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Phytochemical screening and larvicidal efficacy of *Eupatorium capillifolium* (Dog-fennel) against *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract

The primary purpose of this study is to evaluate the larvicidal efficacy of *Eupatorium capillifolium* (Dog-fennel) crude extract on *Aedes aegypti* and *Culex quinquefasciatus*. Phytochemical constituents were identified using qualitative screening. All tests were done at the Microbiology Laboratory of the University of Mindanao, Matina, Davao City. One-way ANOVA was use in analyzing the data. Phytochemical screening of the crude extract revealed the presence of tannins, alkaloids, tri-terpenoids, coumarins, saponins, steroid and phytosteroid, flavanones, flavones, carbohydrates and amino acids. A total of 1,200 3rd and 4th instars larvae of *A. aegypti* and *C. quinquefasciatus* were exposed to different concentrations of control treated with acetone and plant extracts ranging from 25%-100%. Larval mortality was observed after 1, 2, 6, 12, and 24 hours exposures. Highest susceptibility and toxicity with 99% was recorded after 1 hour exposure in 50%, 75% and 100 % concentrations of *E. capillifolium*. The larvae of *A. aegypti* revealed 86.68 % mortality rate in the plant extract while 48.7 % in control. Meanwhile, *C. quinquefascatus* showed 91% mortality rate in the extract and 33.3% in control. This indicates that crude leaf extract of *E. capillifolium* is very effective in exterminating *A. aegypti* and *C. quinquefasciatus* larvaes. This finding may lead to a possible new low cost alternative and environmentally friendly method for mosquito control programs.

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Introduction

Mosquitos (Diptera: Culicidae) is considered as the oldest human enemy that signifies threat to human health due to their pathogenic abilities causing millions of morbidities worldwide (WHO, 1992, 1998). In fact, the World Health Organization (WHO) (1996), declared mosquito as the number one public enemy. Recently, the Department of Health (DOH) Philippines through its Epidemiology Bureau of the Public Health Surveillance Division reported 176,411 cases of mosquito related diseases (specifically Dengue) as of January to November, 2016. Region XI in Mindanao ranked 8th from amongst the regions with the highest rate of Dengue comprising 11,077 cases and 74 casualties.

The availability of commercial inorganic pesticides, insecticides, and larvicides might lessen the increasing cases of mosquito related diseases. However, indiscriminate use of these chemical-based products would result in the development of resistance causing its rebounding vectorial capacity of mosquitos and subsequently giving rise to serious environmental issues (Rathy, 2015). In addition, nature has superbly perfected mosquito's biology, enabling them to survive under the most adverse and diverse of all environmental conditions (Manimegalai and Sukanya, 2014).

Bisht and Kamal (1994) observed that there is a strong need to investigate the phytochemical constituents of many plants to determine their ability to be used as fungicide, insecticide, or larvicide and as a possible source of a new drug. The most important of these constituents are alkaloids, terpenoids, steroids, coumarins, saponins, quinones, flavonoids, flavanones and these biologically active compounds (Sugumar, 2014).

One of the interesting plants is *E. capillifolium* commonly known as Dog-fennel. This is a perennial herbaceous in the family Asteraceae Native in North America (Schmidt and Schilling, 2000; King and Robinson, 1987) characterized by narrow leaf segments, fine-textured leaves, and distinctive odor (Tabanca *et al.*, 2010). At present, data on its phytochemicals are still not available. Mosquito vectors such as *C. quinquefasciatus* also known as the Southern House Mosquito survives in the lower latitudes of temperate regions and throughout the tropics. This brown medium sized opportunistic night time-active blood feeder is a vector of numerous pathogens, several of which affect humans such as St. Louis encephalitis virus, Western equine encephalitis virus, West Nile virus, *Wuchereria bancrofti*, and avian malaria (Hill and Connelly, 2009; Vinogradova *et al.,* 2003). The *A. aegypti* on the other hand is a small sized mosquito that is dark and has a white "violin/lyre" shaped markings on the dorsal surface and has banded legs. They are globally known as the type of mosquito who most commonly transmits the virus that causes dengue.

Identifying constituents for bio-larvicide that are efficient, appropriate, and adaptive to environmental condition is essential to the scientific quests in finding means and continued effective vector control management. The present paper, presented the experimental findings of using *E. capillifolium* crude leaf extract in exterminating *C. quinquefasciatus* and *A. aegypti* lavaes.

Methodology

Research locale

The preparation of extracts, phytochemical screening test, preparation of TLC plate, Thin Layer Chromatography (TLC) assay, larvicidal assay of the 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* were all done at the Microbiology Laboratory of University of Mindanao, Matina Campus, Davao City, Philippines on October 2016, to February 2017. The chemicals were provided by the Chemistry Department, University of Mindanao, Matina Campus.

Biosafety protocols

Bio-safety cabinet, anti-mosquito gear, anti-mosquito lotion, rotary evaporator, thermometer, refrigerator and fume hood were all secured and used to obtain optimum result, zero mosquito bite, and overall protection of the team within the laboratory.

Prior performing the experiment, working conditions in all assay procedures were disinfected using Lysol and 70 % isopropyl alcohol. Surgical mask and sterile gloves were also used in using the fume hood to avoid inhalation and dripping of toxic chemicals. Forceps and droppers were also used to avoid inclusions of other specimen in the samples and proper segregation and obtaining accurate number of larvae to be transferred in the beakers. Washing of hands before and after the experiment with anti-bacterial soap before performing an experiment was also done.

General experimental set-up

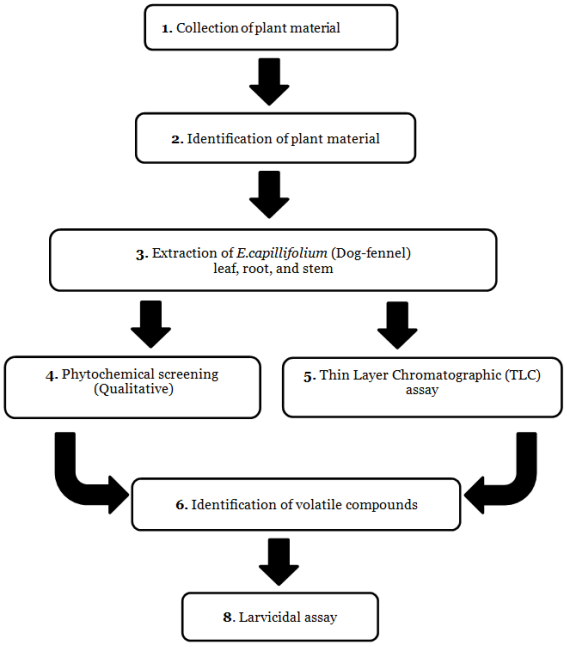

Fig. 1. General experimental setup.

Collection and identification of plant material

The leaves, stem, and roots of *E. capillifolium* (Dog-fennel) used were collected from Sitio Marahan, Barangay Marilog, [6°58"7.34N; 125°14"125.14E] Davao City, Philippines. The collected plant materials were sent to the Philippine National Museum for verification of identification.

Extraction of E. capillifolium (Dog-fennel) Leaf, root and stem

Two hundred grams (200g) of *E. capillifolium* (Dog-fennel) leaf, root, and stem were washed, air dried and cut into small pieces. The cuttings were placed in a 1000 ml beaker with five 500 ml of 95% ethanol to prevent enzyme hydrolysis.

The materials were then macerated for 48 hours at 25° C and filtered using whatman paper. The filtrate was purified using the rotary evaporator machine. The concentrated extracts were refrigerated at 4° Celsius and subjected to phytochemical screening, TLC, and larvicidal assay. The physical characteristics of the extracts were noted including volume, color, odor, and consistency.

Calculation of concentrations follows after Guevara (2004) which presented as follows:

Compute:

$$\frac{\text{200 grams of } \textit{Eupatorium capillifolium} \text{ (Dog-fennel) leaf}}{\text{500 ml of 95\% ethanol}}$$

= 0.4 grams/ml

Compute:

$$\frac{\text{200 grams of } \textit{Eupatorium capillifolium} \text{ (Dog-fennel) root}}{\text{500 ml of 95\% ethanol}}$$

= 0.4 grams/ml

Compute:

$$\frac{\text{200 grams of } \textit{Eupatorium capillifolium} \text{ (Dog-fennel) stem}}{\text{500 ml of 95\% ethanol}}$$

= 0.4 grams/ml

Biological stability of the *E. capillifolium* (Dog-fennel) leaf, root, and stem crude extract were monitored such as length of storage, the physical characteristics, and the effect of temperature. The extract on desired concentration was stored at 4°C and between 24°C to 29°C when test were done.

Phytochemical screening

The following active components were identified: alkaloids, terprenoids, coumarins, flavanoids, tannins, flavones, quinines, saponins, steroids and phytosteroids, anthraquinones, carbohydrates, and amino acids following the procedure of Guevara (2004) and Harbone (2005).

After conducting series of different method in identifying the appropriate plant part of *E. capillifolium* that contains numerous biologically active constituents (Phytochemical Screening test) with presence of volatile compounds (Thin Layer Chromatographic Assay) to be used in identifying

Larvicidal of Efficacy of *E. capillifolium* (Dog-fennel) against 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*, the Leaf crude extract among the other extracts (Stem and root) was chosen since it contains (10) high number of secondary metabolites with identified Volatile compounds (Sesquiterpene Lactones and Pyrrolizidine Alkaloids).

Thin Layer Chromatography

Thin Layer Chromatographic (TLC) was performed to determine the separation of bioactive components (volatile compound) present in *E. capillifolium* (Dog-fennel) leaf, stem and root crude extract using different solvent system with dissimilar fractions; to compute for retention factor.

The 200 ml of *E. capillifolium* (Dog-fennel) leaf, root, and stem crude extract were put in a sterile test tube and heated for 5 to 10 minutes in a water bath; then filtered. As a standard precaution, *E. capillifolium* (Dog-fennel) leaf, root and stem crude extract was properly labeled and stored in the cold temperature between 0-4°C. Glass jars with closely fitted covers were used as developing chambers. An enough solvent was poured in the filter paper to moisten and to use it to have a solvent height of 10 mm in jar. The chamber was equilibrated for 15 to 30 minutes. The Thin Layer Chromatography (TLC) plates were handled carefully to prevent the absorbent silica gel from being dirty. Too much pressing of the pencil into the absorbent silica gel was prevented to avoid destruction of the composition. By using pencil, a line was drawn transversely on the plate at 0.5 cm mark. In this case, the line drawn was used to let the spot appeared.

The diameter of the spot was about 2mm, at most; and a distance not less than 15mm between neighboring spots. Unused capillary tube was used as sample applicator for every spot to avoid mixture of other extracts, then the capillary tube was inclined into the liquid sample; and fill it into an elevation of 20mm; the plate was spotted with an extract for about 15mm from the lower edge of the plate, the spots were air dried between applications.

For additional sample, the used capillary tube was cut with straight edges and reloaded at the opposite end; the intended distance was mark lightly for the travelling of the solvent. The distance was one (1) cm approximately from the topmost edge of the coated plate.

Series of chamber development with different solvent system were prepared using Hexane, Ethyl Acetate and Acetic Acid with the following fraction: Hexane: Ethyl Acetate (9:1), Hexane: Ethyl Acetate: Acetic Acid (5:4:1), (4:4:2), (3:6:1), (2:7:1). The spotted plates were placed in the equilibrated chamber confirming that the points of application were beyond the surface of the solvent. It was cover tightly, allowing the solvent to rise upward until the solvent front reaches the marks. The developed chromatogram was removed from the chamber and the spots were marked immediately. The developed chromatogram was air dried. If the sample is too concentrated, it will run as a smear, streak or tails. The developed chromatogram was visualized under ultraviolet (UV) light, short wave (240nm) and long wave UV (365 nm). The chromatogram was traced with a pencil which indicates the point of each spot. The tracing of chromatogram was labeled with the name of the extract, solvent system and was recorded with the R_f value of each spot.

The R_f (Retention Factor) of the spot was the ratio of the distance traveled by the solvent, the center of the spot and the distance traveled by spot between the point of application. Mathematically expressed as:

$$R_f = \frac{\text{Distance of the solute from point of application}}{\text{Distance of solvent from point of the application}}$$

Identification of volatile compounds

Sesquiterpene lactone

Separate phytochemical screening was conducted to attain the presence of a specific constituent belonging to the group of Terpenoids namely sesquiterpene lactone (C-15) on *E. capillifolium* (Dog-fennel) leaf ethanol extract. The isolation and identification of the said constituent was done through concentrating the ethanol extract about 5-10 times together with the addition of Hexane, 2 separate layer indicates the presences of Sesquiterpene lactones.

Pyrrolizidine alkaloid

Same procedure of Thin layer chromatographic (TLC) assay with different solvent system, chloroform, methanol and acetic acid (80:19:1) was used to verify the presence of pyrrolizidine alkaloid of *E. capillifolium* (Dog-fennel) leaf, stem and root crude extract.

Collection, selection, and identification of mosquito Larvae

A. aegypti larvae were collected from places with clean stagnant water and from artificial containers such as vases, drums, and tires along National Housing Authority (NHA) Bangkal, Davao City, Region XI, Philippines. *C. quinquefasciatus* larvae were also collected from street canals in the same area. Larvaes were all brought back to the University of Mindanao, Microbiology Laboratory, and Matina Davao City. Healthy and highly mobile 3rd-4th instar larvae were selected for the assays.

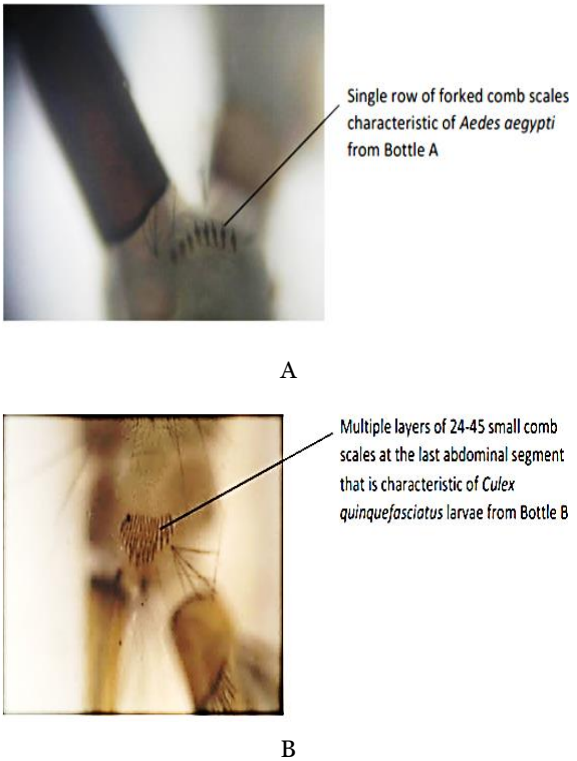

Fig. 2. Distinct morphological characters of vectors: A- Forked comb scales of *A. aegypti*; B- Multiple layers of comb scales of *C. quinquefasciatus*.

Larvicidal assay

Bioassay for the larvicidal activity of *E. capillifolium* (Dog-fennel) leaf crude extract between the 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* was carried out using World Health Organization (1996, 2005) standard procedures on the testing and evaluation of the affectivity of insecticides and/or larvicides with slight variations. From the plant extract, concentrations of 25%, 50%, 75% and 100% were prepared. Twenty-five (25) 3rd and 4th instars larvae were introduced to 250 ml beaker containing 200 ml of distilled with each concentration. A control was set through addition of acetone and H_2O using the same percentage in the treatment. A total of three (3) replicates per three (3) trials were evaluated since, multiple trials showed whether results of each experiment or in every trial exhibited consistency. Consistent findings strengthen the conclusion's value. Mortality was noted after 1, 2,6,12 and 24 hour of exposure to the treatment.

Preparation of Stock and Standard Solution

A total of five treatments were used in treating both 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*. The concentration was varied to twenty five percent (25%) labeled as Treatment two (2), fifty percent (50%) as Treatment three (3), seventy five percent (75%) as Treatment four (4) and one hundred percent (100%) as Treatment five (5). 40 ml pure crude extract of *E. capillifolium (*Dog-fennel*)* leaf was use as 100% concentration, additional of 30 ,20 and 10 ml distilled water was use to obtain 75,50 and 25% concentration, respectively. Acetone was used in the control group with the same percentage of the treatment using plant extract (25%, 50%, 75%and 100%) and labeled as Treatment one (1). The same preparation of solution in plant extract was demonstrated in the control group.

Experimental Design and Treatment

The experiment consisted of five (5) treatments including one (1) Control group with three replicates of different concentrations against the 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* arranged in corresponding group design presented below:

Table 1. Experimental set-up: Treatments, concentrations, and no. of individuals.

Treatments	Concentrations							
	25%		50%		75%		100%	
Control	^{12}Aa	^{12}C.q	^{12}Aa	^{12}C.q	^{12}Aa	^{12}C.q	^{12}Aa	^{12}C.q
E. capillifolium	^{12}Aa	^{12}Cq	^{12}Aa	^{12}Cq	^{12}Aa	^{12}Cq	^{12}Aa	^{12}Cq
TOTAL	150	150	150	150	150	150	150	150

Legend: *Aa- A. aegypti*; *Cq- C. quinquefasciatus*; ^{12} – no. of individuals.

A total of 192 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* were used during the conduct of the study. Larvae were considered to be dead only if they did not show any signs of movement when prodded with a stirring rod. The bioassays could not be done simultaneously due to some certain limitation on the availability of the larvae; therefore treatments were conducted separately over a period of time. Both treated and control larvae were kept under the same conditions (room temperature 28° ±2).

Statistical Treatment of Data

One Way Analysis of Variance (ANOVA) and T-Test for independent variable were used in identifying the larvicidal efficacy of *E. capillifolium* (Dog-fennel) Leaves crude extract against 3rd and 4th instar larvae of *C. quinquefasciatus* and *A. aegypti*. Confidence limits of lower and upper confidence l were calculated using the SPSS software (Statistical Package of Social Sciences). Results with $P < 0.05$ were statistically significance. In cases where the larval mortality in control bioassays ranged between 5% and 20%, the control mortality was amended using Abbott's formula.

$$\frac{\text{Mortality in treatment (\%) - mortality in control bottle (\%)}}{\text{[100\%- mortality in control bottle (\%)}} \text{ X } 100$$

Results and discussions

Phytochemical screening test

Phytochemical screening of different bioactive compounds (alkaloids, terpenoids, coumarins, saponins, steroids and phytosteroids, flavanones, quinones, flavones, tannins, anthraquinone carbohydrates and amino acids) were tested in three (3) different parts (leaves, stems and roots) of *E. capillifolium* (Dog-fennel) crude extract. The analysis of the presence of the different phytochemical compounds was carried out with standard procedure and the results are tabulated in Table 5.

The studies indicate the presence of all constituents in the leaves and stem crude extract of *E. capillifolium* (Dog-fennel) however quinones and athraquinones were not detected in the leaves and amino acids and anthraqiunones were not present in the stem. For the roots only quinones, flavanones, alkaloids, tannins, flavones and carbohydrates were detected, the rest were negative. Photos of the identified secondary metabolites on different parts of *E. capillifolium* (Dog-fennel) were shown in Fig. 9-20.

Table 2. Phytochemical screening of *E. capillifolium* (Dog-fennel) leaf, root and stem crude extract.

Phytochemical constituents	Qualitative test	Indication		
		Leaves	Roots	Stems
I. Test for Alkaloids	Wagner's Test	+	+	+
II. Test for Terpenoids	Liebermann Burchard Test	+	-	+
III. Test for Coumarins	Sodium Hydroxide Test	+	-	+
IV. Test for Flavones	Shinoda Test	+	+	+
V. Test for Tannins	*Gelatin Test*	+	+	+
VI. Test for Flavanones	Sodium Hydroxide Test	+	+	+
VII. Test for Quinones	Sulphuric acid Test	-	+	+
VIII. Test for Saponins	Foam Test	+	-	+
IX. Test for Steroid & Phytosteriod	Liebermann Burchard Test	+	-	+
X. Test for Anthraquinone	Hydrochloric acid Test	-	-	-
XI. Test for Carbohydrates	Benedict's Test	+	+	+
XII. Test for Amino Acids	Ninhydrin test	+	-	-

(+): Present, (-): Absent

Thin-Layer Chromatographic (TLC) Analysis

Thin layer chromatographic assay on the crude extract of the different plant parts (leaf, root and stem) of *E. capillifolium* (Dog-fennel) tried a different number of solvent systems to attain a best resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used. In Solvent system, I Hexane: Ethyl Acetate (9:1) of leaf crude extract, 2 spots were visible with R_f values of 0.31 and 0.82. In root extract, 2 spots were detected with R_f value 0.39 and 0.64. For stem, crude extract 2 spots were also detected with R_f value 0.16 and 0.70. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spots with R_f values 0.79 and 0.92 were visible in leaf crude extract. In root crude extract 2 spots were obtained having R_f 0.28 and 0.85. And for the stem extract, only 1 spot is visible with 0.94 R_f value. Solvent system III Hexane: Ethyl Acetate: Acetic acid (4:4:2), leaf crude extract shows 2 spots with R_f values 0.67 and 0.98.For root, 2 spot were detected with 0.15 and 0.77 R_f value. In stem extract, one spot was detected with 0.87 R_f value. For Solvent system IV Hexane: Ethyl Acetate: Acetic Acid (3:6:1), Leaf crude extract shows 3 spots with correspond ding 0.41, 0.82 and 0.98 R_f value, while only 1 spot were visible in both root and stem extract with 0.77 and 0.87 R_f value respectively. Lastly for solvent system V Hexane: Ethyl Acetate: Acetic Acid (2:7:1) 2 spots were attain in the leaf extract with 0.57 and 0.94 R_f value. 2 spots were also detected in the root crude extract with 0.36 and 0.75 R_f value and one spot with R_f value of 0.82 were visible in stem crude extract (Table 3).

Table 3. R_f values of *E. capillifolium* (leaf, root and stem) crude extract using different TLC solvent systems.

Plant Part	Solvent system I		Solvent system II		Solvent system III		Solvent system IV		Solvent system V	
	No. of Spots	R_f Value	No. of Spots	R_f Value	No. of Spots	R_f Value	No. of Spots	R_f Value	No. of Spots	R_f Value
Leaf	2	0.31 0.82	2	0.79 0.92	2	0.67 0.98	3	0.41 0.82 0.98	2	0.57 0.94
Root	2	0.39 0.64	2	0.28 0.85	2	0.15 0.77	1	0.77	2	0.36 0.75
Stem	2	0.16 0.70	1	0.94	1	0.87	1	0.87	1	0.82

Note: R_f was measured in centimeter (cm).

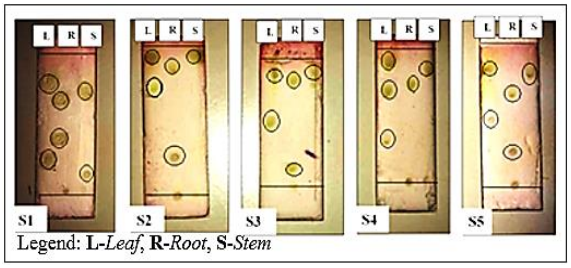

Fig. 3 Visualization of chromatogram on different solvent systems.

Solvent system 1 exhibits Hexane: Ethyl Acetate (9:1), solvent system 2 exhibits Hexane: Ethyl Acetate: Acetic Acid (5:4:1), solvent system 3, Hexane: Ethyl Acetate: Acetic Acid (4:4:2), solvent system 4 Hexane: Ethyl Acetate :Acetic Acid (3:6:1), and solvent system 5 Hexane: Ethyl Acetate :Acetic Acid (2:7:1).

Based on the preliminary identification on the presence of secondary metabolites of *E. capillifolium*, it shows positive result on the Terpenoids. Sesquiterpene lactones are a group of naturally occurring plant terpenoids that signify a unique and diverse class of natural products and are one of the most important plant constituent of essential oils. It is composed of a large and diverse group of biologically active plant chemicals that have been identified in several plant families such as Anacardiaceae, Acanthaceae, Euphorbiaceae, Rutaceae, Apiaceae, Magnoliaceae, Lauraceae, Hepatideae etc Menispermaceae, Winteraceae and (Robles *et al.,* 2005; Zang *et al.,* 2005) Nonetheless, the highest numbers are found in the family of *E. capillifolium* the Asteraceae (Compositae) family with over 3000 reported diverse structures (Modzelewska, 2005, Cho,2006).

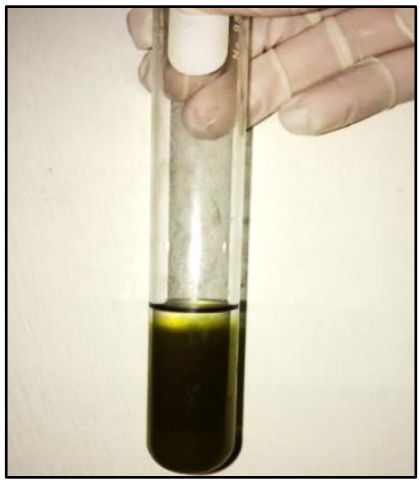

Fig. 4. Two (2) separate layers indicate the presence of Sesquiterpene lactones in *E. capillifolium* (Dog-fennel) leaf crude extract.

Qualitative identification of the Sesquiterpene lactone (Terpenoids Group) showed positive result in the Leaf crude extract of *E. capillifolium*. *E. capillifolium* leaf, root and stem extracted with ethanol as a mobile phase at room temperature (25°Celcius) with 10 ml mixture of chloroform, methanol and acetic acid (49.1:49.1:1) as solvent system showed complexity and diversity of R_f values (Table 7) . Leaf extract shows the highest R_f value followed by stem and root. On comparing the R_f values of the different spot exhibited by the 3 different parts of Dog-fennel with the R_f value of the detected pyrrolizidine alkaloid (senecionine) on the study conducted by Hamid and Kadhim ,2016 , indicates similar R_f value with the Leaf extract of *E. capillifolium*.

Table 4. Retention (R_f) values on the three (3) simultaneous trialson leaves, roots, stems of *E. capillifolium* (Dog fennel crude extract.

Plant extract	Distance of solute			Distance of solvent	R_f value		
	Leaf	Root	Stem		Leaf	Root	Stem
Trial 1	6.1	5.3	5.5	7.1	0.86	0.75	0.77
Trial 2	6.2	4.8	5.1	6.9	0.89	0.70	0.74
Trial 3	6.7	5.8	6.1	7.7	0.87	0.75	0.79

Note: R_f was measured in centimeter (cm).

Thin Layer Chromatography (TLC) is an inexpensive, quick, microscale technique that can be used to determine the quantity of components in a compound and verify substance's identity (Geiss, 1987). This was attained through calculating the number of every spot on the TLC plate, some cases where only one spot appears, retention factor value is determine by the number of components present in the mixture, thus the greater the R_f value the numerous components it contains.

Leaf crude extract of *E. capillifolium* calculated with an average of 0.88 cm R_f value, 0.77cm and 0.73 cm for stem and root respectively. In confirming a substance identity, the R_f value of unknown substance was compared to the R_f value of known substance, hence if the two spots have the same value of retention factor, hence the two substances are of the same particle/molecule (Geiss, 1987). However, just as countless organic molecules have the same color and melting point, some may also contain similar R_f value, therefore, identical R_f values doesn't always necessarily mean identical compounds. Thus, for retention factor value comparison to be valid, TLC plates must be run under the same chromatographic conditions like mobile phase, stationary phase, and temperature.

In the extraction, isolation and characterization of Pyrrolizidine alkaloids present in *Senecio vulgaris* Linn conducted by Hamid and Kadhim (2016) the stationary of TLC plate that they used is silica with 0.25 thickness, for mobile phase they used ethanol and the temperature were secluded at 23-25 ° C or room temperature. Apparently, these three (3) conditions exhibit similarity in the present study. However in the aforementioned study, (85:14:1) of dichloromethane: methanol: ammonia was used as solvent system, while chloroform, methanol and acetic acid (80:19:1) solvent system were utilized in the current study.

But then again, further investigation reveals that dichloromethane and chloroform has almost the same polarity and same goes with the ammonia and acetic acid. Nevertheless, the solvent system was not included in the condition needed to be similar in verifying substance identity.

Pyrrolizidine Alkaloids (PAs) are a class of naturally occurring alkaloids that are manufactured by plants as a defense mechanism against insect herbivores (Haddad and Winchesters, 1990).It is considered as one of the most vital groups of plant toxins in the world and are important cause of poisoning in livestock, resulting in decline of financial and damages of production each year (Kellerman *et al.* 1996). According to Ober and Hartmann (1999) and Dharmananda (2002) more than 95% of plants containing PAs were investigated and confirmed to belonged in the families of Orchidaceae, Boraginaceae , Fabaceae (genus Crotalaria) and Asteraceae (tribes Senecioneae and Eupatorieae). Research conducted by Conner *et al.,* (2000) confirmed the presence of 95% alkaloid content specifically the Pyrrolizidine alkaloid (lycopsamine and intermedine) in *E. capillifolium* . In addition, four (4) small amounts of structurally correlated pyrrolizidine alkaloids are said to be present, were two of which have not yet been identified from elsewhere in nature.

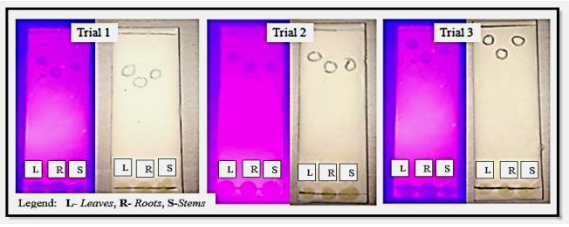

Fig. 5. Visualization of chromatogram under ultraviolet (UV) light, short wave (240 nm) and long wave (365 nm) after 20 minute.

Larvicidal assay

Table 5 shows the average toxicity percentage of the 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* after 1,2,6,12 and 24-hours exposure to the different dosage of *E. capillifolium* (Dog-fennel) leaf crude extract.

Result showed high Mortality rate with 99% significance at 50%, 75% and 100% in both mosquito larvae (*A. aegypti* and *C. quinquefasciatus*) only after 1 hour exposure to the treatment. The succeeding hour of observation were noted to be insignificant because most of the mosquito larvae already died during the first hour of the treatment. Thus, low mortality rate was noted.

Table 5. Significant difference of the mortality rates of both *A. aegypti* and *C. quinquefasciatus* in different treatment concentrations.

Hour	Concentration	N	Mortality Rate	SD	F	p-value
Hour 1	25%	12	7.83	6.631	9.234	0.000**
	50%	12	16.00	9.751		
	75%	12	19.08	6.288		
	100%	12	22.00	3.931		
Hour 2	25%	12	7.42	4.461	1.766	0.168ns
	50%	12	4.42	4.738		
	75%	12	5.75	6.225		
	100%	12	3.00	3.931		
Hour 6	25%	12	2.58	2.811	6.077	0.001**
	50%	12	4.58	5.583		
	75%	12	0.00	0.000		
	100%	12	0.00	0.000		
Hour 12	25%	12	2.83	3.070	10.222	0.000**
	50%	12	0.00	0.000		
	75%	12	0.00	0.000		
	100%	12	0.00	0.000		
Hour 24	25%	12	4.42	4.833	10.022	0.000**
	50%	12	0.00	0.000		
	75%	12	0.00	0.000		
	100%	12	0.00	0.000		

* Significant at Legend: ** Significant at 99%

ns Not Significant 95%

Fig. 6 showed the mortality rate of the 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* in both control and treatment group at different time intervals. After 1 hour observation in the treatment group, 86.68% mortality rate was recorded in *A.aegypti* and 91% in *C.quinquefasciatus* while 48.7% and 33.32% mortality rate was noted in control group. After another hour of exposure (2h) 100% mortality rate was already noted in the treatment group in both

mosquito larvae, calculated as 13.32% and 9% correspondingly. For the control group, 10.32%, 5% and 8% were noted for the larvae of *A. aegypti* and 18.32%, 6.32% and 9.68% mortality rate were calculated for the larvae of *C. quinquefasciatus* after 6, 12, and 24 hours observation respectively.

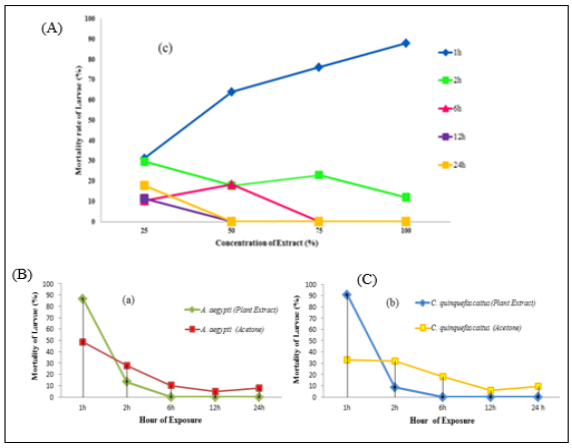

Fig. 6. Average regression for lethal concentration values of *E. capillifoium* leaf extract.

Concentrations of *E. capillifolium* against 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefascatus* treated after 1,2,6,12 and 24 hour exposure (A); Mortality rate of treated and control group against 3rd and 4th instar larvae of *A. aegypti* after 1,2,6,12 and 24 hour exposure (B), and Mortality rate of Treated and Control group against 3rd and 4th instar larvae of *C. quinquefasciatus* after 1,2,6,12 and 24 hour exposure (C).

Table 7. Phytochemical constituents of the three plant organs of *E.capillifolium*.

Active compounds	Leaf	Root	Stem
Amino acid	√	√	√
Steroid or phytosteroid	√	-	√
Flavonones	√	√	√
Carbohydrate	√	√	√
Saponin	√	-	√
Tannins	√	√	√
Anthraquinone	√	-	√
Quinines	√	√	√
Flavones	√	√	√

Legend: √ – present; - absent

Secondary metabolites found in floras are natural candidate for the discovery of new products to help eradicate the problems caused by mosquitoes. It can be extracted from specific portion of the plant or either whole plant, depending on the activity of the derivatives. Some biologically active chemicals found in plant accumulate on its different parts such as stem, leaves, flowers, barks, fruits and roots (Sukumar *et al.* 1991). In the present study, results show that among the three (3) different parts (leaf, root and stem) of *E. capillifolium* (Dog-fennel) tested, leaf and stem crude extract was found to contain numerous phytochemical constituents (Fig. 6), some of which that are believed to have insecticidal property such as alkaloids, terpenoids, coumarins, saponins steroids and phenols (Shaalan *et al.* 2005; Sukumar *et al.* 1991).

Among the 12 secondary metabolites screened, leaves and stem crude extract revealed same number of biologically active constituents both extract showed 10 positive secondary metabolites. On the other hand, root crude extract showed less number of positive secondary metabolites. Only 50 % (6) were detected, and the other 50 % showed negative result. These phytochemicals showed notable high toxicity effects with 99% significance exhibited by *E. capillifolium* (Dog-fennel) leaves crude extract against the 3rd and 4th instars larvae of *A. aegypti* and *C. quinquefasciatus* indicate its potential use as natural larvicides for the control of dengue vector and other mosquito related diseases.

Conclusion and recommendation

Plants comprised immense unexploited pool of biologically active phytochemicals that may be extensively used as alternatives to organic synthetic pesticides/insecticides that offer promising affect in future mosquito control programs. The volatile compounds (sesquiterpene lactone and pyrrolizzidine alkaloid) that were identified demonstrated larvicidal properties with huge potential to be developed as natural insecticide. This proves that *E. capillifolium* (Dog-fennel) can be utilized as mosquito control agent. Moreover, education of the public is still necessary towards mosquito control prevention program.

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