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# PHENOLIC CONTENT, ANTIOXIDANT POTENTIAL AND *AEDES AEGYPTII* ECOLOGICAL FRIEND LARVICIDAL ACTIVITY OF SOME SELECTED EGYPTIAN PLANTS

By

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**Abstract**

Polyphenols constitute a distinct group of natural compounds of medicinal importance exhibiting wide range of physiological activities as antioxidant, immunestimulant, antitumor and antiparasitic. Yellow fever and dengue fever are mosquito-borne infectious diseases transmitted by *Aedes aegyptii*, the presence of yellow fever in Sudan and dengue fever in Saudi Arabia are threats to Egypt with the reemerging of *Ae. aegyptii* in Southern Egypt, larvae control is feasible than flying adults. This work was conducted targeting estimation of the relative levels of total phenolic content, antioxidant potential and larvicidal activity of 110 selected Egyptian plants. The highest total phenolic contents were estimated in aqueous extracts of *Coronilla scorpioides* L., *Forsskaolea tenacissima* L., *Crataegus sinaica* Boiss., *Pistacia khinjuk* Boiss. and *Loranthus acacia* Benth.**;** they were 916.70±4.80, 813.70±4.16, 744.90±4.93, 549.00 ±3.93& 460.80±4.02 mg% while those of methanol extracts were estimated in *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Loranthus acacia* and *Pistacia khinjuk*; they were 915.60±4.86, 664.60±4.16, 659.30±4.80, 590.80±4.49 & 588.00±3.85 mg% respectively. Investigation of the antioxidant potentials revealed that the most potent plants were *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Pistacia khinjuk* and *Loranthus acacia* with calculated values of 454.80±4.83, 418.4±4.16, 399.10 ±4.90, 342.5±2.72 & 239.7±2.91% for aqueous extracts and 452.9±4.94, 389.6 ±4.6, 378.48±3.84, 352.3±3.06 & 346.5±2.98% for methanol extracts respectively while screening of larvicidal activity proved that *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Pistacia khinjuk* and *Loranthus acacia* exhibited highest potency calculated as 22.53±2.01, 23.85±2.07, 28.17±2.06, 31.60±2.93 & 39.73±4.58 mg% aqueous extracts and 18.53±1.95, 18.8±1.67, 20.17±1.85, 23.28±2.7 & 28.48±3.9 mg% methanol ones respectively.

**Keywords:** Egypt**,** Antioxidants, Phenolic content, *Aedes aegyptii*, Yellow fever, Dengue virus, Larval control.

## Introduction

Natural products are derived from the phenomenon of biodiversity in which the interactions among organisms and their environment formulate these diverse complex chemical entities within the organisms that enhance their survival and competitiveness (Nurmikko *et al,* 2007), they are the main source for the majority of FDA-approved agents and are continued to be one of the major sources of inspiration for future drug discovery (Bhuwan *et al,* 2011). Phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, tannins) are one of the main free radical scavenging molecules in plants (Cai *et al,* 2003; Zheng and Wang, 2001). Epidemiological studies have shown that many of these compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Owen *et al,* 2000; Sala *et al,* 2002; Cushnie and Lamb, 2005; El-Hela *et al*, 2011). Numerous physiological and biochemical processes in human body may produce oxygen entered free radicals and other reactive oxygen species as by-products, the overproduction of such free radicals can cause oxidative damage to biomolecules as lipids, proteins and even DNA, eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans (Halliwell, 1994; Niki, 1997; Poulson *et al,* 1998).

The available synthetic antioxidants have been suspected of causing or prompting negative health effects, so strong restrictions encountered their application and there is an urgent trend to substitute them with naturally occurring antioxidants (Chu, 2000; Hosny and Rosazza 2002; Molyneux, 2004) while the intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with age which have the advantage of being almost devoid of side effects (Yang *et al,* 2001; Sun *et al*, 2002).

Yellow fever is endemic in 34 countries of Africa with a combined population of 468 Million (WHO 1996). Dengue fever and dengue hemorrhagic fever are vector-borne diseases of public health importance in tropical, subtropical, and temperate regions of the world infecting millions of people annually (Gubler 1998; Jacobs 2000; Pancharoen *et al,* 2000), dengue vaccine is not available, and the only effective vector intervention involves well organized larval control measures (Swawudhipong *et al,* 1992; Gratz, 1993; Kantachuvessiri, 2002; Pancharoen *et al,* 2002).

The principal vector of dengue and yellow viruses is *Aedes aegyptii* L. (*Stegomia*), Diptera: Culicidae, whose eggs are resistant to desiccation as they remain quiescent during the dry seasons and hatch only when rain fills breeding places (Abdal-magid and Alhusein 2008; Husham *et al,* 2010).

The use of synthetic organic insecticides in larvae control around the world has resulted in damage to the environment, pest resurgence and toxic effects on non-target organisms (Ab-

udulai *et al,* 2001), in addition extensive use of chemical insecticides has made strains of the target insects resistant to most of them (Schaafsma *et al,* 1990) and so attention being diverted in favour of non-chemical methods for insect management.

More than 2,000 species of plants are known to have insecticidal properties (Klocke, 1989) while others have reported the bioactivity of extracts and essential oils from various plants against agricultural pests (Nagpal *et al,* 1996; Abdel-Hady *et al* 2005) and fortunately the plant derived insecticides encompasses an array of chemical compounds thus, the chance of insects developing resistance to such insecticides are less and also they are considered as ideal safe ecological friend insect controllers.

This perspective study aimed to the estimation of the total phenolic contents of the aqueous and methanol extracts of the 110 selected Egyptian plants to determine their antioxidant potentials as natural antioxidant drugs, and screening of the larvicidal activity of *Ae. aegyptii* targeting the discovery of natural ecological friend, cost-effective alternative to the harmful chemical insecticides.

### Material, Equipment and Methods

Plant material: The complete range of wild plant samples were collected at the flowering stage throughout Egypt, at random, from each of the five biogeographic Egyptian regions (Saint Catherine, South Sinai, 2008; Giza Zoo Garden, 2006; Garden of Faculty of Agriculture, Al-Azhar University, Nasr City, 2006; River Nile, 2007 and gardens of the National Gene Bank, the National Institute of Horticulture, Faculty of Agriculture, Cairo University, 2008). The plant samples were identified taxonomically by Dr. Mohamed Tantawy, Prof. of Botany, Faculty of Science, Ain-Shams University and Dr. Moneer Abd El-Ghany, Prof. of Plant Taxonomy, Faculty of Science, Cairo, University.

The collected samples were air-dried, powdered and kept in clean tightly closed amber coloured glass containers in a dark place at low temperature.

Voucher specimens were kept in the Herbarium Museum, Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University.

Material for determination of total phenolic content: Folin-Ciocalteu's reagent (Sigma Chemical Co., St. Louis, MO, USA), and Gallic acid (E. Merck, Darmstadt, Germany).

Material for determination of antioxidant effect: DPPH (Sigma-Aldrich Quimica South Madrid Spain), Silica gel 60-F254 (Merck, Darmstadt, Germany), Mobile phase [butanol: acetic acid: water (40:10:50)] and Butylated hydroxyl toluene (BHT): Sigma-Aldrich Quimica South Madrid Spain.

Material for determination of larvicidal effect: egg rafts of *Aedes aegyptii* were obtained from Aswan and Toshka, they were provided by last author.

Equipment: Soxhlet, Chromatographic glass jars, Rotatory evaporator (BUCHI Rotavapor® R-210/R-215, Germany), 96 Micro-well™ Plates, Conical

Wells, Thermo Fisher Scientific USA, Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for quantitative estimation of total phenolic content and Spectrophotometer (Perkin-Elmer Lambada 3) for quantitative determination of antioxidant effect.

Preparation of extracts: for the total methanol extract 50 g of each dried powdered plant under investigation was extracted separately by soxhlet for 24 h with methanol, after filtration, extracts were concentrated under vacuum then washed within hexane until the chlorophyll was completely removed; the washed methanol extracts were filtered and used for study while for the total aqueous extract 50 g of each powdered plant are infused in boiling distilled water set aside for 2 h, filtered, after filtration, extracts were concentrated under vacuum, washed within hexane where the washed aqueous extracts were used for study.

Determination of the total phenolic content: The concentration of total phenolic compounds in the methanol extract of each plant was determined spectrophotometrically using the Folin-Ciocalteu's reagent which is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic compounds and polyphenol antioxidants to (Singleton and Rossi, 1965; McDonald *et al,* 2001). Standard curve was done using different concentrations of gallic acid (10:60 mcg/ml) in methanol, the concentrated extracts of the tested plants were dissolved each in least methanol volume then completed to 10 ml, 100µl of each extract was separately diluted with 8 ml of distilled water. To each sample 0.5 ml of 50% Folin-Ciocalteu's reagent were added, left 8 min, and then 1.5 ml of 5% sodium carbonate was added, mixed and allowed to stand for 60 min. protected from light; their absorbance was measured at 725 nm using methanol as blank and the concentration of the total phenolic content of was calculated.

Determination of antioxidant potential: Determination of the antioxidant potential of each tested extract was done according to stable DPPH radical technique both qualitatively using thin layer chromatography (TLC) and quantitatively using the spectrophotometric method.

TLC assay: This was performed after Cavin *et al.* (1998) where 20µl aliquot of the tested extract was spotted on silica gel plates and developed using butanol: acetic acid: water (40:10:50) as a mobile phase, after development, the dried TLC plates were sprayed with 0.2% DPPH solution in methanol and examined after 30 min. active antioxidants compounds appeared as yellow spots against purple background.

Spectrophotometric assay: This was performed after Gialvez *et al.* (2005) where the test was carried out on 96 Micro-well plates. Standard curve was done using different concentrations of BHT (butylated hydroxytoluene) in methanol (7 serial 2 fold dilutions to give final range of 60 to 10µg/ml), 50µl of a 0.022% DPPH solution in methanol was added to a range solution of different concentrations (7 serial 3 fold solutions to give final range of

1000 to 1.3µg /ml of each extracts in methanol (230µl) and their absorbance were measured at 517nm after 30 min.

Determination of larvicidal activity: the egg rafts were reared in trays containing tape water and maintained at 28±2°C and when eggs were hatched into first instar larvae, they were fed with yeast powder and glucose, on the third day after hatching the first instar larvae moulted into second instar larvae and on the fifth day the third instar larvae were observed which moulted into fourth instar larvae the seventh day. The method of testing larvicidal action of the crude extracts was slightly modified from those of WHO (1996) where a stock solution was prepared by dissolving a known amount of the crude extract in an appropriate solvent and stored in a refrigerator at 15°C. Twenty healthy, late 3rd- 4th instar larvae were introduced into each testing cup (sterilized plastic drinking cup of 150 ml capacity), which contained 100 ml of de-chlorinated tap water, a measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of five concentrations, each with three replicates, with a final total number of 60 larvae for each concentration; each batch of replicates contained one control of 100 ml of tape-water and another of 100 ml of water containing a volume of solvent corresponding to the maximum volume of extract tested.

As very few larvae succumbed within 24 hours of exposure to the test solutions, mortality was recorded after 48 hour of exposure, during which no food was offered to the larvae.

The mortalities of the larvae were recorded if moribund larvae were incapable of rising to the surface or of showing the characteristic diving reaction when the water was disturbed or they showed discoloration, unnatural position or rigor. The LC50 was determined by a Probit analysis program (Finney, 1971) and mortality was estimated by Abbott formula (1925).

Statistical analysis: The outcome data was carried out using one way analysis of variance (ANOVA) followed by student t-test, P value <0.05 were considered significant **(**Elliott and Woodward, 2007**).**

## Results

The results are shown in table (1) and figures (1-4).

Fig. 1: Standard curve of gallic acid:

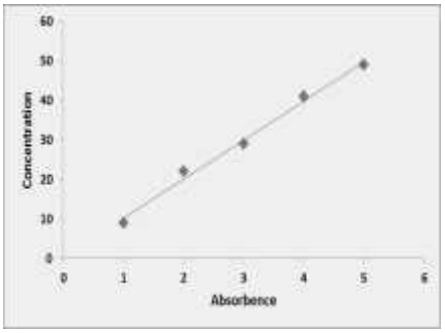


Fig. 2: Standard curve of butylated hydroxy toluene:

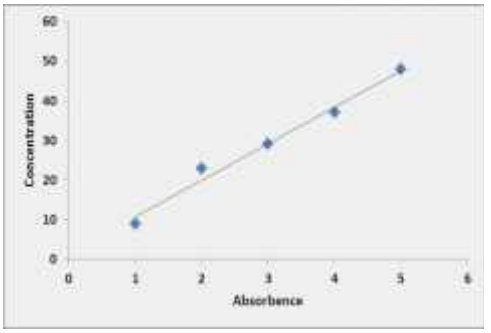

Table 1: Total phenolic content, antioxidant potential and larvicidal activity of the selected Egyptian plants:

| Plant name | Total Phenolic Content (mg %) | | Antioxidant Potential (%Scavenging) | | Larvicidal Activity ($LC_{50}$ mg %) | |
|---|---|---|---|---|---|---|
| | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract |
| **Acanthaceae** | | | | | | |
| *Blepharis ciliaris* Boiss. | 73.10±2.70 | 82.70±2.90 | 28.10±2.03 | 27.40±2.09 | 106.50±3.61 | 98.29±3.40 |
| **Aizoaceae** | | | | | | |
| *Aizoon hispanicum* L. | 48.30±1.93 | 15.10±1.40 | 25.00±2.63 | 12.10±1.03 | 113.82±3.70 | 100.57±3.30 |
| *Aizoon canariense* L. | 38.10±1.50 | 15.90±2.06 | 24.40±2.82 | 13.60±1.85 | 134.40±4.64 | 126.81±4.04 |
| **Anacardiaceae** | | | | | | |
| *Pistacia khinjuk* Boiss. | 549.00±3.93 | 588.00±3.85 | 342.50±2.72 | 352.30±4.06 | 31.60±2.93 | 27.28± 2.70 |
| **Adiantaceae** | | | | | | |
| *Adiantum capillus veneris* | 42.10±1.69 | 49.00±2.06 | 19.40±1.72 | 21.30±1.90 | 107.79±3.20 | 99.26±3.30 |
| **Apiaceae** | | | | | | |
| *Buplerum lancifolium* | 78.60±2.82 | 27.60±1.93 | 27.80±2.04 | 18.00±1.84 | 58.95±3.20 | 41.30±2.93 |
| *Daucus syticus* Murb. | 75.80±2.83 | 49.00±2.72 | 26.90±2.06 | 19.70±1.29 | 61.15±3.09 | 53.77±3.20 |
| *Ferula sinaicus* Boiss. | 160.70±3.50 | 86.70±3.06 | 104.60±3.03 | 88.40±2.92 | 36.73± 1.90 | 19.59±1.99 |
| *Malabaila suaveolens* | 75.60±2.42 | 23.60±2.30 | 23.80±2.63 | 16.00±1.79 | 73.28±4.17 | 68.00±4.35 |
| *Scandex peten-veneris* | 160.70±4.01 | 86.70±3.20 | 124.60±1.04 | 98.40±2.05 | 44.69±3.90 | 31.60±2.92 |
| **Asclepiadaceae** | | | | | | |
| *Calotropis procera* Ait. | 90.20±3.31 | 50.80±2.70 | 29.40±2.15 | 21.70±1.80 | 67.40±3.39 | 59.67±3.50 |
| *Gomphocarpus sinaicus* | 133.30±3.48 | 63.90±2.02 | 25.90±2.10 | 23.80±2.00 | 79.05± 2.68 | 68.17±2.07 |
| *Cynanchum actum* | 91.20±2.96 | 53.80±1.53 | 29.80±2.74 | 21.90±1.26 | 82.58±3.80 | 77.50± 3.62 |
| *Caralluma sinainca* | 79.00±5.09 | 56.80±4.80 | 27.40±1.95 | 22.40±1.62 | 83.40±3.99 | 78.15±3.90 |
| **Asteraceae** | | | | | | |
| *Achillea santolina* L. | 72.60±3.09 | 60.20±2.70 | 23.50±2.95 | 19.2±2.60 | 91.51±3.86 | 84.90±3.60 |
| *Ageratum conyzoides* | 56.9±2.14 | 51.20±2.09 | 18.70±1.96 | 17.10±1.63 | 58.40± 3.90 | 44.28±3.30 |
| *Amberboa leucantha* | 76.70±3.02 | 85.30±2.97 | 33.90±2.80 | 20.50±1.90 | 55.84±3.30 | 49.71±3.20 |
| *Anacyclus alexanderinus* | 63.60±2.62 | 53.20±2.90 | 19.70±2.30 | 18.50±1.90 | 49.39±3.50 | 40.16± 2.80 |
| *Astriscus graveolens* | 55.80±2.90 | 65.30±2.04 | 20.20±2.83 | 19.60±2.90 | 68.20±2.50 | 56.08±3.36 |
| *Carduus getulus* | 189.00±3.94 | 214.90±3.73 | 109.10±3.86 | 122.10±3.70 | 86.94±3.02 | 80.50±2.90 |
| *Carthamus tenuis* | 162.30±9.03 | 61.80±2.10 | 107.30±2.84 | 76.40±2.51 | 100.92±3.28 | 94.60±3.69 |
| *Centaurea ammocy-* | 85.00±2.05 | 50.40±1.79 | 18.20±1.92 | 16.90±1.80 | 68.58±3.50 | 55.40±3.65 |
| *Dittrichia viscosa* | 269.80±3.30 | 247.80±2.82 | 107.30±2.70 | 103.90±2.03 | 59.40±3.20 | 60.29±3.03 |
| *Echinops hussoni* | 100.30±3.07 | 77.80±2.75 | 34.50±2.01 | 19.60±2.30 | 95.96±4.30 | 88.50±3.96 |
| *Helichrysum conglobatum* | 232.30±4.09 | 164.80±3.20 | 111.30±2.80 | 104.70±2.99 | 59.05±4.80 | 60.03±3.88 |
| *Iphiona mucronata* | 65.20±2.04 | 46.20±2.00 | 21.30±2.74 | 1790±2.66 | 110.39±4.18 | 96.40±3.60 |
| *Matricaria recutita* L. | 147.60±3.03 | 142.40±2.14 | 45.90±2.80 | 43.30±1.88 | 58.69±3.05 | 48.20±2.90 |
| *Notobasis syriaca* L. | 64.00±1.85 | 43.80±1.99 | 13.70±1.53 | 11.70±1.15 | 64.10± 3.96 | 55.30±3.94 |
| *Pallenis spinosa* L. Cass. | 55.70±1.70 | 57.10±1.20 | 13.60±2.00 | 12.80±1.69 | 88.44± 4.60 | 78.30±4.75 |
| **Boraginaceae** | | | | | | |
| *Alkanna tinctoria* L. | 157.90±3.37 | 104.20±2.74 | 116.70±3.11 | 101.30±3.22 | 109.20±3.80 | 99.60±3.90 |
| *Anchusa azurea* Mill. | 83.3±2.80 | 88.20±2.27 | 22.30±2.74 | 26.70±2.25 | 120.38±3.06 | 113.59±3.27 |
| *Anchusa aegypt* | 66.7±2.90 | 43.60±2.39 | 19.90±2.80 | 16.40±1.70 | 107.50±3.33 | 98.21±3.95 |
| *Asperugo procu* | 189.80±7.82 | 87.50±4.79 | 96.00±2.74 | 75.70±2.06 | 84.37±3.26 | 80.50±3.03 |
| *Echium lycopsis* L. | 148.90±3.02 | 108.20±2.95 | 82.90±2.64 | 41.20±2.55 | 126.49±4.11 | 118.57±4.09 |
| *Echium setosum* Vahl. | 70.20±3.09 | 71.30±3.21 | 21.70±1.70 | 22.50±1.20 | 117.39±3.20 | 108.30±3.72 |
| *Lappula spinoca* | 227.50±6.18 | 124.30±5.88 | 134.90±2.70 | 96.80±2.53 | 88.07±3.30 | 78.40±3.02 |
| *Paracaryum rugulosum-* | 140.60±6.83 | 98.00±4.55 | 52.70±2.46 | 45.30±2.57 | 95.79±3.91 | 87.90±3.05 |
| **Brassicaceae** | | | | | | |
| *Eruca sativa* Miller | 64.60±2.60 | 34.60±2.11 | 29.00±2.90 | 19.80±1.89 | 102.47±3.20 | 91.30±3.90 |

Cont.,

| Plant name | Total Phenolic Content (mg %) | | Antioxidant Potential (%Scavenging) | | Larvicidal Activity ($LC_{50}$ mg %) | |
|---|---|---|---|---|---|---|
| | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract |
| *Farsetia aegyptia* L. | 49.70±1.42 | 26.30±1.10 | 17.80±0.99 | 18.50±0.76 | 165.40±4.38 | 158.32±4.79 |
| *Zilla spinosa* L. Prantl | 42.90±2.80 | 23.80±2.30 | 17.50±0.81 | 14.90±0.55 | 176.49±4.71 | 160.48±4.30 |
| **Caesalpiniaceae** | | | | | | |
| *Cassia italica* Mill. | 79.00±3.30 | 53.70±2.99 | 34.50±2.30 | 29.30±1.91 | 143.72±4.29 | 130.50±4.80 |
| **Capparaceae** | | | | | | |
| *Capparis aegyptia* L. | 91.80±3.88 | 81.60±3.29 | 28.30±2.10 | 26.3±1.94 | 197.10±4.22 | 186.47±3.90 |
| **Chinopodiaceae** | | | | | | |
| *Hammada eigii lljin* | 49.30±2.60 | 30.60±1.97 | 18.30±1.97 | 14.80±1.70 | 55.86±3.59 | 46.01±2.70 |
| *Salsola kali* L. | 58.40±2.90 | 34.80±2.20 | 19.90±1.29 | 17.30±1.00 | 58.28±3.64 | 46.50±2.94 |
| *Salsola volkensii* Asch. | 41.30±2.30 | 27.50±2.10 | 17.40±0.95 | 15.50±0.80 | 79.57±2.30 | 66.05±2.49 |
| *Salsola vermiculata* L. | 40.80±2.64 | 32.60±2.14 | 17.90±1.07 | 17.50±0.90 | 50.37±3.50 | 41.93±2.49 |
| **Cistaceae** | | | | | | |
| *Helianthemum cilliatum* | 274.20±3.40 | 176.10±3.27 | 188.50±3.45 | 125.80±2.39 | 117.10±3.30 | 102.40±2.77 |
| *Helianthemum lodifolium* | 227.80±3.26 | 155.60±2.80 | 169.40±3.21 | 121.30±2.17 | 148.47±3.80 | 130.15±3.05 |
| *Helianthemum sphaero-* | 141.80±2.99 | 74.90±2.07 | 58.50±2.89 | 38.80±2.50 | 139.48±3.40 | 129.11±3.04 |
| **Cucubitaceae** | | | | | | |
| *Bryonia syriaca* Bioss. | 120.80±3.64 | 65.70±3.00 | 48.40±2.10 | 19.80±1.63 | 115.29±3.19 | 99.20±2.87 |
| *Bryonia cretica* L. | 170.50±5.74 | 22.90±2.47 | 59.60±2.90 | 11.60±0.96 | 130.20±3.28 | 118.27±3.26 |
| *Cucumus prophetarum* | 141.70±3.80 | 79.80±3.15 | 58.90±2.90 | 41.50±2.60 | 143.55±4.09 | 137.74±4.95 |
| **Cycadaceae** | | | | | | |
| *Cycas beddomei* | 218.40±3.30 | 237.50±3.07 | 133.80±2.60 | 141.69±2.49 | 148.39±4.39 | 136.50±4.88 |
| *Cycas circinalis* | 197.49±3.38 | 211.30±2.05 | 127.85±2.39 | 131.20±2.42 | 133.80±4.26 | 127.40±3.09 |
| *Cycas revolute* | 232.57±3.84 | 247.90±3.30 | 137.80±3.01 | 142.95±2.96 | 127.60±4.86 | 116.69±4.70 |
| **Dipsacaceae** | | | | | | |
| *Petrocephalus pappo-* | 143.60±3.09 | 78.50±2.96 | 55.90±3.30 | 37.60±2.90 | 228.98±4.16 | 224.60±3.36 |
| *Petrocephalus sanctus* | 86.50±2.60 | 97.20±3.69 | 39.60±3.11 | 43.10±2.30 | 184.40±3.09 | 175.20±2.04 |
| **Ephedraceae** | | | | | | |
| *Ephedra alata* Decne | 47.50±2.44 | 66.30±2.60 | 18.80±1.88 | 19.20±2.01 | 153.80±4.58 | 139.40±4.10 |
| *Ephedra aphylla* | 49.60±2.70 | 68.70±3.05 | 19.50±1.64 | 20.00±2.50 | 195.72±4.06 | 182.30±4.90 |
| **Eqiusetaceae** | | | | | | |
| *Eqiusetium ramossissinum* | 39.10±2.07 | 56.80±2.30 | 19.50±3.36 | 19.60±1.96 | 185.46±3.61 | 172.59±3.90 |
| **Fabaceae** | | | | | | |
| *Coronilla scorpioides* | 916.70±4.80 | 915.60±4.86 | 354.80±4.03 | 352.90±4.94 | 27.53±2.01 | 18.53±1.95 |
| *Lathyrus aphaca* L. | 44.20±2.40 | 60.50±2.15 | 11.30±1.83 | 18.60±2.07 | 145.20±3.90 | 138.40±3.37 |
| *Melilotus sulcata* Desf. | 60.30±2.46 | 298.7±3.90 | 42.60±3.11 | 105.40±4.03 | 141.40±3.79 | 133.50±3.05 |
| *Ononis veginalis* Vahl. | 48.40±2.57 | 64.60±3.04 | 14.80±1.50 | 18.30±1.92 | 188.20±4.58 | 179.28±3.90 |
| *Ononis serrata* Forssk | 85.90±3.94 | 47.80±3.11 | 21.00±1.80 | 13.60±1.25 | 190.16±4.27 | 177.26±3.85 |
| *Retama raetam* Forssk | 133.30±4.01 | 67.90±2.60 | 29.50±1.99 | 24.30±1.75 | 173.10±4.05 | 166.30±3.81 |
| **Geraniaceae** | | | | | | |
| *Erodium bryoniifolium* | 45.40±2.10 | 25.40±2.07 | 20.10±1.83 | 11.90±1.79 | 67.90±3.69 | 55.38±3.09 |
| *Monsonia nivea* Dence | 45.70±2.70 | 25.90±2.38 | 19.30±1.74 | 12.80±1.92 | 44.92±2.95 | 39.75±2.80 |
| **Ginkgoaceae** | | | | | | |
| *Ginkgo biloba* L.C. | 321.90±4.29 | 278.60±3.30 | 189.50±2.94 | 185.60±2.70 | 57.60±3.58 | 68.90±3.19 |
| **Hypericaceae** | | | | | | |
| *Hypericum sinaicum* | 440.60±3.80 | 535.80±4.23 | 244.60±3.00 | 257.20±2.63 | 79.54±3.70 | 68.27±3.29 |
| **Lamiaceae** | | | | | | |
| *Eremostachys laciniata* | 85.60±2.05 | 89.40±2.06 | 19.20±1.93 | 19.40±2.00 | 77.42±3.90 | 71.38±3.85 |
| *Ballota kaiseri* Tackh. | 90.60±3.43 | 74.80±3.11 | 25.60±2.94 | 21.90±3.29 | 89.18±4.03 | 80.59±3.97 |

Cont.,

| Plant name | Total Phenolic Content (mg %) | | Antioxidant Potential (%Scavenging) | | Larvicidal Activity ($LC_{50}$ mg %) | |
|---|---|---|---|---|---|---|
| | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract |
| *Marrubium alysson* L. | 76.70±2.90 | 53.20±2.63 | 24.10±1.50 | 21.80±1.95 | 76.60±3.37 | 67.61±3.05 |
| *Phlomis aurea* Decne | 59.60±2.66 | 60.50±2.91 | 15.70±1.00 | 16.20±1.41 | 86.11±4.02 | 79.51±3.58 |
| *Phlomis floccose* D. | 54.20±2.40 | 46.90±2.10 | 14.80±0.91 | 13.60±1.18 | 99.61±4.30 | 87.35±4.20 |
| *Salvia aegyptiaca* L. | 29.50±2.04 | 30.30±2.17 | 16.70±1.26 | 16.20±1.03 | 100.82±4.40 | 92.66±4.50 |
| *Salvia lanigera* Poir. | 223.90±3.13 | 155.80±2.50 | 133.90±2.70 | 124.70±2.03 | 75.00±3.30 | 67.10±3.04 |
| *Salvia spinosa* L.D. | 90.60±3.05 | 74.80±2.99 | 23.60±2.30 | 21.90±2.38 | 98.69± 4.28 | 86.4±3.90 |
| *Lamium amplexiccaula* | 85.00±2.60 | 77.50±2.49 | 22.80±2.00 | 21.40±1.30 | 107.64±4.50 | 88.84±3.27 |
| **Liliacaeae** | | | | | | |
| *Asparagus aphyllus* L. | 65.30±2.09 | 37.80±1.74 | 15.60±2.17 | 9.70±0.93 | 105.93±3.07 | 102.00±2.85 |
| *Asparagus stipularis* | 61.20±2.03 | 39.60±1.80 | 17.60±1.93 | 13.20±1.06 | 104.52±3.70 | 96.30±3.38 |
| *Scilla hanburyi* Baker. | 81.40±2.39 | 44.70±2.05 | 21.20±1.96 | 19.66±0.82 | 102.68±3.09 | 99.70±2.95 |
| **Loranthaceae** | | | | | | |
| *Loranthus acacia* | 460.80±4.02 | 590.80±4.49 | 239.70±2.91 | 246.50±2.98 | 28.73±2.58 | 19.48±1.90 |
| **Malvaceae** | | | | | | |
| *Alcea striats* (DC.) | 20.50±2.10 | 24.40±1.97 | 12.30±1.42 | 5.70±0.74 | 202.15±4.06 | 191.10±3.56 |
| **Papavaraceae** | | | | | | |
| *Argemone mexicana* L. | 65.30±2.89 | 29.70±2.09 | 19.30±1.84 | 15.10±1.05 | 153.52±5.48 | 146.20±5.37 |
| *Glaucium corniculatum* | 161.90±3.90 | 81.20±2.70 | 120.60±3.15 | 118.80±2.93 | 127.40±4.47 | 117.35±3.90 |
| *Papver decaisnei* | 77.80±3.01 | 69.40±2.95 | 26.40±1.70 | 23.90±1.54 | 150.18±4.62 | 139.60±4.05 |
| *Papver hybridum* L. | 70.50±3.16 | 58.90±3.07 | 24.60±1.83 | 22.60±1.21 | 147.36±5.86 | 137.52±4.79 |
| **Plantaginaceae** | | | | | | |
| *Plantago lanceolata* | 27.70±2.06 | 43.20±2.38 | 6.60±0.94 | 7.80±0.98 | 145.94±5.50 | 138.61±4.95 |
| *Plantago sianica* L. | 29.40±1.90 | 45.60±2.39 | 7.60±1.17 | 9.00±1.05 | 130.68±4.49 | 119.58±3.63 |
| **Polygonaceae** | | | | | | |
| *Rumex pictus* Forssk | 161.20±3.70 | 60.60±3.17 | 121.30±2.95 | 110.90±3.92 | 139.26±5.38 | 121.67±4.70 |
| **Runumculaceae** | | | | | | |
| *Ranunculus sceleratus* | 14.60±1.09 | 16.80±1.10 | 6.30±0.93 | 8.40±1.03 | 203.75±6.69 | 192.50±5.90 |
| **Resedaceae** | | | | | | |
| *Ochradenus baccatus* | 79.20±3.22 | 45.80±2.75 | 23.70±2.03 | 8.20±0.95 | 176.90±7.70 | 164.61±6.62 |
| **Rhumnaceae** | | | | | | |
| *Ziziphus lotus* L. Lam. | 238.20±3.20 | 200.70±3.19 | 135.60±3.95 | 129.40±3.57 | 148.37±5.59 | 142.40±4.20 |
| **Roseceae** | | | | | | |
| *Crataegus sinaica* | 744.90±4.93 | 659.30±4.80 | 399.10±4.90 | 378.48±3.84 | 28.17±2.06 | 21.17±1.85 |
| *Potentilla supine* L. | 140.50±3.40 | 170.80±3.79 | 22.50±2.30 | 27.30±3.09 | 69.58±3.49 | 52.5±3.09 |
| **Rutaceae** | | | | | | |
| *Ruta tuberculata* For- | 83.60±3.18 | 72.60±3.10 | 21.10±2.39 | 19.50±2.00 | 108.15±4.69 | 99.07±3.93 |
| **Salvadoraceae** | | | | | | |
| *Salvadora persica* L. | 48.80±2.90 | 42.50±2.73 | 18.90±2.25 | 15.30±1.98 | 190.17±5.88 | 180.28±5.20 |
| **Scrophilareaceae** | | | | | | |
| *Kickxia aegyptiaca* L. | 45.20±2.04 | 38.10±1.95 | 16.70±1.96 | 19.10±0.95 | 110.46±3.30 | 102.09±2.93 |
| *Kickxia heterophylla* | 49.70±3.09 | 48.20±2.96 | 18.90±2.28 | 17.60±1,80 | 104.06±3.40 | 98.48±3.96 |
| *Scrophularia arguta* | 79.30±2.90 | 50.10±2.73 | 18.50±2.06 | 12.90±1.70 | 118.67±3.05 | 108.22±3.58 |
| *Verbascum fruticu-* | 68.40±2.72 | 46.20±2.18 | 20.40±2.04 | 17.50±2.10 | 148.37±4.57 | 136.80±4.61 |
| *Veronica anagallis-* | 38.00±2.30 | 38.90±2.38 | 15.40±1.03 | 18.70±0.91 | 109.56±4.20 | 102.88±4.19 |
| **Solanaceae** | | | | | | |
| *Hyoscyamus aureus* L. | 56.90±2.96 | 51.80±2.50 | 18.30±1.90 | 19.10±2.01 | 208.63±4.70 | 201.80±4.28 |
| **Verbenaceae** | | | | | | |
| *Verbena tenara* | 198.50±3.10 | 211.80±3.27 | 95.30±2.36 | 97.20±2.45 | 73.95±4.50 | 67.66±3.37 |

Cont.,

| Plant name | Total Phenolic Content (mg %) | | Antioxidant Potential (%Scavenging) | | Larvicidal Activity ($LC_{50}$ mg %) | |
|---|---|---|---|---|---|---|
| | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract | Aqueous extract | Methanol extract |
| *Verbena rigda* Hand | 143.30±2.85 | 176.80±3.90 | 96.40±2.84 | 97.40±2.39 | 84.62±4.60 | 78.80±3.47 |
| *Verbena venosa* | 199.50±3.41 | 185.90±3.50 | 93.90±2.95 | 90.50±2.85 | 86.92±5.06 | 90.30±4.95 |
| *Lantana camara* L. | 249.38±3.48 | 265.80±3.01 | 106.40±3.60 | 115.73±3.95 | 49.68±1.84 | 46.30±1.59 |
| **Urtiaceae** | | | | | | |
| *Forsskaolea tenacissi-* | 813.70±4.16 | 664.60±3.70 | 418.40±4.16 | 389.60±4.60 | 23.85±2.07 | 18.85±1.67 |
| *Urtica pilulifera* L. | 49.50±3.07 | 20.60±2.11 | 17.90±1.85 | 16.20±0.60 | 68.15±2.99 | 60.28±2.90 |

Results = mean of three measurements ± standard error, Total phenolic content calculated as gallic acid equivalents GAE mg%, % scavenging potential for Butyl hydroxyl toluene (BHT) "Antioxidant synthetic standard= 93± 0.50 at dose level of 0.4 mg/ml, scavenging potential for Quercetin "Antioxidant natural standard= 95.6 ±0.40 at dose level of 0.025 mg/ml.

## Discussion

Phenolic compounds is a generic term that refers to a large number of compounds (<8,000) widely dispersed throughout the plant kingdom characterized by having at least one aromatic ring with one or more hydroxyl groups attached, they range from simple, low molecular-weight, the single aromatic-ringed compounds to large and complex tannins and derived polyphenols (Crozier *et al,* 2006; Pereira *et al,* 2009). Medicinal plant are commonly rich in phenolic compounds with many useful properties for human health as anti-inflammatory, antimicrobial, antiallergic, cytotoxic and antitumor activities, but still the most important action of phenolic compounds is their antioxidant potential (Chu *et al,* 2000; Fukumoto *et al,* 2000; Chi-Tai and Gow-Chin, 2006; Germano *et al*, 2006; Podsedek *et al*, 2006; Podsedek 2007).

Quantitative estimation of the total phenolic content of aqueous and methanol extracts of the selected medicinal plants was done by Folin-Ciocalteu's reagent (Zhou and Yu, 2006) compared to standard gallic acid (Tab. 1, Fig. 1, 3) showed that there was minor difference of their quantities depending on the solvent used for extraction; the highest percent of total phenolic compounds were calculated for taqueous extracts of *Coronilla scorpioides* L., *Forsskaolea tenacissima* L., *Crataegus sinaica* Boiss., *Pistacia khinjuk* Boiss. and *Loranthus acacia* Benth.**;** they were 916.7± 4.80, 813.7±4.16, 744.9± 4.93, 549.0±3.93 & 460.8±4.02 mg% while those of methanol extracts were estimated in *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Loranthus acacia* and *Pistacia khinjuk***;** they were 915.6±4.86, 664.6±4.16, 659.3±4.80, 590.8±4.49 & 588.0 ±3.85 mg% respectively.

The results proved that the solvents for extraction of phenolic compounds varies individually by varying medicinal plant used i.e. total phenolic content of *Crataegus sinaica* aqueous extract was 744.9±4.93 mg% while its methanol one contained 659.30 ± 4.80 mg% whereas methanol extract of *Loranthus acacia* contained 590.80±4.49 mg% of total phenolic compounds; aqueous one showed 460.80±4.02 mg%.

Total phenols were measured in terms of gallic acid equivalent where the standard curve equation is (y=0.05, x±0.0545, r2=0.9873).

Reactive oxygen species (ROS) such as $^{\bullet}O_2$ (superoxide anion), $H_2O_2$ (hydrogen peroxide), and $^{\bullet}OH$ (hydroxyl radical) may cause tissue damage, resulting from the imbalance between such reactive oxygen species generated and the natural scavenging system and seem to be implicated in the pathology of a number of disorders as atherosclerosis, ischemia-reperfusion injury, cancer, malaria, diabetes, inflammatory joint, asthma, cardiovascular diseases, cataracts, immune system decline, and could play a role in neurodegenerative diseases and aging processes (Singh, 1989; Squadrito and Pryor, 1998; Dorman *et al*, 2003; Young *et al,* 2005; De Pascual-Teresa *et al,* 2010).

Many researchers focused on the powerful but non-toxic antioxidants from natural sources, such natural antioxidants could prevent formation of such reactive species-related disorders in human beings instead of synthetic antioxidants that suspected of causing or prompting negative health effects and strong restrictions encountered their application with an urgent trend to substitute them with natural antioxidants (Grice, 1986; Wichi, 1988; Chu, 2000; Hosny *et al,* 2002).

The antioxidant potential of phenolic compounds is related to its chemical structure that confers them redox properties, they can play an important role in adsorbing and neutralizing reactive oxygen species (ROS), quenching singlet and triplet oxygen, or decomposing peroxides and so great variety of natural medicinal plants have been screened for their antioxidant activities and results have shown that the raw extracts or isolated pure compounds among them there were the more effective antioxidants *in vitro* compared to BHT or vitamin E (Gordon and Weng 1992; Gu and Weng 2001; Ross and Kasum, 2002; Pyo *et al*, 2004; Othman *et al,* 2007; Ali *et al*, 2008; Ibrahim *et al*, 2010; Abdel-Hady *et al*, 2011; El-Hela *et al*, 2011) and so medicinal plants are potential source of natural antioxidants (Cesquini *et al*, 2003).

The DPPH is a free radical which has been widely been used to test the free radical scavenging ability of various samples (Cuvelier *et al,* 2000; Shimoji *et al,* 2002; Sakanaka *et al,* 2005), it is a stable free radical has a characteristic absorbance at 517 nm and was used to study the radical-scavenging effects of the extracts as antioxidants donate protons to this radical, the absorbance decreases and so for evaluation of the DPPH scavenging effects of the extracts of the chosen plants percent DPPH inhibition was investigated.

Qualitative TLC-DPPH assay of tested extracts showed that most of them are active compounds as DPPH scavengers appearing as zones with different $R_f$ values at in the chromatogram, these results directed the research to quantitative estimation of the antioxidant capacity of each extract individually.

Quantitative estimation of the antioxidant potential of different extracts was done spectrophotometrically by using

DPPH method revealed that most of the tested extracts possess significant free radical scavenging activity which is proven to be more potent when compared with the reference synthetic antioxidant standard butylated hydroxytoluene (BHT), the most significant percent free radical scavenging potentials (Tab. 1, Figs. 2 & 3) were recorded among the aqueous and methanol extracts of the *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Pistacia khinjuk* and *Loranthus acacia***;** they were 454.8±4.83, 418.4±4.16, 399.10±4.9, 342.50±2.72 and 239.7± 2.91 % for aqueous extracts & 452.9±4.94, 389.6±4.6, 378.48 ±3.84, 352.±3.06 & 346.5±2.98% for methanol ones respectively. Comparing the results of total phenolic contents and antioxidant potential showed a significant linear correlation, which means that phenolic compounds provide the major contribution to the antioxidant activity of these plant extracts evaluated by these assays. This is in line with similar correlations between total phenolic content and antioxidant activity of various plants was reported (Nencini *et al,* 2007; Abdel-Hady *et al*, 2011; El-Hela *et al*, 2011).

Mosquito-borne diseases, as malaria, filariasis and viral hemorrhagic fevers are still major public health problems in the African countries because of their tropical or subtropical climate, poor drainage system especially during the rainy seasons, and presence of many fish ponds, irrigation ditches and rice fields which provide abundant mosquito breeding places and to prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The Yellow fever strikes an estimated 200,000 persons world-wide each year and causes an estimated 30.000 deaths (WHO, 1992), the main vector of Yellow fever is *Ae. aegyptii* the most important mosquito vectors of human disease native of Africa and was introduced to the Americas in the 1600s by the slave trade, and became highly domesticated, adapted to humans, and a highly efficient vector of epidemic yellow fever and dengue. The yellow fever virus is transmitted when a mosquito bites an infected human, then after an incubation period of 12-21 days, bites a susceptible human. *A. aegyptii* breeds readily in all types of the domestic and peridomestic collections of water i.e. flower vases, water drums, tin cans, broken coconut shells and even gutters (Simpson 1996; Su and Mulla, 1998).

The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds but this has not been very successful due to human, technical, operational, ecological, and economic factors so, in recent years, the use of many of the former synthetic insecticides in mosquito control programme has been limited due to lack of novel insecticides, high cost of synthetic insecticides, concern for the environmental sustainability, harmful action on human health, and non-target populations, non-biodegradable nature, higher rate of the biological magnification through ecosystem, and increasing

insecticide resistance on a global scale.

Before the discovery of the synthetic insecticides, natural ones such as pyrethrum, rotenone, nicotine and others have been extensively used for insect control (Balandrin, 1985; Monzon *et al*. 1994; Pedigo, 1996; Shaalan *et al,* 2005).

Some medicinal plant extracts are effective against mosquito larvae, in addition they may greatly reduce the risk of adverse ecological effects, also they do not induce pesticide resistance in mosquitoes by the virtue that these chemicals are taken from medicinal plants, they are expected to have low human toxicity and a high degree of biodegradation abilities (Choochote *et al*, 1999) while other researchers reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potential of several plant derived secondary materials, such as the essential oils, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans and also documented the isolation of several bioactive toxic principles from various plants and reported their toxicity against different mosquito species (Yang *et al,* 2004; Shaalan *et al,* 2005; Kishore *et al,* 2011).

Searching for new control agents from natural sources has gained popularity among researchers in countries with a strong herbal tradition and large numbers of plants have been reported to possess insecticidal activity (Nagpal *el al,* 1996; Abdel-Hady *et al,* 2005; Abdel Halim, 2006; Ibrahim *et al,* Brown 1986; Russell *et al,* 2009; 2010; Kishore *et al,* 2011; Kabir *et al,* 2013). The results gained for the screening of the *Ae. aegyptii* larvicidal activity (tab.1, fig. 4) proved that the *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Pistacia khinjuk* and *Loranthus acacia* exhibited the highest potency expressed as the least $LC_{50}$; the calculated values were 22.53±2.01, 23.85±2.07, 28.17± 2.06, 31.6±2.93 & 39.73±4.58mg% of aqueous extracts & 18.53±1.95, 18.85± 1.67, 20.17±1.85, 23.28±2.70 & 28.48 ±3.9 mg% of methanol extracts respectively.

By comparing the gained results of total phenolic compounds and larvicidal activity (Tab.1, Fig. 4) revealed that there was a linear correlation between larvicidal effect indicated as $LC_{50}$ and total phenolic content of the investigated plants, suggesting that the phenolic compounds have significant larvicidal activity.

In Egypt, the *Aedes* species were encountered, Kirkpatrick (1925) reported *Ae. aegyptii* and Gad (1963) identified *Ae. aegypti, Ae. caspius* and *Ae. detritus*. Holstein (1967) reported complete eradication of *Ae. aegypti* from Egypt. Mostafa *et al.* (2002) reported *Ae. detritus* in governorates of Assiut, Al Fayium, Giza, Aswan, Al Wady Al Gadeed and South Sinai. *Ae. caspius* was found in Assiut and Aswan and as larvae in Kena and Al Wady Al Gadeed. Morsy *et al*. (2003, 2004) found *Ae. caspius* in Qalyoubia, Giza and the Greater Cairo. Shaalan *et al*. (2005a, b) in Aswan found *Ae. aegypti* in water sources. Mikhail *et al.* (2009)

reported *Ae. caspius* and *Ae. detritus* in Greater Cairo, Sharkia, Qualyoubia and Giza. Abdel-Hamid *et al.* (2011) in El Menoufia reported *Ae.* (*O.*) *caspius* and *Ae.* (*O.*) *detritus*. Shoukry *et al.* (2012) in Toshka at southern Egypt identified adults and immature stages of *Ae. aegypti*. Undoubtedly, mosquitoes-borne diseases are threat worldwide (Mikhail *et al*, 2009).

Undoubtedly, re-emerging of the *Ae. aegypti* in Egypt mainly Aswan Governorate (Essam *et al*, 2006) and in Toshka Project (Heikal *et al,* 2011; Shoukry and Morsy, 2011; Shoukry *et al*, 2012; Morsy, 2012), which is the main mosquito-vector of the Yellow fever (CDC, 2010a), the Dengue and Dengue hemorrhagic fever (El-Bahnasawy *et al*, 2011) and Chikungunya fever (CDC, 2010b).

## Conclusion

Searching for the new natural antioxidants become an urgent demand due to thehealth hazards accompanying the use of synthetic ones and the strict need of such antioxidants to decline many the health disasters caused by liberated free radicals.

Moreover, the increasing insecticide resistance require strategies to prolong the use of highly effective vector control compounds, medicinal plants can kill, deform the post-embryonic molting stages of mosquitoes could be a valuable approach in integrated vector management programs to replace synthetic pesticides. The outcome results proved that the most suitable medicinal plants used as antioxidant drugs and larvicidal agents are *Coronilla scorpioides*, *Forsskaolea tenacissima*, *Crataegus sinaica*, *Pistacia khinjuk* and *Loranthus acacia* as all shared in containing high content of total phenolic compounds.

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Fig. 3: Total phenolic compounds content and antioxidant potential of top five potent medicinal plants:

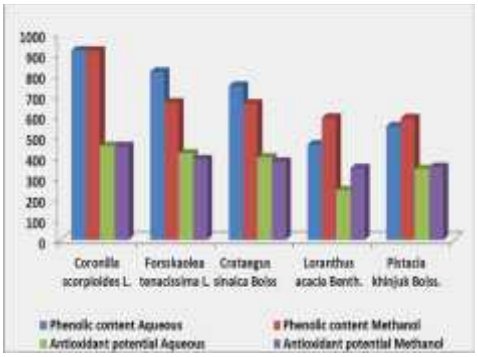


Fig. 4: Aedes aegyptii larvicidal activity of the top five potent medicinal plants: