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Natural Larvicidal Agents: Chemical Composition and Toxicological Evaluation of Sidr Oil and Leaf Extracts Against Aquatic Common House Mosquito Larvae

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ABSTRACT

Rising insecticide resistance and the negative impacts of synthetic pesticides have fueled the pursuit of safer, environmentally friendly alternatives. Among these, plant extracts are gaining attention for their natural insecticidal properties, supported by their antioxidant activity and rich essential oil content. *Ziziphus spina-christi* (known locally as Nabak or Sidr), a subtropical plant with long-standing traditional uses, was investigated in this study for its larvicidal potential against *Culex pipiens* Linnaeus (Diptera: Culicidae), a medically important mosquito species. Bioassays revealed that sidr oil was far more potent than ethanol and methanol leaf extracts, with LC_{50} values of 110.87, 2372.14, and 4421.92 ppm, respectively. Biochemical analyses showed that methanol extract significantly reduced protein levels (57.7%) and slightly lowered lipid content (2.2%), while sidr oil caused the most striking decline in carbohydrate levels (57.3%) after 48 hours. Notably, acetylcholinesterase activity increased dramatically, especially with ethanol extract (576.03%), whereas glutathione (GSH) reserves were depleted, with methanol extract showing the greatest reduction (28.67%). Phytochemical and GC–MS analyses confirmed the presence of bioactive metabolites: methanol and ethanol extracts were rich in phenolics, flavonoids, tannins, and saponins, while key compounds included phytol, octadecatrienoic acid, squalene, and oleic acid (the latter dominating the oil at 66.6%). In conclusion, the antioxidant-rich profile of *Z. spina-christi* confers strong larvicidal activity, positioning sidr oil and leaf extracts as promising eco-friendly alternatives to conventional insecticides for mosquito control.

INTRODUCTION

Mosquitoes spread a wide range of human and animal diseases, making their control a global public health challenge **(Riaz *et al*., 2012)**. *Culex pipiens* L. is recognized among of the most widespread mosquito species across the globe **(Shehata, 2019)**. In Egypt, eleven species of *Culex* are prevalent nationwide between these species, *Culex pipiens* L. (Diptera: Culicidae), the common house mosquito, is the predominant mosquito vector in both rural and urban zones and it is the principal transmitter of lymphatic filariasis, Rift

Valley fever virus and Western Nile virus **(Zahran *et al*., 2017; Shahat *et al*., 2020a**). Reactions that manifest as allergies and dermatological issues resulting from mosquito bites constitute significant health concerns linked to the existence of mosquitoes **(El-Sabrout *et al*., 2020)**.

The utilization of artificial agents like antibiotics for controlling of animal diseases or pesticides for the treatment of phytopathogens represents a favorable option; however, this practice adversely impacts natural biological control mechanisms, fosters antibiotic resistance among microorganisms, and contributes to the accumulation of pesticide residues in agricultural products. Recent investigations have established novel strategies for the management of diseases affecting both humans and plants. The employment of natural substances, including antioxidants and antimicrobial agents, is currently advancing **(Fard *et al*., 2020)**.

Botanical extracts signify a highly promising alternative due to their non-polluting nature, ease of biodegradability, and reduced toxicity to the ecosystem **(Blenau *et al*., 2012)**. From this perspective, researchers turned their focus to the plant kingdom in search of alternative sources of bioactive compounds that could serve as potential insecticides **(Shahat *et al*., 2020b)**. Plant-derived products have gained attention for their biological potential, particularly their antioxidant and insecticidal properties, which are associated with the presence of phenolics, iso-flavonoids, essential oils, alkaloids, and tannins **(Elaloui *et al*., 2022)**.

The botanical genus *Ziziphus*, belonging to the Rhamnaceae family, comprises around 900 species worldwide and is native to Asian tropical zones, Africa, and the Americas **(Fard *et al*., 2020)**. *Ziziphus spina-christi* is a subtropical plant known as Nabak or Sidr, in Arabic. It is a deciduous tree indigenous to zones characterized by warm-temperate and subtropical conditions, notably South and East Asia as well as the Middle East **(Yossef *et al*., 2011)**.

Phytochemical screening of *Ziziphus spina-christi* (sidr) has become essential to validate its strong effectiveness and traditional medicinal applications, its leaves and fruits are valued for anti-inflammatory, hypoglycemic, hypotensive, and pain-relieving effects **(Elaloui *et al*., 2017)**. These biological activities are likely associated with the availability of secondary metabolites, like saponins, flavonoids, alkaloids, lipids, proteins, and phenolic compounds, which have been isolated from the leaves **(Yu *et al*., 2013; Khaleel *et al*., 2016; Ads *et al*., 2017; Rigane *et al*., 2017; Elaloui *et al*., 2022)** in addition to sterols and tannins. The leaves of *Ziziphus spina-christi* exhibited strong insecticidal activity, attributed to the n-hexane and ethyl acetate fractions, emphasizing the potential applications of these extracts in industry **(Elaloui *et al*., 2022)**.

Plant oils are composed of evaporative and aromatic terpenoids that modulate voltage-gated and ligand-gated ion channels in the central nervous system, including tyramine, octopamine, GABAA_AA, TRP-type ion channels, and acetylcholinesterase (**Blenau *et al*., 2012**). Essential oils and their main components have gained significant

interest as potential bioactive component for insect control **(Raghavendra *et al*., 2011; Zahran & Abdelgaleil, 2011)**. Due to their low mammalian toxicity, high biodegradability, and cost-effectiveness, essential oils are considered highly promising candidates for developing potent and environmentally friendly pest control formulation **(Farahat *et al*., 2024)**. Essential oils, produced through secondary plant metabolism, exhibit notable larvicidal potential, with their constituents able to deter insects, decreasing enzymatic activity, and inducing deformities and mortality across different larval stages **(Pavela, 2015; Nathan, 2020)**. *Ziziphus spina-christi* oil was identified as containing carotol, hexadecanoic acid, linoleic acid, vetivenic acid, and valeranone, compounds known for their antioxidant activity **(Fard *et al*., 2020)**. To date, only a limited number of plant-derived products have shown potential for large-scale field application in vector control **(Raghavendra *et al*., 2011)**. This study was designed to assess the larvicidal efficacy of *Z. spina-christi* essential oil and leaves extracts against *Cx. pipiens*.

MATERIALS AND METHODS

Maintenance of *Culex pipiens* larvae

Culex pipiens was maintained under insect laboratory rearing unit conditions at Ain Shams University and Research and the Training Center of Diseases (RTC) following the methods of **Gerberg (1970)**.

Adults were maintained in wooden mesh cages designed for respiration with fabric holes for food and jars entry. Sucrose solution (10%) was continuously administered for adult sustenance through an inverted jar covered with a piece of gauze, allowing the sugar solution to flow when needed. A blood meal was offered to the female mosquitoes before mating by allowing them to feed on pigeons to support the egg formation. Pupae were collected in plastic jars half-filled with water. Eggs were laid in separate water filled-jars placed at the bottom of cages, while emerging adults flew into the upper portion **(Kauffman *et al*., 2017)**. The cages were kept at 26– 29°C with 70–85% relative humidity under a 12:12h light–dark photoperiod according to the **WHO (2005)**. Larval stages were reared in water plates containing a small amount of fish food and water was changed periodically between stages.

Extraction of plant material:

Leaves of *Ziziphus spina-christi* were sampled from Siwa oasis. The plant material was taxonomically identified by Professor Mohamed Tantawy, professor of plant taxonomy and flora faculty of Science Ain Shams university. Leaves were rinsed with tap water to eliminate surface impurities and subsequently dried at room temperature. Leaves powder was pulverized by a milling device to obtain a fine powder. A portion of the leaf powder (150g) was subjected to maceration in 1000mL of 80% ethanol and 80%

methanol. Extraction took place overnight on a shaker at ambient temperature. The solutions were then filtrated with Whatman filter paper to obtain the crude extract. Excess solvent from the filtrate was eliminated under vacuum using a rotary evaporator at 40°C. The crude extracts were transferred to brown bottle stored at deep freezer at -4°C.

Phytochemical screening studies

Estimation of total phenolics

The total phenolic content of the raw samples was quantified by the Folin–Ciocalteu reagent **(Makkar *et al*., 1993)**. The reaction mixture contained 0.2mL of crude extract (ethanolic or methanolic) or gallic acid standard solution (0– 100mg/ L), 1.8mL of distilled water, and 0.2mL of 10% Folin–Ciocalteu reagent. Following a 5-minute interval, an addition of 0.2mL of 7% Na_2CO_3 solution and 0.8mL of distilled water was conducted, followed by 90min of dark incubation at room temperature. The absorbance was read against the blank at 750nm using spectrophotometer (Spectronic 601, Milton Roy Company) and the total phenolic levels were standardized to gallic acid equivalents (mg GAE) per gram of dry mass.

Estimation of total flavonoids

The aluminum chloride colorimetric method described by **Chang *et al*. (2002)** was applied to determine the total flavonoids in the ethanolic and methanolic crude extracts. The reaction mixture contained 1ml of crude extract with 0.1ml of 1M potassium acetate, 1.5ml of methanol, 0.1ml of 10% aluminum chloride and 2.8ml of distilled water. This mixture was incubated at room temperature for 30min. The absorbance of this mixture was measured against the blank at 415nm using spectrophotometer (Spectronic 601, Milton Roy Company). Total flavonoid concentration was quantified using a quercetin standard curve prepared in 80% (v/v) methanol. The findings were standardized to milligrams of quercetin equivalents per gram of dry matter.

Estimation of tannins

Tannins were measured by Folin-Ciocalteu method **(Chandran & Indira, 2016)**, with minor modification. An aliquot of the crude plant extract was transferred to a measuring flask containing 4mL of distilled water, followed by the addition of 0.5mL Folin–Ciocalteu phenol reagent and 2mL of 17% sodium carbonate solution. The mixture was thoroughly mixed and was left under ambient conditions for 20 minutes. Absorbance was compared with the blank at 760nm with an UV/ Visible spectrophotometer (Spectronic 601, Milton Roy Company). Tannin amount was determined in three replicates and standardized to milligrams of tannic acid equivalents per gram of dry matter.

Estimation of saponins

The crude plant extracts were vigorously shaken, developing a voluminous froth that persisted for nearly an hour, indicating the presence of saponins **(Moftah, 2001)**.

Total anti-oxidation activity (radical scavenging activity)

The radical scavenging activity of crude extracts were detected by 2,2, diphenyl-1-picrygydrazyl (DPPH) as a free radical the method of **Hatano *et al.* (1988)**. We used 0.01 mM of DPPH prepared as 0.004g of DPPH in 00ml methanol. The mixture was 0.5ml of (0.01 mM) DPPH and 1.5ml of crude extracts. A decrease in absorbance in tests extracts was measured at 517nm using an UV/ Visible spectrophotometer (Spectronic 601, Milton Roy Company). The blank consisted of a solution lacking the extract. The percentage inhibition of DPPH free radicals was evaluated using the following formula:

$$\% \text{ scavenging activity} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Gas chromatography/ mass spectrometry (GC- MS)

GC–MS analysis was conducted using an Agilent 7890B GC system with a 5977A mass detector and an HP-5MS capillary column (30m × 0.25mm × 0.25µm). Helium was used as the carrier gas at 8.2psi with a 1µL splitless injection at 300°C. The oven program started at 60°C (3min), increased to 300°C at 20°C/ min, and was kept for 5min. Mass spectra were recorded in the 50– 550m/z range under EI ionization (70 eV) with an 8.0min solvent delay, using BSTFA with trimethylchlorosilane as the silylating agent.

The reaction was carried out by adding 300µL of BSTFA to each extract, succeeded by heating in a water bath at 80°C for two hours. Constituents were identified by comparing their mass fragmentation patterns with reference spectra from the NIST/EPA/NIH Mass Spectral Library (Version 2.2, June 2014) using the NIST Mass Spectral Search Program."

Toxicological evaluation

Larvicidal activity of ethanolic and methanolic leaf extracts and oil of *Ziziphus spina-christi* was evaluated against third-instar *Culex pipiens* larvae using the immersion method. (**WHO, 2005**) as follows:

Groups of twenty late third-instar *Culex pipiens* larvae were transferred in disposable test cups holding 100mL of water, maintained under laboratory conditions (27 ± 2 °C, 70 ± 10% RH, and a 14:10 h light–dark cycle). Five different concentrations of each crude extracts range from 1400 to 6000 ppm were prepared in ethanol or methanol

and different concentrations of sidr oil ranging from 50 to 170ppm. The experiment was carried out by using three replicates for each treatment, and for control an addition of 1ml of ethanol or methanol to distal water was performed; mortalities ranged from 20% to 90%. Tiny amount of fish food Tetramine was added to both treated and control replicates. Mortality data were recorded at 24, 48, 72 and 96-hours post-treatment and correction for control mortality was carried out through the Abbotts formula described in the study of **Abbott (1925)**.

Biochemical studies

Biochemical activities were assessed by homogenizing whole larvae exposed to the LC_{50} concentration of each compound, followed by centrifugation at varying speeds. The obtained supernatant served as the source for determining total protein, carbohydrate, lipid, acetylcholinesterase, and glutathione transferase (GSH) activities **(David *et al*., 2002)**.

Determination of total protein

Total protein concentration was estimated by **Bradford**'s **(1976)** method using bovine serum albumin (BSA) as the standard. Supernatants were centrifuged at 12,000 rpm for 20min, and BSA standards (0.2– 0.8mg/ mL) were prepared in test tubes to a total volume of 1.0mL with distilled water. Each tube received 5mL of Coomassie Brilliant Blue (CBB) reagent, vortexed, and the optical density was recorded at 595nm within 1h by a spectrophotometer (UNICO SP2100 UV, China). A reagent blank was prepared with 1mL distilled water and 5mL CBB, and each measurement was carried out three times. A standard calibration curve was generated by plotting protein concentration (mg/mL) against absorbance, which was subsequently used to quantify protein levels in the unknown samples.

Determination of total carbohydrates

Total carbohydrate content was determined following the method of **Singh and Sinha (1977)**. Diluted homogenate samples (100µL; 1:4), centrifuged at 4000 rpm for 20min, were transferred to test tubes and were mixed with 5mL of anthrone reagent (prepared by combining 360mL of 75% sulfuric acid with 140mL distilled water, followed by the addition of 250mg anthrone while maintaining warm and vigorous stirring). The mixtures were vortexed, and absorbance was recorded at 620nm. Each measurement was performed in triplicate.

Determination of total lipids

Total lipid content was determined from supernatants centrifuged at 8000 rpm for 20min using method of the Sulfo-Phospho-Vanillin (SPV) of **Frings and Dunn (1970)**. The phosphovanillin (PV) reagent was produced by dissolving 6mg vanillin in 100mL

hot water and diluting to 500mL with 85% phosphoric acid. For analysis, 20µL of each sample (with or without oleic acid) was mixed with 180µL concentrated sulfuric acid, maintained at 100°C for 10 minutes, followed by cooling, and treated with 0.5mL PV reagent for color formation. Following 15min incubation at 37°C, samples were placed in 96-well microplates, kept in darkness for 45min, and optical density was measured at 490nm using a multilabel plate reader (Biotek-800TS, USA). Data were represented as absorbance values.

Determination of acetylcholine esterase

Acetylcholinesterase (AChE) activity was assayed following **Simpson *et al*. (1964)** using acetylcholine bromide (AChBr) as the substrate. Supernatants centrifuged at 5000 rpm for 10min were used in a reaction mixture containing 200µL enzyme solution, 0.5mL of 0.067 M phosphate buffer (pH 7), and 0.5mL of 3mM AChBr. The mixture was incubated at 37°C for 30min, after which 1mL of alkaline hydroxylamine (1:1 mixture of 2M hydroxylamine chloride and 3.5M NaOH) was added, followed by 0.5mL HCl (1:2 dilution of concentrated HCl in distilled water). The mixture was stirred thoroughly and left to stand for 2min, after which 0.5mL of ferric chloride (0.9M in 0.1M HCl) was added to the mixture. The hydrolysis of AChBr by AChE was then quantified spectrophotometrically at 515nm. The solution was intensely agitated and left to stand for 2min, then 0.5mL ferric chloride solution (0.9 M $FeCl_3$ in 0.1 M HCl) was added and thoroughly mixed. The reduction of AChBr by hydrolysis catalyzed by AChE was measured spectrophotometrically at 515nm.

Determination of GSH enzyme

Glutathione (GSH) levels were measured according to **Ellman *et al*. (1961)**. Tissue extracts were made in 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 2000 rpm for 10min. The supernatant was reacted with 10 mM DTNB in 0.1 M phosphate buffer (pH 8.0), kept at ambient conditions for 10min, and optical density was recorded at 412nm. The level of GSH was calculated from the standard curve and was reported as µg per mg protein.

Statistical analysis

Mortality data were examined using probit regression to estimate LC_{50}, LC_{95}, and slope values **(Finney, 1971)**. Correction for control mortality was carried out through the Abbotts formula according to **Abbott (1925)**. Toxicity index and relative potency were measured according to the method described by **Sun (1950)** to compare the potencies of the tested extracts. Data were expressed as the mean ± standard error (SE). Statistical analyses were conducted using SPSS software (version 19). Comparisons among treatment groups were performed using one-way analysis of variance (ANOVA),

followed by least significant difference (LSD) post hoc tests. A *P*- value of less than 0.05 was considered statistically significant.

RESULTS

1. Phytoconstituents of sidr leaves extracted by ethanol and methanol

The results indicate that sidr leaves extracted using ethanol and methanol as solvents contain phenols, flavonoids, tannins, and saponins as major phytoconstituents as shown in Table (1).

The data presented in Table (2) showed clear differences between the ethanol and methanol leaves extractions. The methanol extract contained higher levels of phenolic and flavonoid compounds, while the ethanol extract showed greater tannin content. In terms of antioxidant performance, the ethanol extract exhibited slightly stronger DPPH radical scavenging activity, suggesting a higher overall antioxidant potential compared to the methanol extract.

Table 1. Phytoconstituents analysis of sidr leaves extracted by ethanol and methanol

Phytochemical component	Ethanolic extract	Methanolic extract
Phenol	+	+
Flavonoid	+	+
Tannin	+	+
Saponins	+	+

Table 2. Quantitative analysis of phytoconstituents and DPPH scavenging capacity of sidr leaves

Antioxidant component (mg/g dry weight)	Ethanolic extract	Methanolic extract
Phenolic (mg GAE/g dw)	21.50 ±2.5	20.27 ±0.08
Flavonoid (mg QE/g dw)	76.71 ±7.6	54.25 ±0.63
Tannin (mg CE/g DW)	331.75 ±16.3	124.76 ±62.3
DPPH Scavenging (%)	67.71 ±2.90	66.55 ±031

Values are presented as mean ± SE.

2. Gas Chromatography of sidr oil, ethanol and methanol leaves extracts

Qualitative analysis was done to determine the constituents of sider oil, ethanol and methanol leaves extract by using GC-MS. Tables (3, 4, 5) show the chemical components, the RT, area of the peak concentration (%), molecular formula and molecular weight of the identified components, which led to the identification of natural chemical components.

2.1. Chemical composition of ethanol sider leaves extract

Gas chromatography examination of the ethanol extract indicated the existence of 51 chemical components as shown in Table (3). The major components included phytol (18.78%), a diterpenoid; (9,12,15)-octadecatrienoic acid (Z,Z,Z) (15.93%), recognized as an omega-3 fatty acid (α-linolenic acid); and n-hexadecanoic acid (10.75%), a saturated long-chain fatty acid. Additionally, phytol-TMS derivative (9.96%) was also classified as a diterpenoid, while squalene (5.45%) belongs to the terpenoid and isoprenoid class. Neophytadiene (3.29%) was categorized as a diterpene, vitamin E (2.85%) and tricosane (1.29%) as members of the tocopherol and tocotrienol groups, and isophytol (1.14%) as a terpenoid alcohol. Notably, some components were uniquely detected in the ethanol extract, including vitamin E (2.85%) and stigmast-5-ene, 3β-(trimethylsiloxy)-, (24S) (1.47%), which belongs to the sterol class.

Table 3. Gas chromatography-mass analysis of ethanol sidr leaves extract

No.	RT	Area%	Compound name	Formula	Molecular weight
1	8.4817	0.0605	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	$C_9H_{27}O_3Si_4$	**295.65**
2	9.6261	0.1496	Acetic acid, bis[(trimethylsilyl)oxyl]-, trimethylsilyl ester	$C_{11}H_{28}0_4Si_3$	**308.59**
3	10.0896	0.0685	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	**384.84**
4	10.4386	0.0515	1-Octadecanesulphonyl chloride	$C_{18}H_{37}CIO_2S$	**353**
5	10.5073	0.184	Thiophene, 2-decyl-	$C_{14}H_{24}S$	**224.41**
6	10.9593	0.0819	Eicosane	$C_{20}H_{42}$	**282.5**
7	11.171	3.263	Neophytadiene	$C_{20}H_{38}$	**278.5**
8	11.2969	1.0107	3,7,11,15-Tetramethyl-2-	$C_{20}H_{40}O$	**296.5**

			hexadecen-1-ol		
9	11.3942	2.4354	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	$C_{10}H_{18}$	**138.25**
10	11.5201	0.6262	Phthalic acid, isobutyl octadecyl ester	$C_{30}H_{50}O_4$	**474.7**
11	11.6517	0.278	8-(2-Nitrophenoxy)octan-1-ol	$C_{14}H_{21}NO_4$	**267.32**
12	11.749	1.1429	Isophytol	$C_{20}H_{40}O$	**296.5**
13	11.955	1.3537	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	**284.5**
14	12.0007	10.7568	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	**256.42**
15	12.5958	18.7899	Phytol	$C_{20}H_{40}O$	**296.5**
16	12.7503	9.9657	Phytol, TMS derivative	$C_{23}H_{38}OSi$	**368.7**
17	12.8647	15.9397	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	**278.4**
18	13.2081	0.343	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	**402.5**
19	13.6544	0.4059	Oleic Acid	$C_{18}H_{34}O_2$	**282.5**
20	13.723	0.3651	4,8-Decadien-3-ol, 5,9-dimethyl-	$C_{12}H_{22}O$	**182.3**
21	14.0378	0.2215	Pentacosane	$C_{25}H_{52}$	**352.7**
22	14.095	0.2188	Carbonic acid, 2-ethylhexyl heptadecyl ester	$C_{26}H_{25}O_3$	**412.7**
23	14.3696	1.7286	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	**390.6**
24	14.5356	0.0905	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	**917.2**
25	14.7701	0.2783	Hexadecane, 1-iodo-	$C_{16}H_{33}I$	**352.34**
26	14.8274	0.2602	Carbonic acid, 2-ethylhexyl heptadecyl ester	$C_{26}H_{52}O_3$	**412.7**
27	14.9475	0.3215	Octocrylene	$C_{24}H_{27}NO_2$	**361.5**
28	15.0677	0.1962	Cyclotetradecane	$C_{14}H_{28}$	**196.37**
29	15.1192	0.5875	Octacosane	$C_{28}H_{58}$	**394.8**
30	15.2909	5.4537	Squalene	$C_{30}H_{50}$	**410.7**
31	15.3824	0.4341	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	**222.37**
32	15.4682	0.9708	alpha.-Tocospiro A	$C_{29}H_{50}O_4$	**462.7**

33	15.5483	0.6285	alpha.-Tocospiro B	$C_{29}H_{50}O_4$	**462.7**
34	15.6227	0.8358	Ethyl 3-hydroxy-2,6-di-p-chlorostyrylisonicotinate	$C_{24}H_{19}CI_2NO_3$	**440.3**
35	15.8402	0.7452	Eicosane	$C_{20}H_{42}$	**282.5**
36	15.9489	1.0668	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	$C_{25}H_{48}$	**348.6**
37	16.2979	0.5814	Cedran-diol, (8S,14)-	$C_{15}H_{26}O_2$	**238.3657**
38	16.4238	0.8873	gamma.-Tocopherol	$C_{28}H_{28}O_2$	**416.7**
39	16.5955	0.8712	Phytyl dodecanoate	$C_{32}H_{62}O_2$	**478.8**
40	16.6527	0.3182	Cholestane, 2-formyl-3-(2-methylbenzylidene)-	$C_{36}H_{54}O$	**502.8**
41	16.7843	2.8519	Vitamin E	$C_{29}H_{50}O_4$	**430.7**
42	17.2306	1.2926	Tricosane	$C_{23}H_{48}$	**324.6**
43	17.4366	0.6095	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	**207.27**
44	17.6025	0.9035	Stigmasterol, TMS derivative	$C_{32}H_{56}OSi$	**484.9**
45	17.6884	0.5801	(E)-Tetradec-11-en-1-yl 2,2,3,3,4,4,4-heptafluorobutanoate	$C_{18}H_{27}F_7O_2$	**408.4**
46	17.8657	0.5293	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	**917.2**
47	17.9859	1.4803	Stigmast-5-ene, 3.beta.-(trimethylsiloxy)-, (24S)-	$C_{32}H_{58}OSi$	**486.9**
48	18.0717	0.8518	1-Bromo-11-iodoundecane	$C_{11}H_{22}BrI$	**361.1**
49	18.3578	0.4073	Hexadecanoic acid, 2-hydroxy-, methyl ester	$C_{17}H_{34}O_3$	**286.4**
50	18.5238	0.1968	Benzonitrile, 2-fluoro-4-(4'-propyl[1,1'-bicyclohexyl]-4-yl)-	$C_{22}H_{30}FN$	**327.5**
51	19.2104	0.2497	4-n-Hexylthiane, S,S-dioxide	$C_{11}H_{22}O_2S$	**218.36**

(RT) retention time

2.2. Chemical composition of methanol leaves extract

Gas chromatography examination of the methanol extract detected the existence of 25 chemical constituents as shown in Table (4). The major constituents included phytol-TMS derivative (37.27%), classified as a diterpenoid; n-hexadecanoic acid (10.19%), a saturated long-chain fatty acid; and neophytadiene (5.35%), a diterpene. Other notable compounds included tetradecanal (4.38%), a long-chain fatty alcohol; hexadecanoic acid, methyl ester (3.35%), known as methyl palmitate and classified as a fatty acid methyl ester; and 9-octadecenoic acid, methyl ester (E) (1.14%), another fatty acid methyl ester. Additionally, the methanolic extract was characterized by the presence of unique components such as tetradecanal (4.83%), dibutyl phthalate (2.91%) and bis(2-ethylhexyl) phthalate (2.66%), both grouped as phthalates, and cyclodecasiloxane, eicosamethyl (1.79%), a cyclic siloxane.

Table 4. Gas chromatography-mass analysis of methanol sidr leaves extract

No.	**RT**	**Area %**	**Compound name**	**Formula**	**Molecular weight**
1	8.476	0.3815	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	458.99
2	10.0667	0.4011	Pentanedioic acid, 2,4-bis[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester, (R*,S*)	$C_{17}H_{40}O_6Si_4$	452.8
3	10.4329	0.2579	Eicosane	$C_{20}H_{42}$	282.5
4	10.5016	0.7577	Heptane, 1,7-dibromo-	$C_7H_{14}Br_2$	257.99
5	11.1653	5.3586	Neophytadiene	$C_{20}H_{38}$	278.5
6	11.2912	2.1966	1-Hexadecyne	$C_{16}H_{30}$	222.41
7	11.3885	4.8388	Tetradecanal	$C_{14}H_{28}O$	212.37
8	11.5143	2.9173	Dibutyl phthalate	$C_{12}H_{22}O_4$	278.34
9	11.6517	3.3568	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
10	11.7489	1.9171	Isophytol	$C_{20}H_{40}O$	296.5
11	11.8004	1.3322	7-Thiabicyclo[4.2.1]nonane	$C_8H_{14}S$	142.26

12	12.0122	10.1941	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
13	12.3154	1.2121	2,5-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370.66
14	12.4928	1.3284	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5
15	12.5386	0.9154	Oleic Acid	$C_{18}H_{34}O_2$	282.5
16	12.5786	1.1408	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5
17	12.7274	37.2785	Phytol, TMS derivative	$C_{23}H_{48}OSi$	368.7
18	13.0078	5.3167	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{13}H_{39}O_5Si_6$	443.96
19	13.1966	2.9609	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	402.5
20	13.8375	0.6139	m,p'-DDD	$C_{14}H_{10}Cl_4$	320
21	14.3639	2.6632	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6
22	14.9475	0.583	2-(Acridin-9-ylamino)-3-methyl-butyric acid	$C_{18}H_{18}N_2O_2$	294.3
23	15.0848	1.3351	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.4
24	17.2191	1.7925	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741.5
25	18.0545	1.0052	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	577.2

(RT) retention time

2.3. Chemical composition of sidr oil

Data in Table (5) show that the gas chromatography analysis of sidr oil revealed that it is primarily composed of oleic acid (66.6%), a long-chain fatty acid; 9,12-octadecadienoic acid (Z,Z) (22.55%), classified as an omega-3 fatty acid (linolenic acid); and n-hexadecanoic acid (2.02%), a component found in all three sidr extracts.

Table 5. Gas chromatography–mass analysis of sidr oil

No.	RT	Area%	Compound name	Formula	Molecular weight
1	12.4586	0.0946	benzoic acid, 4-[[(dimethylamino)methylene]amino]-	$C_{16}H_{16}N_2O_2$	**268.31**
2	14.6787	2.0213	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	**256.4**
3	15.6229	66.6935	Oleic Acid	$C_{18}H_{34}O_2$	**282.5**
4	16.7158	22.5597	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	**280.4**
5	17.2594	0.1824	6-Nonynoic acid	$C_9H_{14}O_2$	**54.21**
6	18.8558	0.1378	Silane, (butoxymethyl)trimethyl-	$C_8H_{20}OSi$	**160.33**
7	19.3364	0.1672	D-Arabino-Hexonic acid, 3-deoxy-2,5,6-tris-O-(trimethylsilyl)-, .gamma.-lactone	$C_{15}H_{34}O_5Si_3$	**378.68**

(RT) retention time

3. Toxicological activity

The data presented in Tables (6,7,8,9) indicate that sidr oil and both ethanol and methanol extracts of sidr leaves exhibited larvicidal activity against *C. pipiens* 3rd instar larvae. The LC_{50} values after 48 hours were 110.87 ppm for sidr oil, 2372.14 ppm for the ethanol extract, and 4421.92 ppm for the methanol extract. Sidr oil showed the highest toxicity, with mortality increasing significantly after 96 hours. Similarly, both extracts demonstrated enhanced larval mortality over time, indicating a latent toxic effect.

Table 6. Mortality percentage of *Culex pipiens* 3rd larval instar treated with varying concertation of ethanol sidr leaves extract

Conc. (ppm)	Percentage mortality (%) ± SE			
	24hr	**48hr**	**72hr**	**96hr**
Control	3.3± 1.7[a]	6.7± 1.7 [a]	6.7± 1.7 [a]	6.7± 1.7 [a]
1400	6.7± 1.7 [b]	9.5± 2.9 [b]	11.2± 1.7 [b]	13± 1.7 [b]
1800	20± 2.9 [c]	20± 2.9 [c]	26.7± 4.4 [c]	34.3± 3.3 [c]
2200	21.7± 1.7 [c]	28.6± 1.7 [d]	37.8± 4.4 [d]	41.4± 1.7 [d]
2600	53.3± 3.3 [d]	53.8± 3.3 [e]	57.4± 2.9 [e]	64.4± 1.7 [e]

3000	81.7± 4.4 [e]	89± 2.9 [f]	92.8± 4.4 [f]	94.6± 2.9 [f]
LC25	1984.51	1908.83	1798.19	1708.18
LC50	2471.26	2372.14	2247.70	2242.59
LC95	4219.32	4029.80	3873.06	3723.22

ANOVA and LSD tests indicated that column values denoted by different letters vary significantly ($P< 0.05$).

Table 7. Mortality percentage of *Culex pipiens* 3rd larval instar treated with varying concertation of methanol sidr leaves extract

Conc. (ppm)	**Percentage mortality (%) ± SE**			
	24hr	**48hr**	**72hr**	**96hr**
Control	3.3± 1.7[a]	3.3± 1.7 [a]	3.3± 1.7 [a]	5± 0.0 [a]
2200	3.3± 1.7 [a]	10± 2.9 [b]	26.6± 1.7 [b]	41.6± 1.7 [b]
3000	6.7± 1.7 [b]	18.3± 1.7 [c]	38.3± 3.3 [c]	56.6± 6.0 [c]
4200	13.3± 1.7 [c]	46.7± 1.7 [d]	65± 2.9 [d]	86.7± 3.3 [d]
5200	56.6± 4.4 [d]	71.7± 4.4 [e]	86.7± 4.4 [e]	96.7± 3.3 [e]
6000	70± 2.9 [e]	86.7± 1.7 [f]	95± 2.9 [f]	100± 0.0 [f]
LC25	4068.82	3118.17	2347.08	1904.20
LC50	5185.07	4421.91	3210.53	2566.53
LC95	9365.18	8086.57	6092.21	5314.72
Slope±SE	6.4 ±0.61	5.60 ±0.45	4.9 ±0.42	5.2 ±0.46

ANOVA and LSD tests indicated that column values denoted by different letters vary significantly ($P < 0.05$).

Table 8. Mortality percentage of *culex pipiens* 3rd larval instar treated with varying concertation of sidr oil

Sidr oil Conc. (ppm)	**Percentage mortality (%) ± SE**			
	24hr	**48hr**	**72hr**	**96hr**
Control	3.3± 1.7[a]	6.7± 1.7[a]	6.7± 1.7 [a]	6.7± 1.7 [a]
50	6.7± 1.7[b]	41.4± 2.9 [b]	57.4± 2.9 [b]	64.4± 4.4 [b]
100	20± 2.9[c]	46.8± 2.9 [c]	59± 1.7 [c]	62.7± 2.9 [c]
130	35± 5.0[d]	66.2± 4.4 [d]	80.4± 1.7 [d]	87.5± 1.7 [d]
150	70± 2.9[e]	73.4± 2.9 [e]	82.2± 3.3 [e]	89.3± 2.9 [e]

170	80± 5.0[f]	85.7± 4.4 [f]	92.8± 1.7 [f]	96.3± 1.7 [f]
LC25	105.95	36.16	21.64	19.61
LC50	139.16	110.87	48.34	40.98
LC95	288.24	462.34	342.93	247.41
Slope±SE	4.76 ±0.49	2.09 ±0.31	1.93 ±0.31	2.10 ±0.33

ANOVA and LSD tests indicated that column values denoted by different letters vary significantly ($P< 0.05$).

Table 9. The toxicity data of sidr oil, ethanol extract and methanol extract on *Culex pipiens* 3rd larval instar after 48 hours of exposure

Treatments conc.(ppm)	LC_{50}	LC_{95}	Slope ±SE	Toxicity index	Relative potency
Sidr oil	110.87	462.34	2.09 ±0.31	100%	39.8
Ethanolic extract	2372.14	4029.80	7.14 ±0.63	4.6%	1.8
Methanolic extract	4421.92	8086.57	5.60 ±0.45	2.5%	1

Values are presented as mean ± SE.

4. Biochemical studies

Data presented in Fig. (1) illustrate the effect of LC_{50} concentrations of sidr oil, ethanol and methanol leaf extracts on the biochemical composition of *C. pipiens* third instar larvae. The results revealed that larvae treated with the methanol extract exhibited the greatest reduction in protein levels, with a decrease of approximately 57.7%, from 29.67 ± 0.132 to 12.55 ± 0.015 mg/ml. This extract also led to a slight reduction in lipid levels by 2.2% in contrast to the control group. Conversely, treatment with sidr oil resulted in the most pronounced decrease in carbohydrate levels, showing a reduction of approximately 57.3%, from 2.18 ± 0.010 to 0.93 ± 0.007 mg/ml.

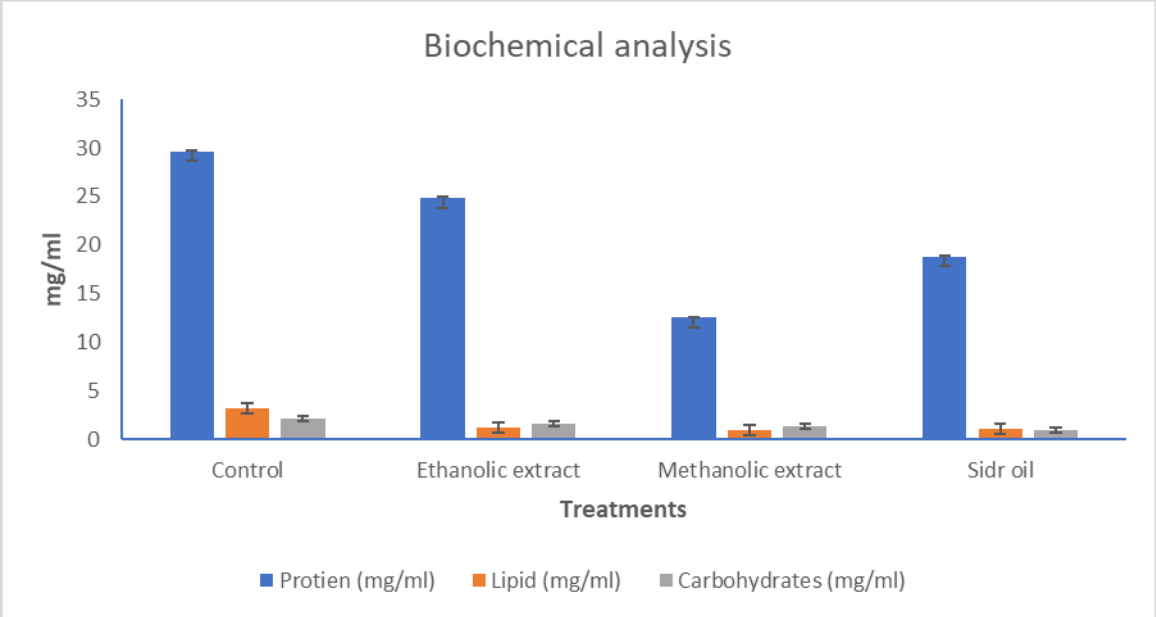

Fig. 1. Impact of LC_{50} concentrations of sidr oil, ethanol and methanol extracts on protein, lipid, and carbohydrate levels in treated *Culex pipiens* third instar larvae

Results in Fig. (2) show a great increase in Acetylcholinesterase enzyme activity about 576.03% in larvae treated with ethanol extract, and larvae treated with methanol extract. The Acetylcholinesterase enzyme activity increased about 449.83% compared with control larvae. On the other hand, the elevation in Acetylcholinesterase enzyme activity is about 131.8% more than control larvae.

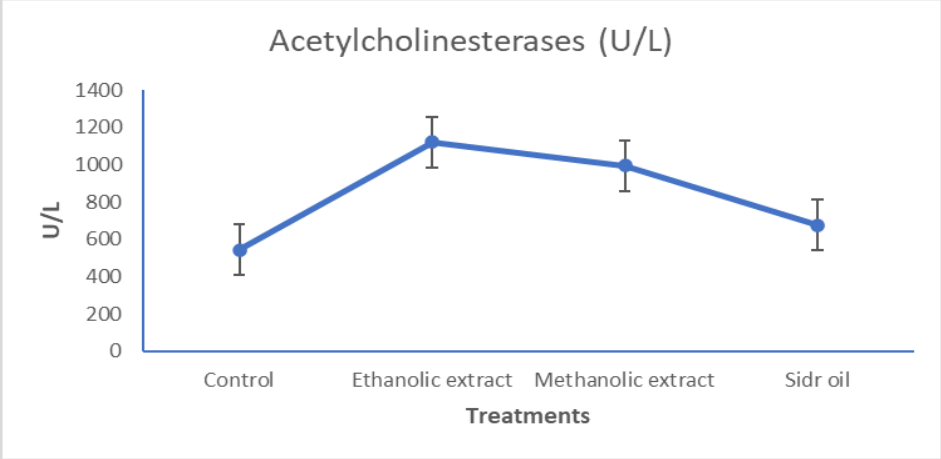

Fig. 2. Impact of LC_{50} concentrations of sidr oil, ethanol and methanol extracts on Acetylcholinesterase enzyme levels in treated *Culex pipiens* third instar larvae

The activity levels of glutathione were measured in both treated and control larvae. As shown in Fig. (3), a decrease in activity level was detected in larvae treated with the

methanol extract, sidr oil, and ethanol extract, with percentage decreases of approximately 28.67, 28.10, and 26.73%, respectively

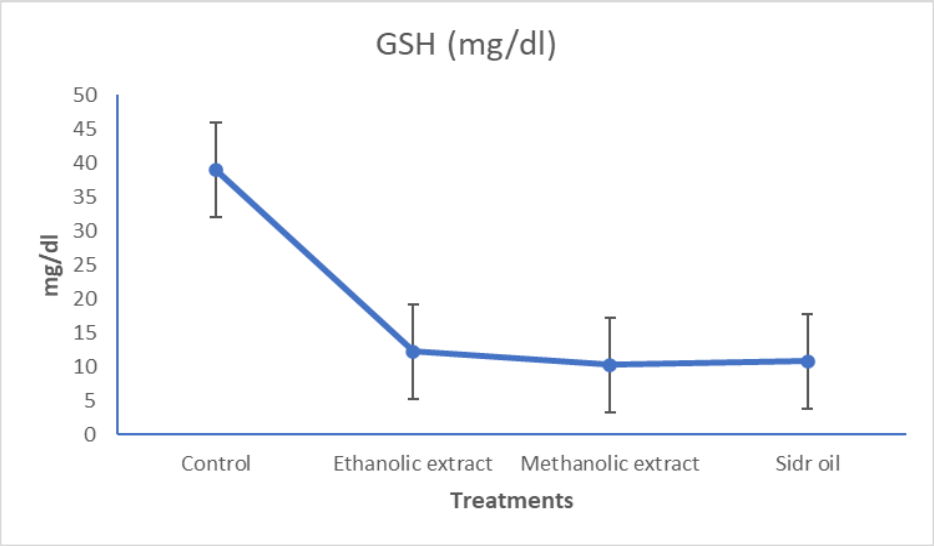

Fig. 3. Impact of LC_{50} concentrations of sidr oil, ethanol and methanol extracts on glutathione levels in treated *Culex pipiens* third instar larvae

DISCUSSION

This study emphasizes the significance of the insecticidal uses of *Ziziphus spina-christi* leaf extracts because of its high phenolic content. Such extracts have potential uses as insecticidal, antioxidant, and allelopathic agents **(Elaloui *et al*., 2022)**. The leaves of *Z. spina-christi* (sidr) are reported to possess a broad spectrum of biologically active substances, including saponins, alkaloids, flavonoids, lipids, proteins, and free sugars. In the current work, relatively high levels of phenolic compounds were detected in both ethanolic (21.50 ± 2.5 mg/g Dw) and methanolic (20.27 ± 0.08 mg/g Dw) extracts. Our findings are within range for those obtained by **Khaleel *et al*. (2016)** and **Elaloui *et al*. (2022)** who recorded lesser and higher values of phenolic contents, extracted by methanol.

Such discrepancies may reflect variations in extraction methods, solvent polarity, and seasonal differences in leaf collection. Additionally, interactions between phenolic compounds, flavonoids, and antioxidant activity may influence the measured levels **(Khaleel *et al*., 2016; Noutarki *et al*., 2017)**. The obtained variation implies that phenolic yield is highly dependent on both environmental conditions and methodological factors. Therefore, careful optimization of extraction procedures and consideration of ecological influences are essential for standardizing *Z. spina-christi* extracts for potential insecticidal applications.

Our findings further verified the existence of tannin and flavonoids in *Z. spina-christi* leaves. Flavonoid content reached 76.71 ± 7.6 mg/g Dw in ethanol extracts and

54.25 ± 0.63 mg/g Dw in methanol extracts. Similarly, tannins were detected at 331.75 ± 16.3 mg/g Dw and 124.76 ± 62.3 mg/g Dw in ethanol and methanol extracts, respectively. In comparison, **Elaloui *et al*. (2022)** reported much lower values of tannins and flavonoids in methanol extracts, with 4.42mg/ g Dw of flavonoids and 1.62mg/ g Dw of tannins. On the other hand, **Barakat *et al*. (2024)** detected considerably higher flavonoid contents in *Z. spina-christi* (sidr) powder, ranging from 411.72 to 455.03mg/ 100g Dw, while in sidr pulp and seeds extracted with water, flavonoid levels ranged from 96.54 to 152.47mg/ 100g Dw. Antioxidant activity, obtained by the DPPH assay, further supports these findings. **Barakat *et al*. (2024)** reported antioxidant activity ranging from 53.02 to 64.59% in sidr leaf powders and aqueous extracts, which closely resembles our results for ethanolic (67.71 ± 2.90%) and methanolic (66.55 ± 0.31%) extracts. This similarity suggests that the oxidative capacity of *Z. spina-christi* is strongly linked to its phenolic and flavonoid contents. Indeed, a positive association between total phenols, flavonoids, and antioxidant activity has been emphasized by **Badr *et al*. (2020)**. Collectively, these findings suggest that *Ziziphus spina-christi* leaves represent a rich source of bifunctional compounds with potent free radical scavenging activity, though quantitative variations across studies may result from differences in plant material, solvent polarity, and extraction methods.

The GC–MS analysis of *Ziziphus spina-christi* (sidr leaves) revealed a wide range of phytochemical constituents, with 51 compounds identified in ethanol extracts and 25 compounds in methanol extracts. The primary components in both extracts included n-hexadecanoic acid (a saturated long-chain fatty acid), phytol TMS derivative (diterpenoid), squalene (a terpenoid and isoprenoid), neophytadiene (diterpene), vitamin E (a tocopherol), tricosane (long-chain hydrocarbon), and isophytol (a terpenoid alcohol). These compounds are widely recognized for their bioactive and antioxidant activities.

Comparable findings were documented by **Elaloui *et al*. (2016)**, who detected β-sitosterol and stigmasterol as dominant constituents in *Z. jujuba*. Across different ecotypes, α-linolenic acid (42.04%) was the dominant component, followed by palmitic acid (23.04%), oleic acid (13.08%), linoleic acid (12.19%), stearic acid (9.8%), behenic acid (3.47%), and myristic acid (2.5%). Moreover, triterpene alcohols and methyl sterol fractions such as squalene, cycloartenol, and citrostadienol were identified as key constituents. This indicates that *Z. spina-christi* shares common phytochemical features with *Z. jujuba* while retaining unique chemical profiles.

The larvicidal bioassay revealed that ethanol extracts were more toxic to *Culex pipiens* larvae than methanol extracts, with LC_{50} values of 2372.14 and 4421.92 ppm, respectively, after 48h of exposure. These observations agree with **Elaloui *et al*. (2022)**, who found higher insecticidal activity of ethanol extracts compared with methanol extracts, due to differences in phytochemical composition. In contrast, fractions from *Zizyphus oxyphylla* (n-hexane and ethyl acetate) exhibited only mild insecticidal activity

against *Callosobruchus analis*, suggesting that insecticidal potential varies both among *Ziziphus* species and with extraction solvent **(Nisar *et al*., 2010)**.

The larvicidal activity of sidr leaf extracts can be attributed to their phytochemical composition. **Collares *et al*. (2023)** emphasized that terpenoids, terpenes, and carbonyl compounds in plant extracts act as insecticides against mosquitoes, beetles, and caterpillars. Furthermore, the high content of triterpene alcohols and methyl sterols, such as citrostadienol, may provide antioxidant activity, while saponins present in sidr leaves (as indicated in our results) may physically impair larval respiration. Saponins lower water surface tension, preventing the hydrophobic hairs around the siphon from attaching to air bubbles, thereby blocking respiration. Excessive foaming may also coat spiracles, leading to impaired gas exchange, CO_2 accumulation, immobility, and eventually death **(Elhag Hammed, 2009)**.

Phytochemical profile of *Ziziphus spina-christi* essential oil further supports its bioactivity. Major compounds included carotol, hexadecanoic acid, linoleic acid, vetivenic acid, and valeranone, which are associated with antioxidant effects **(Fard *et al*., 2020)**. Our GC–MS analysis also revealed high levels of oleic acid (66.69%), linoleic acid (22.55%), and n-hexadecanoic acid (2.02%). Notably, oleic acid, an unsaturated fatty acid, has been reported to exhibit larvicidal activity against *C. pipiens* and *C. quinquefasciatus* **(de Meloa *et al*., 2018; Abdel-Haleem *et al*., 2023)**. This supports our bioassay results, where sidr oil displayed higher toxicity (LC_{50} = 110.87 ppm) against third instar larvae compared to ethanol (2372.14 ppm) and methanol (4421.92 ppm) extracts. Previous studies have also confirmed stronger insecticidal activity of oils compared with solvent-based extracts. For example, **El-Husseiny *et al*. (2014)** reported that *Z. jujuba* oil was more toxic than its ethanol and petroleum ether extracts, while **Alouani *et al*. (2025)** observed significant larvicidal effects of *Z. spina-christi* oil (LC_{50} = 686 and 536mg/ L at 48 and 72h, respectively), with residual activity lasting up to 12 days.

The larvicidal activity of sidr oil may be linked to its high fatty acid content, which enhances penetration and accumulation in larval tissues **(Boutjagualt *et al*., 2022; Aly *et al*., 2023; Eltak *et al*., 2023)**. Biochemical assays revealed that treatment with LC_{50} concentrations of sidr extracts caused reductions in proteins, lipids, and carbohydrates in third instar larvae after 48h. Methanolic extracts induced the greatest inhibition of protein levels (a 17.12% reduction from 29.67 ± 0.132 to 12.55 ± 0.015 mg/ml), along with decreases in lipids and carbohydrates. These findings agree with **Simmonds (2003)**, who reported that phenolic compounds exert post-ingestive effects in the insect midgut through oxidative mechanisms, leading to superoxide radical formation and oxidative stress **(Barbehenn, 2002)**. Fatty acids may further alter protein metabolism in mosquito larvae **(Sugumar *et al*., 2014)**.

The extent to which insects tolerate phenolics depends on antioxidant defenses, detoxifying enzymes (cytochrome P450 monooxygenase, esterases), and midgut pH **(Rey**

***et al*., 2000)**. During oxidative stress induced by plant extracts exceeds larval antioxidant capacity, protein oxidation and lipid peroxidationtake place, leading to mortality. In contrast, **Devi and Bora (2017)** reported that phenolic extracts of *Z. jujuba* increased protein levels in *Aedes aegypti* larvae by 15.44% compared to controls, although lipids and glycogen decreased by 5.52 and 12.32%, respectively. They attributed protein elevation to the overproduction of detoxifying enzymes and stress proteins such as heat-shock proteins **(Zhao & Jones, 2012)**. Similarly, decreases in lipid and carbohydrate levels may reflect metabolic shifts, where glycogen is mobilized as an immediate energy source **(Arrese *et al*., 2010)**, followed by lipid catabolism when carbohydrate reserves are depleted **(Sharma *et al*., 2011)**. Such depletion of essential biomolecules ultimately weakens larval development and survival **(Aly *et al*., 2023)**.

Acetylcholinesterase (AChE) serves as a main enzyme responsible for hydrolyzing choline esters and has significant role in nervous system regulation. In the present study, AChE activity increased markedly in *Culex pipiens* larvae following treatment with *Ziziphus spina-christi* extracts and oil, rising by 576.03% in ethanolic, 449.83% in methanolic, and 131.8% in oil-treated larvae compared to controls. This elevation suggests a disruption of nervous system function, likely as a compensatory response to the neurotoxic effects of plant derived secondary metabolites. Like compounds including saponins, flavonoids, alkaloids, and essential oils may inhibit acetylcholinesterase, causing acetylcholine accumulation, and in turn, larvae may upregulate AChE as a detoxification or adaptive mechanism. In contrast, glutathione (GSH), a major antioxidant responsible for neutralizing reactive oxygen species (ROS) and acting as a cofactor for glutathione S-transferase (GST), was significantly depleted, reflecting oxidative stress and high consumption during detoxification. The observed reduction in GSH likely results from excessive ROS production triggered by phytochemicals that impair mitochondrial function or promote oxidative imbalance. Consistently, GSH activity decreased after 48 h of treatment, with the strongest inhibition observed in methanolic extracts (28.67% below control), followed by sidr oil (28.1%) and ethanolic extracts (26.73%). These findings differ from those of **Huang *et al*. (2013)** and **Aly *et al*. (2023)**, who observed increased GSH level and reduced acetylcholinesterase activity in larvae exposed to polyphenolic-rich extracts. However, they align with the explanation of **Mills *et al*. (2004)**, who suggested that essential oils exert their insecticidal effects mainly through neurotoxicity. Impacts include acetylcholinesterase inhibition, disruption of GABA receptor function **(Priestley *et al*., 2003)**, in addition to a stimulatory effect on the octopamine system **(Enan, 2005)**.

CONCLUSION

Overall, this study demonstrates that *Ziziphus spina-christi* leaves and oil are rich in bioactive compounds, particularly fatty acids, phenolics, and flavonoids, which

collectively contribute to their strong antioxidant, insecticidal, and toxicological stress effects on *Culex pipiens* larvae. Both ethanolic and methanolic extracts exhibited significant larvicidal activity, with the ethanol extract being more potent, while sidr oil exhibited the highest efficacy, reflected by lower LC_{50} values and stronger biochemical impacts. The enhanced potency of sidr oil suggests that essential oils may provide an environmentally friendly and sustainable substitute to synthetic insecticides, while also offering natural antioxidant benefits. Biochemical assays confirmed that the treatments induced oxidative stress and neurotoxic effects, evidenced by increased Acetyl cholinesterase activity and glutathione depletion thereby disrupting larval detoxification and metabolism. Collectively, these findings highlight *Z. spina-christi* as prospective source of natural compounds in mosquito control, warranting additional research into optimized formulations and potential field applications for integrated pest management.

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