For each fifty grammes of *E. milii* plant powder used for soxhlet extraction, 4.0 equivalent of oil with pH 3.4 was obtained which translated to 8% yield. For *C. occidentalis* 5.0 g equivalent of oil with pH 3.9 was obtained which translated to 10% oil yield.

The GC-MS analysis of *E. milii* oil revealed twenty-four peaks showing that the oil contained twenty-four compounds (Table 3). These compounds ranged from; phenols, acyclic olefins, esters, ketones, carboxylic acids and alcohols. The compounds with the highest concentrations in *E. milii* oil 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.

For *C. occidentalis* oil, a total of eighteen peaks representing eighteen compounds were observed (Table 4). Among these chemical compounds the ones with the highest concentration include; Decane; Oleic acid**;** 4,7-methano-1H-indene,3a,4,7,7a-tetrahydro and n-Hexadecanoic acid. Other compounds found in moderate concentrations include; O-xylene**;** 1, 3, 5, 7-cyclooctatetraene**;** benzene, 1-ethyl-2-methyl and 1-propyne, 3 phenyl.

The result of the contact insecticidal activity of both plant extracts and SWAN showed that both extracts possess commendable insecticidal effects (Table 5). While SWAN has been isolated and formulated into an industrially and commercially active form, *E.milii and C.occidentalis* oils if formulated into an industrially pure and active form, may perform even better.

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

Treatment	**Mean mortality rate (%)**		
Amount of oil extract(g)	***P. americana***	***T. viridissima***	***A. gambaiae***
E. milii (0.5)	76.67 ± 5.77^{e}	66.67 ± 5.77^{d}	70.0 ± 10^{d}
C. occidentalis (0.5)	60.00 ± 10^{d}	56.67 ± 5.77^{d}	53.33 ± 5.77^{c}
A. montanus (0.5)	36.67 ± 5.77^{c}	43.33 ± 5.77^{c}	43.33 ± 5.77^{c}
I. sarifolia (0.5)	23.33 ± 5.77^{b}	23.33 ± 5.77^{b}	23.33 ± 5.77^{b}
E. africana (0.5)	33.33 ± 11.55^{c}	16.67 ± 5.77^{b}	26.67 ± 5.77^{b}
Control (no treatment)	6.67 ± 5.77^{a}	6.67 ± 5.77^{a}	3.33 ± 5.77^{a}

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

Table 2. Percentage yield of Soxhlet extracted oil from leaves

Sources of oil	**pH**	**Weight of plant powder used (g)**	**Weight equivalent of oil extrac oil obtained**	**% yield of oil**
E. milii	3.4	50	4.0	8
C. occidentalis	3.9	50	5.0	10

Table 3. Chemical composition of *E. milii* oil as revealed by GC-MS analysis

S/N	Name of compound	Retention time(RT)	Peak area concentration (%)
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 phosphate	22.30	14.60
20.	4, 8, 12, 16-Tetramethylheptadecan-4-olide.	22.94	2.09
21.	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	24.46	29.46
22.	6,9,12,15-Docosatetraenoic acid, methyl ester	24.61	14.58
23.	Hexadecanoic acid,2-(acetyloxy)-1-[(acetyloxy) methyl]ethyl ester	25.32	3.76
24.	Hexadecanoic acid,2,3-dihydroxypropyl ester	26.33	3.29

Table 4. Chemical composition of *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	1.13

Table 5. Lethal effect derived from exposing test insects to 0.2, 0.4 and 0.6 mg of *E. milii*, *C. occidentalis* and SWAN treatments

Source of oil	Lethality (mg/kg)					
	P. americana		*T. viridissima*		*A. gambaie*	
	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

The insecticidal screening test of the selected plants showed that *E. 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. viridissima* and *A. gambaie* respectively. *C. 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. viridissima* and *A. gambaie* respectively. These two plants exhibited the strongest insecticidal effects among screened plants. *C. occidentalis* also showed promising insecticidal potentials as can be seen from the result (Tables 1) followed by *A. montanus* which caused mean mortality rates of 36.67% ±5.77, 43.33%±5.77 and 43.33% ±5.77 *for P. americana, T. viridissima and A. gambaie* respectively. *I. asarifolia* and *E. africana 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.

4. DISCUSSION

The insecticidal screening test revealed that *E. millii* and *C. occidentalis* exhibited promising insecticidal potential. 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* [27]. *C. occidentalis* has also been reported to suppress wood damage by termites causing mortality of worker termites within the shortest duration of application [28]. There are also reports that *C. occidentalis* oil possesses significant larvicidal potential against the malaria vector *Anopheles stephensi* [29]. *C. occidentalis* was reported to be the probable cause of hepatomyo-encephalopathy in children in western Ultar Pradesh [30]. No study has however investigated or reported the active principles likely responsible for this insecticidal action.

The insecticidal activities observed with *E. milii* oil is likely as a result of the presence 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 but present in *E. milii* oil, thus the oils may exert their insecticidal activities through different routes. Also, the mechanism of action of *E. milii* oil may be largely different from that of *C. occidentalis*. According to Dalia [31] hexadecanoic acid and its methyl ester was instrumental to the insecticidal activity exhibited by *Casimiroa edulis* leaf extract and its fractions against *Spodoptera litteralis* larvae. Oladipupo et al. [32] also observed that the presence of hexadecanoic acid and n-hexadecanoic acid methyl ester in significant concentrations in the leaf extracts of *Chromolaena odorata* was accountable for the insecticidal action of the leaf towards adults of *S. zeamays*. Hexadecanoc acid and its derivatives was observed to be abundantly present in both plant extracts, this may account for some similarities in their mechanism of action e.g both were observed to be electrolyte disruptors.

Pthalic acid; 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. Pthalic acid has been reported to exhibit insecticidal activity against *G. mellonella* in a dose-dependent manner. Also, one hundred percent insect mortality was observed at 108 hours after injection of 1M pthalic acid [33]. Since pthalic acid and 6,9,12,15-docosatetraenic acid, methyl ester are major chemical components contained in *E. milii* oil but not in *C. occidentalis* oil, the oil may belong to different classes of insecticides. For *C. occidentalis* oil its insecticidal efficacy is likely due to the presence of 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.

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 [34]. Decane has been classified as an organo-chlorine pesticidal agent contained in Mirex (an organochlorine pesticide) [35]. This means that *C. occidentalis* oil may belong to the organo-chlorine class of insecticides. Another compound discovered in *C. occidentalis* oil extract but not in *E. milii* oil 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 [36]. Chordene is an organo-chlorine pesticide whose acute toxic action is on the CNS [37].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 [38]. 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. No wonder the oil reduced acetyl cholinesterase activity in test insects. Thus the oil extract may also double as a neuro-toxic insecticide. The presence of a wide variety of biological compounds in natural plants is one of the advantages they possess over the synthetic insecticides which in most cases, 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. The 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 [39]. This may affect fertility and reproduction negatively in mammals.

Oleic acid, another major component found in *C. occidentalis* oil is classified as a monounsaturated omega-9 fatty acid [40] used as an acidifier, acidulant, inhibits arachidonic acid and uric acid production, and increases aromatic amino acid decarboxylase activity [41]. Tare and Sharma [42] compared the larvicidal properties of different fatty acid constituents against *Aedes aegypti* and found that oleic acid was the most effective. In another study by Oladipupo et al. [32], leaf extract of *Chromolaena odorata* 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 *Pogono myrmex* 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 [43].Interestingly, n-hexadecanoic acid and its derivatives were found in both plants analyzed. 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 [44]. Its ester; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester has been reported as haemolytic, pesticidal, nematicidal, and as an inhibitor of 5-alpha reductase by Akpuaka et al. [45]. Note that hexadecanoic acid is the IUPAC nomenclature for palmitic acid [46].

The values of the LD_{50}s and LD_{90}s obtained for SWAN (the positive control) and *E. milii* oil on test insects were quite close. This shows that the oil from *E. milii* could compete effectively with SWAN given the same conditions. The LD_{50} and LD_{90} values obtained for *C. occidentalis* oil was slightly higher than the other two treatments (SWAN and *E. milii*). Thus even though *C. occidentalis* oil have shown evidence of strong insecticidal activity, it may not be as potent as *E. milii* oil 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 is a more potent insecticidal combination than; decane**;** oleic acid; 4,7-methano-1H-indene,3a,4,7,7a-tetrahydro and n-hexadecanoic acid as combined naturally in *C. occidentalis* oil. It is also very likely that a combination of both plant extracts will make for an even stronger and more effective insecticidal agent possibly stronger than the positive control

(SWAN).This is worth trying and calls for further research.

E. milii oil holds promising potentials as an insecticidal agent. *C. occidentalis* also proved to be a potent insecticidal agent. If the active compounds in these oils are carefully isolated, purified and reconstituted into an industrially active and commercially available form, we may have in our hands another potent insecticide. Also resistance to SWAN has developed quickly in insects exposed frequently and can render it ineffective [47]. In contrast, resistance to these natural oils is not likely to occur very easily given the complexity of natural chemicals that combine together to make them effective. They may therefore serve as good alternatives to synthetic insecticides.

5. CONCLUSION

E. milii and *C. occidentalis* oils could be exploited and utilized as potent insecticidal agents, since their activities favorably compare with the positive control (SWAN) a synthetic insecticide. *A. montanus* also 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-insecticidal agent. Also phthalic acid and octadecanoic acid and their derivatives tend to possess strong insecticidal actions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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