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Evaluation of the Aqueous Leaf Extract of *Ocimum gratissimum* (Scent Leaf) against Larvae of *Musca domestica*

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Abstract

This research focused on evaluating the larvicidal effects of aqueous extracts from the leaves of *Ocimum gratissimum* (commonly known as scent leaf) against the larvae of *Musca domestica*. The leaves of *O. gratissimum* were harvested, thoroughly washed, dried in the shade, and ground into a fine powder. Extraction of the plant material was carried out using the maceration technique and Whatman filtration for use in larval toxicity testing. The larvae were cultured following the procedure outlined by Pavela. Both qualitative and quantitative analyses of the extract were conducted using established analytical methods. Phytochemical screening of the extracts confirmed the presence of saponins, flavonoids, alkaloids, tannins, steroids, and proteins. Four different concentrations (100, 250, 500 and 1000 ppm) of extracts were used. Four (4) replicates were maintained for each concentration and mortality (Moribund and dead) was recorded after 24, 48 and 72 hours. Findings of this study indicated 6.25 (25.00 %); 8.50 (34.00 %) and 5.25 (21.00%) mortality of the larvae within 24; 48 and 72hrs respectively of exposure. Mortality rate was concentration dependent as highest mortality 12(48.00%) was recorded in 1000 ppm after 48 hours; The Concentration-response larvicidal bioassay of the extracts against larva of *Musca domestica* results showed that aqueous extract have at 72hours 5.25 (21.00 %) mortality at 115.536 ppm (minimum lethal concentration LC50) and 113.90 ppm (maximum lethal concentration LC95). The control group (larvae maintained in distilled water and Quaker oat without the extract) exhibited no signs of toxicity. The findings of the study demonstrated that the tested plant extracts possess notable potential for controlling houseflies. *Ocimum gratissimum* extracts, in particular, could serve as an effective means of housefly management. Nonetheless, additional research focusing on the mortality of pupae and adult flies is advised.

Keywords: *Toxicity, Larvicidal, Aqueous Extract, Phytochemical, Musca*

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Introduction

A vast variety of secondary metabolites, agrochemicals, flavors, pigments, colors, biopesticides, and food additives can all be found in plants, which are also valuable sources. Pharmaceuticals are based on compounds produced from plants where chemical synthesis predominates (Glaser *et al*., 1999). Recent studies by Umoh *et al*. (2020) and Saravanan et al. (2022) describes the use of plants with larvicidal properties in the management of various vectors. *Ocimum gratissimum*, (scent leaves) is a perennial aromatic herb that is commonly cultivated in Nigeria and is a member of the Lamiaceae family (Ifemeje et al., 2014). It is utilized to make tea, make infusions, and cure epilepsy, high fevers, and diarrhea (Akinmoladun *et al*., 2007; Adebolu *et al*., 2005). Fungal infections, colds, catarrh, influenza, pneumonia, and conjunctivitis are all treated with *Ocimum gratissimum*. It has inflammatory-fighting, antiseptic, antitussive, antiplasmodial, diaphoretic, and antiseptic effects (Kabir *et al*., 2005; Ijeh *et al*., 2005).

Houseflies and similar species are a mechanical vector for more than 100 pathogens, including those that cause typhoid, cholera, shigellosis, bacillary dysentery, tuberculosis, anthrax, ophthalmia, poliomyelitis (Cirillo, 2016; Ekesiobi, 2025), and pyogenic cocci, making them a particular problem for hospitals and during disease outbreaks. Houseflies are a mechanical vector for more than 100 pathogens, including those that cause typhoid, cholera, shigellosis, bacillary dysentery, tuberculosis, anthrax, ophthalmia, poliomyelitis (Cirillo, 2016. However, houseflies do not act as a secondary host. The maximum number of eggs laid by a single female of

the housefly, *Musca domestica* L (Diptera Muscidae), were found in the larval media of the control treatment, where no plant extract was applied, according to research by Sohail *et al*., (2015) on the effects of aqueous extracts of various plants on life cycle and population build up parameters.

According to a study by Morey and Khandale (2012) the essential oils of *Cinnamomum verum* and *Z. officinalis* had the least deterrent effects on *M. domestica* but had significant larvicidal and repellent effects. According to Ebe *et al.,* (2014) the analysis of the toxicity of *Occimum grattissimum* leaf extract on several mosquito species' developmental stages, the lower the percentage of mortality, the higher the developmental stages.

Due to the high rate of breeding of *Musca domestica* in the environment, which act as mechanical disease vectors of diseases, constituting a public health problem, and the lack of effective chemical control, there is a need to investigate some medicinal plants that can have an activity on housefly larvae, so as to break their life cycle and serve as a means of control. The research effort and its findings was intended to add to the existing knowledge and to the baseline information about the larvicidal activities and effects of aqueous leaf extract in controlling *Musca domestica* as a mechanical vector of major parasitic intestinal diseases.

Materials and Methods

Study Area

The study was carried out in Igbariam town in Anambra East Local Government Area, of Anambra State, Southeastern Nigeria. Igbariam community, popularly known as Okalakwu Kingdom, is a semi-rural community located in Anambra East LGA, Anambra State with latitude 6° 23' 26" N and Longitude 6° 56' 38" E. It experiences two seasons: wet (April to october) and dry seasons (November to March) with a short period of harmattan season.

The relative humidity of the area is about 70% in the dry season reaching 80% during the wet season (Microsoft encarta, 2009). The annual rainfall is between 2,250 to 2,500mm. The average daily temperature range of the area during the dry season is 27-33^{0}C and 21.1^{0}C-30^{0}C in wet season.

Collection and Identification of Plant Materials

Fresh leaves of *Ocimum gratissimum* were purchased from the market in study area, Anambra State, Nigeria. They were identified by a botanist in the Department of Biological Science of Chukwuemeka Odumegwu Ojukwu University, Uli Anambra State, Nigeria.

Plant Collection and Drying

Only healthy leaves were collected and washed with sterilized water to remove surface dust and contaminants. The leaves were stored under shade and protected from light exposure in the lab for three weeks to completely dry under room temperature and humidity. After drying, they were grinded using a grinder and fully powdered samples were obtained with a greater surface area for a better extraction process.

Extraction of Plant (Water extract)

The cold maceration method performed of Perry *et al*. (2019) was used for plants extraction. Indeed, 500 g of plant leaf powder was macerated in 2.5 L of water for 3 days in the glass jar (5 L) and agitated twice a day. Then, the maceration was filtrated through Whatman No.1 filter paper and filtrate was evaporated under reduced pressure and was dried using a rotary evaporator at 55 °C. The dry plant extracts were stored at -4 °C until its use for phytochemical screening and bioassays. The extraction yield of each extract was determined using the following formula:

$$Extraction\ yield\ (\%) = \frac{Weight\ of\ the\ extract\ obtained\ (g)\ \times 100}{\text{Weight of the plant powder used (g)}}$$

Phytochemical Screening in the Early Stages

Screening of qualitative phytochemicals

In order to identify the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, and reducing sugars in the chosen plant extracts, a procedure based on the earlier reports by Banso and Adeyemo (2016) and Stankovic (2011) was used.

Test for tannins

Each powdered sample was individually cooked for five minutes in a water bath with 20 cc of distilled water, then immediately filtered. A few drops (2–3) of 10% ferric chloride were added to 1 ml of cool filtrate after it had been distilled to 5 ml with distilled water, and the mixture was then checked for precipitate development and colour changes. To establish the presence of tannins, the reaction mixture was examined for a brownish green or blue-black colouring.

Test for saponins

One gram of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10 mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

(a) Demonstration of frothing: 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 mins, formation of froth which is stable for some minutes indicate the presence of saponin in the filtrate.

(b) Demonstration of emulsifying properties: 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

Test for steroids

(a) About 0.2 g of each portion of the powdered sample was dissolved in 2 ml of chloroform.0.2 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown colour at the interface between the layer indicates the deoxy-sugar characteristics of cadenolides which indicates the presence of steroid

(b) Two (2) ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2 ml of concentrated H_2SO_4. The colour change from violet to blue or green in some samples is an indication of the presence of steroids.

Test for alkaloids
One gram of each powdered sample was separately boiled with water and acidified with 5 ml of 1 % HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed.
(a) Mayer's Test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids in the extract.
(b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown or reddish- brown precipitate was regarded as evidence for the presence of alkaloids in the extract.
(c) Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide), Formation of orange-brown precipitate was regarded as evidence for the presence of alkaloids in the extract.
(d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution), Formation of yellow coloured precipitate was regarded as evidence for the presence of alkaloids in the extract.

Test for cardiac glycosides
a) Five (5) ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.
b) Ten (10) ml of 50 % H_2SO_4 was added to 1 ml of the filtrate in separate test tubes and the mixtures heated for 15 mins followed by addition of 10 ml of Fehling's solution and boiled. A brick red precipitate indicated presence of glycosides.

Test for free anthraquinones
Five (5) ml of chloroform was added to 0.5 g of the powdered dry seeds of each sample. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

Test for combined anthraquinones
One gram of powdered sample of each sample was boiled with 2 ml of 10 % hydrochloric acid for 5 mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinone.

Test for flavonoids
(a) One gram of powdered sample of each sample was separately boiled in 20 ml of water and then filtered. 5 ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4. A yellow coloration was indicative of the presence of flavonoids.
(b) One gram of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Test for terpenoids
Five (5) ml of each extract was mixed in 2 ml of chloroform. Three (3) ml of concentrated H2SO4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Test for phlobatannins
Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobatannins.

Test for carotenoids
One gram of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Phenolics
Half gram of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 mins and filtered while hot .Then 1ml of ferric chloride solution was added. Formation of blue-black or brown colouration indicated the presence of phenol.

Test for reducing sugars
To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5 mins. The mixture was filtered while hot and the cooled; 5 ml of mixture of equal volume of Fehling"s solution (A and B) was added to 2 ml of the filtrate in a test tube and the resultant mixture was boiled for 2 mins. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar.

Test for phenol
Ferric chloride test: 2 drops of neutral ferric chloride solution was added to 1ml of diluted aqueous solution of the test sample. A greenish purple color indicates the presence of phenolic compounds.

Quantitative Phytochemical
Determination of Alkaloids (using Harbone Method, 2010)
5 grams of the sample was weighed into a 250 ml beaker and 20 ml of 20 % acetic acid in ethanol was added and covered and allowed to stand for 4 hours at room temperature. This was filtered with filter paper and the filtrate was heated to one quarter of the original volume. 5 ml of concentrated ammonium hydroxide was added drop wise until the precipitate was complete. Then, filter with the pre weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven at 80 ^{o}C. The alkaloid content was

calculated and expressed as a percentage of the weight of the sample analyzed. Then was calculated using the formula.

$$\% \, Alkaloid = \frac{(Weight \, of \, filter \, paper + \, residue) - (Weight \, of \, filter \, paper) \, x \, 100}{\text{weight of the sample analyzed}}$$

Determination of Tannins by Titration (according to Person method, 2004)

Ten gram of sample was weighed in a conical flask and 100mls of n hexane or petroleum ether was added and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes for the solvent to evaporate. It was then re-extracted by soaking 100mls of 1% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected. 25 ml of ammonium hydroxide were added to the filtrate to precipitate the alkaloids. The alkaloid was heated with electric hot plate to remove some of ammonium hydroxide still in solution. The remaining volume was measured and 5 ml of this was taken and 20 ml of ethanol was added to it. It was titrated with 0.IM NaOH using 1ml of phenolphthalein as indicator until a pink end point is reached. Tannin content was calculated in percentage (C1V1 = C2V2) molarity

Determination of Saponins (according to the method of AOAC Method, 2005)

Ten grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber fitted with a condenser and flask. 250 ml of methanol was put into the flask. Extraction continued for 1hr, The saponin was exhaustively extracted by heating the flask on a heating mantle. After the thimble and its content was removed and the methanol recovered leaving the saponin and little quantity of methanol in the flask. It was then taken to an oven and kept at slanting position at a temperature of 70ºC to evaporate the residual methanol. The flask and content was weighed and the difference between the flask plus saponin and flask alone was the mass of saponin extracted. The saponin content was weighed and calculated in percentage.

Calculation:

$$\% \text{ Saponin} = \frac{\text{(Weight of beaker + sample) – (Weight of empty beaker) x 100}}{\text{weight of the sample analyzed}}$$

Determination of Flavonoids (according to the method of AOAC, 2005)

Ten (10) of the plant sample was put in a beaker with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Calculation:

$$\% \, Flavonoids = \frac{(Weight \, of \, crucible \, + \, residue) - (Weight \, of \, crucible) \, x \, 100}{\text{weight of the sample analyzed}}$$

Determination of phenolic (according to the method of AOAC Method, 2005)

Twenty (20) grams of the sample was weighed into 250 ml conical flasks. The sample was soaked in 100 ml of 2 % concentrated HCl for 3 hours, the sample was then filtered. 50 ml of the filtrate was placed in 250 ml beaker and 100 ml distilled water added to the sample. 10ml of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (III) chloride solution l.

$$\% \text{ Phenolic acid} = \frac{\text{Titre Value x 0.00195 x 1.19 x 100}}{\text{sample analyzed}}$$

Gas chromatography/mass spectroscopy analysis conditions

The crude ethanolic extract was dissolved in ethanol and votex mixed for 1 minutes and then centrifuged at 3000 rpm for 10 minutes. 1µL of the supernatant was injected into the GC and run for 30 minutes. The volatile compounds in the sample were analyzed by Gas Chromatography Mass Spectrometer (GC/MS) on an Agilent 7890B Gas Chromatography (GC) directly coupled to the Mass Spectrometer system (MS) of an Agilent 5977A. The ion source was set at 230 ℃ and the ionization voltage at 70 eV. The GC oven temperature was programmed from 80 ℃, with an increase of 15 ℃/min, to 200 ℃, then 5 ℃/min to 280 ℃, ending with a 5 min isothermal at 28.

Rearing of Housefly Colony

The culture of housefly from ogiri (fermented castor oil seed) samples was maintained at a temperature of 28 ± 2 ℃ and 60–70 % relative humidity (RH) in plastic jars (35 × 15 cm), covered with cheese cloth. Hatched larvae were transferred to jars containing larval media (yeast, dry milk powder, wheat bran and water according to the method described by Pavela (2018) and checked daily until the pupal stage. Pupae moved into plastic jars containing wood dust for the emergence of adults. The obtained larvae were used in the larvicidal bioassays.

Larvicidal Efficacy

The larvicidal bioassays were carried out by following the methods of Busvine (2011) and Palacios *et al*., (2019), respectively, with few modifications. The water and ethanol extracts were dissolved in 0.5 mL of Tween-80 and different concentrations of 1000, 500, 250 and 100 PPM for plant extracts in the plastic cups. Twenty five larvae were transferred into the each test solutions prepared.

Four (4) replicates were maintained for each concentration and mortality of the larvae was recorded after 24, 48 and 72 h for plant extracts. Dead larvae were detected when appendages did not move when probed with needle. The mortality rate of the treated larvae was calculated after 24 h of treatment by using below mentioned formula.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae} \times 100}{\text{number of larvae tested}}$$

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \%} - \text{Control mortality \%} \times 100}{100 - \text{Control mortality \%}}$$

LC_{50} and LC_{95} for Larvicidal Activity

The dose-response tests were performed in the range of 10–90 % mortality. Twenty five larvae were separated per dose (in triplicate), requiring a minimum of 8 doses to perform the curve. The larvae were placed in 500 ml cups containing 249 ml of dechlorinated water, along with 1 ml of the extract in the desired concentration, diluted in ethanol. For the control

group, Twenty five larvae were used in 250 mL of dechlorinated water. The concentrations of extracts used for assaying were 1000, 500, 250 and 100 PPM, respectively.
Mortality was recorded every 10 min during the first hour and 24 h after the start of the test, as recommended by the World Health Organization (1981). Larvae that did not spontaneously move, even if subjected to mechanical stimulation, were considered dead. The LC calculation was performed using the Polo Plus program (see item on statistical analysis).

Statistical Analysis
The data were organized in spreadsheets using Microsoft Excel software (version, 2007). Lethal concentrations (LC) 50 %, 95 % and slope were obtained through Probit analysis with the aid of the Polo Plus software (Raymond, 1985). Significant differences in the LC50 and LC90 values were based on non-overlap of 95% confidence intervals (Hematpoor *et al*., 2017; Wang *et al*., 2019). The analysis of variance (Anova) and the Tukey test, at a 95 % significant level were carried out.

Results

Qualitative Phytochemical
The qualitative results are presented in Table 1. The result showed that aqueous extract of scent leaf contained alkaloids, saponins, phenol, flavonoid, protein, tannin and steroid compounds while terpenoid, glycosides, reducing sugar and anthraquinnones were absent.

Quantitative Phytochemical
The quantitative phytochemical analysis of the scent leaf powder were presented in Table 2 showed that alkaloid recorded highest concentration (26.80%), saponin (26.73mg/100g), phenol (24.36mg/100g), tannin (13.77mg/100g), steroid (5.40mg/100g) flavonoids (3.05mg/100g) and terpenoids (2.06mg/100g).

Table 1: Qualitative Phytochemical Profile of *Ocimum gratissimum* aqueous leaf Extract

Phytochemicals	Aqueous extract
Saponin	++
Flavonoid	++
Alkaloid	+
Tannin	+
Steroids	+
Terpeniods	-
Glycosides	-
Protein	+
Reducing sugar	-
Anthraquinnones	-

Key: +++ = Present in high concentration; ++ = Present in moderate concentration; + = Slightly or sparingly present; - = Absent.

Table 2: Quantitative Phytochemical Profile of *Ocimum gratissimum* aqueous leaf Extract

Phytochemicals	Mean Value (mg/100g)
Alkaloid	26.8600±0.643[a]
Flavonoid	3.0550±0.077[e]
Phenol	24.3667±18.520[b]
Saponin	26.7367±5.200[a]
Tannin	13.7733±9.24[c]
Steroid	5.4000±3.810[d]
Terpenoid	2.0633±0.590[e]

*Values are mean scores ± Standard deviation of three (3) replicates
*Data in the same column bearing different superscript differ significantly ($p < 0.05$).

Gas Chromatography-Mass Spectrometric analysis
The result from the Gas Chromatography-Mass Spectra as shown in Table 3 revealed eleven (11) peaks indicating eleven (11) different phyto compounds were present.

Table 3: Gas Chromatography-Mass Spectrometric Analysis of *Scent* leaf

S/N	Retention Time(RT)	Molecular Weight	Name of Compound
1.	14.242	206	3, 5-bis 1, 1 dimethylethyl (Phenol)
2.	17.808	228	Tetradecanoic acid
3.	18.417	240	1, 2-epoxyhexadecane(Oxirane)
4.	19.442	270	Methylhexadecanoate (Palmitic acid)
5.	20.142	242	Hexadecanoic acid (Eicosanoic acid)
6.	21.800	294	9, 12-octadecadienoic acid (Linoleic acid)
7.	22.133	296	3, 7-dimethyldodecan-1-ol (Phytol)
8.	22.850	282	6-octadecenoic acid(Oleic acid)
9.	23.217	284	octadecanoic acid(Stearic acid)
10.	28.117	386	Cholest-5, 3-ol, 5-acetate (Cholestane)
11.	28.350	390	1,2-Benzenedicarboxylic acid (Di-n-octyl phthalate)

Mortality rate of the larvae of *Musca domestica* at different concentrations of the plant extracts
The effects of the aqueous leaf extract against the larvae of *Musca domestica* at after 24, 48, and 72, hours of exposure were shown in Table 4. There was 6.25 (25.00%) mortality of the larvae within 24hrs of exposure in aqueous extract. Also, 8.50 (34.00%) mortality after 48hrs of exposure in aqueous leaf extract. Finally, 5.25 (21.00%) mortality after 72hrs of exposure in aqueous extract. Result also showed that 5.00 (20.00%) of the larvae were still alive after 72hrs of exposure in in aqueous extract.

Table 4: Mortality rate of the larvae of *Musca domestica* at different concentrations of leaf extracts of *Ocimum grattisimum*

Scent leaf	No of larva of *Musca domestica* used	Mortality rate (%) at 24hrs	Mortality rate (%) at 48 hrs	Mortality rate (%) at 72hrs	No (%) of alive larvae
Aqueous extract					
100ppm	25	3 (12.00%)	5(20.00%)	7(28.00%)	10 (40.00%)
250 ppm	25	5(20.00%)	7(28.00%)	8 (32.00%)	5(20.00%)
500 ppm	25	7(28.00%)	10(40.00%)	4(16.00%)	4(16.00%)
1000 ppm	25	10(40.00%)	12(48.00%)	2 (8.00%)	1 (4.00%)
Mean	25.00	6.25 (25.00%)	8.50 (34.00%)	5.25 (21.00%)	5.00 (20.00%)
Control					
0.00mg/L	25	0	0	0	25.00 (100%)

The result showed that the mortality rate is dose dependents as highest mortality 21 (84.00%) was observed in 1000ppm ethanol after 24hrs, SE (sample E) containing 50mg/ml concentration of the extracts. SD (Sample D) containing 37.5mg/ml concentration of the extract produced 100% mortality against the larvae at 48 hours of exposure and 4 (16.00%) after 48hrs indicating 100% mortality. The control which contained only the larvae in distilled water and Quaker oat with no extract, showed no toxicity. The percentage mortality rate of the larva at different concentrations is also shown in Table 5.

Table 5: Concentration response larvicidal bioassay of scent leaf extracts against larva of *Musca domestica*

Scent leaf	Time	% Mortality	LC_{50} (ppm)	LC_{95} (ppm)
Aqueous	24	6.25 (25.00%)	131.459	1295.96
	48	8.50 (34.00%)	129.668	1278.3
	72	5.25 (21.00%)	115.536	1139.0

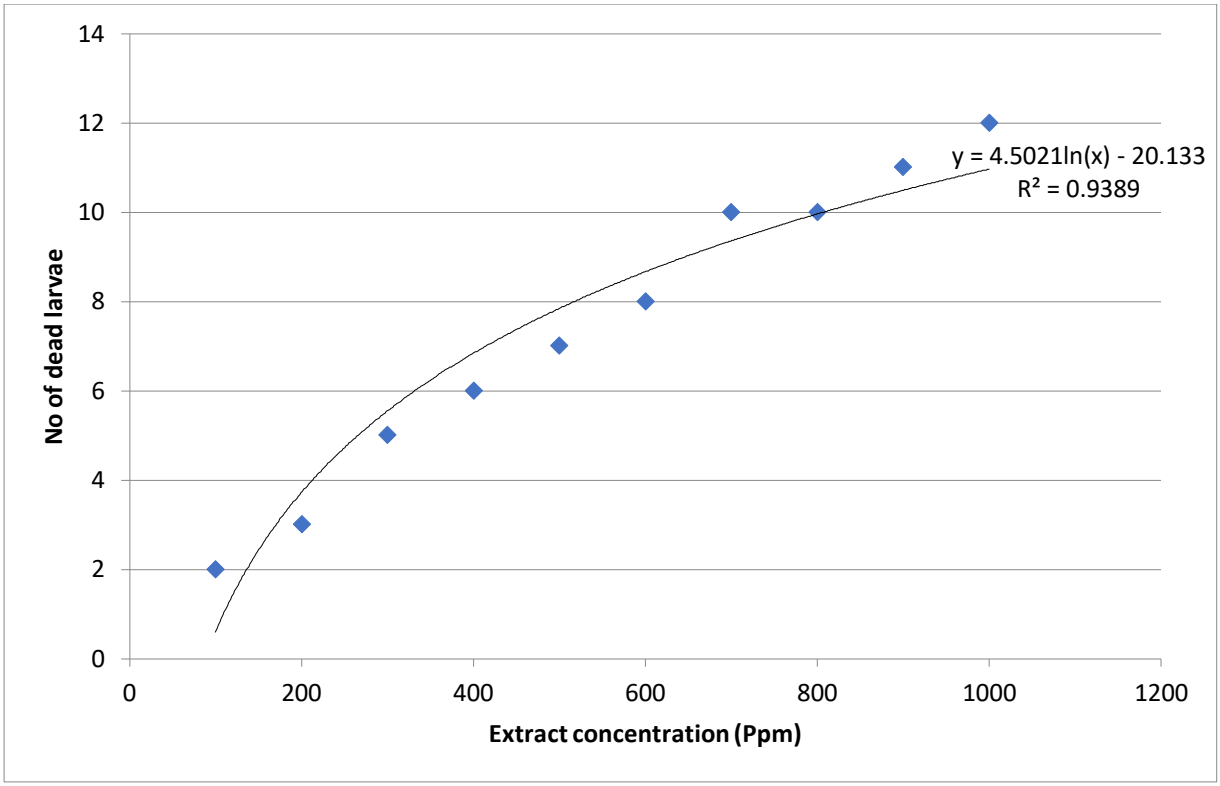

Figure 1: Mortality rate of the larva of aqueous scent leaf extracts against larvae of *Musca domestica*

The analysis of the LC_{50} and LC_{90} of all the concentrations against larva of *Musca domestica* (Table 5) indicated comparable LCs values among them, indicating activity of scent leave regardless of insect resistance. The results agree

with previous studies of plant extracts and highlights their potential of acting as efficient larvicides on housefly that are resistant to different types of insecticides, whose use has already led to the development of resistant populations in Nigeria (for e.g.: Temephos (Valle *et al*., 2019) pyrethroids (Costa *et al*. 2020).

Discussion

This study indicated that the aqueous extracts of scent leaf contained phenols, glycosides, alkaloids, flavonoids, steroids, and saponins, and the larvicidal activity of the plant could be attributed to these bioactive compounds. This is in line with an earlier study on the ethanolic extract (Ekesiobi and Onebunne, (2025). Various studies have reported the presence of tannins, saponins, flavonoids, steroids, phenols, and cardiac glycosides in the aqueous extracts of scent leaf (Azando *et al*., 2011).

In this study, the GC-MS analysis identified eleven compounds in the ethanol leaf extract fraction of scent leaf, while the quantitative phytochemical screening revealed that alkaloids had the highest concentration (26.80%), followed by saponins (26.73mg/100g), phenols (24.36mg/100g), tannins (13.77mg/100g), steroids (5.40mg/100g), flavonoids (3.05mg/100g), and terpenoids (2.06mg/100g). Alkaloids are known to be beneficial to plants, acting as repellents to predators and parasites (Ojo *et al*., 2013), which likely contributes to their antimicrobial activity.

Saponins are thought to interact with the cholesterol-rich membranes of cancer cells, inhibiting their growth and viability. These compounds are also known to precipitate and coagulate red blood cells. Some characteristics of saponins include their ability to form foams in aqueous solutions, hemolytic activity, cholesterol-binding properties, and bitterness (Okwu, 2011). In medicinal plants, saponins are responsible for many biological effects related to cell growth and division in humans, and they exhibit anti-inflammatory effects (Okwu, 2011).

The presence of phenol in the scent leaf indicates that it has biological properties such as antiapoptosis, antiaging, anticarcinogenic, antiinflammatory, antiatherosclerotic, cardiovascular protective effects, and the ability to improve endothelial function, as well as inhibit angiogenesis and cell proliferation activities (Egba *et al*., 2012; Abiodun et al., 2025). The identification of tannins suggests the plant's potential role as an antidiarrheal and antihemorrhagic agent (Asquith and Butler, 1986). Steroidal compounds are significant in pharmaceuticals due to their connection to substances used as sex hormones (Okwu, 2011). The tannins found in coconut oil and water may contribute to its sharp taste and have been reported to speed up the healing of wounds and inflamed mucous membranes. Cardiac glycosides are recognized for lowering blood pressure and are beneficial in treating heart diseases. Therefore, the findings from this study imply that spices are a valuable source of bioactive compounds with significant socioeconomic potential (Egba *et al*., 2012).

The results (Table 4) revealed that both extracts demonstrated effective larvicidal activities against the larvae of *Musca domestica*, with varying levels of susceptibility. This variability aligns with previous reports indicating that different species of houseflies exhibit differential susceptibility to various plant extracts (Pathak *et al*., 2020). The aqueous sample with a 1000ppm concentration showed the highest mortality rate among all the aqueous samples. The average mortality rate suggests that the extract had a significant effect on the larvae of *Musca domestica*. The bioassay indicated that the toxic effect of the extracts was concentration-dependent, with the highest concentration being the most effective, consistent with the findings of Okigbo *et al*. (2020).

Research investigating plant extracts as potential sources of new ovicides, larvicides, adulticides, and repellents has become a significant strategy for managing agricultural pests, medical and veterinary vectors, or urban viruses. The housefly (*Musca domestica*; Diptera: Muscidae) is a major medical and veterinary pest that causes irritation, contaminates food, and serves as a vector for over 100 species of pathogens, posing a serious threat to human health and livestock (Morey and Khandagle, 2012). Considered a cosmopolitan pest, the housefly is found worldwide. However, since the World Health Organization does not establish specific criteria to define larvicidal activity, the current study adopted a 90–100% larvicidal activity range for five different concentrations, as previously recommended.

The analysis of the LC50 and LC90 values for all concentrations against *Musca domestica* larvae (Table 4.5) revealed comparable LC values, suggesting that scent leaf maintains its activity regardless of insect resistance. These findings align with earlier studies on plant extracts and emphasize their potential as effective larvicides against houseflies resistant to various insecticides, which have already led to the development of resistant populations in Nigeria (e.g., Temephos Valle *et al*., 2019; pyrethroids Costa *et al*., 2020).

Conclusion

The phytochemical analysis of the plant extracts identified several bioactive secondary metabolites that may contribute individually or in combination to the observed larval toxicity. The results of this study showed mortality rates of 6.25% (25.00%), 8.50% (34.00%), and 5.25% (21.00%) after 24 hours, 48 hours, and 72 hours, respectively, in the aqueous extract, with mortality being concentration-dependent. These findings highlight the potential of scent leaf aqueous extracts as an alternative method for controlling *Musca domestica* larvae, including both susceptible and insecticide-resistant strains. The study also confirms that the aqueous extracts of *Ocimum gratissimum* contain chemical compounds that are active against *Musca domestica* larvae.

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