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Research Article

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GC-MS analysis of bioactive compounds in phenolic extracts of leaves and flowers of *Chrysanthemum cinerariaefolium* and their efficacy against larvae of *Culexquin quefaciatus Say(Diptera: Culicidae)*

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ABSTRACT

The bioactive compounds of phenolic extract of leaves and flowers of (pyrethrum) Chrysanthemum cinerariaefolium have been investigated and evaluated using GC-MS analysis. (Perkin Elmer GC clarus 500) Gas Chromatography - Mass Spectrometry, The mass spectra of bioactive compounds were matched according to National Institute of Standards and Technology (NIST) library. The phenolic extract of leaves contains four compounds, while the flowers extract has 13 compounds, The analysis showed also that3,7,11,15-Tetramethyl-2-hexadecen-1-ol,3-Buten-2-one, and 4-(2-hydroxy-2,6,6-trimethylcyclohexyl) were found in both leaves and flowers extract. Then bioassay of these two phenolic extract were tested against all larval instar of Cx. quinquefaciatus and showed that the first larval instar was more sensitive than other preceding instars and the mortality percentages of these larval instars were high in phenolic extract of flowers.

Keywords: GC-MS, *C. cinerariaefolium*, Phenolic compounds, *Cx. quinquefaciatus*

INTRODUCTION

Culex quinquefaciatus is distributed abundantly in Iraq, especially in the central and southern parts, This species is vector of some pathogens such as *Wuchereriabancrofti* ,viruses such as causing West Nile Fever, Japanese Encephalitis as well as Dengue Fever [1],[2]. Botanical pesticides is one of promising caudate to control vectors disease[3]and are environmentally safe, degradable and target-specific[4]. *C.cinerariaefolium* It is an important source of pyrethrins [5],[6]. Phenols are harmful because of their ability to join with proteins by hydrogen bonds.[7]

EXPERIMENTAL SECTION

Insect cultuer

The cultuer of *Culex quinquefaciatus* were maintained at entomology laboratory, college of science, Qadisiyah University, at a temperature of 28C º ± 5 % for 14–h photoperiod. The larvae of *Cx. quinquefaciatus* were maintained in separate enamel containers.

Plant samples

Leaves and flowers of the plant were collected in the early morning (before sunrise), and then were washed and cleaned and dried naturally at lab temperature. The dry leaves and flowers were ground separately using an electric grinder and kept in dark plastic containers ready for use.

Preparation of crude phenolic extract

The phenolic extract was attended according to [8] as follows: 20 gms of dried powder of leaves and flowers of *C.cinerariaefolium* was blended separately 400 ml solution of concentrated hydrochloric acid HCL 36% (2ml).

Extraction was done using reflex condenser in a water bath of boiling water (100 ºC) for one hour, then left until cool and then filtered through Whattman No.1 filter paper. The filtrate was transferred to the separating funnel and the same volume of n- propanol was added. Then a sufficient quantity of NaCl was added until saturation. After separation the top layer (phenolic) taken and dried in rotary evaporator at 45Cº, and then left to dry at lab. temperature. The resulting product was then collected and kept in sealed glass tubes in the refrigerator at 4 Cº ready for use.

GC-MS method

GC-MS analysis was carried out on a GC clarus 500 PerkinElmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument under the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µ Mdf, composed of 100% Dimethyl polysiloxane),operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was set at 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time was 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The software adapted to handle mass spectra and chromatograms was Turbo Mass Ver 5.2.0 [9].

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology(NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components were ascertained.

Bioassay of phenolic extract

In order to estimate the biological activity of the extract of crude phenolic compounds of *C.cinerariaefolium* leaves and flowers was attended the Stock solution by dissolving 4 gms of dry matter for each of the leaves, flowers separately in 20 ml of ethanol 96% and finished size to 100 with distilled water, bringing to the Stock solution 4% or (40 mg /ml), and from the last solution attended concentrations (2.5, 5, 10, 20, 40) . The control treatment was 5ml ethanol added to it 95 ml of distilled water. The experimental tubes was 3 replications each containing 100 ml from each concentration and explained where 20 larvae were mortality ratio calculated after 24 hours. and corrected ratios according to the equation and subjected all experiments for statistical analysis by using Least significant Differences (L.S.D) $p \leq 0.05$ using Abbott's formula [1925][10].

$$\% \text{ mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} X\ 100$$

RESULTS AND DISCUSSION

Four phenolic compounds were identified in *C. cinerariaefolium* leaves and thirteen compounds were also identified in flowers. The name, retention time (RT), molecular formula, molecular weight(MW) are presented in (Table1, and Fig.1, Table 2, Fig.2). Also it turns out that some of the compounds were present in all of the leaves and flowers, but differ in the retention time, such as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)-.

Table 1: GC-MS analysis of *Chrysanthemum cinerariaefolium* leaves extract

Peak #	R. time	Area %	Name	Molecular formula	Molecular weight
1	12.62	10.28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
2	13.56	7.72	1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-	$C_{16}H_{30}$	222
3	18.57	76.07	3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)-	$C_{13}H_{22}O_2$	210
4	20.42	5.92	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390

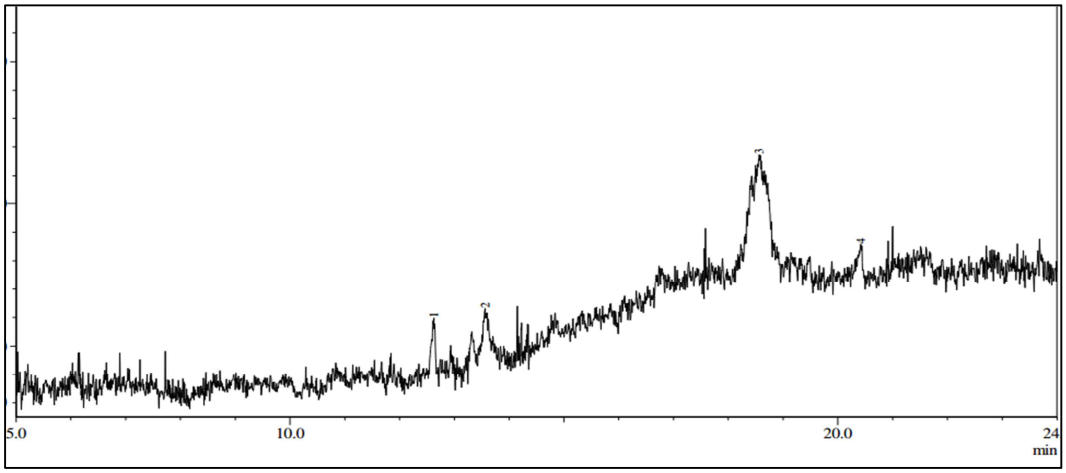

Fig.5: Chromatogram obtained from the GC/MS leaves extract of *C. cinerariaefolium*

Chemical detection using GC-MS demonstrated that the plant extract of *Boerhavia diffusa L.* contains Steroles, Tannins and Flavonoids as well as Phenolic compounds [11]. [12] selected methanol extract compounds of roots, stems and leaves of *Labisia paucifolia*, where the results indicate that the leaves extract contains a large amount of phenolic compounds and flavonoids compared with the roots and stems extract, and was all of gallic acid, Kaempfferol are more flavonoids and phenols presence.

Table 2: GC-MS analysis of *Chrysanthemum cinerariaefolium* flowers extract

Peak #	R. time	Area %	Name	Molecular formula	Molecular weight
1	5.87	28.07	Acetic acid	C2H4O2	60
2	6.98	7.11	Camphor	C10H16O	152
3	9.39	8.04	Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-	C11H20O	168
4	10.45	2.01	2-Butenoic acid, 2-methyl-, (Z)-	C5H8O2	100
5	11.63	1.77	1-Hexadecanol	C16H34O	242
6	12.58	7.22	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296
7	13.31	1.39	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	C9H14	122
8	13.60	6.10	Diazoacetic acid, 2-isopropyl-5-methylcyclohexyl ester	C12H20N2O2	224
9	16.87	5.68	3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	C10H18O	154
10	17.22	2.93	3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-	C10H16O2	168
11	18.00	2.20	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-1R*,3E,7E,11R*)]-	C15H24O	220
12	18.42	14.81	3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)-1	C13H22O2	210
13	22.88	12.66	Thujone	C10H16O	152

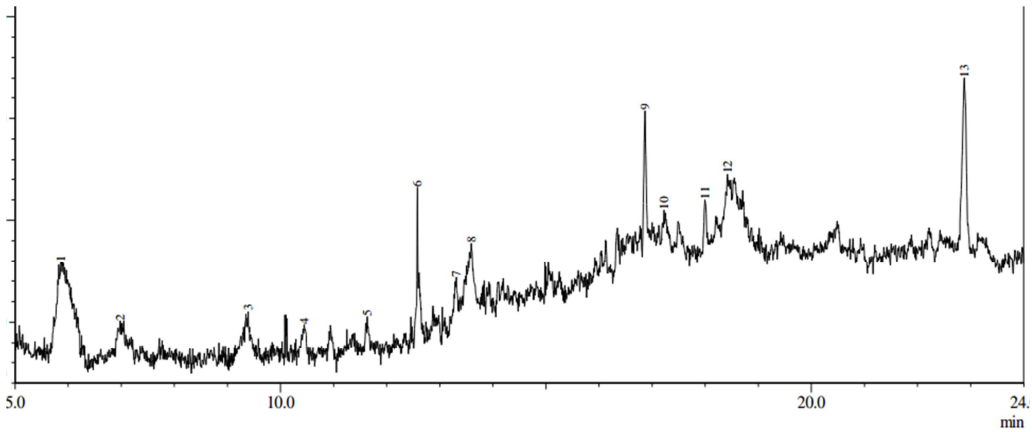

Fig.6: Chromatogram obtained from the GC/MS with the extract of *C. cinerariaefolium* flowers

GC-MS analysis used to explained that content of plant *Dolichandrone atrovirens* leaves extract , where it found container on saponins, phenols, flavonoids and vitamin C, so that this plant use of pharmaceuticals and pharmaceuticals purposes [13].Phenols are the secondary metabolites that a ubiquitously present in plants. They have been suggested to play a role in the antioxidant function . Phenolic compounds have antioxidant properties because of their ability to scavenge free radicals.

The phenolic compounds of plant origin showed their antioxidative effect by various mechanisms, including their ability to scavenge free radicals or activate various antioxidant enzymes and inhibit oxidizes[14].[15]investigated the presence of phytochemical contents of the selected medicinal plants. Proteins, carbohydrates, phenols, tannins, flavonoids, saponins were detected in all of the plants tested.[16] was determined the larvicidal activity of the ethanol extract of *E. rutaecarpa* unripe fruits and the isolated constituents against the larvae of the Culicidae mosquito *Ae. albopictus*. The presence of phytochemical compounds of carbohydrates, terpenoids, steroids, flavonoids, phenols was screened by qualitative method.[17] reported 49 phytoconstituent in ethanolic extract of *Maranta arundinacea. L* subjected to GC MS analysis.

BIOASSAY

The phenolic extract of flowers record high mortality was 77.79 % .The results also indicate the sensitivity of the larval instar to ward the extract . Where the first instar was the most sensitivity to the extract and in the all concentration used compared to the other larval stages .The first stages record ratios of mortality of (77.33-84.24)% compared tp the second stages (68 .10 – 80. 54)% , third (64. 66 – 74.22)% and fourth (62.99 – 72.47) in the phenolic extract of leaves and flowers respectively.

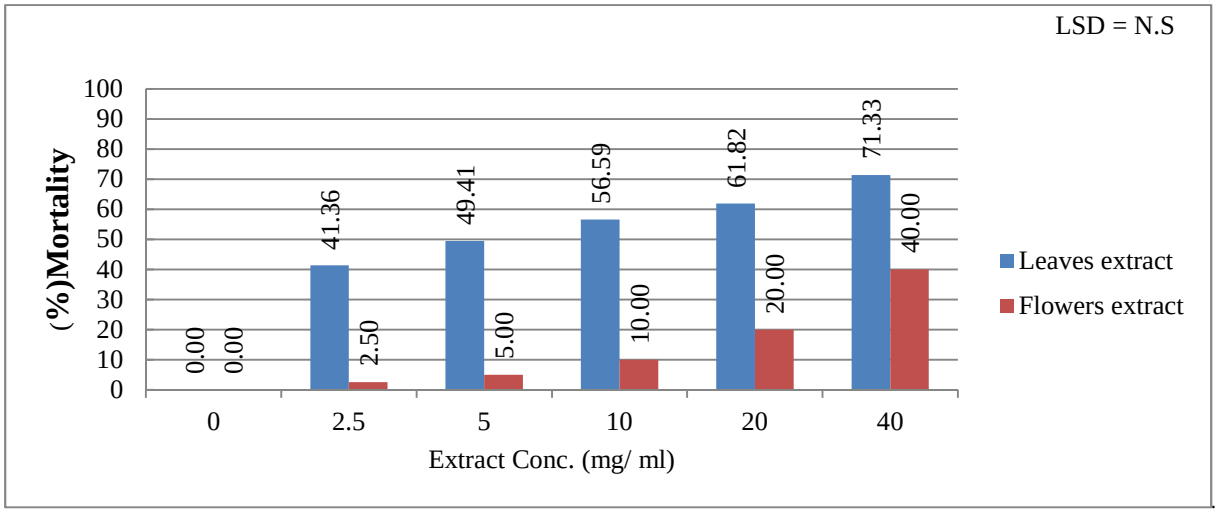

Fig. 1: effects of phenolic compounds extract of the leaves and flowers of *C. cinerariaefolium*on mortality of first larval instar of *Cx .quinquefaciatus*

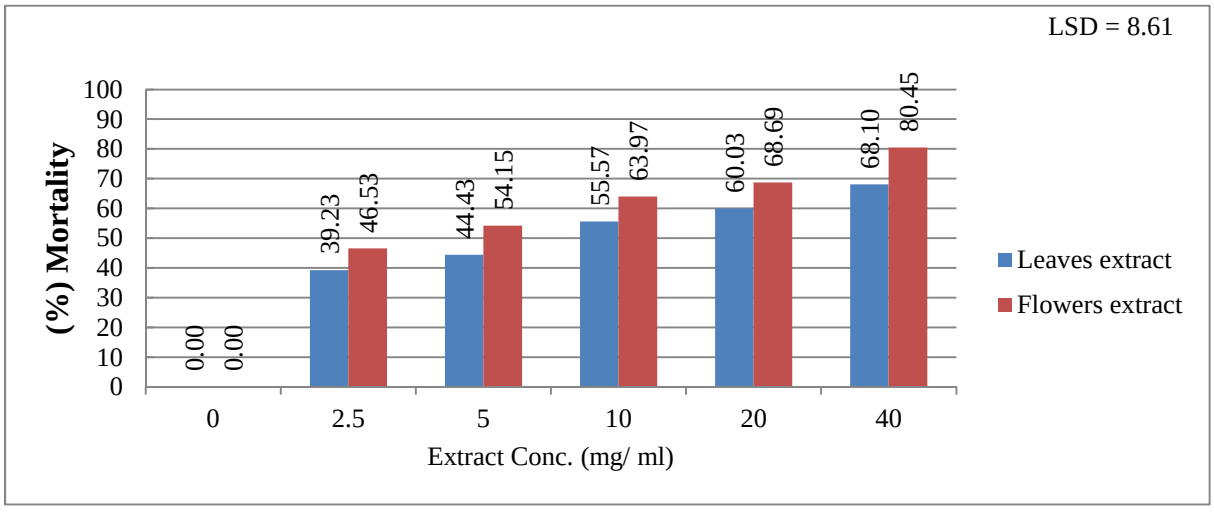

Fig. 2: effects of phenolic compounds extract of the leaves and flowers of *C. cinerariaefolium* on mortality of second larval instar of *Cx .quinquefaciatus*

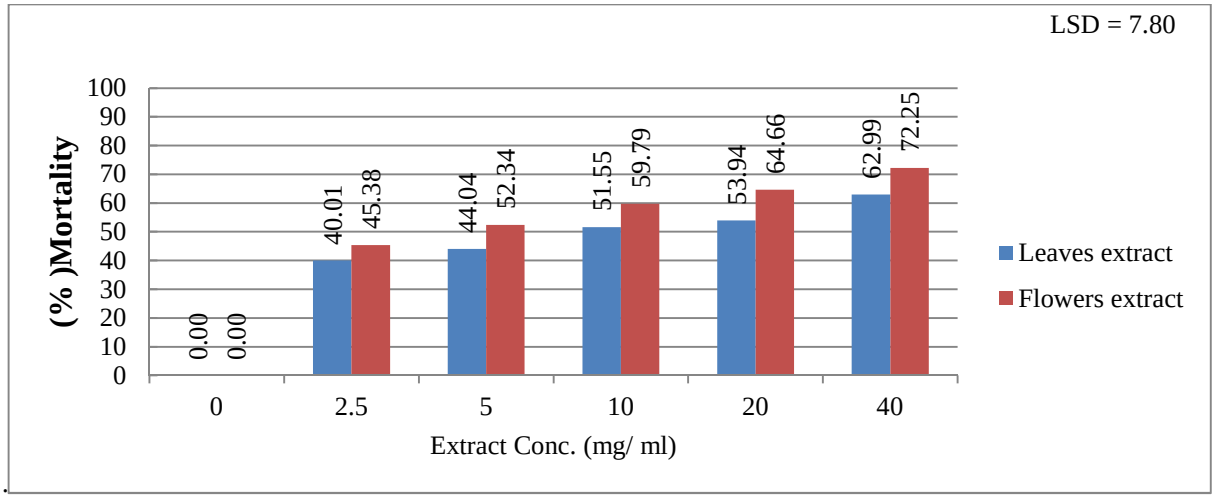

Fig. 3: effects of phenolic compounds extract of the leaves and flowers of *C. cinerariaefolium* on mortality of third larval insrar of *Cx .quinquefaciatus*

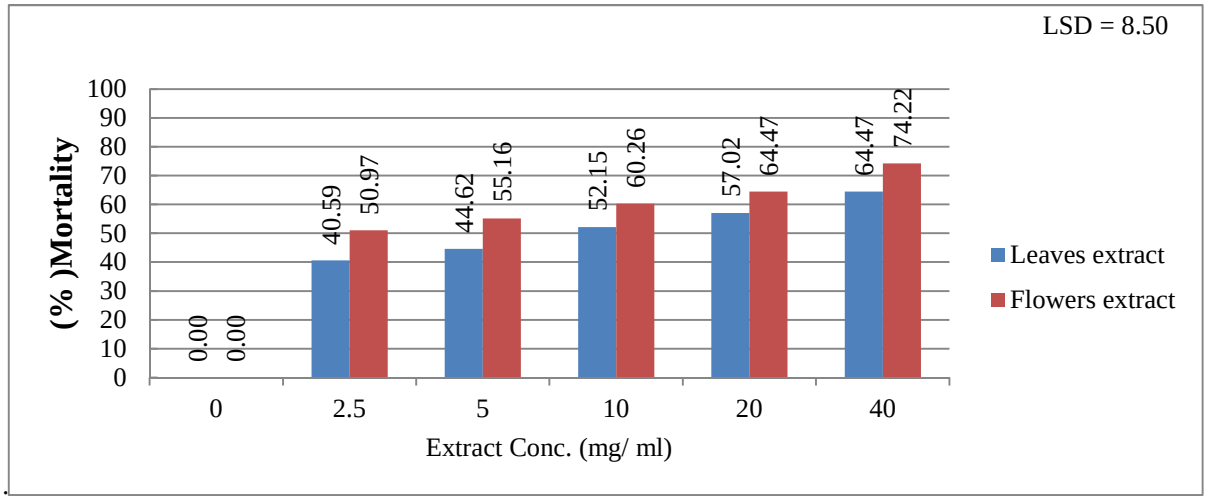

Fig. 4: effects of phenolic compounds extract of the leaves and flowers of *C. cinerariaefolium* on mortality of fourth larval instar of *Cx .quinquefaciatus*

Phenolic compounds cause two types of physiological effects in the larvae tissues were toxic effect in directly disrupt secretion in the nervous system , or a direct effect through the spread of theentry into force of these compounds in the target tissue [18],[19].It may return the cause of the effectiveness of phenols on other secondary metabolites to the different active substancesin the *C. cinerariaefolium* plant and it contains tannins that are toxic compounds to insects as associated with saliva and digestive enzymes in cluding trypsin and chymotrypsin and then inhibited and thus the insects start to lose weight and then death [20].

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