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Development of an Eco-Friendly Mosquitocidal Agent from *Chrozophora oblongifolia* Against the Dengue Vector Disease, Antimicrobial and Phytochemical Analysis

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Abstract: Dengue fever is a viral disease caused by dengue virus which can transmit via the bite of *Aedes aegypti* mosquito. Dengue fever is an endemic disease in more than hundred countries. It is a real problem worries the world where 390 million dengue infections occur every year. A 500,000 of dengue cases develop to severe and 25,000 deaths. As the failure of chemical insecticides to combat and control of mosquitoes due to their resistance it was an urgent necessary to search for an effective natural product can control and combat mosquitoes. *Chrozophora oblongifolia* is a herbal and medicinal plant which has many pharmacological activities. In this study, we evaluated the effect of methanol and acetone leaves extracts of *Chrozophora oblongifolia* plant against 3rd instar larvae and adults of *Ae. aegypti* mosquito using five concentrations of 1000 ppm, 800 ppm, 500 ppm, 250 ppm and 125 ppm. The mortality rates of larvae, pupae and adult stages resulted during larvae treatment were calculated. Methanol and acetone extracts had lethal toxic effects on larvae, pupae and adult stages of mosquito. Both extracts showed completely larval mortality by 100 % at highest concentration of 1000 ppm. Methanol extract revealed more effect with LC50 339.87 ppm compared to acetone extract with LC50 372 ppm. As the increasing in the strains number of multi-drug resistant microbes, we tend to find a natural product to overcome multi-drug resistant microbes. We tested methanol extract of *Chrozophora oblongifolia* leaves using three concentrations 10000 ppm, 5000 ppm and 2500 ppm against pathogenic microbes, the bacteria, *Staphylococcus aureus*, *Escherichia coli* and the yeast, *Candida albican.* Methanol extract exhibited antimicrobial activity against these pathogenic microbes. The Minimum inhibition concentration (MIC) against *S. aureus* and *E. coli* was 5000 ppm while against *C. albicans* was 10000 ppm. Our phytochemical analysis of methanol leaves extract of *C. oblongifoli* plant by Gas Chromatographic Mass Spectroscopy (GC-MS) indicated more than twenty chemical compounds. Out of these components, nine prevailing major compounds are revealed. The major identified compounds had high larvicidal activity against the larvae of dengue vector, *Aedes aegypti* mosquito as well as antimicrobial activity against the pathogenic microbes, *S. aureus*, *E. coli* and *C. albicans*. Based on this current study, it is suggested that *C. oblongifolia* plant could be used as a natural insecticide against the dengue vector *Ae. aegypti* mosquito as well as a natural antimicrobial agent.

Keywords: mosquitocidal agent; *Chrozophora oblongifolia*

Introduction

Dengue fever is a viral infection disease caused by dengue virus (positive-stranded RNA virus) can be transmitted to humans via the bite of the Egyptian tiger mosquito, *Aedes aegypti* (1, 2). This

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type of mosquitoes is highly distributed worldwide and found in subtropical and tropical regions, South America, Southeast Asia and parts of Africa. *Ae. aegypti* mosquito is active during day and often bites early in the morning and in the evening at dusk but it can bite human at any time of the day. Although, *Ae. aegypti* transmits dengue fever and many viral diseases, it causes skin irritation in the bite site given annoying condition to human. The risk of transmission of dengue is high in the rainy season. In recent decades, transfer of people between the cities in the endemic country or abroad has increased the number of circulation and outbreaks of dengue virus. Dengue fever is became an endemic disease in more than hundred countries and in 2019, dengue virus could invade more than120 countries (3). Dengue is a real problem worries the world where 390 million dengue infections occur every year and 500,000 of dengue cases develop to severe and 25,000 deaths (4, 5). It is became a public health concern increasing year by year. In the beginning of 2023, the dengue cases are increased and deaths are reported in endemic area with further prevalence in free dengue area. More than 5 million dengue cases and over 5000 dengue- associated deaths are recorded in all six WHO regions. In 2024, the Americas Region had 13,027,747 cases recorded by 50 countries and territories, where 6,906,396 cases were confirmed by laboratory diagnosis and 22,684 cases were described as severe dengue cases with 0.17% and 8,186 were fatal cases with 0.063% (6). In the Kingdom of Saudi Arabia, the dengue fever is started in 1994 with numerous outbreaks in Geddah and Makkah and in 2010, about 710 cases are recorded by the Ministry of Health as well 6512 cases are recorded in 2013 and 3000 of dengue cases reported in 2019 (7). Higher dengue cases recorded in Jeddah following by Jazan and Makkah regions (8). About four billion people in 130 countries have risk of *Aedes*- borne infections. The typical symptoms of dengue disease are high temperature, nausea, rash, retro-orbital pain, myalgia, and arthralgia. Dengue fever can develop to sever disease characterized by bleeding, extreme low blood pressure, low level of blood platelets and blood plasma leakage (9,10). The Appropriate medical care to dengue patients can reduce the mortality rates. Accurate vaccine for dengue is not available yet but the best way for prevention is avoid mosquito bites and control the vector to minimize virus transmission. Plants are basic sources for natural chemical compounds used by human for different biological properties such as antimicrobial, anticancer, antidiabetic, insecticides, cosmetics, dyes, food additives, and drug discovery (11,12, 13, 14). Almost misquotes are became resistant to several synthesized insecticides furthermore the chemical insecticides cause harmful side effects to humans, non-target organisms and environment. These aspects encouraged the researchers to search and find natural insecticides (15,16,17).. Many studies found that the bioactive compounds of plant extracts have biological effect to combat and control mosquitoes. (18,19,20,21). The increasing in the strains number of microbial resistance to commercial antimicrobial agents result in increase in the morbidity and mortality rates, so that there was an urgent necessity to find a natural agent has antimicrobial effect able to overcome the problem of multi-drug resistant microbes. The medicinal plant, *Chrozophora* genus is a plant belongs to the family Euphorbiaceae with 300 genera and nearly 7,500 species. They are annual small shrubby monoecious herbaceous plants with simple, alternate, oval to diamond-shaped, hairy leaves and their fruits are capsules (trilobite) covered in scaly warts. These plants grow in habitats involving sandy soils, stony places, cultivated places, and waste grounds worldwide. They are found in Asia, West Africa, and in the Middle East countries (22, 23). The species, *Chrozophora oblongifolia* belongs to this family has various biological activities like antioxidants, antimicrobial, antiviral, antidiabetes, antiseptic for wounds, hemorrhoids treatment and also it was reported to increase sex hormone (androgen) in the serum of adult male rats (24). In the current study, we evaluated the biological activity of methanol and acetone leaves extracts of *C. oblongifolia* on various stages of the dengue virus vector, *Ae. aegypti* mosquito. Also we tested the methanol leaves extract activity against the pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* and against the yeast, *Candida albicans.* The results appeared lethal toxic effects against life stages of the mosquito, *Ae. Aegypti* as well as against the pathogenic microbes, *S. aureus*, *E. coli* and *C. albicans.* Our phytochemical investigation of methanol leaves extract of *C. oblongifoli* plant indicated more than twenty chemical compounds. The major prevailing chemical compounds identified by GC- MS analysis exhibited high larvicidal

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activity against the larvae of dengue vector, *Aedes aegypti* mosquito as well as antimicrobial activity against the pathogenic microbes, *S. aureus*, *E. coli* and *C. albicans*. It is suggested that *C oblongifolia* plant could be used as a safe natural insecticide to combat and control of *Ae. aegypti* mosquito instead of harmful synthesized insecticides to minimize the outbreak of dengue disease as well could be used as a natural antimicrobial agent for treatment and overcome the multi-drug resistant microbes, *S. aureus*, *E. coli* and the yeast, *C. albicans*.

Material and Methods

The *Aedes aegypti* Larvae were got from Centre of Vector-Borne Diseases in Jazan (Figure 9). larvae allowed to bred in the lab. for six generations in Department of Biology, Faculty of Sciences, Jazan University under optimum conditions of (27±2ºC), RH (70-80%) and (12-12) light-dark. The Adult of mosquitoes were put in (30×30×30cm) wooden cages. The adult was daily supported with cotton pieces soaked in 10.0% sucrose solution for three days. Then, the adult female mosquitoes were allowed to take meal of blood from the pigeon, this is a necessary for eggs laying. A plastic oviposition cup (15×15cm) containing dechlorinated tap water was placed in the cage for facilitate eggs laying. Finally, the egg clusters picked up from the plastic cup and moved into plastic pans (25×30×15cm) filled by three liters of tap water and leftover for 24 hours. Daily the emerged larvae were supplied.

1-Plant collection:

In April 2024, *Chrozophora oblongifolia* leaves (Family: Euphorbiaceae) (Figure 1) were collected from Al-Hashr Mountain place in the Jazan region of Saudi Arabia (17°26′34.80″ N, 43°2′34.79″ E). The plant was identifier at Biology Department, faculty of Sciences, Jazan University, Saudi Arabia, and kept in the herbarium collection according to the guidelines of Tackholm (25) and Boulos (26).

Figure 1. Morphology of *Chrozophora oblongifolia* leaves.

2-Extract preparation for biological effect against *Aedes aegypti* mosquito.

The collected leaves were washed and put under the shade at room temperature (27–31 °C) for draying up to become a brittle texture. Then the dried leaves was transferred to hammer mill for grinding to make a powder. For extraction, two solvents were used, methanol and acetone. The leaves powder (100 g) were extracted with methanol and acetone separately three times with 300 ml at room temperature. After 24hour, the supernatant of extraction was aspirated and filtered through Whatman filter paper No. 5. and put in evaporator at 40 °C for concentration . Finally we obtained

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12.9 g and 9.1 g of semi-solid crude leaves extracts. After that, dried extracts were stored in a deep freezer at - 4 °C for further using.

2.a. Larval Toxicity assay:

Two drops of Tween 80 was put in the extracted crudes to make an emulsifier for testing the extracts toxicity. various concentrations of methanol and acetone leaves extracts were prepared in parts per million (1000 ppm, 800 ppm,500 ppm, 250 ppm, and 125 ppm). Thirty late 3rd instar larvae were dipped in 300 ml plastic cups containing 200 ml of dechlorinated tap water and they were maintained till the emergence of adults under the optimum conditions of 27 ± 2 °C, 70±10% RH, and 12–12 LD. two drops of Tween80 were added in 100 ml water having larvae for control. Larval mortality was reported and the dead larvae and pupae were collected. All experiments were done in triplicates.

2.b. Experimental bioassay:

After the treatment of larvae with extracts , the development of larvae were noted daily until become pupation and adult emergence. Then the estimation were calculated as the following parameters:

Larval mortality was indicated by a failure to respond to mechanical stimulation (27). Larval mortality percent was estimated by using the following equation (28): Larval mortality % = AB/ A× 100 (Where: A = number of tested larvae. B = number of resulted pupa).Percentage of pupation was estimated by using the following equation: Pupation % = A / B × 100 (Where: A=number of resultant pupae. B = number of tested larvae). Pupal mortality was observed by a failure to developing to adult stage. The pupal mortality percent was determined using the following equation: Pupal mortality % = A / B × 100 (Where: A = number of dead pupae. B = number of resulted pupae. Adult emergence estimation: The emerged males and females adults were counted and the adult emergence percent was calculated by using the following equation: Adult emergence % = A / B × 100 (Where: A = number of emerged adults. B = number of resulted pupae).

2.c LC50 and LC90 calculation

The LC50 value which represents the lethal concentration for 50 % larval mortality and the LC90 value which exhibits the lethal concentration for 90 % larval mortality were calculated through linear regression analysis using Microsoft Excel sheet (29).

3. Extract preparation for Antimicrobial Activity

A 2.2 Kg of air-dried leaves of *C. oblongifolia* plant were extracted in 70 % methanol through maceration method three times for each 6 Liters overnight till complete exhaustion . Then the methanolic extract was filtered and concentrated under reduced pressure to make 400 g of dark brown syrupy.

4. Antimicrobial Activity

Antimicrobial activity of methanol leaves extract of *C. oblongifolia* was studied against two standard pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* and one standard pathogenic fungi, the yeast, *Candida albicans* which were maintained in the microbiology laboratory of the department of Biology, College of Sciences, Jazan University.

4.a. Microbial culture

The two bacterial strains, *S. aureus* and *E. coli* were inoculated in nutrient agar at 37 °C for 24 h. The yeast strain, *C. albicans* was inoculated on YPD agar at 30 °C for 24 h. After 24 h pure colonies for each culture were picked up separately and put in 10 ml of 0.9 % sterile normal saline to make cells suspension and turbidity was matched to the 0.5 McFarlands turbidity standard tubes (1.5 x 10^8 CFU/mL).

4.b. Antimicrobial assay

The cells of each prepared suspension was spread separately on sterile Muller and Hinton agar (MHA) plates using sterile cotton swab. By using sterile metal borer, the Wells on plates agar were

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dug. Methanolic extract of *C. Oblongifolia* leaves was dissolved in DMSO. Then, three concentrations were prepared from this stock were prepared as 10000 ppm,5000ppm and 2,500 ppm. A100µl of extract was added in each corresponding wells. Standard antibiotic disc of Amoxicillin used as antibacterial positive control and Cyclohexamide used as antifungal positive control. The inoculated plates were incubate at 37°C for 24 h. By using measuring ruler, the inhibition zone was measured in millimeters (mm). Minimum inhibition concentration (MIC) was calculated as the lowest concentration showing zone of inhibition. All the experiments were done in triplicates.

5. Gas Chromatographic Mass Spectroscopy (GC-MS) analysis

The methanol extract of *C. oblongifolia* leaves was analyzed using a Shimadzu QP2010 Gas-Chromatography–Mass. The device employed a fused silica column packed with Elite -5 ms (5% Diphenyl 95% Dimethyl poly siloxane, 30 mm × 0.25 mm × 0.25 µm df). Then the components were separated by using helium as carrier gas at a constant flow of 1 ml/min. A 2 µL of sample extract was injected into the instrument and it detected by the turbo gold mass detector with aid of turbo mass 5.2 software. The oven during GC Process was maintained at 110°C with 2 min holding and the injector temperature was set at 250°C. The temperature of inlet line was 200°C and source temperature was 200°C. Mass spectra were taken at 70 eV, a scan period of 0.5 S and fragment from 45 to 450 Da. The MS detection was done within 48 min. The mass spectrum GC-MS interpretation was performed using the database of National Institute Standard and Technology (NIST and WILEY) spectroscopy which contain more than 62,000 patterns. The spectrum of unknown components was kept in the NIST and WILEY library (30).

Statistical Analysis:

Statistical analysis of the data was performed using One-way analysis of variance. ANOVA was applied to find the differences between the activity of tested extracts using Tucky's test at 0.005 probability level, where means with P>0.05 are not statistically significant. Statistical analysis was done using SPSS software.

Results

The biological activity of methanolic and acetone leaves extracts of *Chrozophora oblongifolia* leaves against various stages of the dengue virus vector, *Aedes aegypti* mosquito at concentrations of (1000 ppm, 800 ppm, 500 ppm, 250 ppm, 125 ppm) had toxic effects.

1.Biological effect of methanolic extract on life stages of *Aedes aegypti* mosquito

The methanolic extract effects against the 3rd instar larvae of *Ae. aegypti* showed lethal toxicity effects. A hundred percent of larval mortality was observed at the concentration of 1000 ppm (Table 1, Figure 2). At the concentrations of 800 ppm, 500 ppm, 250 ppm, the larval mortality were 80.00±0.00 %, 72.00±0.00% and 62.00±5.00% respectively. At the low concentration, 125 ppm the larval mortality was 38.00±6.00 % compared to the untreated larvae (control)(Table 1 , Figure 2). The LC50 value of larva mortality was 339.87 (ppm) and LC90 was 830.06 (ppm) (Table 3, Figure 7) this represented a significant toxicity of methanol extract against larvae. The larva duration at concentration 800 ppm was 6.33±1.00 days while at low concentration 125 ppm was 7.00±0.00 days compared to control (5.00±0.00 days) (Table1). The extract also had effect on pupation rate, at the concentration of 800 ppm, the pupation percent was 20.00±0.00 %, whereas at 125 ppm the pupation increased to 62.00±6.00 %.compared to the control (100.00±0.00 %) (Table 1, Figure 3). Methanolic extract effects on pupae life was observed. At concentration of 800 ppm, the pupal mortality was 33.33±3.00 % this value did not exceed the 50% indicted that there was not significant toxicity showed of extract against pupae life (Table 1, Figure 4). The methanol extract effects on adult emergency and adult mortality also was recorded. At concentration of 125 ppm, the adult emergence percent was 90.00±2.00 % compared to the control (100.00±0.00 %) but at the concentration, 800 ppm, the adult emergence reduced to 67.67±2.00 % (Table 1, Figure 5). A 35.00±0.00 % of adult mortality was showed at concentration of 800 ppm this value did not exceed the 50% indicated not significant toxicity of methanol extract against adult mosquito (Table1, Figure 6).

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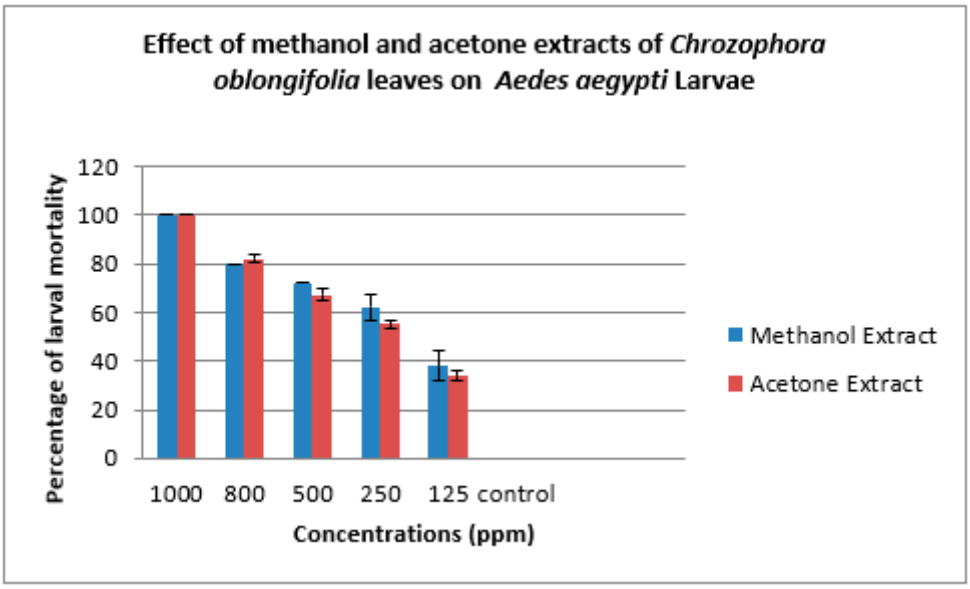

Figure 2. Biological activity of methanolic and acetone leaves extract of *Chrozophora oblongifolia* on *Aedes aegypti* larvae.

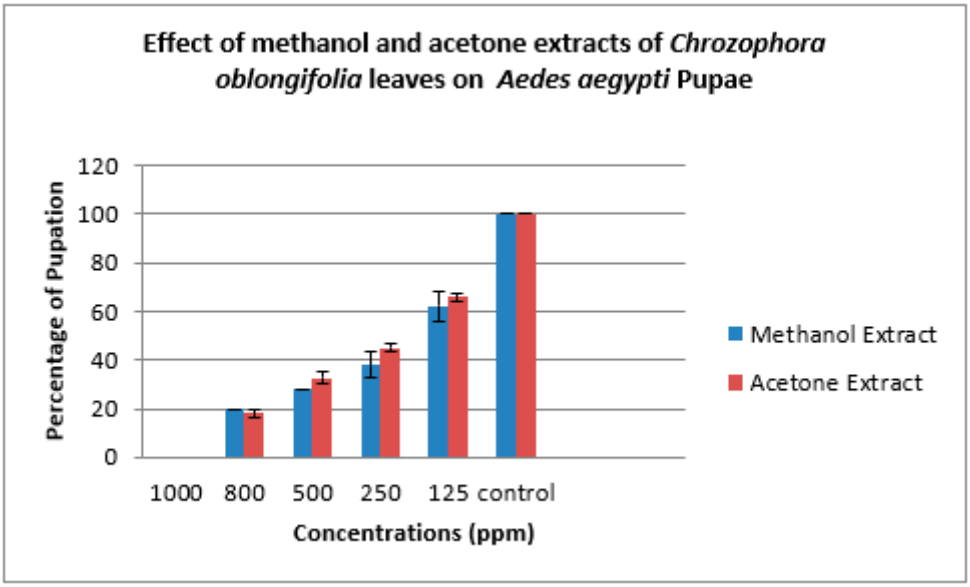

Figure 3. Biological activity of methanolic and acetone leaves extract of *Chrozophora oblongifolia* on *Aedes aegypti* pupation.

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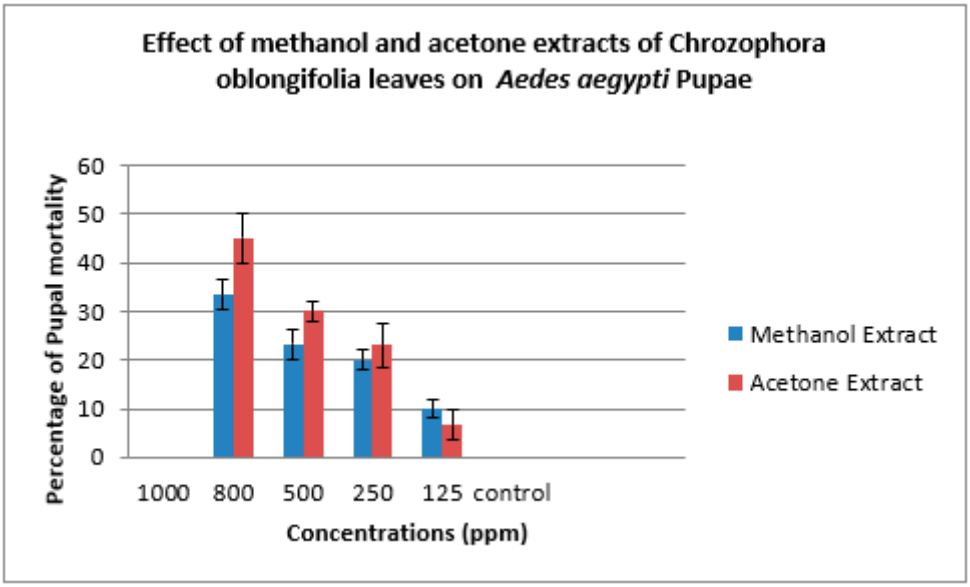

Figure 4. Biological activity of methanolic and acetone leaves extracts of *Chrozophora oblongifolia* on *Aedes aegypti* pupae.

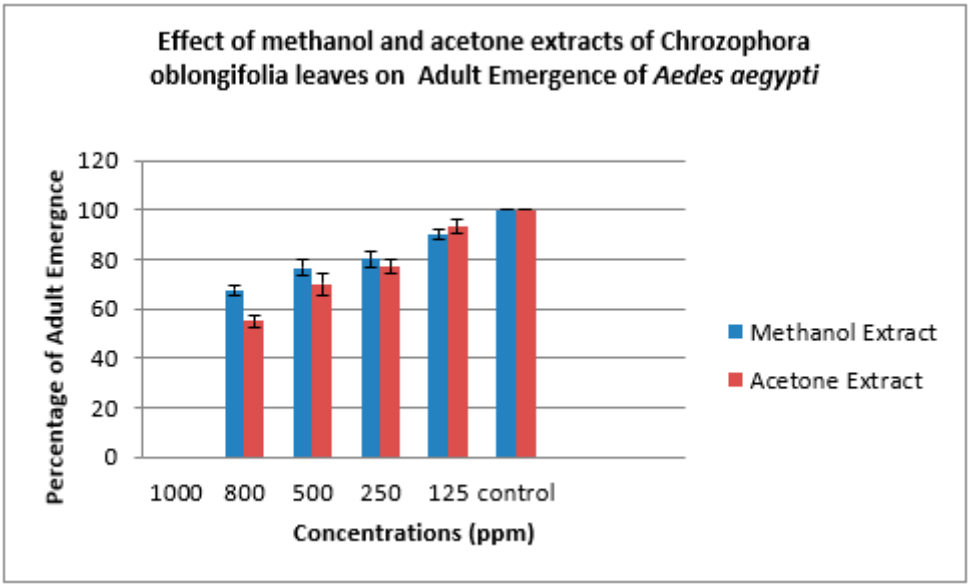

Figure 5. Biological activity of methanolic and acetone leaves extracts of *Chrozophora oblongifolia* on Adult Emergence of *Aedes aegypti.*

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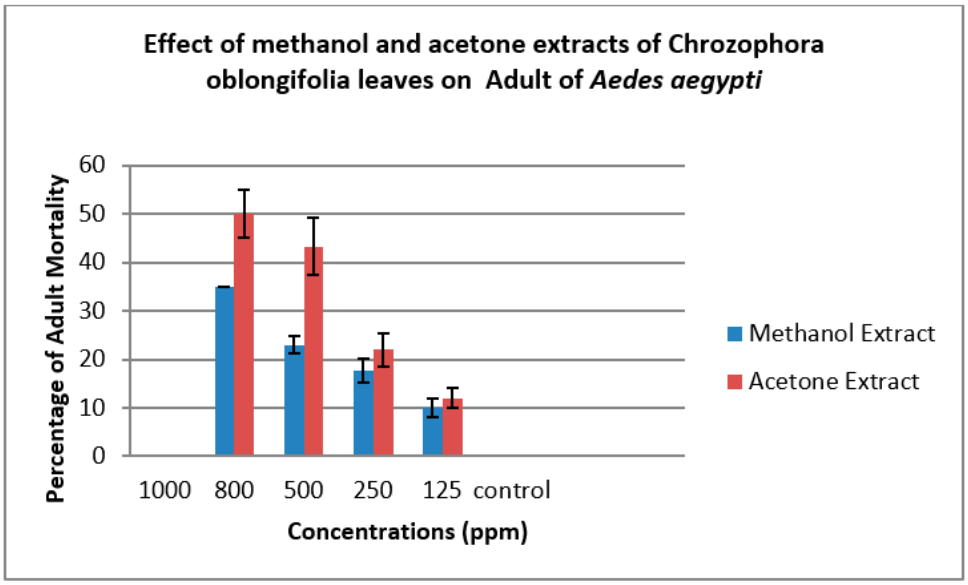

Figure 6. Biological activity of methanolic and acetone leaves extracts of *Chrozophora oblongifolia* on Adult of *Aedes aegypti.*

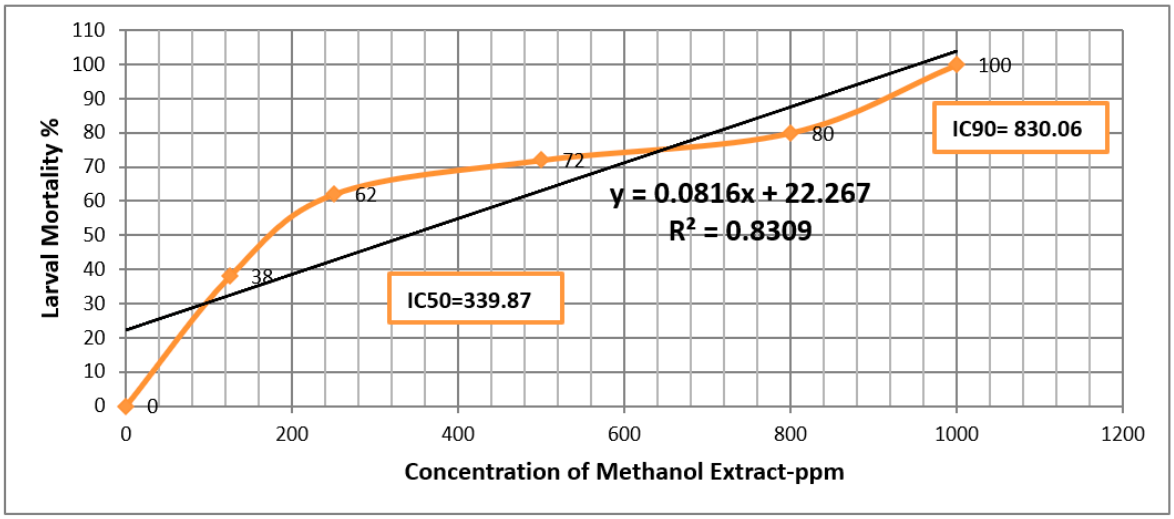

Figure 7. LC50 and LC90 values (ppm) of methanol leaves extract of *Chrozophora oblongifolia* plant against larvae of *Aedes aegypti* mosquito.

Table 1. The biological effect of Methanolic and Acetone leaves extract of *Chrozophora oblongifolia* plant on different stages of *Aedes aegypti* mosquito.

Methanol Extract						
Concs. ppm	Larva Mortality%	Larva duration/ days	Pupation%	Pupal Mortality%	Adult Emergency%	Ault Mortality%
1000	100.00±0.00	----	------	------	-------	----
800	80.00±0.00	6.33±1.00	20.00±0.00	33.33±3.00	67.67±2.00	35.00±0.00
500	72.00±0.00	7.33±1.00	28.00±0.00	23.33±3.00	76.67±3.00	23.00±2.00
250	62.00±5.00	6.00±1.00	38.0±5.00	20.00±2.00	80.00±3.00	17.67±3.00

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125	38.00±6.00	7.00±0.00	62.00±6.00	10.00±2.00	90.00±2.00	10.00±2.00
Control	0	5.00±0.00	100.00±0.00	0	100.00±0.00	0

2.Biological effect of acetone extract on life stages of *Aedes aegypti* mosquito

Acetone extract against the 3rd instar larvae of *Ae. aegypti* showed lethal toxicity effects. At the highest concentration (1000 ppm), the larval mortality was 100.00±0.00 % (Table 3, Figure). At the concentrations of 800 ppm ,500 ppm, 250 ppm and 125 ppm, the larval mortality were 82.00±2.00 %,67.33±2.00 %, 55.00±2.00 respectively (Table 2, Figure 2). At 125 ppm the larval mortality was 34.00±2.00 %% compared to the control (Table 2, Figure 2). The LC50 value of larva mortality was 372 (ppm) and LC90 was 839.30 (ppm) (Table 2, Figure 8). The duration larva at concentration 800 ppm was 5.33±1.00 days while at low concentration 125 ppm was 6.67±1.00 days compared to control (5.00±0.00 days) (Table 1). Acetone extract also had effect on the rate of pupation, at 800 ppm, A 18.00±0.00 % pupation percent was observed, while at 125 ppm the pupation rate increased to 66.00±2.00 %.compared to the control (100.00±0.00 %) (Table 2, Figure 3). Acetone extract effects on pupae life was done. At concentration of 800 ppm, the pupal mortality was 45.00±5.00 % this value did not exceed the 50% indicted that there was not significant toxicity showed of extract against pupae life (Table2, Figure 4). The extract effects on adult emergency and adult mortality also was observed, where at 125 ppm, the adult emergence percent was 93.33±3.00 % compared to the control (100.00±0.00 %) but at the higher concentration, 800 ppm, the adult emergence reduced to 55.00±3.00 % (Table2, Figure5). The adult mortality was 50.00±5.00 % at concentration of 800 ppm this value represented 50% toxicity of acetone extract against adult mosquito but did not exceed the 50% as a significant toxicity (Table2, Figure 6).

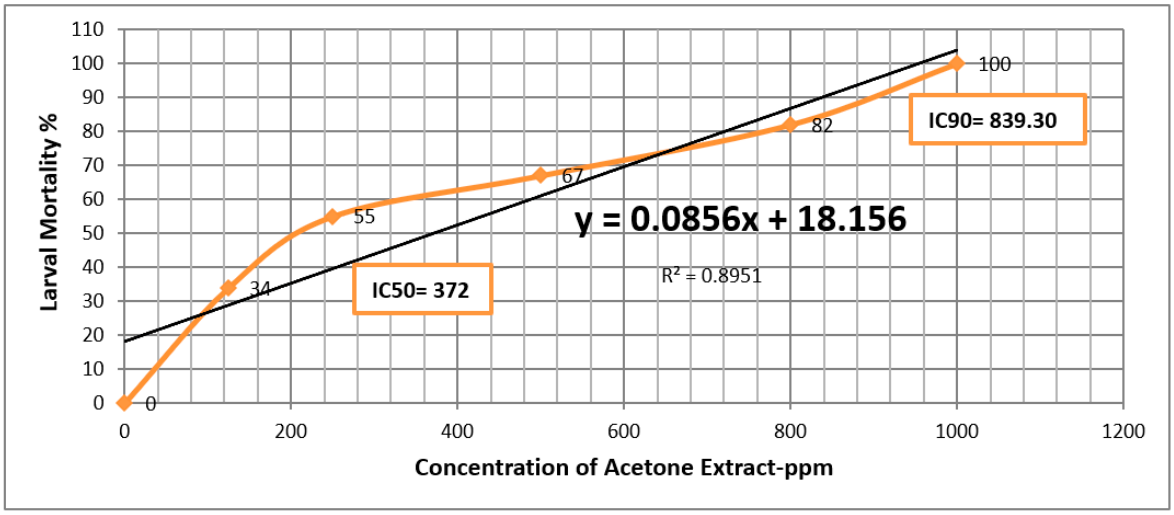

Figure 8. LC50 and LC90 values (ppm) of acetone leaves extract of *Chrozophora oblongifolia* plant against larvae of *Aedes aegypti* mosquito.

Table 2. The biological effect of Acetone leaves extract of *Chrozophora oblongifolia* plant on different stages of *Aedes aegypti* mosquito.

Acetone Extract						
Concs. ppm	Larva Mortality%	Larva duration/ days	Pupation%	Pupal Mortality%	Adult Emergency%	Ault Mortality%
1000	100.00±0.00	------	-----	----	------	-----
800	82.00±2.00	5.33±1.00	18.00±0.00	45.00±5.00	55.00±3.00	50.00±5.00

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500	67.33±2.00	6.33±1.00	32.67±2.00	30.00±2.00	70.00±4.00	43.33±6.00
250	55.00±2.00	6.67±1.00	45.00±2.00	23.00±4.00	77.00±3.00	22.00±3.00
125	34.00±2.00	6.67±1.00	66.00±2.00	6.67±3.00	93.33±3.00	12.00±2.00
Control	0	5.00±0.00	100.0±0.00	0	100.00±0.00	0

Table 3. LC50 and LC90 values (ppm) of methanolic and acetone extracts of *Chrozophora oblongifolia* leaves against larva of *Aedes aegypti* mosquito.

Extract	LC50	LC90
Methanol	339.87 (ppm)	830.06 (ppm)
Acetone	372 (ppm)	839.30 (ppm)

3.Antimicrobial activity of methanolic leaves extract of *Chrozophora oblongifolia*

The methanol extract against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* had inhibition growth, where the Minimum inhibition concentration (MIC) against *S. aureus* and *E. coli* was 5000 ppm (Table 4, Figures 10 and 11), while against *Candida albicans* was 10000 ppm (Table 4, Figure 12).

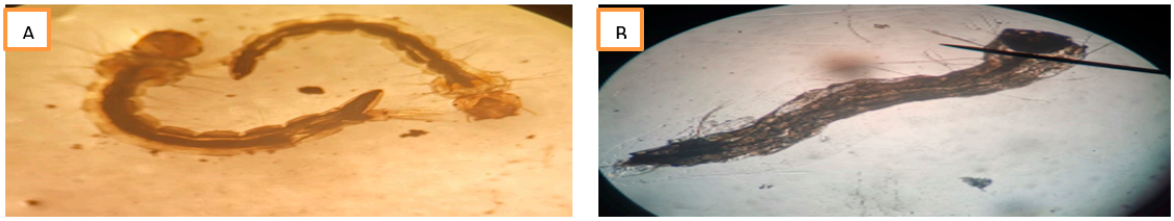

Figure 9. The effect of *Chrozophora oblongifolia* Leaves extracts on *Aedes aegypti larva*: a) untreated larva, b) larva after treatment.

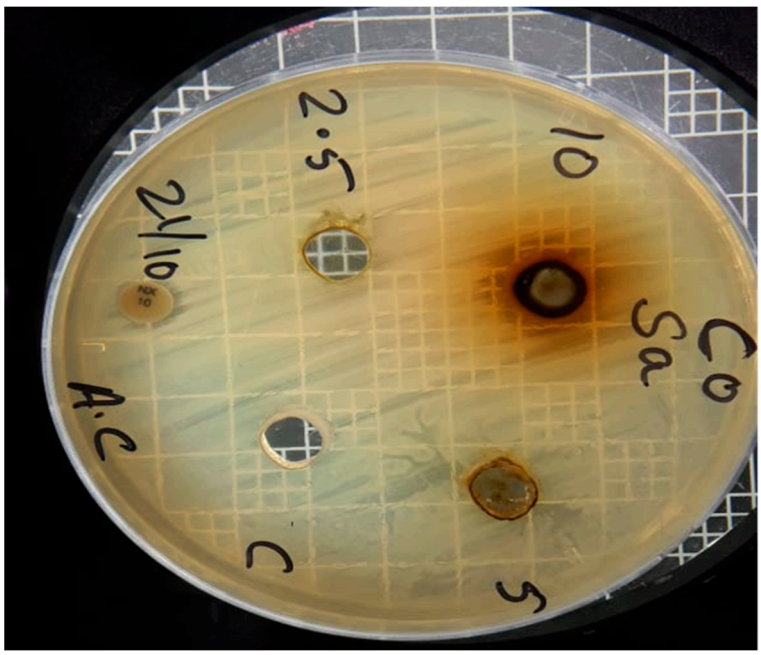

Figure 10. Antibacterial activity of methanol leaves extract of *Chrozophora oblongifolia* against *Staphylococcus aureus* growth.

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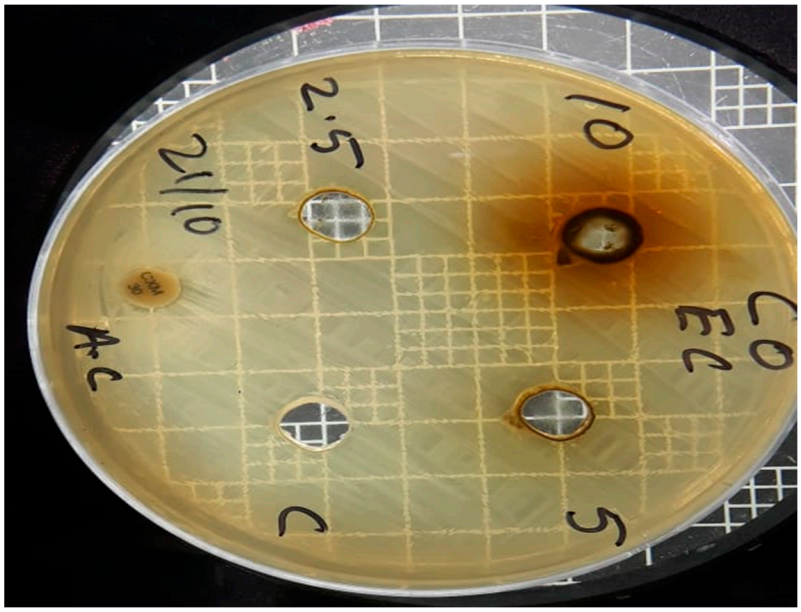

Figure 11. Antibacterial activity of methanol leaves extract of *Chrozophora oblongifolia* against *Esherichia coli* growth.

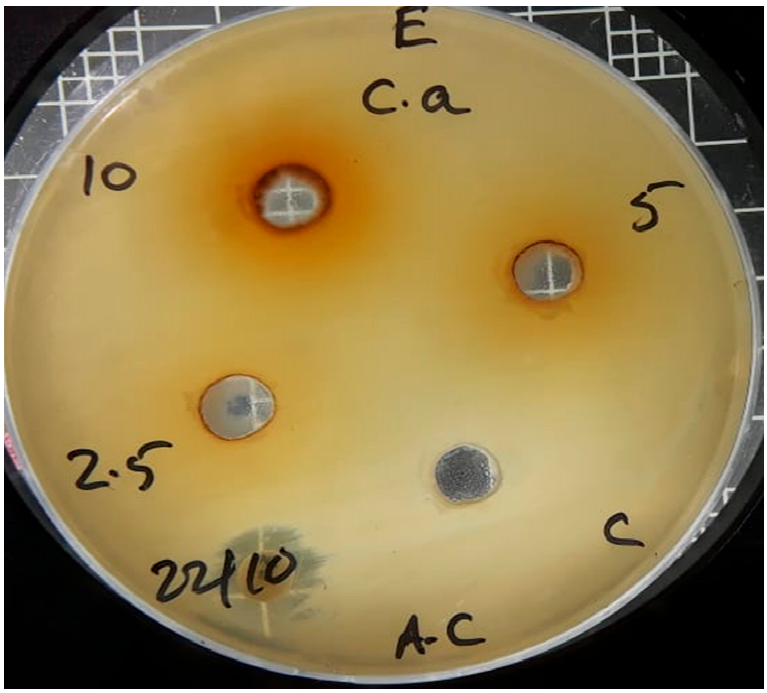

Figure 12. Antifungal activity of methanol leaves extract of *Chrozophora oblongifolia* against *Candida albicans* growth.

Table 4. Antimicrobial activity of methanol extract of Chrozophora oblongifolia leaves against Staphylococcus aureus, Escherichia coli and Candida albicans.

		Methanol leaves extract of *Chrozophora oblongifolia*		
Sr no.	**Microbial strains**	Concentrations (ppm)		
		10000	5000	2500
1	*S. aureus*	14 mm	11 mm	0

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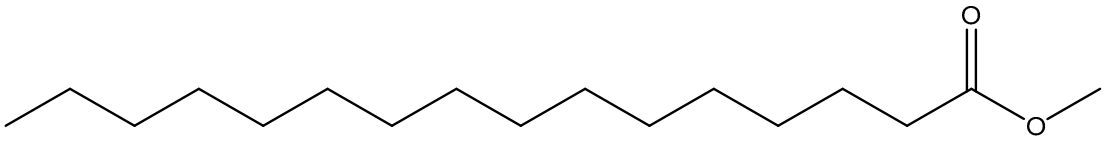
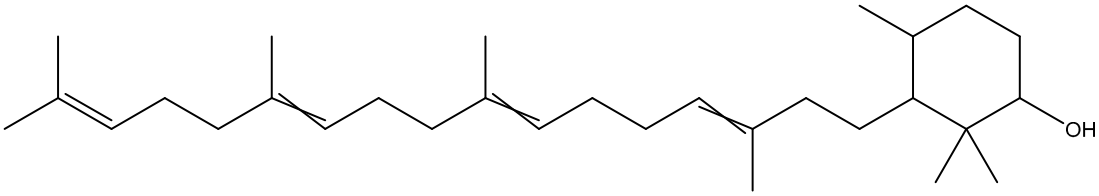
2	*E. coli*	17 mm	13 mm	0
3	*Candida albicans*	16 mm	0	0

4.GC-MS analysis of methanolic leaves extract of *Chrozophora oblongifolia*.

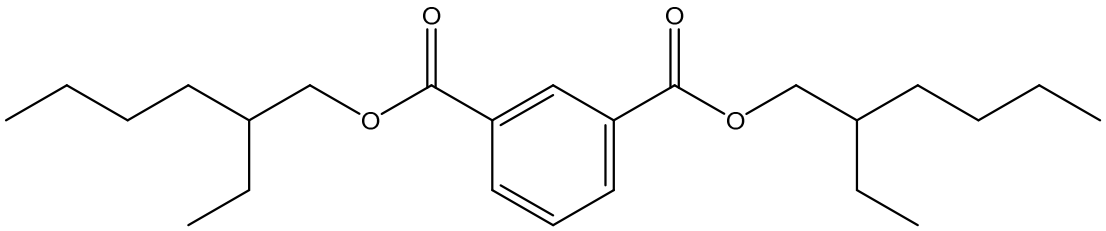
The phytochemical analysis of methanol leaves extract of *Chrozophora oblongifolia* using Gas Chromatographic Mass Spectroscopy (GC-MS) was identified more than twenty chemical compounds. The chemical compounds with their Retention Time (RT), Molecular Formula (MF) and concentration (Peak area %) are tabulated in Table 5. The major prevailing compounds were Hexanedioic acid, bis(2-ethylhexyl) ester (28.18%), Hexanedioic acid, bis(2-ethylhexyl) ester (15.59%), 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca--3,7,11,15-tetraenyl-cyclohexanol (4.06 %), Hexadecanoic acid, methyl ester (3.74%), 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (3.6 %), Dasycarpidan-1-methanol, acetate (ester) (3.44 %), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- (2.67%), 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (1.76%), and 3-Methyleicosane (1.01 %) (Table 5, Figure 13).



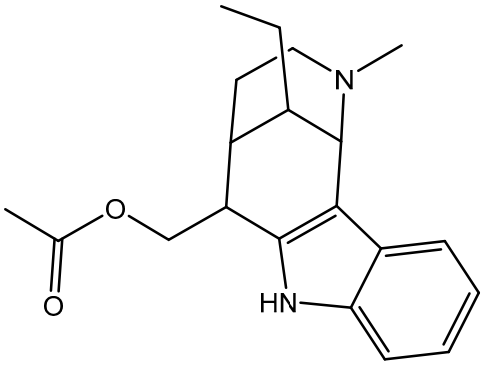
Hexadecanoic acid, methyl ester



2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol



1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) este



Dasycarpidan-1-methanol, acetate (ester)

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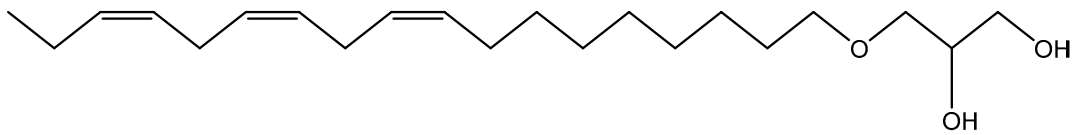
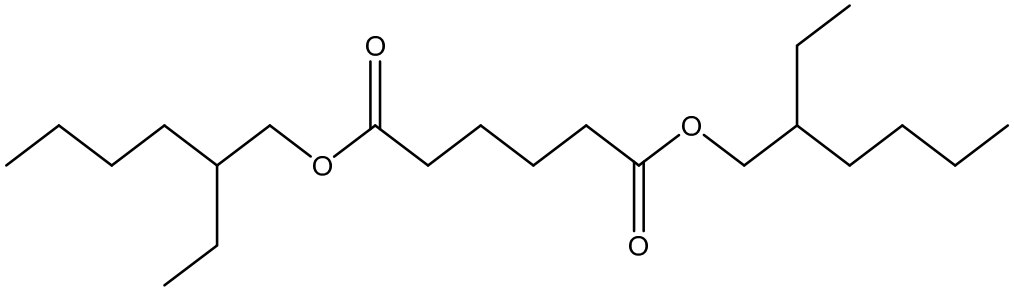
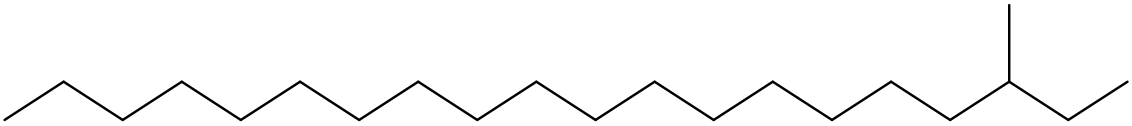


9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-



Hexanedioic acid, bis(2-ethylhexyl)ester



3-Methyleicosane

Figure 13. Structures of major compounds in the methanol leaves extract of *Chrozophora oblongifolia* identifies by GC-MS.

Table 5. Gas chromatography-mass spectrometry analysis (GC-MS) of methanol leaves extract of *Chrozophora oblongifolia* plant.

RT	Peak Area %	Compound Name	Molecular Formula
11.38	1.01	3-Methyleicosane	C21H44
16.92	0.51	2-Myristynoyl pantetheine	C25H44N2O5S
17.35	0.68	Methyl 10-methylundecanoate	C13H26O2
18.95	0.52	Tetradecanoic acid, methyl ester	C15H30O2
19.29	0.54	Estra-1,3,5(10)-trien-17á-ol	C18H24O
19.47	0.48	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	C24H46O2
19.68	0.51	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	C24H46O2
20.37	3.74	Hexadecanoic acid, methyl ester	C17H34O2
21.52	2.67	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
21.96	3.44	Dasycarpidan-1-methanol, acetate (ester)	C20H26N2O2
22.14	15.59	Hexanedioic acid, bis(2-ethylhexyl) este	C22H42O4
22.92	0.52	3,4',5,6'-tetra-tert-butylbiphenyl-2,3'-diol	C28H42O2
23.2	28.18	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4

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23.8	0.44	Oleic acid, eicosyl ester	C38H74O2
24	0.47	9-(2',2'-Dimethylpropa noilhydrazono)-3,6-dic hloro-2,7-bis-[2-(dieth ylamino)-ethoxy]fluore	C30H42Cl2N4O3
24.14	0.48	Digitoxin	C41H64O13
24.75	3.6	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) este	C24H38O4
25.08	1.76	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) este	C24H38O4
25.4	4.06	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-hep tadeca--3,7,11,15-tetra enyl)-cyclohexanol	C30H52O
25.95	0.81	Cholesterol margarate	C44H78O2
26.44	0.63	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	C28H44O4
27.47	0.53	9,11,18-Trihydroxy-6,18-epoxypimara-5,8(1 4),15-trien-7-one, 2Ac derivative	C24H30O7
28.13	0.59	Ethyl iso-allocholate	C26H44O5

Discussion

The Controlling and combating of mosquitoes is considered as an urgent necessity to minimize prevalence of diseases which are transmit by mosquitoes. Dengue fever is one type of diseases transmits by *Ae. aegypti* mosquito. In the recent years as the resistance of mosquitoes to different industrial insecticides, the researchers tend to find natural insecticides from plant source are harmless and safe to humans and environment for control and combat mosquitoes. The chemical insecticides have undesirable side effects on environment, animal, humans and no-targets organisms (41,42). Also these chemical insecticides can cause carcinogenic, endocrine, and reproductive problems to human (43,44). Many plants have biological effect against mosquitoes with safe manner than the chemical insecticides (45). Around 1200 species of plants have mosquitocidal activity (46). From thousands of years, the natural products from plant origin played a basic role for treatment many diseases in human. The remedies by natural products based on different sources such as terrestrial plants, microorganisms, sea macro and microorganisms, terrestrial invertebrates and vertebrates. *Chrozophora* genus belongs to family Euphobiaceae are monoecious, shrubby herb and annual plants. The leaves, stems and fruits and whole plant of *Chrozophora* genus are used as food and traditional medicine for treatment of many diseases. The *Chrozophora* species have high content of protein and oil with high percentages of fatty acids (47). The *Chrozophora oblongifolia* is one of the *Chrozophora* genus plants have biological activity used as antimicrobial, emetics, cathartic and for treatment diverse of ailments (48). The immature fruits of *C. oblongifolia* are used for treatment of ulcers, blisters, burns and anal fissures (49). In Egypt, *C. oblongifolia* plant is used in folk medicines for antidiabetic because it has hypoglycemic activity. In Sudan, the stem and leaves extract is used for treatment of the bacterial infection, gonorrhea (50). Methanol extract of *C. oblongifolia* parts exhibited highest antioxidant, flavonoids and hepatoprotective activities. It is considered as a valuable biological source for drugs enhances fertility (51). In this manuscript, we investigated the methanolic and acetone leaves extracts of *C. oblongifolia* against 3th larva stars of the dengue vector, *Aedes aegypti* mosquito as well against the pathogenic microbes, *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Methanol and acetone leaves extracts had variable lethal toxic effects against life stages of

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Aedes aegypti mosquito at various concentrations. The used extracts had highest mortality percent against larvae at concentration of 1000 ppm by 100 % (Table 1&2 ,Figure 2). At concentration of 1000 ppm, the pupae, adult emergency and adult stages were disappeared due to completed death of larvae. This indicated that both methanol and acetone extract had larvicidal, pupacidal, and adultcidal at 1000 ppm (Table 1&2 ,Figure 2). At the concentrations of 800 ppm, 500 ppm, and 250 ppm, methanol had larval mortality with 80.00±0.00 %, 72.00±0.00%, and 62.00±5.00 % respectively (Table 1, Figure 2) while acetone extract showed larval mortality by 82.00±2.00 %, 67.33±2.00 % and 55.00±2.00 % respectively (Table2, Figure2). At low concentration 125 ppm, methanol and acetone extract had not significant effect on larvae because the larval mortality percent did not exceed 50 % (Table1&2, Figure2). The duration time of acetone extract against larva at 800 ppm showed short time with 5.33±1.00 day (Table 2) compared to methanol extract with 6.33±1.00 day (Table 1). Rates of pupation were increased gradually at the concentrations of 800 ppm,500 ppm, 400 ppm, and 200 ppm due to decreasing in larval mortality at both extracts effect (Tables 1&2, Figure 3). At 800 ppm, both extracts had not significant effects on pupae life because the pupal mortality percent did not exceed 50% (Tables 1&2, Figure 4). At concentration of 800 ppm, Acetone extract had a 50 % percent mortality against adult mosquito by 50.00±5.00% (Table 2, Figure6) compared to methanol extract with 35.00±0.00%. (Table 1, Figure 2). Although the acetone extract represented 50% toxicity against adult mosquito but this value did not exceed the 50% as a significant toxicity against adult mosquito (Table2, Figure6). However methanol extract revealed more effective with LC50 339.87 ppm (Table 3 , Figure7) compared to acetone extract with LC50 372 ppm (Table 3,Figure 8`). The obtained results indicated that methanolic extract had more biological effect compared to acetone extract, this may be due to its polarity with high solubility for extracting various polar compounds such as flavonoids, terpenoids, phenolic compounds and vitamins (52, 53). The microbial resistance to antimicrobial agents is became a critical health problem around the world. In US, two million people acquired bacterial infections in hospitals each year and 70% of cases included strains have resistant to at least one drug (54). So that it was necessary to get a new antimicrobial agent from plant origin able to inhibit and overcome multi-drug resistant microbes to solve microbial resistance of antimicrobial agent and minimize or stop mortality rates. The methanol extract of *C. oblongifolia* leaves exhibit antimicrobial activity against the pathogenic bacteria, *S. aureus* and *E. coli* and the yeast, *Candida albicans*. The MIC against *S. aureus* and *E. coli* was 5000 ppm (Table 4, Figure 10 &11) while against *C. albicans* was 10000 ppm (Table 4, Figure 12). The flavonoids in plant are known to have antibacterial, antiviral, antifungal and insecticidal activity (55). Many studies are reported that *C. oblongifolia* has flavonoid compounds, therefore it has antimicrobial activity (56). Our phytochemical analysis of methanol leaves extract of *C. oblongifoli* plant by GC.MS indicated more than twenty chemical compounds (Table5). Out of these analyzed components, nine prevailing major compounds are revealed (Table 6, Figure 13). The literatures exhibited that the major identified compounds have various biological activities (Table 6). The biological activities of these nine major identified compounds are tabulated in the table 6 as follow, the compounds, Hexanedioic acid, bis(2-ethylhexyl) ester and Hexanedioic acid, bis(2-ethylhexyl) ester have antioxidant and antibacterial activity (31). 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca--3,7,11,15-tetraenyl-cyclohexanol has antidiabetic effect (32). Hexadecanoic acid, methyl ester has antimicrobial activity (33). The compounds, 1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester and 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester have anticancer, larvicidal, antibacterial, antifungal, antimutagenic and cytotoxic effect (34,35, 38, 39). Dasycarpidan-1-methanol, acetate (ester) has antioxidant, anti-inflammatory, antibacterial, antimicrobial anticancer, angiogenesis and analgesic property (36). The compound, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- has antioxidant, antimicrobial, anticancer, and anti –inflammatory activity (37). The compound, 3-Methyleicosane has antifungal, antimicrobial, anti-inflammatory, antioxidant and cytotoxic effect. (40). The methanol leaves extract of *C. oblongifoli* plant exhibited larvicidal effect may be due to it has the compounds, 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester and 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester. In additionally the antimicrobial activity of methanol extract against the tested

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pathogenic microbes, *S. aureus*, *E. coli* and *C. albicans* may be due almost nine prevailing major compounds (Table 6, Figure 13) except the compound,2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca--3,7,11,15-tetraenylcyclohexanol which has antidiabetic effect (Table 6, Figure 13). The major prevailing compounds identified by GC-MS analysis had high larvicidal effect against larvae of dengue vector, *Aedes aegypti* mosquito as well as antimicrobial activity against the pathogenic microbes, *S. aureus*, *E. coli* and *C. albicans*. It is suggested that *C. oblongifolia* plant could be used as a safe natural insecticide to combat and control of *Ae. aegypti* mosquito instead of harmful synthesized insecticides for minimize outbreak of dengue disease as well could be used as a natural antimicrobial agent for treatment and overcome the multi-drug resistant microbes, *S. aureus*, *E. coli* and the yeast, *C. albicans*. Further study can be conducted to isolate and purify the natural larvicidal and antimicrobial compounds from the medicinal plant, *Chrozophora oblongifoli*.

Table 6. The biological activity of nine major prevailing compounds analyzed by GC-MS..

Sr. no.	Compound	Peak Area %	Biological activity
1	Hexanedioic acid, bis(2-ethylhexyl) ester	28.18%	Antioxidant and antibacterial activity (31)
2	Hexanedioic acid, bis(2-ethylhexyl) ester	15.59 %	Antioxidant and antibacterial activity (31)
3	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca--3,7,11,15-tetraenyl-cyclohexanol	4.06 %	Antidiabetic effect (32)
4	Hexadecanoic acid, methyl ester	3.74%	Antimicrobial (33)
5	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	3.6 %	Anticancer, larvicidal, antibacterial, antifungal, antimutagenic and cytotoxic effect (34, 35)
6	Dasycarpidan-1-methanol, acetate (ester)	3.44%	Antioxidant, anti-inflammatory, antibacterial, antimicrobial anticancer, angiogenesis and analgesic property (36)
7	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	2.67%	Antioxidant, antimicrobial, anticancer, and anti –inflammatory activity (37)
8	1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	1.76%	Antimicrobial and larvicidal (38, 39)
9	3-Methyleicosane	1.01 %	Antifungal, antimicrobial, anti-inflammatory, antioxidant and cytotoxic effect. (40)

Conclusion

The viral disease, Dengue fever is became a public health problem worries the world as increasing in the deaths annually. This disease is transmit to human by bites of *Aedes aegypti* mosquito. Therefore control and combat of *Aedes aegypti* mosquito is an urgent necessity to minimize prevalence of dengue. The resistant of almost mosquitoes to chemical insecticides and side effects of these chemical compounds tend the researchers to find safe natural insecticides from plant source to control and combat of mosquitoes. In the current study, it concluded that methanol leaves

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of *Chrozophora oblongifolia* tree exhibited more effective against *Aedes aegypti* mosquito life stages compared to acetone extract with LC50 339.87 ppm and LC90 830.06 ppm. Additionally, because present of new strains of multi-drug resistant microbes it became there is an urgent work to find a new antimicrobial agent from plant origin able to overcome the multi-drug resistant microbes. Herein also it concluded that the methanol extract of *C. oblongifolia* leaves showed inhibition effect on the growth of pathogenic microbes *S. aureus*, *E. coli* and *C. albicans.* The major chemical compounds identified by GC MASS analysis revealed high larvicidal activity against the larvae of the dengue vector, *Aedes aegypti* mosquito as well as antimicrobial activity against the pathogenic microbes, *S. aureus*, *E. coli* and *C. albicans* It is demonstrated that this *Chrozophora oblongifolia* plant could be used as a natural insecticide against the dengue vector *Ae. aegypti* mosquito as well as a natural antimicrobial agent against pathogenic microbes.

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Data Availability Statement: Upon request, the appropriate author can supply the data utilized in this investigation.

Competing Of Interest: The authors are declare no competing interest

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