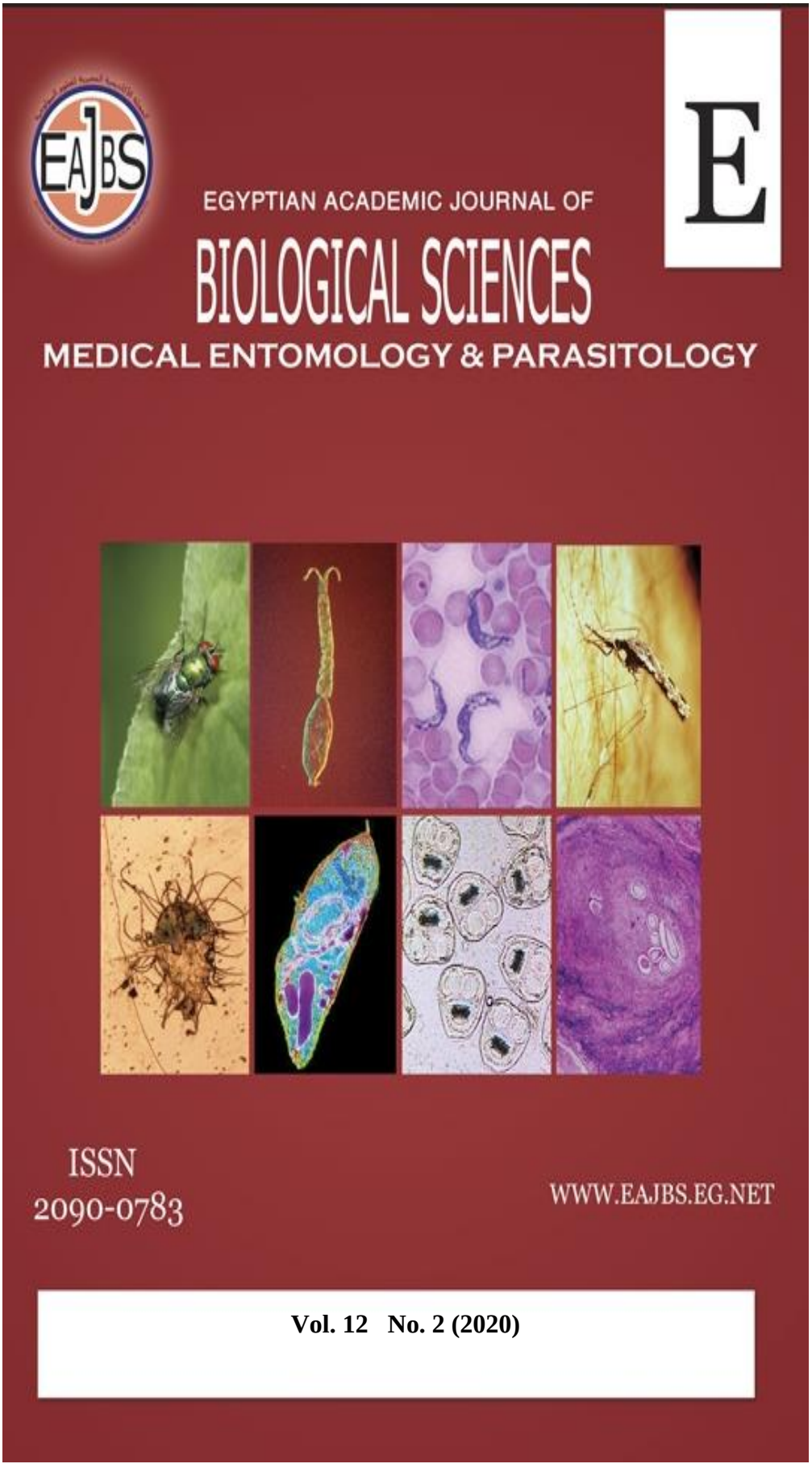
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Effect of Some Plant Extracts on The Biochemical Parameters, AChE and GST Activities of The Mosquito, *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

The present study was undertaken in order to investigate the effect of median lethal concentration (LC_{50}) of methanol, ethyl acetate, chlorobenzene and hexane extracts from leaves of *Origanum syriacum*, *Pergularia tomentosa*, *Senna italica*, and *Otostegia fruticose* against different biological aspects of the 3rd instar larvae of *Culex pipiens*. The effects were examined on the biochemical composition of the larval body and the activities of acetylcholinesterase (AChE) and glutathione S-transferase (GST). Based on obtained results, hexane extract showed more activity against 3rd instar larvae of *C. pipiens* followed by chlorobenzene, ethyl acetate, and methanol extract. Tested extracts caused a modification in biochemical composition. The LC_{50} of each treatment decreased the total carbohydrate, protein, and lipid contents as compared with the control. Tested extracts also showed a neurotoxic activity as evidenced by the inhibition effect of the AChE activity that was significantly ($P<0.001$) decreased. Stimulation of the detoxification system of tested larvae was evidenced by the promoted activity of GST as compared with the untreated group. In general, tested extracts used in the present study may be considered as promising control agents against the mosquito vector, *C. pipiens.*

INTRODUCTION

Mosquitoes play a serious role as vectors of numerous vertebrate blood pathogens. *Culex pipiens* is a common mosquito species in Egypt. It is the overwhelming vector of *Wuchereria bancrofti* that causes filariasis, Rift Valley fever virus and West Nile virus. Common control methods, which largely rely on chemical insecticides, are not always effective due to the prevalent resistance of the mosquitoes to these insecticides. In addition, chemical insecticides have adverse effects on the environment, health and food chain through biomagnification of chemicals to the levels that exceed normal (Hassan *et al.*, 2003; Kumar *et al.*, 2012; Fouda *et al.*, 2013; Hassan *et al.*, 2013).

Due to handling ease, immature stages are usually targeted by synthetic insecticides for mosquito control. Although highly efficacious of these insecticides, mosquito control is facing a threat due to the development of resistance to these chemical insecticides resulting in rebounding vectorial capacity (Bream *et al.,* 2018), besides its hazards on human health, non-target organisms, and the environment. From this point of view, researchers diverted their attention towards the plant kingdom to find alternative agents that possess bioactive chemicals that may act as potential insecticides, growth inhibitors as well as inducers of AChE or GST activities (Tripathy *et al.,* 2011; Abdel-Haleem *et al.,* 2020).

One of the safest methods of control of insect pests and vectors is through the application of easily degradable plant compounds. The Lamiaceae family for example, is one of the most popular plants grown extensively in many continents around the world. Additionally, botanical compounds have abroad-spectrum activity, relative specificity in their mode of action and easy to process and use as well as they tend to be safe for animals and the environment (Rajput *et al.*, 2020).

The present study was carried out under laboratory conditions to assess the efficacy of different tested extracts from leaves of *Origanum syriacum, Pergularia tomentosa, Senna italica* and *Otostegia fruticosa* on the biochemical profile of the 3rd instar larvae of *Culex pipiens*. In addition, we tested the biochemical response following exposure to these extracts by measuring acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities which consider biomarkers of neurotoxicity and detoxification.

MATERIALS AND METHODS

Mosquito Colony:

The mosquito used in this study was *Culex pipiens* L., which was provided by the Medical Entomology institute. It was reared for several generations in the Medical Entomology Insectary, Animal house, Faculty of Science, Al-Azhar University, under controlled conditions at a temperature of 27±2°C, relative humidity 70±10% and 12-12 light-dark regimen. Adult mosquitoes were kept in wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days post-emergence. After this period, females were allowed to take a blood meal from a pigeon host which is necessary for laying eggs.

Preparation of Plant Materials:

Leaves of *Origanum syriacum* (Lamiaceae), *Pergularia tomentosa* (Apocynaceae), *Senna italica* (Fabaceae) and *Otostegia fruticosa* (Lamiaceae) were collected from their natural habitats and left to dry away from sun rays at room temperature (27-31^{o}C) for 14 days, then it was pulverized to powder in a hammer mill. The extraction was performed using methanol, ethyl acetate, chlorobenzene and hexane solvents according to the procedure described by Bream *et al,* (2018).

Biochemical Parameters of *C. pipiens* Larvae*:*

The activity of tested plant extracts on the main body metabolites was estimated using the lethal concentration (LC_{50}) levels in order to determine the total carbohydrate, protein and lipid content in addition to the activity of Acetylcholinesterase (AChE) and Glutathione S-transferase (GST). Selected concentrations were calculated for each tested extract according to the methods used briefly by Shahat *et al.,* (2020). Twenty-five 3rd instar larvae were treated with tested concentrations of each extract and maintained at room temperature for 24 h. The larvae were collected and immediately subjected to biochemical estimations procedures. Biochemical tests were carried out by Al-Ahram Lab., Cairo, Egypt, according to the details given in the kit's instructions. The spectrophotometric measurements were performed using a Shimadzu UV-VIS Recording 2401 PC (Japan).

1. Determination of Total Carbohydrate Content:

The total carbohydrate content of the whole-body homogenate was determined according to the method of Singh and Sinha (1977).

2. Determination of Total Protein Content:

The total protein content was determined using folin phenol reagent according to the method of Lowry *et al.*, (1951).

3. Determination of Total Lipid Content:

The total lipid contentwas determined by the phospho-vanillin method that briefly described by (Frings and Dunn 1970).

Determination of Acetylcholinesterase (AChE) Activity:

Thiocholine resulting from acetylcholine hydrolysis by ChE reacts with dithio-bis (nitrobenzoate) forming 2-nitro-5-mercaptobenzoate which measured spectrophotometrically at 405 nm, using bio diagnostic kits. For AChE determination. Three batches of larvae obtained from applying previously specified concentration were homogenized using a homogenizer, with 10 ml solution of 0.1 M-phosphate buffer, pH 7.5 (KH_2PO_4-NaOH), containing 1% Triton X-100. The homogenates were centrifuged for 60 min at 4^oC at 15.000 rpm, using Heraeus Labofuge 400R, Kendro Laboratory products GmbH, Germany. The resulting supernatant was used without further purification for in vitro inhibition assay of the enzyme. The activity of acetylcholinesterase (AChE) was determined by the method of Ellman *et al.*, (1961). Ten microliter aliquots of supernatant were taken for spectrophotometric analyses according to the pamphlet instructions and AChE activity was expressed in term U/L.

Determination of Glutathione S-Transferase (GST) Activity:

Glutathione S-transferase (GST) activity was measured spectrophotometrically by the method of Habig *et al.*, (1974), using bio diagnostic kits. The principle of the method is based on the measurement of the conjugation of S-2, 4-dinitrophenyl glutathione (CDNB) with reduced glutathione. The formation of CDNB adduct was monitored by measuring the net increase in absorbance at 340 nm against the blank. For assay of GST, the treated larvae were homogenized by homogenizer with 10 ml of sodium phosphate buffer (ph. 8.0) with 1% Triton X-100. The homogenate was centrifuged for 60 min at 4°C at 15,000 rpm, using Heraeus Labofuge 400R, Kendro laboratory products GmbH, Germany. Fifty microliter aliquots of supernatant were taken for spectrophotometric analyses according to the pamphlet instructions and GST activity was expressed in terms of U/g tissue.

Statistical Analysis:

Data represented as Mean±SD. One-way analysis of variance ANOVA was applied to find the differences between tested extracts using Tucky's HSD test at 5% probability level, where the means with $P>0.05$ are not statistically significant. Statistical analysis was carried out using GraphPad InStat software.

RESULTS

1. Evaluation of the LC_{50} values

In this study, we assessed the efficacy of tested extracts against the third-instar larvae of *C. pipiens* by determining the median lethal concentration (LC_{50}) -a concentration that kills 50% of the tested population- for methanol, ethyl acetate, chlorobenzene and hexane extracts for each plant. Depending on the data presented in table (1) the toxicity of tested extracts may be arranged in descending order as follows: hexane > chlorobenzene > ethyl acetate > methanol. This experiment was undertaken to determine the median concentration that will be used in further investigations for each extract.

Table 1: Lethal concentrations (LC_{50}) of different tested plant extracts against the mosquito, *Culex pipiens* larvae.

Tested plant	Extracts	LC_{50} (ppm)	95% Confidence Limits		$\chi 2$
			Lower	Upper	
Origanum syriacum	Methanol	530.30	481.26	579.34	1.43 n. s.
	Ethyl Acetate	480.69	460.80	500.59	0.57 n. s.
	Chlorobenzene	117.17	114.310	120.04	1.85 n. s.
	Hexane	83.41	76.38	90.44	1.75 n. s.
Pergularia tomentosa	Methanol	1435.76	1238.30	1453.20	2.49 n. s.
	Ethyl Acetate	1402.60	1318.10	1487.20	1.32 n. s.
	Chlorobenzene	1087.10	1051.50	1122.8	2.39 n. s.
	Hexane	361.26	325.20	397.33	0.25 n. s.
Senna italica	Methanol	1039.47	999.36	1079.6	3.03 n. s.
	Ethyl Acetate	1024.53	922.50	1126.6	0.57 n. s.
	Chlorobenzene	441.52	422.72	460.32	1.21 n. s.
	Hexane	174.73	166.13	183.34	1.11 n. s.
Otostegia fruticosa	Methanol	683.28	653.00	713.57	1.96 n. s.
	Ethyl Acetate	585.48	578.07	592.90	0.79 n. s.
	Chlorobenzene	261.13	242.14	280.09	1.21 n. s.
	Hexane	129.92	126.27	133.57	1.00 n. s.

ppm = particle per million; $\chi 2$ = Chi-square; n. s. = non-significant, (Alpha = 0.05).

2. Effect of tested plant extracts on total carbohydrate, protein and lipid contents of *Culex pipiens* larvae

The data given in table (2) indicate the effect of tested plant extracts on the total carbohydrate contents of the tested larvae. Tested extracts showed a non-significant ($P>0.05$) decrease of the total carbohydrate levels when compared with the control group 24 h and 48 h post-treatment. Total carbohydrate levels were significantly ($P<0.05$) decreased by all tested extracts except methanol extract of *P. tomentosa*, *S. italica*, and *Ot. Fruticosa* where, it recorded 0.59±0.01, 0.51±0.02 and 0.49±0.09 mg/ml, compared with 0.63±0.06 mg/ml for the control. Additionally, ethyl acetate of *P. tomentosa* decreased total carbohydrate to 0.53±0.05 mg/ml. The lowest carbohydrate values (0.28±0.09 and 0.33±0.01 mg/ml) were recorded by hexane extract of *O. syriacum* and *Ot. Fruticosa* with change percent equal to -55.56 and -47.62% (table 2).

Table 2: Effect of tested plant extracts on the total carbohydrate content of *Culex pipiens* third instar larvae.

Tested plant	Extract	Time (hrs)			
		24 (mg/ml)	Change %	48 (mg/ml)	Change %
	Control	0.49±0.11^{a}	-	0.63±0.06^{a}	-
Origanum syriacum	Methanol	0.41±0.03^{a}	-16.33	0.44±0.02^{b}	-30.16
	Ethyl acetate	0.34±0.08^{a}	-30.61	0.37±0.06^{c}	-41.27
	Chlorobenzene	0.29±0.13^{a}	-40.52	0.32±0.05^{d}	-49.21
	Hexane	0.25±0.03^{b}	-48.98	0.28±0.09^{d}	-55.56
Pergularia tomentosa	Methanol	0.46±0.01^{a}	-6.12	0.59±0.01^{a}	-6.35
	Ethyl acetate	0.44±0.02^{a}	-10.20	0.53±0.05^{a}	-15.87
	Chlorobenzene	0.41±0.06^{a}	-16.33	0.48±0.06^{b}	-23.81
	Hexane	0.37±0.13^{a}	-24.49	0.42±0.02^{c}	-33.33
Senna italica	Methanol	0.43±0.04^{a}	-12.24	0.51±0.02^{a}	-19.05
	Ethyl acetate	0.40±0.13^{a}	-18.37	0.46±0.08^{b}	-26.98
	Chlorobenzene	0.38±0.07^{a}	-22.45	0.43±0.03^{c}	-31.75
	Hexane	0.34±0.02^{a}	-30.61	0.39±0.06^{c}	-38.10
Otostegia fruticosa	Methanol	0.42±0.12^{a}	-14.29	0.49±0.09^{a}	-22.22
	Ethyl acetate	0.36±0.06^{a}	-26.53	0.41±0.04^{c}	-34.92
	Chlorobenzene	0.30±0.03^{a}	-38.78	0.34±0.07^{c}	-46.03
	Hexane	0.29±0.08^{a}	-40.82	0.33±0.01^{d}	-47.62

Within each column, different letters denote significant differences, ($P<0.05$)

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Methanol and ethyl acetate extracts exhibited a non-significant (P>0.05) decreased in the total protein contents of *C. pipiens* 3rd instar larvae except *S. italica* ethyl acetate extract that significantly (P<0.05) reduced protein content from 0.85±0.02 mg/ml for the control to 0.77±0.04 mg/ml (table 3). The total protein content of *C. pipiens* 3rd instar larvae after 48 h from exposure was significantly (P<0.05) decreased as compared with control except for methanol extract of *S. italica* which decreased non-significantly (P>0.05) the total protein content after 48 h of treatment. The total protein content recorded 0.59±0.03, 0.72±0.02, 0.65±0.06 and 0.60±0.04 mg/ml by hexane extract of *O. syriacum, P. tomentosa, S. italica* and *Ot. Fruticosa,* respectively, compared with 0.96±0.07 mg/ml for the control group.

Table 3: Effect of tested plant extracts on the total protein content of *Culex pipiens* third larval instar.

Tested plant	Extract	Time (hrs)			
		24 (mg/ml)	Change %	48 (mg/ml)	Change %
	Control	0.85±0.02[a]	-	0.96±0.07[a]	-
Origanum syriacum	Methanol	0.73±0.09[a]	-14.12	0.69±0.01[d]	-28.13
	Ethyl acetate	0.71±0.02[a]	-16.47	0.66±0.04[d]	-31.25
	Chlorobenzene	0.67±0.08[b]	-21.18	0.64±0.07[d]	-33.33
	Hexane	0.64±0.06[b]	-24.71	0.59±0.03[d]	-38.54
Pergularia tomentosa	Methanol	0.84±0.04[a]	-1.18	0.80±0.06[b]	-16.67
	Ethyl acetate	0.81±0.06[a]	-4.71	0.76±0.06[c]	-20.83
	Chlorobenzene	0.80±0.01[a]	-5.88	0.73±0.05[c]	-23.96
	Hexane	0.78±0.01[a]	-8.24	0.72±0.02[c]	-25.00
Senna italica	Methanol	0.80±0.01[a]	-5.88	0.78±0.07[a]	-18.75
	Ethyl acetate	0.77±0.04[b]	-9.41	0.71±0.05[b]	-26.04
	Chlorobenzene	0.74±0.04[c]	-12.94	0.69±0.10[c]	-28.13
	Hexane	0.73±0.02[c]	-14.12	0.65±0.06[c]	-32.29
Otostegia fruticosa	Methanol	0.77±0.05[a]	-9.41	0.72±0.03[c]	-25.00
	Ethyl acetate	0.74±0.03[a]	-12.94	0.69±0.06[c]	-28.13
	Chlorobenzene	0.70±0.09[a]	-17.65	0.66±0.07[d]	-31.25
	Hexane	0.68±0.08[b]	-20.00	0.60±0.04[d]	-37.50

Within each column, different letters denote significant differences, (P<0.05)

According to the data presented in table (4), tested extracts remarkably induced the total lipid content of the 3rd instar larvae 24 h post-treatment. Total lipid content recorded 0.26±0.03, 0.29±0.10, 0.30±0.09 and 0.35±0.05 mg/ml by methanol, ethyl acetate, chlorobenzene and hexane extracts, respectively for *O. syriacum* compared with 0.71±0.13 mg/ml for the control. In addition, tested extracts recorded a significant (P<0.05) inhibition in total lipid contents 48 h post-treatment except for methanol extract of *P. tomentosa* that non-significantly (P>0.05) reduced the total lipid content from 0.80±0.04 mg/ml for the control group to 0.67±0.04 mg/ml (table 4). The lowest lipid content values (0.29±0.06 and 0.32±0.01mg/ml) were from hexane and chlorobenzene extracts of *O. syriacum* after 48 h from treatment with change percentages equal to -63.75 and -60.00.

Table 4: Effect of tested plant extracts on the total lipid content of *Culex pipiens* third larval instar.

Tested plant	Extract	Time (hrs)			
		24 (mg/ml)	Change %	48 (mg/ml)	Change %
	Control	0.71±0.13[a]	-	0.80±0.04[a]	-
Origanum syriacum	Methanol	0.35±0.05[c]	-50.70	0.41±0.04[d]	-48.75
	Ethyl acetate	0.30±0.09[c]	-57.75	0.36±0.09[d]	-55.00
	Chlorobenzene	0.29±0.10[c]	-59.15	0.32±0.01[d]	-60.00
	Hexane	0.26±0.03[d]	-63.38	0.29±0.06[d]	-63.75
Pergularia tomentosa	Methanol	0.64±0.05[a]	-9.86	0.67±0.04[a]	-16.25
	Ethyl acetate	0.55±0.09[a]	-22.54	0.62±0.09[b]	-22.50
	Chlorobenzene	0.53±0.10[a]	-25.35	0.59±0.01[c]	-26.25
	Hexane	0.47±0.03[b]	-33.80	0.54±0.06[c]	-32.50
Senna italica	Methanol	0.57±0.05[a]	-19.72	0.61±0.04[b]	-23.75
	Ethyl acetate	0.52±0.09[a]	-26.76	0.56±0.09[c]	-30.00
	Chlorobenzene	0.50±0.10[a]	-29.58	0.53±0.01[d]	-33.75
	Hexane	0.46±0.03[b]	-35.21	0.50±0.06[d]	-37.50
Otostegia fruticosa	Methanol	0.45±0.05[b]	-36.62	0.49±0.04[d]	-38.75
	Ethyl acetate	0.43±0.09[b]	-39.44	0.46±0.09[d]	-42.50
	Chlorobenzene	0.38±0.10[c]	-46.48	0.40±0.01[d]	-50.00
	Hexane	0.32±0.03[c]	-54.93	0.37±0.06[d]	-53.75

Within each column, different letters denote significant differences, (P<0.05)

2. Effect on tested plant extracts on Acetylcholinesterase (AChE) and Glutathione S-transferase activities (GST).

Depending on the data given in table (5), tested extracts from leaves of *O. syriacum, P. tomentosa, S. italica* and *Ot. Fruticosa* significantly (P<0.001) decreased the AChE activity of *C. pipiens* 3rd instar larvae as compared with the control group. After 24 h of treatment, AChE activity recorded 5.91±0.09, 6.39±0.07, 6.25±0.07 and 6.03±0.16 U/l by hexane extract of *O. syriacum, P. tomentosa, S. italica* and *Ot. Fruticosa,* respectively compared with 7.22±0.18 U/l for the control group. Additionally, after 48 h of treatment, it recorded 5.94±0.11, 6.49±0.05, 6.30±0.05 and 6.09±0.10 U/l by the same extracts compared with 7.65±0.19 U/l for the control.

Obtained data revealed that, tested extracts significantly (P<0.001) promoted the GST activity after 24 and 48 h of treatment as compared with control. The GST activity increased from 0.86±0.09 U/g for the control group to 1.71±0.02, 1.50±0.04, 1.53±0.01, 1.59±0.11 and 1.53±0.06, 1.46±0.09, 1.47±0.05, 1.53±0.08 U/g for methanol extract of *O. syriacum, P. tomentosa, S. italica* and *Ot. Fruticosa,* respectively after 24 and 48 h of treatment (table 6).

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Table 5: Effect of tested plant extracts on the acetylcholinesterase activity for *Culex pipiens* third instar larvae.

Tested plant	Extract	Time (hrs)			
		24 (U/L)	Change %	48 (U/L)	Change %
	Control	7.22±0.18[a]	-	7.65±0.19[a]	-
Origanum syriacum	Methanol	6.17±0.15[d]	-14.54	6.29±0.04[d]	-17.78
	Ethyl acetate	6.11±0.06[d]	-15.37	6.23±0.12[d]	-18.56
	Chlorobenzene	5.99±0.13[d]	-17.04	6.08±0.07[d]	-20.52
	Hexane	5.91±0.09[d]	-18.14	5.94±0.11[d]	-22.35
Pergularia tomentosa	Methanol	6.61±0.11[d]	-8.45	6.70±0.13[d]	-12.42
	Ethyl acetate	6.55±0.02[d]	-9.28	6.63±0.18[d]	-13.33
	Chlorobenzene	6.48±0.15[d]	-10.25	6.56±0.10[d]	-14.25
	Hexane	6.39±0.07[d]	-11.50	6.49±0.05[d]	-15.16
Senna italica	Methanol	6.51±0.11[d]	-9.83	6.58±0.13[d]	-13.99
	Ethyl acetate	6.46±0.02[d]	-10.53	6.53±0.18[d]	-14.64
	Chlorobenzene	6.36±0.15[d]	-11.91	6.44±0.10[d]	-15.82
	Hexane	6.25±0.07[d]	-13.43	6.30±0.05[d]	-17.65
Otostegia fruticosa	Methanol	6.40±0.08[d]	-11.36	6.47±0.11[d]	-15.42
	Ethyl acetate	6.31±0.12[d]	-12.60	6.38±0.07[d]	-16.60
	Chlorobenzene	6.22±0.09[d]	-13.85	6.26±0.14[d]	-18.17
	Hexane	6.03±0.16[d]	-16.48	6.09±0.10[d]	-20.39

Within each column, different letters denote significant differences, (P<0.05)

Table 6: Effect of tested plant extracts on the glutathione S-transferase activity for *Culex pipiens* third instar larvae.

Tested plant	Extract	Time (hrs)			
		24 (U/g tissue)	Change %	48 (U/g tissue)	Change %
	Control	0.86±0.09[a]	-	0.77±0.11[a]	-
Origanum syriacum	Methanol	1.71±0.02[d]	98.84	1.53±0.06[d]	98.70
	Ethyl acetate	1.69±0.06[d]	96.51	1.52±0.09[d]	97.40
	Chlorobenzene	1.66±0.09[d]	93.02	1.50±0.14[d]	94.81
	Hexane	1.52±0.06[d]	76.74	1.49±0.05[d]	93.51
Pergularia tomentosa	Methanol	1.50±0.04[d]	74.42	1.46±0.09[d]	89.61
	Ethyl acetate	1.46±0.12[d]	69.77	1.42±0.02[d]	84.42
	Chlorobenzene	1.41±0.07[d]	63.95	1.38±0.11[d]	79.22
	Hexane	1.39±0.15[d]	61.63	1.36±0.05[d]	76.62
Senna italica	Methanol	1.53±0.01[d]	77.91	1.47±0.05[d]	90.91
	Ethyl acetate	1.48±0.06[d]	72.09	1.41±0.03[d]	83.12
	Chlorobenzene	1.44±0.09[d]	67.44	1.39±0.10[d]	80.52
	Hexane	1.45±0.08[d]	68.60	1.41±0.03[d]	83.12
Otostegia fruticosa	Methanol	1.59±0.11[d]	84.88	1.53±0.08[d]	98.70
	Ethyl acetate	1.57±0.03[d]	82.56	1.49±0.02[d]	93.51
	Chlorobenzene	1.50±0.01[d]	74.42	1.45±0.07[d]	88.31
	Hexane	1.46±0.04[d]	69.77	1.41±0.09[d]	83.12

Within each column, different letters denote significant differences, (P<0.05)

DISCUSSION

Culex pipiens mosquito transmits many vertebrate blood pathogens and its control is an essential issue to prevent or decrease the prevalence of these diseases and to decrease the rate of transmission of other emerging and re-emerging diseases (Elango *et al,* 2009). The increased use of chemical insecticides in the past decades to control mosquito vectors have resulted in serious consequences such as insect resistance, mammalian toxicity, bioaccumulation through food chains, environmental contamination as well as the toxicity of insecticides to non-targeted organisms (Wattanachai and Tintanon 1999; Rohani *et al.,* 2001).

Chemical insecticides for mosquito control have become a daily lifestyle that makes the control of mosquitoes more complicated due to the increased resistance to these synthetic compounds. Therefore, new materials from the natural origin are needed to avoid the hazards of these synthetic chemicals. One of the safest methods of control of insect pests and vectors is through the application of easily degradable plant compounds (Rajput *et al.,* 2020).

The larvicidal activity of tested extracts was found to be solvent-dependent. The hexane extract was more effective against 3rd instar larvae of *C. pipiens* than those of chlorobenzene, ethyl acetate and methanol. These results are in agreement with the previous results recorded by Vahitha *et al*., (2002) using *Pavonia zeylanica* and *Acacia ferruginea* leaves extract against *Culex quinquefasciatus*, where LC_{50} values recorded 2214.7 and 5362.6 ppm; Prabakar and jebanesan, (2004) using *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* extracts against *C. quinquefasciatus* with LC_{50} values of 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm, respectively; Coria *et al.,* (2008) using extracts from *Melia azedarach* against *Aedes aegypti*; Maurya *et al.,* (2009) using petroleum ether extract from leaves of *Ocimum basilicum* against *An. stephensi* and *C. quinquefasciatus*; Samuel *et al.,* (2014) found that *Ipomoea cairica* and *Ageratina adenophora* extracts were found to be effective against *C. quinquefasciatus* third larval instar causing 77-100% mortality; Shehata (2019) found that petroleum ether extract from leaves of *Prunus domestica* and *Rhamnus cathartica* was more effective against *C. pipiens* (LC_{50} 33.3 and 63.4 ppm) than chloroform (LC_{50} 70.8 and 192.1 ppm) and methanolic extracts (LC_{50} 132.7 and 273.5 ppm).

The present study has shown that the median lethal concentration (LC_{50}) of each tested extract insignificantly ($P>0.05$) decreased the total carbohydrate contents when compared with the control group. The depletion in carbohydrate content may be due to the utilization of the reserved carbohydrate sources of the larval tissues as a result of plant extract stress (Shehata 2018). The effect of *A. annua* extract on carbohydrate content of treated larvae is species-specific, depending on the variation in the physiology of the larvae species, further, during treatments, the alimentary canal of *Culex* sp. was ruptured comparatively less seriously and it was found blocked with the extract (Sharma *et al.,* 2006).

Tested plant extracts showed a reduction in total protein content of *C. pipiens* third larval instar. Similarly, Sharma *et al.,* (2011) attributed this decline in protein content to the interference of the plant extracts with the hormones regulating the protein synthesis. Also, Senthilkumar *et al.,* (2009) reported that, the protein level in *An. stephensi* larvae treated with some phytoextracts was reduced and resumed that it was the result of interference of the plant extracts with normal protein synthesis mechanism. Also, the obtained results come in an agreement with those reported by (Huang *et al.,* 2004; El-Sheikh *et al.,* 2010; Shehata 2018).

Tested extracts recorded an inhibition in the total lipid content of *C. pipiens* third

larval instar after 24 and 48 h of treatment. This reduction in lipid content indicates a negative effect of extracts on lipid metabolism and peroxidation. This decline may be due to a shift in energy metabolism towards lipid catabolism as the result of insecticidal stress induced by the extracts (Sharma *et al.,* 2009).

In the present study, we tested also the biochemical response following exposure to these extracts by measuring acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities. Tested extracts significantly ($P<0.001$) decreased the AChE activity of *C. pipiens* 3rd instar larvae, as compared with the control group. The AChE is abundant in the insect's nervous tissue and considers the biomarker of neurotoxicity. The inhibitory effect of tested plant extracts on AChE activity is recorded by many authors using different insecticides against different insect species, such as Kim *et al.,* (2008) for wood vinegar mixed with insecticides on *Nilaparvata lugens* and *Laodelphax striatellus;* Abdul-Aziz (2012) for chlorpyrifos, deltamethrin and methomyl on *Musca domestica*; Dris *et al.,* (2017) who found that *C. pipiens* larvae treated with LC_{50} of *Ocimum basilicum* revealed a neurotoxic activity as evidenced by inhibition of AchE; Shehata (2018) using α-cyper, chlorpyrifos, and methomyl on *C. pipiens;*.

On the other hand, GSTs play an important role in exogenous substances biotransformation, drug metabolism and protection of the organism against oxidative damage. The increased activity in GST revealed a stimulation of the detoxification system of the targeted larvae. Here, effect of tested plant extracts on the GST activity is in agreement with those reported by Jiang *et al.,* (2003) using α-terthienyl on *Helicoverpa armigera* and *Ostrinia furna*calis; Dris *et al.,* (2017) using the median lethal concentration of *O. basilicum* against the fourth-instar larvae of *C. pipiens.*

Conclusion

Based on obtained results, hexane extract showed more activity against 3rd instar larvae of *C. pipiens* than those of chlorobenzene, ethyl acetate and methanol. Tested extracts caused a modification in biochemical composition, the median lethal concentration (LC_{50}) of each tested extract decreased the total carbohydrate, protein and lipid contents as compared with the control. Tested extracts presented a neurotoxic activity as evidenced by the inhibition effect on the AChE that was significantly ($P<0.001$) decreased. It also promoted GST activity of *C. pipiens* 3rd instar larvae as compared with the untreated group. In general, tested extracts used in the present study can be considered as promising control agents against the mosquito vector, *C. pipiens.* Further studies are needed to recommend the development of ecofriendly chemicals from these plant-based extracts for mosquito control.

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