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Larvicidal Activity of Ethanol Extracts of Mixtures of Some Egyptian Plants against *Culex quinquefasciatus* Say (Diptera: Culicidae) Larvae Collected from Zaria in Nigeria

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ABSTRACT

Preliminary phytochemical screening studies of ethanol extracts of three mixtures of Egyptian plants (1- Ethanol extract of mixture of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum* and *Cassia fistula*) in equal ratios, 2- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia Carterii*) in equal ratios and 3- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia Carterii*) in equal ratios except *Caryphyllus aromaticus* in a double ratio compared to the other four plants) showed that, all these extracts are rich in flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances, with special reference to mixture number. 3. Estimation of total phenolic contents of ethanol extractas of these mixtures under investigation revealed also that, these extracts are rich in phenolics, with special reference to mixture number.3 (50.833±0.003 mg/ml). Results indicated that, all ethanol extracts of these mixtures under investigation are potent larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) against the 3rd instar larvae of *Culex quinquefasciatus*, with special reference to all examined concentrations of ethanol extracts of mixture number.3 (% of mortality=100±0.000, 100±0.000, 100±0.000, 97.778±0.001 and 86.667±0.001 respectively, LC_{50}=1.000±0.001 and LC_{80}=1.600 ±0.003 mg). This effect is dose dependent in all used concentration of ethanol extracts of these three examined mixtures under investigations. LC_{50} and LC_{80} of ethanol extracts of these mixtures under investigation against the larvae are ranged between 1.000±0.001 to 1.895±0.002 and 1.600±0.001 to 3.033±0.001 mg respectively. It could be concluded that: These mixtures could be alternative larvicidal agents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects.

Key words: Larvicidal activity, *Culex quinquefasciatus*, Egyptian Plants, Mixtures, Phenolics, Phytochemical Screening.

1. INTRODUCTION

Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insect well known for their public health importance. Mosquito borne diseases are still a major problem in the world particularly in tropical and subtropical regions and WHO has declared the mosquitoes as "Public enemy number one". They are still representing the world's number one vector of human and domestic animals comprising approximately 3500 species (Rajan and Dhivya, 2018). Although it was highly efficient against the target species, insecticide applications are facing numerous threats due to the development of resistant strains. Other undesirable effects include hazardous effects against non-target animals, environmental problems and human health concerns (Farag *et al*., 2018). Lymphatic filariasis stands next to malaria as the most important vector-borne disease in India. *Culex quinquefasciatus* (*Cx. quinquefasciatus*), a vector of lymphatic filariasis affects 119 million people

living in 73 countries. A successive change in the insecticides result in multiple insecticides resistant was developed for vectors. The phytochemicals derived from plant resources can act as larvicides, adulticides, repellents, and ovipositional attractants, having deterrent activities observed by different researchers and may be alternative sources of mosquito larval control agents (Kamaraj, C. and Abdul Rahuman, 2010). Ethanolic extracts of leaves of different species of *Artemisia* showed toxic effects against *Culex quinquefasciatus* larvae (Masotti *et al.,* 2012). Ethanol and hexane crude extracts of *Cassia fistula* reduce pupation, egg production, hatchability and increased the percentage of sterility in the cotton leaf worm, *Spodoptera littoralis*. The efficacy of the fruit pulp extracts of *Cassia fistula* Linn (Caesalpiniodae: Leguminosae) extracted with three solvents (viz. water, acetone and n- hexanes) was studied against the 4th instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae) in the laboratory. Larval mortality was observed after 36 hours (khan *et al.,* 2017). In spite of the antimosquito activities of several studied species of plants, relatively little work has been done on the larvicidal activities of essential oils extracted from spices, such as clove and cinnamon. A recent study carried out in Nigeria assessed the activity of clove essential oils against *Aedes aegypti* and *Culex. quinquefasciatus* and achieved over 85% larval mortality within 24-hours post-exposure (Thomas *et al.*, 2017). *Boswellia sp.* extracts of acetone, chloroform and ethanol were tested against the eggs of *Culex pipines* at different concentrations. Results revealed that, acetone extract of *B. sacra* possessed strong ovicidal activity. Phytochemical profiling of the extracts showed the presence of many secondary metabolites, which might be reason for its high efficacy (Rajan and Dhivya, 2018).*Syzygium aromaticum* (Clove) essential oil has also shown larvicidal activity against field collected larva of *Aedes aegypti* with LC_{50} of 92.56 and 62.3 ppm in two different reports. Use of clove essential oil as a green larvicide against *Anopheles stephensi*is preferred compared with its major constituent; Eugenol (Osanloo *et al.,* 2018). Crude and chloroform: methanol (1:1 v/v) extracts of some common spices (*Cuminum cyminum* and others) can be recommended effectively in mosquito control programs. Crude plant extracts were more cost effective and may be employed in localized situation. Chloroform: methanol (1:1 v/ v) extract of these materials were very effective as mosquito larvicide at very low concentration (Singha, S. and Chandra, G., 2011). As compared with the herbal extract, *Moringa oleifera* seed extract also act as larvicidal agent and studies have been reported on water extracted *M. oleifera* seeds (WEMOS) against *Aedes aegypti* larvae and methanol-extracted *M. oleifera* roots against *Culex quinfasciatus* and *Aedes albopictus*. The obtained larval mortality may be due to active chemical compounds present in *M. oleifera*. Highest larval mortality was observed at highest dose (100 mg/l) concentration i.e. 93.33±0.58 % at 48 h of exposure time in leaf extracts of *Moringa oleifera* against *Anopheles stephensi*. Larval mortality was highest in 48 h (86.67±0.50 per cent) of exposure time as compared to the 24 h (83.33±0.58 per cent) in 80ml/l concentration (Sharma *et al*., 2013). The promising essential oils, with larvicidal activity demonstrating LC_{50} ranging between 1-258.5 ppm, are derived from a large number of plants, including *Pimpinella anisum, Cuminum myrrham, Cinnamomum camphora*, *Syzigium aromaticum* and others. *Syzigium aromaticum* was found to contain 1.5 % essential oils; these oil was found to have larvicidal activity against *Aedes aegypti* mosquitoes. This larvicidal activity demonstrating LC_{50}, LC_{95} and LC_{99} with values 124.690, 179.720 and 220.600 ppm respectively (Sutthanont *et al*., 2010). Black seed oil, *Nigella sativum* induced 3, 10, 23, 33 and 50% larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively against *Culex pipiens*. After 48 h of exposure, this oil elicited 7.22, 14.34, 30.93, 45.36 and 76.92 % larval mortality 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. While after 72 h of exposure, it induced 7.45, 22.34, 36.17, 64.89 and 100 larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively (Abo El-Mahasen, M. M.And Mahmoud, S. H., 2016). The peel powder of *Punica granatum*, extracted with petroleum ether, was proved to have potential toxicological effects against third instar larvae of *Culex pipiens*. The median lethal dose (LC_{50}) value was found to be 95.6632 ppm. Qualitative phytochemical screening of pomegranate peel extract was assessed by standard methods. The phytochemical constituents present in petroleum ether extract of *Punica granatum* peel were phenols and saponins. The outcome data proved that petroleum ether extract of pomegranate peels is a promising ecological friend mosquito larvicide (Farag *et al*., 2018). Pomegranate contains high levels of phytochemicals including polyphenols, sugars, fatty acids, aromatic compounds, amino acids, tocopherols, sterols, terpenoids and alkaloids (Taher *et al.,* 2012). Phytochemical screening of the *Trigonella foenum-graceum* leaves extract shows the presence of alkaloids, flavonoids, saponins, tannin, glycosides and steroid. The results showed that *T. foenum-graceum* leaves extract has significant larvicidal activity against *Aedes aegypti* and *Anopheles stephensi*. The results of larvicidal activity of *T. foenum-graceum* leaves

extract against *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* for the duration of 24 and 48 hours were showed that, the highest mortality (100%) was detected against *A. aegypti* at the both 24 and 48 h. Significant activity was observed against *A. stephensi* (93-97% after 24 and 48 h exposure). *T. foenum graceum* leaves extract exhibits considerable (59-80%) larvicidal activity against *C. quinquefasciatus* (Prabakaran, K. and Rajalakshmi, S., 2018). Three mixtures of Egyptian plants (1- Ethanol extract of mixture of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) in equal ratios, 2- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios and 3- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios except *Caryphyllus aromaticus* in a double ratio compared to the other four plants) will be studied in this work regarding their phytochemical constituents and larvicidal activity against the 3rd instar larvae of *Culex quinquefasciatus*in order to introduce new efficient larvicidal botanical products.

2. MATERIAL AND METHODS

2.1. Plant materials:

Ten Egyptian plants were collected from the Egyptian markets to be studied after mixing them in equal ratios (Mixture number. 1).

Table - 1: Plants included in mixture number 1.

Sample name	Used Parts
Artemisia monosperma	Shoot systems
Nigella sativum	Seeds
Syzygium aromaticum (Caryphyllus aromaticus)	Fruits
Cassia fistula	Fruits
Cinnamomum sp.	Stem bark
Punica granatum	Peels of Fruits
Moringa oleifera	Seeds
Trigonella foenum graecum	Seeds
Pimpinella anisum	Seeds
Cuminum cyminum	Seeds

Five Egyptian plants were collected from the Egyptian markets to be studied after mixing them in equal ratios (Mixture number. 2). Meanwhile Mixture number.3 is composed of the same five Egyptian plants included in the mixture number. 2 in equal ratios except *Caryphyllus aromaticus,* which is added in a double ratio compared to the other four plants.

Table - 2: Plants included in mixtures number 2 and 3.

Plant Number	Used Parts
1-Cassia fistula	Fruits
2-Artemisia monosperma	Shoot systems
3- Cinnamomum sp.	Stem bark
4-Syzygiumaromaticum (Caryphyllusaromaticus)	Fruits
5-Boswellia Carterii	Gum

2.2. Phytochemical studies

2.2.1. Extraction:

All samples are cleaned, air dried and extracted by ethanol (80 %), then filtered; each 1 ml of ethanol extract of different mixtures contains 50 mg Dry weight (Alam, A. E., 2019). These ethanol extracts of these mixtures were studied regarding their phytochemical screening, total phenolics and larvicidal activity.

2.2.2. Preliminary Phytochemical Screening:

Carbohydrates and/ or Glycosides:

The ethanolic extract (5ml) was mixed with 0.5 ml of ethanolic α- naphthol reagent, then 1ml of sulphuric acid was carefully poured on the walls of the test tube. A violet ring was formed at the interface indicating the presence of carbohydrates and / or glycosides (Stank *et al.,* 1963).

Saponins:

Saponins were determined according to the methods adopted by Hungund and Pathak, (1971).

Forth test:

About 3 grams of the dried sample were extracted with boiling water then filtered. After cooling, the aliquot was shacked vigorously until forth was obtained, then allowed to stand for 15-20 minutes and classified according to their saponin contents (No forth means negative, forth less than 1cm height = weakly positive and forth 1-2 cm or higher means positive).

Blood haemolysis test:

About 5 grams of the dried sample were extracted with hot ethanol (95%). One ml aliquot portion was added to 10 ml of 1:4 suspensions of

erythrocytes in physiological saline solution and haemolysis was observed indicating the presence of saponins.

Tannins:

About 5 grams of the dried sample were extracted with ethanol (50%) and filtered. The addition of ferric chloride reagent to the filtrate gave a green color, then changed to a bluish black color or precipitate indicates the presence of tannins (Trease and Evans, 1978).

Unsaturated sterols and / or Triterpenes:

The alcoholic extract (corresponding to 2 grams of the dried sample) was evaporated. The residue was treated with anhydrous chloroform (10 ml) and filtered; the filtrate was divided into two portions and subjected to the following reactions:

Liebermann- Burchardt's test:

To the first portion, 1 ml of acetic anhydride was added, followed by 2 ml of H_2SO_4 down on the wall of the test tube. If a reddish - violet ring was produced at the junction of two layers, then the solution become bluish- green in color in the acetic acid layer it indicates the presence of unsaturated sterols and / or triterpenes (Claus, 1967).

Salkowiskit's test:

To the second portion, an equal volume of sulphuric acid was added, if a red color was produced it indicates the presence of unsaturated sterols and / or triterpenes (Schmidt, 1964).

Alkaloids and / or Nitrogenous bases:

About 10 grams of the dried sample were extracted with 100 ml of dilute hydrochloric acid. The acidic extract was filtered, adjusted to be alkaline with ammonium hydroxide solution and extracted with chloroform. The chloroformic extract was evaporated to dryness and the residue was dissolved in about 2 ml of hydrochloric acid. The acidic solution gave faint brown precipitate with Wagner's reagent {1.3 grams of Iodine, 2 grams of Potassium iodide, dissolved in 100 ml dist. water} and very slight yellow precipitate with Mayer's reagent {1.36 grams of Mercuric chloride, 5 grams of Potassium iodide, dissolved in 100 ml dist. water }(Shellard, 1957).

Cardiac glycosides:

About 2 grams of the dried sample were boiled with 15 ml of 70 % methyl alcohol for five minutes and filtered. The filtrate was diluted with distilled water and 0.5 ml of concentrated solution of lead acetate was added (to remove chlorophyll and other pigments) and filtered, to remove the excess of lead acetate, H_2SO_4 (10%) was added drop wise until no further precipitate was formed, then filtered. The filtrate was extracted with 10 ml chloroform. The chloroform extract was evaporated to dryness and the following tests were carried out according to Balbaa *et al*., (1981).

Killer –Kiliani test :

About 1ml of ferric chloride solution (3.5 %) in glacial acetic acid was added to one portion of chloroform residue and left, concentrated sulfuric acid was added carefully down the wall of the test tube. On standing, a brown or red layer appeared at the interface (due to the aglycone) and the upper acetic acid layer becomes blue to green (due to desoxy sugar).

Kedde's reaction:

To another portion of the chloroform residue, 3.5-dinitrobenzoic acid (2%) in 90% methanol and one drop of NaOH (2%) were added. The solution acquired a violet color on standing.

Libermann's reaction:

The third portion of the chloroform residue was dissolved in glacial acetic acid, then acetic anhydride (2 ml) was added. Concentrated H_2SO_4 was added carefully down the wall of the test tube. On standing, two layers were afforded, pink color (upper layer) and green color (lower layer).

Flavonoids:

About 5 grams of the dried sample were soaked for one day with 150 ml of 1% HCl and filtered. The filtrate was tested for flavonoid compounds as follows:

About 10 ml of the filtrate were adjusted to be alkaline with sodium hydroxide. The formation of a yellow color indicates the presence of flavonoids. About 5 ml of the filtrate were mixed with 5ml HCl and small pieces of magnesium metal (0.5 g). The formation of red color after 3 minutes, indicates the presence of flavonoids (Mabry *et al* ., 1970).

Anthraquinones:

About 2 grams of the dried sample were boiled for few minutes with 0.5 N KOH (10 ml) to which 1ml of diluted H_2O_2 was added. After cooling, the mixture was filtered and acidified, then extracted with benzene (10 ml). The benzene extract was shacked with NH_4OH (5ml). The presence of anthraquinones was indicated by the formation of red color in the alkaline layer (Farnsworth *et al.,* 1969).

Coumarins:

A small amount (5 g) of the moistened dried sample was placed in a test tube that covered with a filter paper moistened with diluted NaOH (0.1 N) solution. The tube was then removed and examined under U.V. light and any fluorescence is indicated for the presence of coumarins (Feigl, 1960).

Irodoids:

About 2 grams of fresh samples were cut into small pieces and placed in a test tube with 5 ml of 1% aqueous HCl. After 3-6 hours, 0.1 ml of the macerate was decanted into another tube containing 1 ml of the Trim and Hill reagent (10 ml acetic acid, 1 ml 0.2 % Cu SO_4 in water and 0.5 ml conc. HCl). When the tube is heated for a short time on a flame, a blue color is produced if a certain irodoid is present (Weiffering, 1966).

Chlorides and Sulphates:

Chlorides and Sulphates were determined according to the methods adopted by Islam *et al*., (1993).

Chlorides:

Silver nitrate solution gives with a solution containing chlorides a white flocculent precipitate of silver chloride which dissolves in ammonium hydroxide solution and does not dissolve in dilute nitric acid.

Note: The color of the precipitate changes gradually in direct sunlight to violet.

Sulphates:

Barium chloride solution gives a white precipitate of barium sulphate which does not dissolve in mineral acids.

Sublimation:

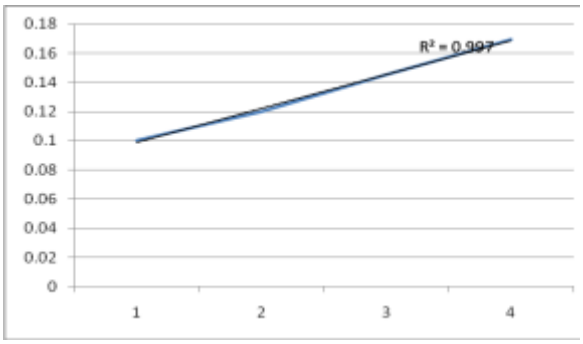
One gram of each sample was carefully subjected to microsublimation in dry crucible, covered with a clean slide. Dark yellowish-brown fumes were evolved and condensed on the lower surface of a slide as a dark brown oily condensate which dissolved in potassium hydroxide solution producing red color indicating the presence of anthraquinons (Afifi, 1972).

2.3. Assay for total phenolics:

Total phenolics were estimated using following the method of Gursoy *et al.,* (2009) involving Folin–Ciocalteu reagent and Gallic acid as standard. 1 ml of ethanol extract of each mixture (contains 50 mg Dry Weight) was added to 1 ml Folin–Ciocalteu reagent in a volumetric flask then 45 ml distilled water was added. The flask was shaken vigorously. After 3 minutes, 3 ml of Na_2 CO_3 (2%) solution was added and the mixture was allowed to stand for 2 hours by intermittent shaking. Each sample was done in triplicate. Absorbance was measured at 760 nm (by using UV 2401 Pc, UV-VIS recording spectrophotometer, Shimazu, Germany). Concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard Gallic acid graph.

The calibration curve of reference standard (Gallic acid) was made using four different concentrations.

Absorbance = 0.0167 Gallic acid (µg) + 0.017 (R^2 = 0.997).



1= GA (25 ppm), 2= GA (50 ppm),

3= GA (100 ppm), 4= GA (200 ppm).

Figure – 1: Standard curve of Gallic acid (Total phenolic contents).

2.4. Larvicidal Activity Studies:

Study Location:

This study was carried out at Zaria, Nigeria, located at Latitude 11.085541 and Longitude 7.719945. Zaria is an old large city formerly called Zazzau, situated in the central part of Nigeria, in the state of Kaduna. The area is known for its hot climate; however the city is a center of agriculture and cultivating a few local crops important for national economy. The population of Zaria is about 700,000 people, and it is one of the most crowded cities in the country. There is a large university, Ahmadu Bello University, in the city, which is considered to be one of the best higher educational establishments in Nigeria.

Tested mosquito:

Adult *Culex quinquefasciatus* Say (Diptera: Culicidae) mosquitoes were trapped using test tube from class rooms in the main campus of Ahmadu Bello University, Zaria, Nigeria. Collected samples of mosquito species were transported to the Entomology and Parasitology Laboratory of Zoology at Ahmadu Bello University in plastic containers. The adult *Culex quinquefasciatus* Say (Diptera: Culicidae) mosquitoes were released

into the Entomological Cages directly containing 200ml of tap water in a 700 ml of bowl plastic containers for oviposition. Blood fed female mosquitoes laid eggs on water which hatched into larvae and were identified up to species level using keys developed by Hopkins (1952). Cyclic generations of the mosquito species were sustained as described by Raveen *et al.* (2014) using restrained quail birds in the cages.

Larvical Bioassays:

The guidelines for laboratory and field testing of mosquito larvicides recommended by WHO (2005) with little modifications were followed in this study. The larval tests were conducted in plastic bowl (700 ml). A series of concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) from the stock solutions of ethanol extracts of three mixtures of investigated Egyptian plants were prepared. Fifteen larvae in triplicates (the 3rd instar larvae) were introduced into each plastic bowl containing 100 ml (10 ml of each tested extract were added to 90 ml of tap water). Mortality was observed for 24 hours after treatment. The larvae were considered dead when they showed no sign of movement when probed using a needle (Raveen *et al.*, 2014). Tap water was used as untreated control (C). The percentage of mortality of larvae was calculated using the following equation:

% Mortality =

$$\frac{\text{Number of dead larvae in a treatment} - \text{Number of dead larvae in a control}}{\text{Total number of larvae in a treatment}} \times 100$$

Additionally $LC_{50}$and LC_{80}were calculated also for each treatment.

2.4. Statistical analysis:

Statistical analysis was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5 and 1% probability level to determine the differences among treatment means (Steel and Torrie, 1984). The CO-STAT computerized package program was subjected to the regular statistical analysis of variance (Nissen *et al.,* 1985), using two designs - 1- Anova-1 completely randomized design (CRD) - 2- Factorial implemented in completely randomized design. Each reading = mean of three replicates + SE for all experiments.

3. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical Screening:

Data in Table. 3 represented preliminary phytochemical screening of ethanol extract of three mixtures of some Egyptian plants. Results revealed that, these extracts are rich in flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances, with special reference to the mixture number. 3.

(1- Ethanol extract of mixture of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) in equal ratios, 2- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios and 3- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios except *Caryphyllus aromaticus*in a double ratio compared to the other four plants).

Table – 3: Preliminary phytochemical screening of ethanol extracts of three mixtures of Egyptian plants.

Experiment	1	2	3
1-Carbohydrates and/or Glycosides	++	++	++
2-Tannins	++	++	++
3-Anthraquinones	++	++	++
4-Sublimable Substances	++	++	++
5-Flavonoids	++	++	++
6-Unsaturated sterols and/or Triterpenoids	++	++	++
7-Alkaloids	++	++	++
8- Saponins	++	++	++
9-Cardiac Glycosides	++	++	++
10-Iridoids	++	++	++
11-Chlorides	+	+	++
12-Sulphates	++	++	++
13- Coumarins	++	++	++

3.2. Assay for total phenolics:

Data in Figure. 2 represented total phenolic contents of ethanol extract of these three mixtures under investigation. Results revealed that, these extracts are rich in phenolics, with special reference to mixture number.3 (50.833±0.003 mg/ml), followed by mixture numer.1 (47.667±0.002 mg/ml). Meanwhile the

least phenolic contents were found to be in mixture number.2 (15.667±0.002 mg/ml).

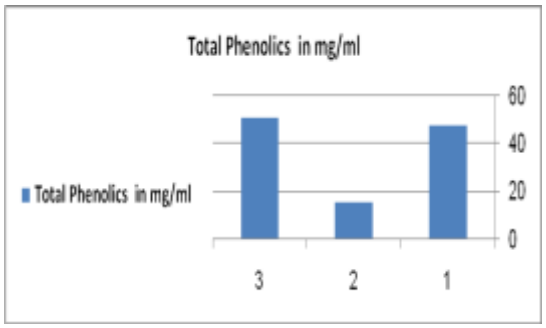


Figure.2: Assay for total phenolics (in mg/ml) of ethanol extracts of three mixtures of Egyptian plants.

(1- Ethanol extract of mixture of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) in equal ratios, 2- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios and 3- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios except *Caryphyllus aromaticus* in a double ratio compared to the other four plants).

3.3. Larvicidal Activity Studies:

Results in Figures 3 and 4 indicated that, all ethanol extracts of the three mixtures under investigation are potent larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml)against the 3rd instar larvae of *Culex quinquefasciatus*, with special reference to all examined concentrations of ethanol extracts of mixture number.3 (% of mortality=100±0.000, 100±0.000, 100±0.000, 97.778±0.001 and 86.667±0.001 respectively, LC_{50}=1.000±0.001 and LC_{80}=1.600 ±0.003 mg), followed by all examined concentrations of ethanol extracts of mixture number. 1 (% of mortality=100.000±0.000, 71.111±0.000, 57.778±0.000, 57.778±0.001 and 57.778±0.001 respectively, LC_{50}=1.406±0.001 and LC_{80}=2.250 ±0.003 mg). Meanwhile all examined concentrations of ethanol extracts of mixture number. 2 have the least larvicidal effect against the larvae (% of mortality=100.000±0.000, 91.111±0.002, 26.667±0.001, 24.444±0.001 and 13.333±0.001 respectively, LC_{50}=1.895±0.002 and LC_{80}=3.033 ±0.003 mg). This effect is dose dependent in all used concentration of ethanol extracts of these three examined mixtures under investigation.

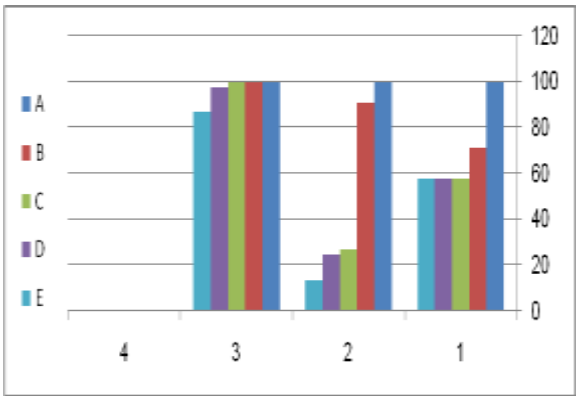


Figure - 3: Larvicidal activity (% of died larvae) of 1- Ethanol extract of mixture of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) in equal ratios, 2- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios and 3- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios except *Caryphyllus aromaticus* in a double ratio compared to the other four plants and 4-Control and A= 5.000 mg/ml, B= 2.500 mg/ml, C=1.250 mg/ml, D= 0.625, E=0.3125 mg/ml.

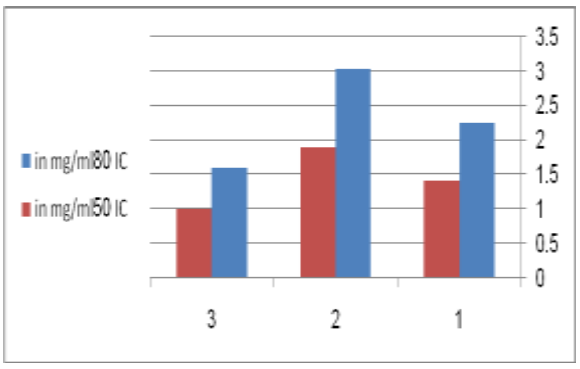


Figure.4: Larvicidal activity (LC_{50} and LC_{80}) of 1- Ethanol extract of mixture of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) in equal ratios, 2- Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp.,*

Syzygium aromaticum (Caryphyllus aromaticus) and *Boswellia carterii*) in equal ratios and 3-Ethanol extract of mixture of five Egyptian plants (*Cassia fistula, Artemisia monosperma, Cinnamomum sp., Syzygium aromaticum (Caryphyllus aromaticus)* and *Boswellia carterii*) in equal ratios except *Caryphyllus aromaticus* in a double ratio compared to the other four plants and 4-Control and A= 5.000 mg/ml, B= 2.500 mg/ml, C=1.250 mg/ml, D= 0.625, E=0.3125 mg/ml.

These results are agreed with findings of others related to the studied larvicidal and insecticidal activity of plants included in the studied mixtures (Prabakaran, K. and Rajalakshmi, S., 2018, Farag *et al*., 2018,Rajan and Dhivya, 2018, Osanloo *et al.,* 2018, khan *et al.,* 2017,Thomas *et al.*, 2017, Abo El-Mahasen, M. M. and Mahmoud, S. H., 2016, Sharma *et al*., 2013, Masotti *et al.,* 2012,Taher *et al.,* 2012, Singha, S. and Chandra, G., 2011 and Sutthanont *et al*., 2010).

4. CONCLUSION:

In the light of the obtained results, all studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) of ethanol extracts of these three mixtures are potent larvicidal agents (LC_{50} and LC_{80} of ethanol extract of these mixtures under investigation against the 3rd instar larvae of *Culex quinquefasciatus* are ranged between 1.000±0.001 to 1.895±0.001 and 1.600±0.001 to 3.033±0.001 mg respectively). These results can be explained on the basis that these ethanol extracts of these mixtures are rich in total phenolics and phytochemical screening of these extracts indicating the presence of flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in these extracts in good quantities. These mixtures could be alternative larvicidal agents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects.

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