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Asafetida plant extract as potential antioxidant, antimicrobial, and odor retardant insecticidal agent against *Culex pipiens*

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Hundreds of species of mosquitoes are reported to be capable of transmitting diseases to humans. As a result, mosquito control is essential worldwide, especially in locations with large concentrations of freshwater sources. *Asafetida* plant extract was prepared and studied their phytochemical characteristics to be used as insecticidal agent against c. pipiens. The total carbohydrate contents, polyphenolic contents, flavonoid contents and protein contents were found 51.90, 129.0, 10.27, and 366.67 mg/g, respectively. The greatest mortality percentage was observed at the concentration 1000 ppm (86.7%) and (80%) after 12 h and 24 h post treatment. In respect to the mortality rate the LC 50, LC 90 and LC 95 were found to be 352.1, 3154.8 and 4028.8 ppm. The total antioxidant values were significantly higher at the concentrations of 125 ppm and 1000 ppm (1.52, 1.49) through the first 12 h of the treatment as well as lipid per oxidation levels showed higher enzymatic levels of 3.6, 3.7 at 125, 250 ppm than those of the control group (0.11).The phenolic concentration increased gradually from 0 to 250 ppm (0.32, 1.32) then suppressed to (0.58) at 500 ppm. DPPH levels showed noticeable elevation through 12 h were (97.0) at 125 ppm while declined at 250 ppm till (95.6) to rise again at 500 ppm (97.3). SOD gradually increase at 500 ppm at 12 and 24 h (0.37, 0.36) than control while superoxide anion radical concentration levels presented as fluctuated from 0 to 1000 ppm.

Keywords *Culex pipiens*, *Asafetida*, Insecticidal, Antimicrobial

In all tropical and sub-tropical countries, mosquitoes (Diptera: *Culicidae*) serve as carriers of life-threatening diseases such as encephalitis, filarial nematode, malaria, and the West Nile Viruses. Mosquitoes pose a serious threat to both human and animal health[1–5]. More than 100 species of mosquitoes are reported to be capable of transmitting diseases to humans. As a result, mosquito control is essential worldwide, especially in locations with large concentrations of freshwater sources[6]. The principal vector of filariasis (also known as Elephantiasis) in humans and various other animal illnesses in Egypt are mosquitoes of *Culex pipiens* (*Cx. pipiens*, Diptera: *Culicidae*)[7–9]. Because of mosquito proliferation and the potential for disease transmission, the use of synthetic pesticides has been a crucial element in mosquito control management. The extensive use of synthetic pesticides such as organophosphates and organochlorines has resulted in pollution, pest resistance, and destruction of non-target beneficial fauna, in turn resulting in fostered environment and human health concern. Thus, natural pesticide substitutes have garnered global medical and environmental interest because of their low toxicity, biodegradability, and capacity to overcome insecticide resistance[10,11]. Due to its acceptance in homes, cities,

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and other sensitive areas, insecticides based on botanical extracts and essential oils are becoming more popular among organic producers and ecologically concerned customers[11]. Plant essential oils (EOs) contain substances that can trigger lethality in the mosquito larvae through diverse mechanisms, such as oxidative stress induction, making them a promising alternative with significant biotechnological potential. Moreover, essential oils (EOs) are the most extensively utilized locally, internationally and very effective at controlling a variety of insects[12]. The chemical compounds used to eradicate insects in a variety of ways, including repellents[13] attractants[14] feeding deterrents/antifeedants, toxicants, growth retardants, and chemosterilants[15] are affected by plant compounds like flavonoids, alkaloids, esters, glycosides, and fatty acids[16,17].

Asafoetida (*Asafetida*) is an oleo-gum obtained from some botanical species of Ferula such as, *Ferula assafoetida* and *Ferula narthex*[18]. Recent developments with bioactive metabolites, such as asafoetida (ASF), have sparked interest in pest management. This oleo-gum resin has piqued human curiosity since it was revealed to have important medicinal and nutritional properties. Recent research has shown a variety of important assets, including antibacterial, anthelmintic, and insecticidal activities. ASF alone has been utilized as a bio-insecticide; for example, oil extracts from *Ferula asafoetida* L. have been employed as an insect pest repellant against thrips, feeding deterrent, and an insect oviposition barrier[19].

Insects cannot tolerate the strong smell of ASF and will therefore avoid grain containers. Therefore, it is most practical for controlling pests. Oxidative stress occurs when the production of reactive oxygen species (ROS) is out of balance with the antioxidant defense system. ROS, such as hydrogen peroxide and superoxide anion radicals, can damage cells. Elements, such as proteins and lipids, have a sophisticated antioxidant defense system with both enzymatic and non-enzymatic components to counteract oxidative stress. Researchers frequently use biomarkers to determine the level of oxidative stress[20]. Two commonly used biomarkers are superoxide anion radicals, which are extremely reactive oxygen species that can damage cellular components and lead to oxidative stress. Lipid peroxidation is a chain reaction initiated by ROS that results in the generation of lipid hydroperoxides[21,22]. These compounds can harm cell membranes and lead to a variety of disorders. The antioxidant capacity of a biological system indicates its ability to neutralize ROS and avoid oxidative damage. The antioxidant capacity may be measured using a variety of methods: total antioxidant capacity: this measurement gives a worldwide evaluation of the antioxidant potential of a sample, taking into account both enzymatic and non-enzymatic antioxidants. Phenolic compounds are a diverse group of plant secondary metabolites with potent antioxidant properties. They can directly scavenge ROS and chelate metal ions, thereby preventing oxidative damage[23,24].

In the context of the research hypothesis, phenolic compounds can serve as both a biomarker of oxidative stress and a potential therapeutic agent. By measuring the concentrations of phenolic compounds under various experimental circumstances. Furthermore, finding phenolic compounds with high antioxidant activity may open the way for the creation of new antioxidant therapies and functional foods. In addition to assessing their effectiveness by estimating their capacity for enzymatic and non-enzymatic antioxidants, the goal of this study was to evaluate the larvicidal effects of ASF extract against *Cx. pipiens in vitro*.

In the context of the research hypothesis, phenolic compounds can serve as both a biomarker of oxidative stress and a potential therapeutic agent. By measuring the levels of phenolic compounds in different experimental conditions. Additionally, identifying specific phenolic compounds with potent antioxidant activity can pave the way for the development of novel antioxidant therapies and functional foods. In addition to analyzing their effectiveness by estimating their capacity for enzymatic and non-enzymatic antioxidants, the objective of this study was to assess the larvicidal impact of ASF extract against *Cx. pipiens* in vitro. As a result of the active ingredients contained in the ASF extract, which is reflected in its ability to act as an antioxidant, as well as its ability to inhibit the growth of many bacteria, in addition to some phenolic and sulfur components that give it a pungent smell, we hope that all these qualities will be combined to make not only an environmentally friendly insect repellent in the event of its proximity to the living environment, but more than that, it has a high ability to kill insects at low concentrations.

Materials and methods

Synthesis of asafetida extract

Assorted Asafetida is a well-known medicinal plant. The dried latex (gum oleoresin) exuded from the rhizome or tap root of several species of ferula was purchased from Harraz for medical plants, Cairo, Egypt, and the extract was prepared as follow: exactly, 10 g of the dried latex was soaked in 100 ml of distilled water for 24 h. The extract was stirred for an extra 24 h. The insoluble fibers were filtered to obtain a thick, brown, pungent extract with a predominant garlic scent. The extract was placed in a deep freezer at -20 °C until completely frozen. Lyophilization was then performed for two days at -48 °C. The resulting powdered extract represents a 100% concentration, and the required quantity was weighed directly and dissolved in a prober quantity of water to achieve the desired concentration.

Identification of polyphenols and flavonoids active ingredients by high performance liquid chromatography (HPLC)

HPLC analysis was carried out using an Agilent 1260 series. The separation was done using Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1–11 min (75% A); 11–18 min (60% A); 18–22 min (82% A) ; 22–24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each sample solution. The column temperature was maintained at 40 °C.

Identification of the volatile compounds by gas chromatography mass spectroscopy (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977 A). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using Hydrogen as the carrier gas at a flow rate of 1.1 ml/min at a splitless, injection volume of 1.0 µl and the following temperature program: 40 °C for 1 min; rising at 10 °C /min to 200 °C and held for 1 min ; rising at 20 °C /min to 220 °C and held for 1 min ; rising at 30 °C /min to 320 °C and held for 3 min. The injector and detector were held at 250 °C, 320 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50–550 and solvent delay 2.00 min. The mass temperature was 230 °C and Quad 150 °C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Determination of the total phenolic contents

The total Phenolic concentration was determined using the Folin-C Assay according to the method of[25]. The method is composed of water extraction of dried extracts with sonication followed by reaction with the Folin-C reagent. The resulting colorimetric reaction was measured spectrophotometrically at 765 nm and compared with a standard curve. Briefly, 1 ml of the tested extract were dissolved in 2 ml of methanol, and 500 µl aliquots of extract were mixed with 2.5 ml Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 ml (75 g/L) sodium carbonate. The tubes were vortexes for 10 s and allowed to stand for 2 h at 25 °C. After incubation at 25 °C for 2 h, absorbance was measured at 765 nm against blank reagent. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gm.

Determination of the total flavonoid contents

A modified calorimetric method was used. Exactly 1 ml of the extract was dissolved in 2 ml of methanol in a 10 ml of volumetric flask. A 5% $NaNO_3$, 5% NaOH and 7% $AlCl_3$ solutions were prepared by using water in a 25 ml volumetric flask. A 200 µl of the extract were taken in a sealed glass vial within 75 µl of 5% $NaNO_3$ and kept at room temperature for 5 min. Later on, 1.25 ml of $AlCl_3$ and 0.5 ml NaOH were added to each vial. The solutions were sonicated and incubated at room temperature for 5 min then the absorbance was measured to all working and standard solutions against methanol blank at 510 nm. The flavonoid content of the extract was estimated by using the quercetin standard calibration curve and the obtained results were expressed as micrograms of quercetin equivalent (Qu) per 1gm of dry extract[26].

Determination of the total carbohydrates

Precisely 100 mg of the dried extract were weighed and placed in boiling tubes. The boiling tubes were kept in the water bath for 3 h then removed and cooled at room temperature. After cooling, the contents of tubes were neutralized by adding solid sodium carbonate until the effervescence ceases. distilled water was added till 100 ml of volume reached then the tubes were centrifuged. Supernatants were kept for use in further reactions. A (0.2, 0.4, 0.6, 0.8, and 1 ml) working standard with 0.1 mg/ml of glucose were added in boiling tubes and the final volumes of each tube were made a 1 ml by adding distilled water. A 1 ml of phenol (5%) and 5 ml of sulphuric acid (96%) were added to each tube one by one then shake well until complete mixing with the working standards. After 10 min, all tubes were placed in water bath at 25–30 °C for 15 min. The blank was added with 1 ml of distilled water in a clean cuvette and the OD of each tube were taken at 490 nm with the help of Biosystem 310 plus spectrophotometer. The phenol and sulphuric acid method was repeated with 0.2 ml of different samples and the OD was taken[27].

Determination of the antioxidant activity by DPPH

The free radical activity of plant leaves extracts was evaluated using the α-diphenyl-β-picrylhydrazyl (DPPH) colorimetric assay according to Blois method[28]. The reported capacity of samples to inhibit the production of reactive oxygen species is expressed as a percentage. A 1 ml of DPPH was added to 3 ml of different extracts in ethanol at different concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/ml). Only extracts that are solubilized in ethanol are utilized here, and their varied concentrations were prepared by the dilution procedure. The mixture was then shaken vigorously and kept at room temp for 30 min. The absorbance was then measured at 517 nm by using a spectrophotometer (UV-VIS Milton Roy). The reference standard used compound was ascorbic acid, and the experiment was performed in triplicate. The sample's IC 50 value, which is the concentration necessary to block 50% of the DPPH free radical, was obtained using a log dose inhibition curve. Lower absorbance of the reaction mixture indicated increased free radical activity. The percent DPPH scavenging effect was calculated using the following equation: DPPH scavenging effect (%) or Percent inhibition = A_0 - A_1 / $A_0 \times 100$. Where A0 was the absorbance of control reaction and A1 was the absorbance in presence of test or standard sample. The DPPH assay was based on the measurement of the scavenging capability of free radicals in the sample. Also, the total protein concentration of samples was determined spectrophotometrically according to the method of Bradford[29]. All these experiments were conducted three times.

Determination of oxidative stress biomarkers

Body homogenates from larvae mosquitoes were used for measuring the concentrations of endogenous lipid peroxides and superoxide anion radicals spectrophotometrically according to the method described by Chen and Li[30]. Lipid peroxides concentration was measured according to Hermes-Lima, et al.[31] and the $O_2^{\bullet-}$ production rate was expressed as OD/mg protein/min. The tissues of insect *Cx. pipiens* were homogenized in ice-cold methanol (1:5, w/v) and mortar (10 strokes/ 30 s) with phosphate buffer (pH 7.0, 0.5 mM) containing epinephrine, NADPH and potassium phosphate buffer. The homogenate samples were then centrifuged at 2000 rpm at 4 °C for 10 min. An amount of 5 ml of the resulting supernatant was mixed with the following components $FeSO_4$, H_2SO_4, and

xylenol orange sequentially. The resulting samples were then incubated in dark conditions at room temperature for 3 h. Then, 10 µl of 0.5 mM Cumene hydroperoxides (an internal standard) were added to each sample. The samples were kept at room temperature for 1 h before they determined spectrophotometrically at 580 nm using an UV Visible Spectrometer. The change in absorbance due to the addition of internal standards was calculated. Lipid peroxides concentration was expressed as mM Cumene hydroperoxides/mg protein.

Assessment of the antioxidant capacity

The antioxidant capacity of insects exposed to the different experimental conditions was assessed by measuring (i) the total antioxidant capacity, (ii) the non-enzymatic and (iii) the enzymatic antioxidant capacities of the samples.

Total antioxidant capacity

The total antioxidant capacity of the insect's tissues was measured according to the procedure described by Prieto, et al. 30. The insect's tissues were mixed with sulfuric acid, sodium phosphate, and ammonium molybdate solution. Then, samples were incubated at 95˚C for 90 min. The absorbance was determined spectrophotometrically at 695 nm.

Non-enzymatic antioxidant capacity

The α-diphenyl-β-picrylhydrazyl (DPPH) antioxidant activity was determined according to Blois (1958) and reported the capacity of a sample to inhibit the production of reactive oxygen species (expressed as a percentage). The DPPH assay was based on the measurement of the scavenging capability of the sample. The test measured 525 nm absorbance. The total protein concentration of samples was determined spectrophotometrically according to the method of Bradford 27. All these experiments were done with three replicates.

Enzymatically based antioxidant capacity

The superoxide dismutase (SOD) was measured according to the method of Misra and Fridovich[32]. The homogenized sample mixed with 5 ml of cold buffer (100 mM potassium phosphate buffer (PMS) and 2 mM EDTA). 2 ml of 10% w/v homogenate was centrifuged at 4,000 rpm for 15 min at 4 °C and the supernatant was mixed with sodium carbonate buffer and freshly prepared epinephrine. The absorbance of samples was measured at 480 nm at room temperature. The activity of SOD is expressed as OD/µg protein/min.

Phenolic concentration

The concentration of phenolic in insects' tissue were performed using colorimetric kit according to methodology of Kupina, et al.[25].

Colony of *Cx. Pipiens*

Cx. pipiens larvae were provided from Medical Research Institute, Dokki, and Giza, Egypt. Mosquito larvae were reared at 27 ± 2 °C and 75–80%, relative humidity (RH) and under a photoperiod of 14:10 h (light/dark) in the insectary room for six generations, according to Alkenani, et al.[33].

Exposure to extract

The Extract were tested to evaluate their larvicidal activity against early 4th larval instar *Cx. pipiens*[34]. The desired concentration was prepared by weighing the solid-lyophilized ASF extract and dissolving at a specific quantity oof distilled water with addition of small amount of tween 20 to help solubility. Concentrations of extract (0, 125, 250, 500, and 1000 ppm) were performed according to Abdel-Meguid[35]. About sixty mosquito larvae were placed in a 500 mL glass beaker containing 250 mL. Each experiment was conducted three times, with the control group receiving only the solvent. Larval mortality was measured at 1, 6, 12, and 24 h post treatment (PT). At the end of the exposure period, the insects were homogenized in potassium phosphate buffer (pH = 7.0) at 4 °C. The samples were further stored at -20 °C until being processed for the below-described physiological measurements.

Antimicrobial evaluation

The most-used technique for antimicrobial susceptibility testing is the well-diffusion test, which yields a quantitative result (zones of inhibition in millimeters) and a qualitative interpretive category (e.g., susceptible or resistant). The CLSI has proposed a well diffusion method for mold susceptibility testing. One significant advantage of this disk diffusion method is that results can be obtained after 16 to 48 h of incubation[36,37]. The CLSI disk method uses non supplemented Mueller-Hinton agar or routine bacteriology laboratory Mueller-Hinton agar plates (pH, 7.2–7.4 after gelling) because this agar provides suitable optimum growth of mold at either 24–48 h. However, the suitability of any new batch of Mueller-Hinton medium should be checked by following CLSI recommendations, because some batches may not support adequate growth of some organisms. Therefore, zones obtained by the well (disk) diffusion test usually will be larger than expected and may exceed the acceptable control limits[36]. The well method employs an inoculum suspension adjusted as described for the broth dilution standard method, and agar plates should be inoculated within 15 min after adjusting the suspension. The entire dried agar surface is evenly streaked in three different directions. Allowing the agar surface to dry for no more than 15 min, then a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a 20–100µl of the antimicrobial agent or extract solution at desired concentration is introduced into the well.

Statistical analysis

All statistical analyses were executed using the statistical software package SPSS Version 23.0.2.0 (copyright IBM Corporation). Data were checked for normal distribution using the Kolmogorov–Smirnov test. Normally distributed variables were investigated using two-way ANOVA, compared using Duncan's tests and were presented as mean ± standard error of mean (SE). Probit analysis was estimated using NCSS Version 2007.

Results

Phytochemical analysis

Identification of polyphenols and flavonoids active ingredients by high performance liquid chromatography (HPLC)

The asafetida extract was examined for phenolic and flavonoid components using about 18 standards and by analyzing the chromatogram data by comparing the chromatograms (Fig. 1) of both the sample and the results were placed in Table 1. The ASF extract contains Chlorogenic acid, Gallic acid, Syringic acid, and Caffeic acid in high concentration of 134.16, 44.23, 30.09 and 19.31 µg/g.

Identification of the volatile compounds by gas chromatography mass spectroscopy (GC-MS)

The attempt to predict the volatile components presented in the ASF aqueous extract was done by gas chromatography-mass spectroscopy (Fig. 2) and the most abundant compounds were listed in Table 2. Linoleic acid ethyl ester, Ethyl Oleate, hexadecenoic acid ethyl ester were represented the most abundant compounds.

Total phenols

The total phenols contents measurement of the *ASF extract* was recorded in (Table 3) of 129 mg/g based on the gallic acid (as a measure of polyphenols contents) standard curve presented in (Table 4) and (Fig. 3).

Total flavonoids

The total flavonoids contents measurement of the *ASF extract* was recorded in (Table 5) of 51.90 mg/g based on the quercetin (as a measure of flavonoids contents) standard curve presented in (Table 6) and (Fig. 4).

Total carbohydrates

The total carbohydrates contents measurement of the *ASF extract* was recorded in (Table 7) of 10.27 mg/g based on the glucose (as a measure of carbohydrates contents) standard curve presented in (Table 8) and (Fig. 5).

Total proteins

The total proteins contents measurement of the *ASF extract* was recorded in (Table 9) of 366.67 mg/g based on the albumin (as a measure of protein contents) standard curve presented in (Table 10) and (Fig. 6).

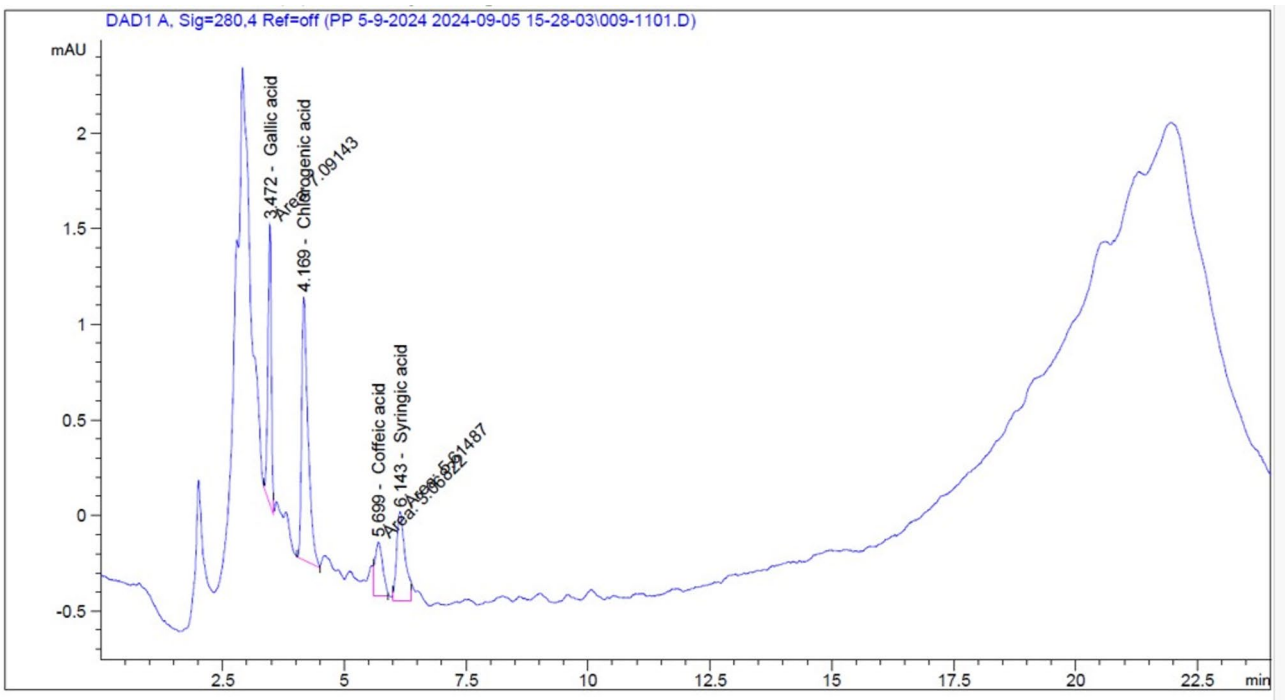

Fig. 1. HPLC chromatogram of the ASF aqueous extract.

Active phenolic or flavonoid compound	Standards		ASF extract (2 g/10 ml diluted 1:20)		
	Injected Conc. (µg/ml)	Area	Area	Detected Conc. (µg/ml)	Detected Conc. (µg/g)
Gallic acid	20	320.66	7.09	8.85	44.23
Chlorogenic acid	50	465.45	12.49	26.83	134.16
Catechin	75	413.52	0.00	0.00	0.00
Methyl gallate	15	360.47	0.00	0.00	0.00
Coffeic acid	18	285.99	3.07	3.86	19.31
Syringic acid	17.2	320.93	5.61	6.02	30.09
Rutin	50	399.99	0.00	0.00	0.00
Ellagic acid	70	705.83	0.00	0.00	0.00
Coumaric acid	20	723.95	0.00	0.00	0.00
Vanillin	12.9	440.50	0.00	0.00	0.00
Ferulic acid	20	445.94	0.00	0.00	0.00
Naringenin	30	414.13	0.00	0.00	0.00
Rosmarinic acid	50	604.54	0.00	0.00	0.00
Daidzein	20	405.06	0.00	0.00	0.00
Quercetin	20	301.82	0.00	0.00	0.00
Cinnamic acid	10	704.45	0.00	0.00	0.00
Kaempferol	20	358.24	0.00	0.00	0.00
Hesperetin	20	528.59	0.00	0.00	0.00

Table 1. The concentration detection of the polyphenols and flavonoids presented in the Asf aqueous extract.

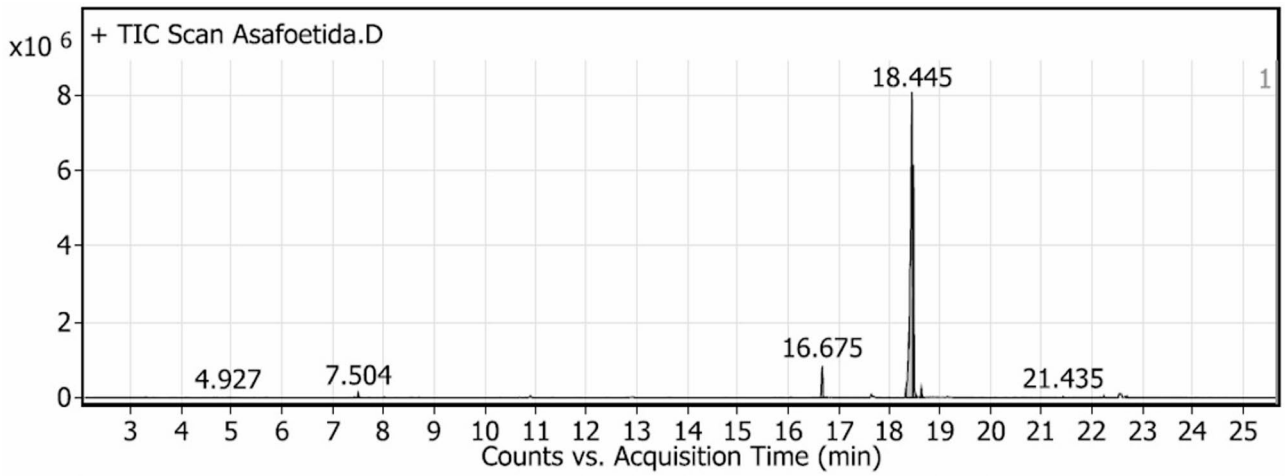

Fig. 2. GC-MS chromatogram of the ASF extract.

DPPH scavenging activity

The DPPH antioxidant activity of the *ASF extract* was evaluated against the ascorbic acid as standard antioxidant compounds and the results were listed in (Tables 11 and 12) of 30.09 ± 0.34 2.64 ± 0.11 µg/ml and (Figs. 7 and 8), respectively.

Insecticidal activity

Effect on phenolic concentrations

The changes in phenolic concentrations are displayed (Table 13). The time and dose exhibited significant effects on the phenolic levels except for insignificant effect ($p = 0.264$) of time on the phenolic concentration at 0 ppm of *ASF extract*.

At 1 h post-treatment, the highest phenolic concentrations were detected at 125 and 250 ppm of EC. After 6 h of treatment, there was no remarkable difference in phenolic concentration between the 0 ppm and 125 ppm followed by gradual elevation from the 250 ppm till 500 ppm, then remarkably reduced to 1000 ppm of *ASF extract*. At 12 h post-treatment, phenolic concentration gradually increased from 0 ppm till 1000 ppm of EC. At 24 h post-treatment, phenolic concentration gradually elevated from 0 till 250 ppm and followed by a marked decline at 500 ppm then followed by a remarkable elevation again at the 1000 ppm concentration of *ASF extract*.

Peak	RT	Name	Formula	Area	Area Sum %
1	4.927	Hexane, 2,3,4-trimethyl-	C9H20	5432.83	0.02
2	7.348	n-Propyl sec-butyl disulfide	C7H16S2	2688.62	0.01
3	7.423	(Z)-sec-Butyl propenyl disulfide	C7H14S2	28735.64	0.09
4	7.504	(E)-sec-Butyl propenyl disulfide	C7H14S2	178504.67	0.53
5	8.017	Disulfide, bis(1-methylpropyl)	C8H18S2	31310.53	0.09
6	16.049	Hexadecanoic acid, methyl ester	C17H34O2	26184.9	0.08
7	16.675	Hexadecanoic acid, ethyl ester	C18H36O2	1,629,828	4.85
8	18.445	Linoleic acid ethyl ester	C20H36O2	24,242,334	72.1
9	18.476	Ethyl Oleate	C20H38O2	7090204.3	21.09
10	18.633	Octadecanoic acid, ethyl ester	C20H40O2	362363.82	1.08
11	21.435	Docosanoic acid, ethyl ester	C24H48O2	22231.19	0.07
12	22.173	Ethyl tetracosanoate	C26H52O2	5270.44	0.02

Table 2. GC-MS parameters of the expected volatile active ingredients of the *ASF* extract.

Code	Total phenolic (con. mg /gm)			Mean	STD	SE
ASF extract	127	129	131	129.00	2.000	0.651

Table 3. The total phenolic content in the *ASF extract*.

Phenolic St. Gallic Acid µg/ml	500	250	125	62.5	31.15	15.62
Abs	1.305	0.634	0.317	0.211	0.115	0.078

Table 4. The total phenolic content in the *ASF extract*.

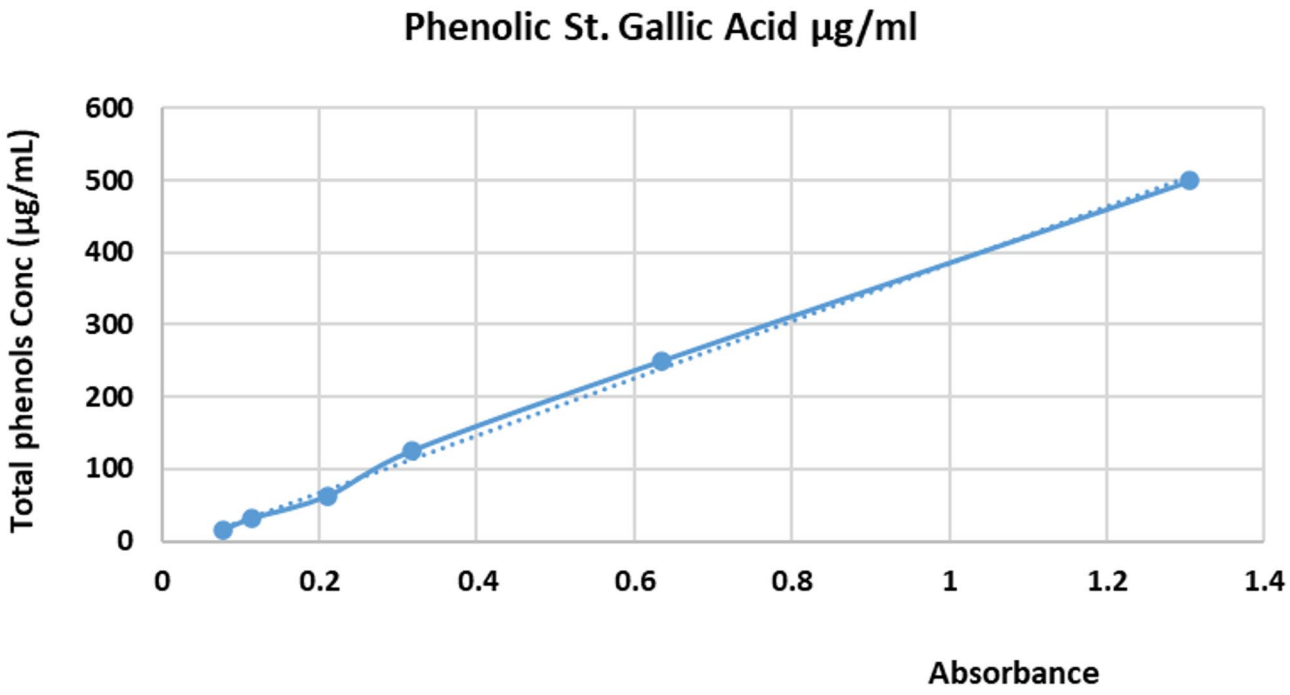

Fig. 3. Total phenol content standard curve.

Code	Flavonoid con. mg/gm			Mean	STD	SE
ASF extract	52	51.8	51.9	51.90	0.100	0.033

Table 5. The total flavonoid content in the *ASF extract*.

Flav St. Qu. µg/ml	500	250	125	62.5	31.15	15.62
Abs	1.79	1.07	0.543	0.324	0.195	0.067

Table 6. The total flavonoid (quercetin) standard curve.

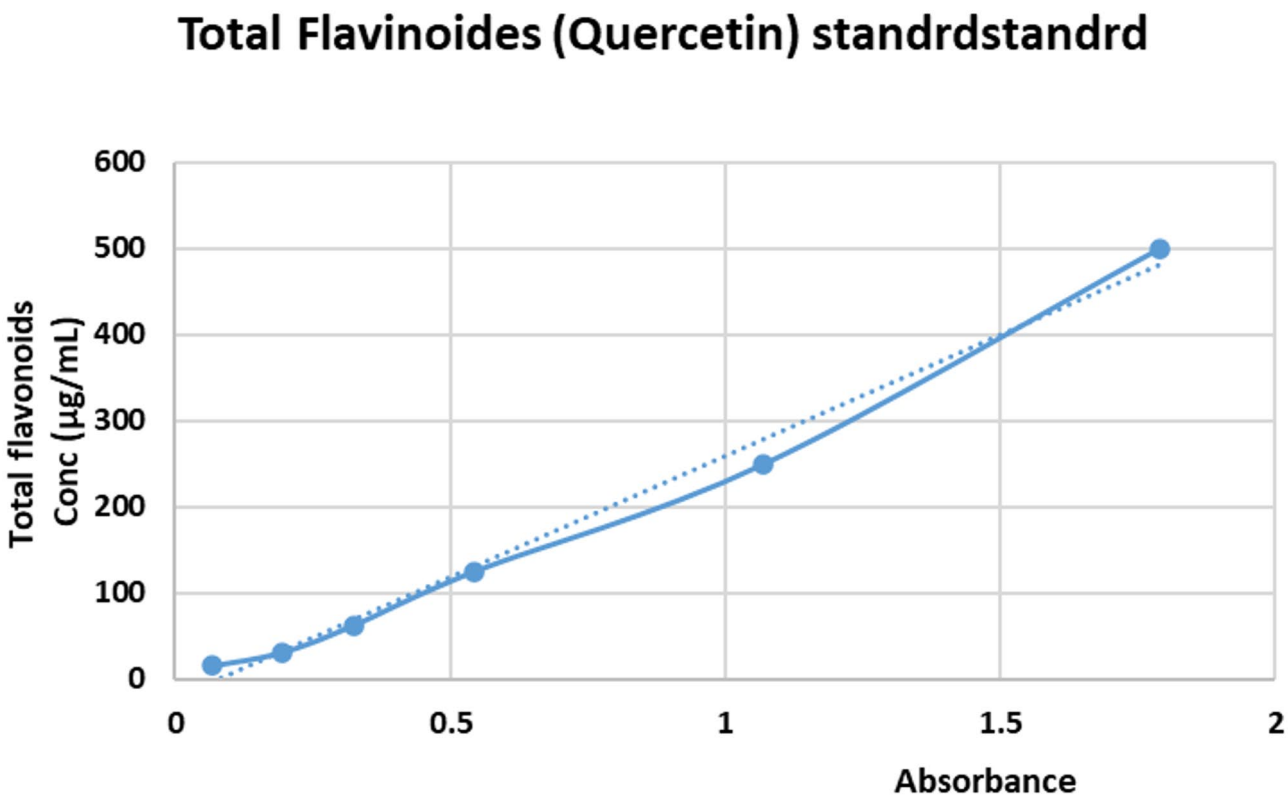

Fig. 4. Total flavonoids standard curve.

Code	Total Carbohydrate (glucose conc. mg/gm)			Mean	STD	SE
ASF extract	10.1	10.3	10.4	10.27	0.153	0.050

Table 7. The total carbohydrates content in the *ASF extract*.

Total Carb.(glucose St. µg/ml	20	40	80	120	160	200
Abs	0.17	0.243	0.475	0.677	0.852	0.985

Table 8. The total carbohydrates (Glucose) Standrd curve.

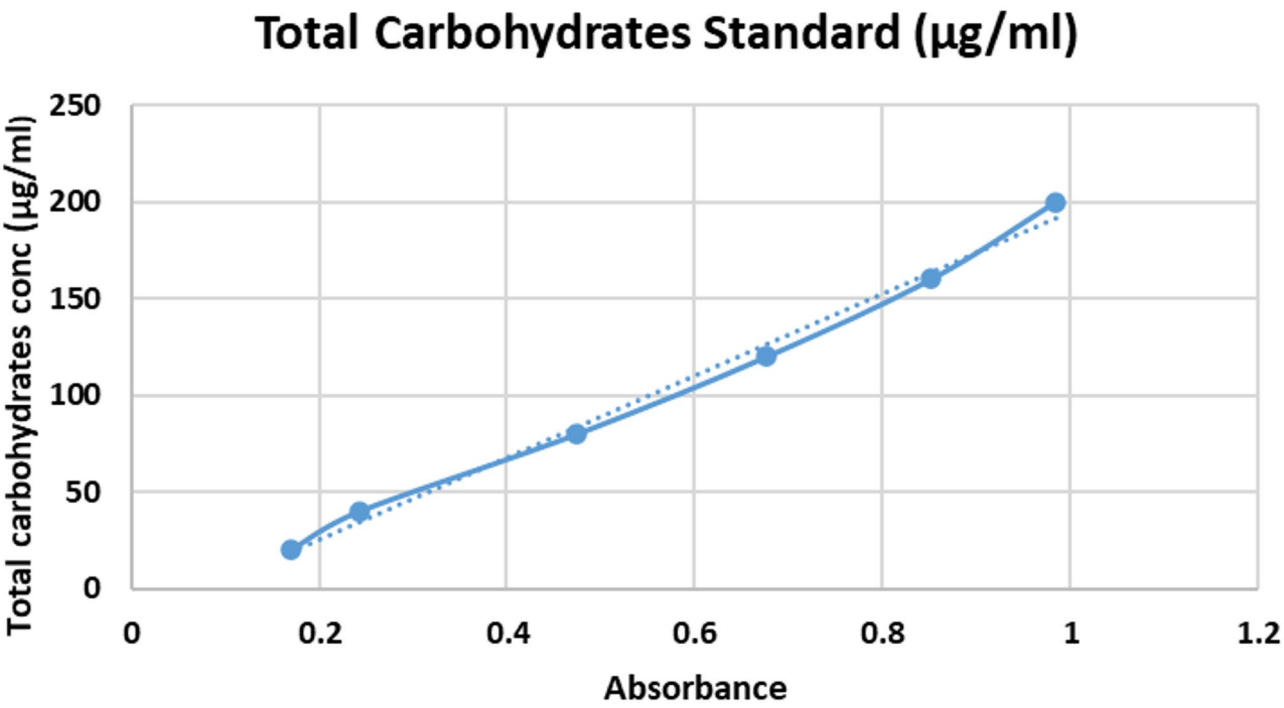

Fig. 5. Total carbohydrates standard curve.

Code	Total Protein (mg/gm)			Mean	STD	SE
Sample	368	366.2	365.8	366.67	1.172	0.382

Table 9. The total proteins content in the *ASF extract*.

Conc. (mg/ml)	10	20	40	60	80
Total protein (Alb. Stand conc)	0.134	0.248	0.422	0.631	0.822

Table 10. Standard curve of total proteins as albumin concentration.

Effect on superoxide anion radical concentration

In Table 14; Fig. 8, the effect of *ASF extract* on the O_2^- levels are reported. Time post treatment significantly affected the level of superoxide anions at any concentration of EC except for insignificant change at 0 ppm of the *ASF extract*.

After 1 h of treatment, the greatest level of superoxide anion was reported at 250 ppm of EC. At 6 h post-treatment, a concentration of 125 ppm caused a significant decline in the O_2^- level, as compared to the 0 ppm. However, at 250 and 500 ppm, the O_2^- level was remarkably greater than at 0 and 1000 ppm. On the contrary, at 0 and 1000 ppm, O_2^- level was significantly higher than the rest of concentrations, at 12 h post treatment. At 24 h post-treatment, a gradual elevation in O_2^- level was recorded from the concentration of 0 ppm till 125 ppm followed by remarkable decline till the concentration of 500 ppm and markedly elevated again at 1000 ppm.

Effect on superoxide dismutase activity

The SOD activity in all groups is displayed (Table 15). The activity of SOD was significantly affected by both time and dose except for insignificant effect of time at 0 and 1000 ppm of EC.

At 1 h post-treatment, SOD activity at 125, 500 and 1000 ppm was significantly higher than the control. After 6 h of treatment, the highest SOD activity was reported at 250 ppm of *ASF extract*. However, the maximum SOD activity detected at 500 ppm of the *ASF extract*, after 12 h and 24 h post-treatment.

Effect on total antioxidant concentration

The results of TAC at different concentrations of *ASF extract*, throughout 24 h post treatment are presented (Table 16). Time significantly affected TAC at all EC concentrations except for insignificant change at 0 ppm.

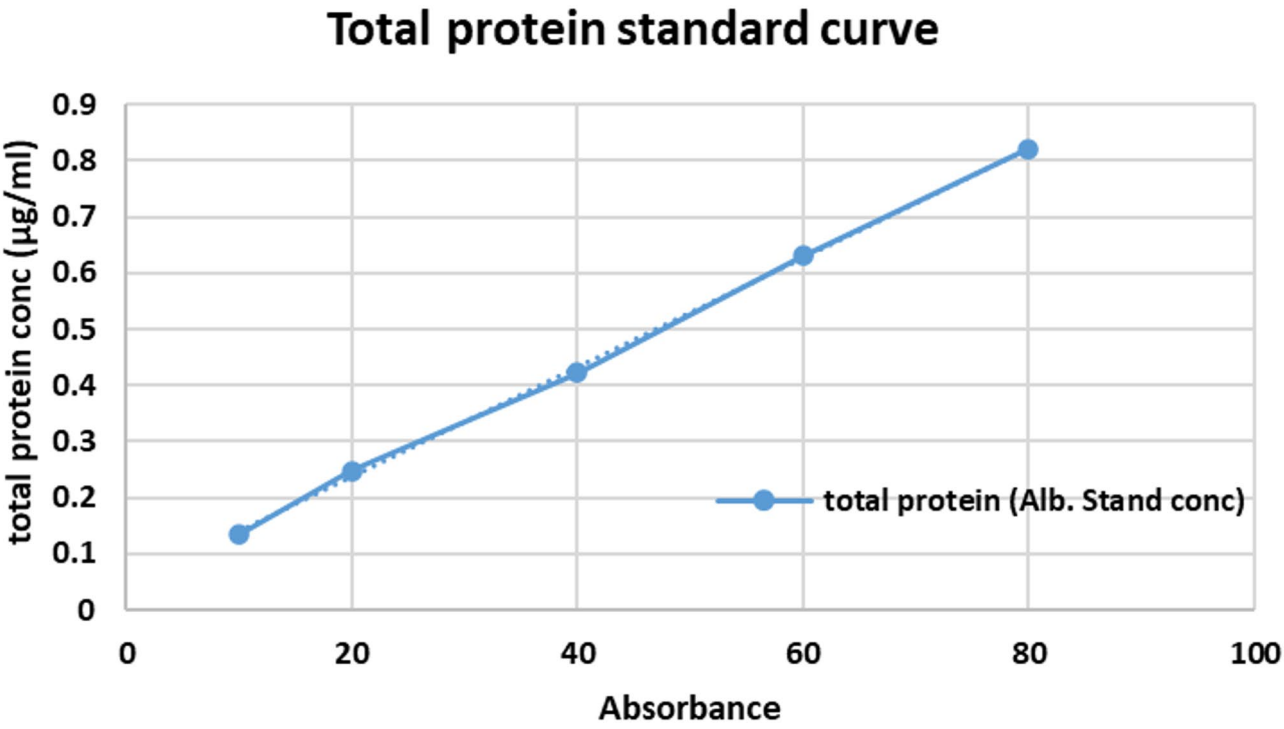

Fig. 6. Total protein standard curve.

(Conc. µg/ml)	OD R1	OD R2	OD R3	OD Mean	DPPH scavenging%	SD	SE	IC_{50}
1000	0.175	0.176	0.182	0.178	86.6	0.004	0.001	
500	0.232	0.238	0.226	0.232	82.5	0.006	0.002	
250	0.339	0.332	0.335	0.335	74.6	0.004	0.001	
125	0.436	0.441	0.439	0.439	66.8	0.003	0.001	
62.5	0.543	0.538	0.545	0.542	59.0	0.004	0.001	
31.25	0.624	0.634	0.632	0.630	52.3	0.005	0.002	30.09 ± 0.34
15.625	0.76	0.745	0.748	0.751	43.2	0.005	0.002	
7.8125	0.877	0.873	0.881	0.877	33.7	0.004	0.001	
3.9	0.967	0.988	0.973	0.976	26.2	0.011	0.004	
1.95	1.082	1.066	1.086	1.078	18.5	0.011	0.003	
0	1.319	1.326	1.321	1.322	0.0	0.004	0.001	

Table 11. DPPH scavenging activities of the *ASF extract* at different concentrations.

(Conc. µg/ml)	OD R1	OD R2	OD R3	OD Mean	DPPH scavenging%	SD	SE	IC_{50}
1000	0.029	0.031	0.031	0.030	97.7	0.001	0.000	
500	0.055	0.058	0.053	0.055	95.8	0.003	0.001	
250	0.091	0.093	0.097	0.094	92.9	0.003	0.001	
125	0.175	0.172	0.176	0.174	86.8	0.002	0.001	
62.5	0.226	0.232	0.229	0.229	82.7	0.003	0.001	
31.25	0.332	0.331	0.327	0.330	75.0	0.003	0.001	2.64 ± 0.11
15.625	0.429	0.425	0.423	0.426	67.8	0.003	0.001	
7.8125	0.542	0.541	0.539	0.541	59.1	0.002	0.000	
3.9	0.619	0.633	0.632	0.628	52.5	0.008	0.003	
1.95	0.752	0.748	0.755	0.752	43.1	0.004	0.001	
0	1.319	1.326	1.321	1.322	0.0	0.004	0.001	

Table 12. DPPH scavenging activities of the ascorbic acid (standard curve).

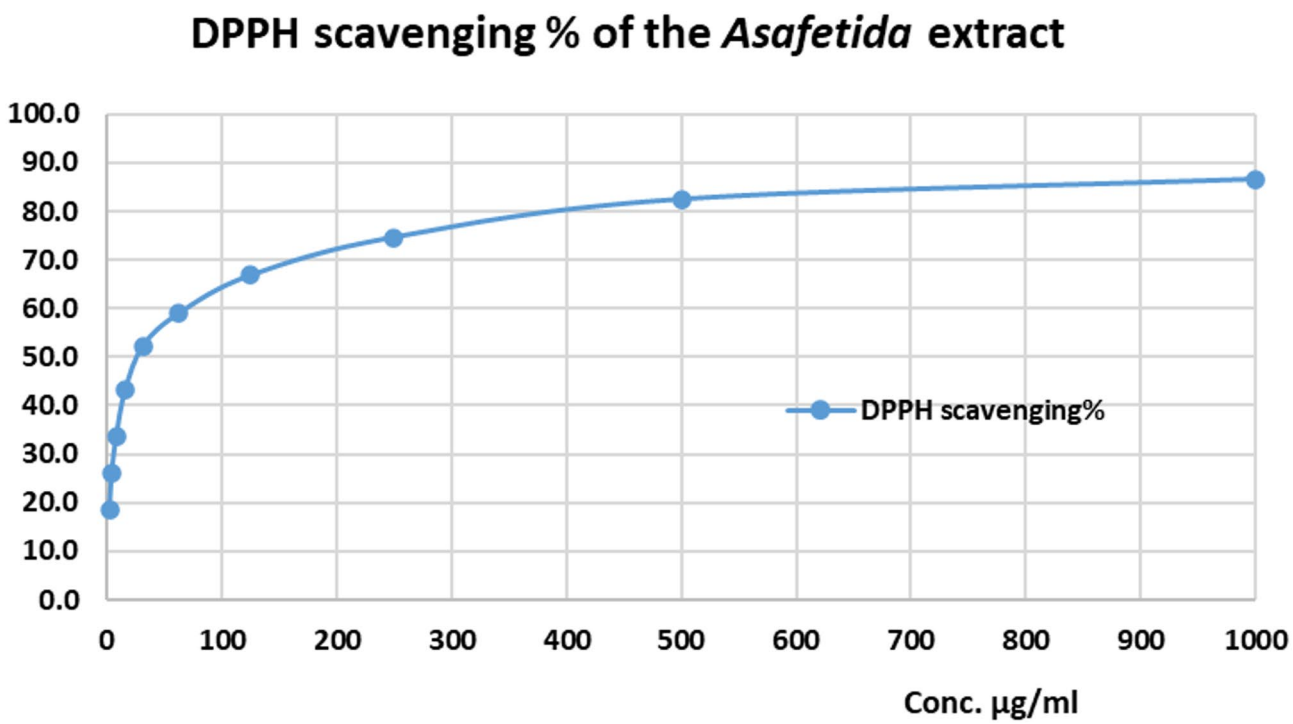

Fig. 7. DPPH scavenging activity of the *ASF extract*.

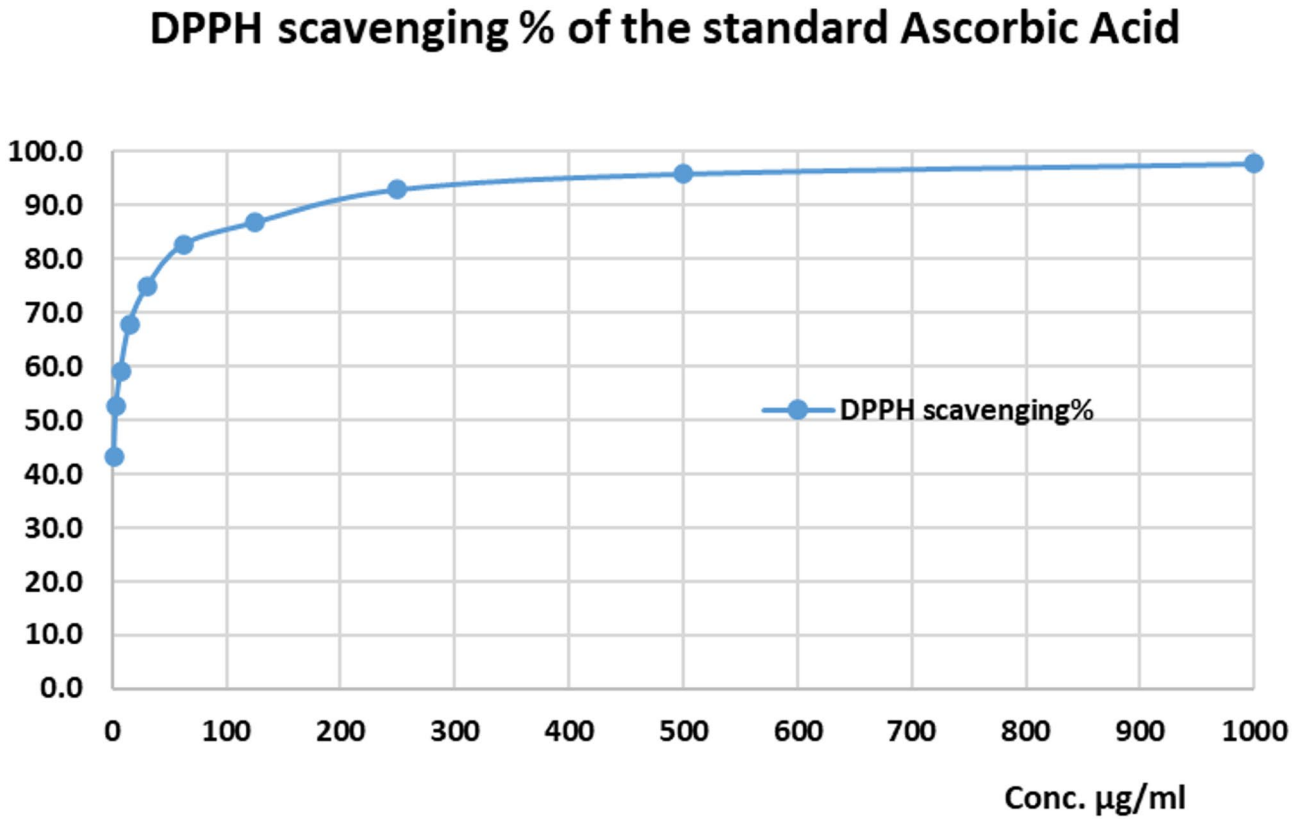

Fig. 8. DPPH scavenging activity of the ascorbic acid standard.

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
Phenolics concentration (PPM)	1	0.32 ± 0.004^{Aa}	1.32 ± 0.063^{Cc}	1.28 ± 0.03^{Bc}	0.58 ± 0.011^{Bb}	0.67 ± 0.015^{Bb}	0.000
	6	0.32 ± 0.006^{Aa}	0.32 ± 0.004^{Aa}	0.63 ± 0.004^{Ac}	0.81 ± 0.005^{Cd}	0.49 ± 0.009^{Ab}	0.000
	12	0.31 ± 0.003^{Aa}	0.51 ± 0.009^{Bb}	0.59 ± 0.039^{Ab}	0.52 ± 0.110^{Ab}	1.47 ± 0.014^{Cc}	0.000
	24	0.31 ± 0.004^{Aa}	0.54 ± 0.03^{Bb}	0.62 ± 0.017^{Ac}	0.37 ± 0.031^{Aa}	1.76 ± 0.016^{Dd}	0.000
Time effect		0.264	0.000	0.000	0.004	0.000	

Table 13. Effect of *ASF extract* on the phenolics concentrations (PPM) in Culex pipiens. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
Superoxide anion concentration (OD/mg protein)	1	0.37 ± 0.015^{Ab}	0.47 ± 0.005^{Dd}	0.53 ± 0.007^{Ce}	0.44 ± 0.009^{Dc}	0.25 ± 0.006^{Aa}	0.000
	6	0.38 ± 0.011^{Ab}	0.33 ± 0.003^{Ba}	0.40 ± 0.006^{Bc}	0.41 ± 0.006^{Cc}	0.37 ± 0.012^{Bb}	0.000
	12	0.38 ± 0.008^{Ac}	0.26 ± 0.007^{Ab}	0.14 ± 0.006^{Aa}	0.26 ± 0.006^{Ab}	0.39 ± 0.005^{Bc}	0.000
	24	0.40 ± 0.006^{Ab}	0.44 ± 0.012^{Cc}	0.40 ± 0.006^{Bb}	0.35 ± 0.011^{Ba}	0.44 ± 0.007^{Cc}	0.000
Time effect		0.305	0.000	0.000	0.000	0.000	

Table 14. Effect of *ASF extract* on the superoxide anion radical concentration (OD/mg protein) in *Cx pipiens*. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
SOD activity (OD/mg protein/min)	1	0.11 ± 0.016^{Aa}	0.18 ± 0.007^{Ab}	0.14 ± 0.005^{Aa}	0.18 ± 0.007^{Ab}	0.20 ± 0.010^{Ab}	0.000
	6	0.11 ± 0.009^{Aa}	0.22 ± 0.007^{Bb}	0.28 ± 0.013^{Bc}	0.20 ± 0.012^{Ab}	0.20 ± 0.015^{Ab}	0.000
	12	0.12 ± 0.006^{Aa}	0.22 ± 0.013^{Bb}	0.28 ± 0.010^{Bc}	0.37 ± 0.015^{Bd}	0.23 ± 0.005^{Ab}	0.000
	24	0.14 ± 0.010^{Aa}	0.19 ± 0.010^{Ab}	0.30 ± 0.011^{Bc}	0.36 ± 0.010^{Bd}	0.22 ± 0.018^{Ab}	0.000
Time effect		0.34	0.045	0.000	0.000	0.337	

Table 15. Effect of *ASF extract* on the superoxide dismutase activity (OD/mg protein/min) in *Cx. pipiens*. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
Total antioxidant concentration (mg/mg protein	1	0.38 ± 0.029^{Aa}	1.52 ± 0.062^{Ac}	1.89 ± 0.087^{Bd}	1.17 ± 0.077^{Ab}	1.49 ± 0.078^{Ac}	0.000
	6	0.42 ± 0.040^{Aa}	3.81 ± 0.147^{Cd}	2.86 ± 0.080^{Cc}	2.13 ± 0.038^{Bb}	2.16 ± 0.078^{Bb}	0.000
	12	0.46 ± 0.014^{Aa}	3.14 ± 0.040^{Bd}	1.42 ± 0.049^{Ab}	2.03 ± 0.019^{Bc}	2.78 ± 0.095^{Cd}	0.000
	24	0.50 ± 0.039^{Aa}	4.00 ± 0.192^{Ce}	2.11 ± 0.095^{Bb}	2.67 ± 0.019^{Cc}	3.10 ± 0.102^{Dd}	0.000
Time effect		0.122	0.000	0.000	0.000	0.000	

Table 16. Effect of *ASF extract* on the total antioxidant concentration (mg/mg protein) in *Cx. pipiens*. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

The greatest TAC values were detected at 250 and 125 ppm, after 1 h and 6 h post-treatment, respectively. At 12 h post-treatment, at 125 ppm and 1000 ppm, TAC concentration was remarkably higher than at the rest of *ASF extract* doses. After 24 h of treatment, TAC concentration reached its maximum value at 125 ppm of EC.

Effect on lipid peroxide concentration

The changes in lipid peroxide concentration are presented (Table 17). Time post-treatment showed an insignificant effect on the lipid peroxide concentration at most *ASF extract* concentrations.

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
Lipid peroxidation concentration (mM cumene hydroperoxide/mg protein)	1	0.11 ± 0.006^{Aa}	3.67 ± 0.087^{Ac}	3.79 ± 0.131^{Ac}	2.75 ± 0.138^{Ab}	3.73 ± 0.252^{Ac}	0.000
	6	0.14 ± 0.012^{Ba}	3.71 ± 0.113^{Ab}	3.69 ± 0.110^{Ab}	3.45 ± 0.158^{Bb}	3.82 ± 0.178^{Ab}	0.000
	12	0.13 ± 0.004^{Aa}	3.82 ± 0.092^{Ab}	3.90 ± 0.124^{Ab}	3.78 ± 0.236^{Bb}	4.01 ± 0.099^{Ab}	0.000
	24	0.15 ± 0.006^{Ba}	4.01 ± 0.316^{Ab}	3.81 ± 0.165^{Ab}	3.65 ± 0.298^{Bb}	3.71 ± 0.102^{Ab}	0.000
Time effect		0.027	0.581	0.75	0.041	0.593	

Table 17. Effect of *ASF extract* on the lipid peroxide concentration (mM cumene hydroperoxide/mg protein) in *Cx. pipiens*. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
DPPH inhibition (%)	1	62.00 ± 1.53^{Aa}	84.67 ± 0.88^{Ab}	96.67 ± 1.20^{Ac}	95.67 ± 2.40^{Ac}	95.67 ± 1.67^{Ac}	0.000
	6	69.33 ± 5.55^{Aa}	97.00 ± 0.58^{Bb}	97.67 ± 0.33^{Ab}	94.67 ± 0.33^{Ab}	95.33 ± 1.86^{Ab}	0.000
	12	65.67 ± 4.06^{Aa}	97.00 ± 0.58^{Bb}	95.67 ± 1.67^{Ab}	97.33 ± 0.88^{Ab}	96.00 ± 1.16^{Ab}	0.000
	24	67.33 ± 2.91^{Aa}	97.67 ± 0.88^{Bb}	95.33 ± 0.33^{Ab}	95.67 ± 1.45^{Ab}	96.67 ± 1.45^{Ab}	0.000
Time effect		0.596	0.000	0.441	0.658	0.937	

Table 18. Effect of *ASF extract* on the DPPH Inhibition (%) in *Cx. pipiens*. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

	Time (h)	Doses (ppm)					Dose effect
		0	125	250	500	1000	
Mortality (%)	1	6.67 ± 6.67^{Aa}	26.67 ± 6.67^{Ab}	26.67 ± 6.67^{Ab}	46.67 ± 6.67^{Ab}	33.33 ± 6.67^{Ab}	0.022
	6	13.33 ± 6.67^{Aa}	33.33 ± 13.33^{Aa}	40.00 ± 11.55^{Aa}	40.00 ± 20.00^{Aa}	53.33 ± 24.04^{Aa}	0.552
	12	0.00 ± 0.00^{Aa}	46.67 ± 6.67^{Ab}	66.67 ± 6.67^{Ac}	66.67 ± 13.33^{Ac}	86.67 ± 13.33^{Ac}	0.001
	24	13.33 ± 6.67^{Aa}	26.67 ± 17.64^{Aa}	40.00 ± 11.55^{Ab}	53.33 ± 13.33^{Ab}	80.00 ± 11.55^{Ab}	0.032
Time effect		0.363	0.624	0.085	0.606	0.124	

Table 19. Effect of EC on the mortality (%) in *Cx. pipiens*. Data are presented as mean ± standard error of mean. Different capital letters (column) and lower-case letters (row) indicate that the differences between the groups are significant by (Two-way ANOVA, Duncan's test).

Throughout the 24 h post-treatment, lipid peroxide level was significantly elevated at all *ASF extract* concentrations, as compared to the 0 ppm.

Effect on DPPH Inhibition

In Table 18, the changes in DPPH levels are displayed. The levels of DPPH were significantly affected by the *ASF extract* concentration. Throughout the 24 h post-treatment, a gradual elevation in the DPPH concentration was detected.

Effect on mortality

The mortality percentage at different time intervals post-treatment of different concentrations of *ASF extract* are displayed (Table 19).

Time post-treatment did not exhibit any significant effect on the mortality percentage. At most time intervals, the morality percentage was significantly affected by the *ASF extract* concentration. The greatest mortality percentage was observed at 1000 ppm (86.7%), after 12 h post-treatment followed by that at 1000 ppm (80.0%) after 24 h post-treatment.

The LC50, LC90 and LC95 were calculated for the *ASF extract* (352.1, 3154.8 and 4028.8 ppm. Chi-squared for the mortality percentage data was 0.41 ($p > 0.05$), indicating the regression model is fitting. The relationship between mortality percentage and the *ASF extract* concentration was best represented with a power equation with R2 = 0.965 (Table 20).

	LC50	LC90	LC95	Chi-squared (Sig.)	Equation	R2
Estimate (95%CI)	352.11 (178.4-525.8)	3154.8 (-803.12-5506.5)	4028.77 (-2723.5-10781)	0.41 (0.81)	$y = 0.007 \times^{6.8655}$	0.9658

Table 20. LC50, LC90 and LC95 (ppm) values of EC against *Cx. pipiens* larvae, 24hpost-treatment.

Sample Microorganism	ASF extract	Control
B. subtilis (ATCC6633)	25 ± 0.2	26 ± 0.2
S. aureus (ATCC6538)	23 ± 0.2	23 ± 1
E. coli (ATCC 8739)	18 ± 2	22 ± 0.2
K pneumonia (ATCC13883)	15 ± 1	22 ± 0.1
C. albicans (ATCC 10221)	19 ± 2	27 ± 0.2
Aspergillus Niger	No activity	32 ± 2

Table 21. The antimicrobial evaluation of the *ASF extract* against different microbial strains.

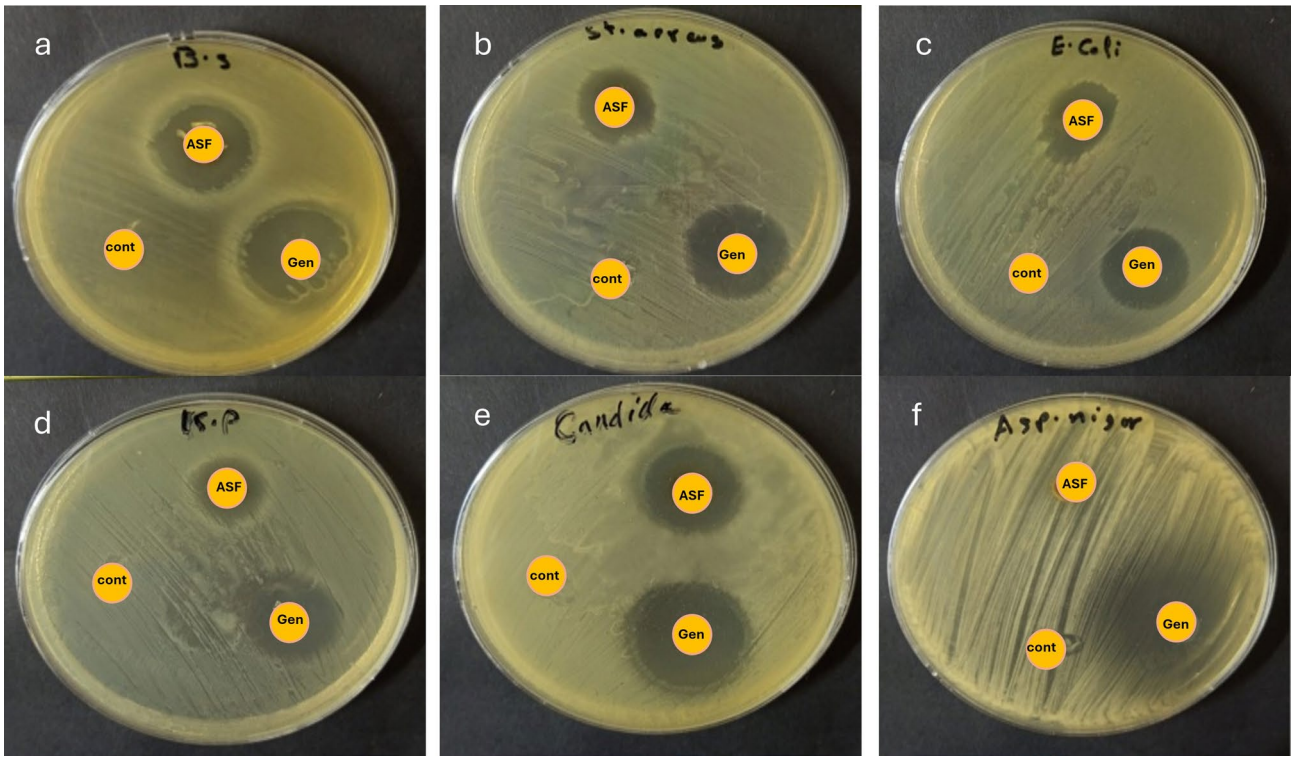

Fig. 9. The inhibition zones of diameter of *ASF extract* with the untreated negative control, and gentamycin positive control (1.0 gm/ml) for bacterial strains (**a**) *B. subtilis*, (**b**) *S. aureus*, (**c**) *E. coli*, (**d**) *K. pneumonia*, (**e**) *C. albicans*, (**f**) *A. niger*.

Antimicrobial evaluation

Inhibition zone diameter

The antimicrobial effect results of *ASF extract* bacterial strains are represented at Table 21; Fig. 9 as inhibition zones (mm). The antibiotic Control for Bacteria was Gentamycin and for fungi was Fluconazole at concentration 1.0 mg/ml. According to antibiotics screening results, the zone of inhibition for *Bacillus subtilis* and *S. aureus* showed potential antimicrobial activity with inhibition zone diameter 25 and 23 mm, respectively.

Minimal inhibitory concentration (MIC) and minimal bactericidal activity (MBC)

Minimal inhibitory concentration and minimal bactericidal concentration were determined for every single strain against *ASF extract* and the results presented in Table 22.

Microbial strain	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC index
B. subtilis (ATCC6633)	31.25 ± 0.0	62.5 ± 0.0	2
S. aureus (ATCC6538)	31.25 ± 0.0	31.25 ± 0.0	1
E. coli (ATCC 8739)	125 ± 0.0	250 ± 0.0	2
K. pneumonia (ATCC13883)	250 ± 0.0	500 ± 0.0	2
C. albicans (ATCC 10221)	250 ± 0.0	250 ± 0.0	1
Aspergillus Niger	non	non	non

Table 22. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the *ASF extract*.

Discussion

This study is considered from pioneer studies on examining the effect of *ASF extract* on the larval lethal and sublethal mortality, enzymatic and non-enzymatic antioxidant capacity of the mosquito *Cx. pipiens*. The internal composition and the active substances contained in the extract, were examined and the chromatographic analysis confirmed that the ASF extract sample was rich in Chlorogenic acid, Gallic acid, Syringic acid, and Caffeic acid in addition to the linoleic acid ethyl ester, Ethyl Oleate, hexadecenoic acid ethyl ester compounds which determines the extent of the extract's activity in fighting various diseases or even disease vectors such as mosquitoes, bacteria, fungi and all pathogens in humans and may cause high toxicity to many insects, according to Pavela[38] who reported acute toxicity of *C. quinquefasciatus. ASF extract*s are one of these extracts that combines many properties, making it a fertile environment and the focus of researchers interested in human health affairs. In addition to the antioxidant properties of *ASF* extract, which were confirmed by DPPH analyses for the extract itself.

At most time intervals, the morality percentage was significantly affected by concentration. The greatest mortality percentage was observed at 1000 ppm (86.7%), after 12 h post-treatment followed by that at 1000 ppm (80.0%) after 24 h post-treatment. The LC_{50}, LC_{90} and LC95 were calculated for the *ASF extract* 352.1, 3154.8 and 4028.8 ppm respectively. Chi-squared for the mortality percentage data was 0.41 ($p > 0.05$), indicating the regression model is fitting. These values are much greater than those of many essential oils that have been examined for larvicidal activity against mosquitoes[39–42]. The greater LC_{50} values may reflect a higher safety margin for the plant extract towards the normal cells.

Around the world, *ASF extract* is utilized as a traditional remedy for a variety of ailments and as a flavoring ingredient[43–45]. Additionally, *ASF extract* leaf extracts are moderately toxic to *Aedes aegypti* larvae ($LC_{50} = 65.02$ ppm for acetone extracts and $LC_{50} = 62.61$ ppm for petroleum ether extracts) and effective against a range of cotton pests[46,47]. Our findings thus show that the essential oil of *ASF extract* is more effective against mosquito larvae and may be used to create a new type of mosquito larvicide.

As a good substitute for commercial pesticides, plant-derived insecticides successfully suppressed mosquito larvae and pupae[48]. Baz, et al.[8] evaluated 32 oils against *Cx. pipiens*. *Thymus vulgare* and *C. sinensis* found that the most effective oils against adults were *An. graveolens* and *V. odorata* followed by *T. vulgaris* then *N. sativa*. The data revealed that *F. vulgare* is a highly potent larvicide. Similarly, the AS oils possess larvicidal properties, hence augmenting their effectiveness and durability. Agreeing with our findings, Fennel oil also appears to be effective against the larvae and adults of *Culex quinquefasciatus*[49,50] *Anopheles atroparvus*[51] and *Aedes aegypti*[52]. Fennel oil (40 mg/L) was adequate in another investigation to result in 50% mortality in *Cx. pipiens* second larval stage following two hours of PT[53]. A practical larvicide action against *Anopheles arabiensis* and *Anopheles gambiae* was also demonstrated by green tea leaf extract[54] which resulted in 100% larval death at 1000 ppm and adult repellent effects against *Cx. pipiens*. Caffeine and other chemical compounds in green tea make it an efficient larvicide against *Drosophila prosaltans*[55].

The laboratory and field strains of *Cx. pipiens* in Egypt were susceptible to the larvicidal effects of local essential oils such *Nigella sativa*, *Allium cepa*, and *Sesamum indicum* (LC50 = 247.99 and 108.63; 32.11 and 2.87; and 673.22 and 143.87 ppm, respectively); these oils negatively impacted rates of pupae and adult emergence and caused anomalies in the development of larvae[56]. Furthermore, mosquito larvae *Cx. pipiens* were successfully reduced by the oils of *ASF* (*Trigonella foenum-grecum*), mustard (*Brassica compestris*), ground almond (*Cyperus esculentus*), frankincense (*Boswellia serrata*), watercress (*Eruca sativa*), and parsley (*Carum Petroselinum)* (LC50 = 32.42, 71.37, 47.17, 83.36, 86.06, and 152.94 ppm)[57]. Certain oils can repel adult mosquitoes; *Z. piperitum* oil including 5% vanillin was discovered in the field to be more effective than 25% DEET + 5% vanillin for protecting against *Aedes gardnerii*, *Anopheles barbirostris*, *Armigeres subalbatus*, *C. tritaeniorhynchus*, *C. gelidus*, *C. Vishnu* group, and *Mansonia uniformis*[58]. According to earlier research, the main components of *ASF extract* that give it its larvicidal properties are allyl disulfide and diallyl[59]. As minor components of *ASF extract* essential oil, allyl disulfide (7.38%) and diallyl trisulfide (4.55%) was found in essential oil. Therefore, both molecules are probably among the minor components determining the toxicity of *ASF extract* essential oil, even though they are not very abundant. To ascertain the primary elements' contribution to the overall toxicity of *ASF extract* essential oil, more research is required.

Some essential oils contain bioactive compounds that may interact synergistically or antagonistically against mosquitoes when combined[60–62]. EOs could serve as suitable alternatives to synthetic insecticides because they are relatively safe, available, and biodegradable[15]. Finding mixtures of essential oils that work well together could be a key step in creating safe, affordable, and efficient substitutes for synthetic pesticides. However, essential oils

from garlic and *ASF extract* behaved antagonistically against *Cx. pipiens* and *C. restuans*, making them more effective when administered separately rather than together. According to these results, mixing two extremely toxic essential oils might not always produce the best outcomes. Instead, knowledge-guided decision-making procedures should be employed to choose essential oil combinations that don't jeopardize vector control initiatives[62].

Most egg rafts from the essential oil treatments of garlic (73.1%) and *ASF extract* (55.8%) did not hatch when exposed to oil concentrations that killed 50% of the larvae (LC50)[39]. A significant component of essential garlic oil, has been shown to hinder egg hatch in certain beetle species and may have had a role in this effect[63]. Either only trace amounts of allyl disulfide and diallyl trisulfide are needed to inhibit egg hatch, or that other components of asafetida essential oil also contribute to egg hatch inhibition.

Additionally, it has been demonstrated that garlic extracts have ovicidal effects on mosquitoes[64] and the red spider mite, *Oligonychus coffeae* (Nietner)[65] and that garlic essential oils have ovicidal effects on the diamondback moth *Plutella xylostella* (L.)[66].

Reactive oxygen species (ROS) mediate the toxicity of numerous plant extracts by oxidatively damaging cells. ROS is eliminated from cells when antioxidant defense systems are functioning correctly. However, excessive ROS production may upset the equilibrium between ROS generation and elimination, resulting in molecular damage and other diseases[67].

The volatile compound detected by the GC-MS and polyphenols detected by the HPLC of the *ASF extract* affects the oxidative stress markers. Due to their defence mechanisms against certain insecticides, insects produce more detoxifying and antioxidant enzymes[68].

To explain the role of the SOD enzymes, these discoveries centre on the oxidation of superoxide anion radicals (O − 2) to oxygen and H_2O_2[69]. CAT enzymes transform the highly produced H_2O_2 into oxygen and water[70,71]. Oxidative stress indicators have been used in numerous research to examine the biomonitoring of pesticide treatments[72,73]. One internal and external element that raises the synthesis of H_2O_2 in living organisms is the treatment of plant extracts, which raises the levels of ROS generation. The current data has demonstrated a notable rise in the H_2O_2 generation rate at the highest concentration *ASF extract*, which is consistent with previous findings. Fridovich[74] that endogenous toxicants produce ROS when they enter an insect's body and boost H_2O_2 levels[51].

When the production and removal of reactive oxygen species (ROS) are out of balance, oxidative stress occurs[75–77]. In the presence of oxygen, the SOD enzymes become active. Together with ascorbate peroxidase, ascorbate, the main non-enzymatic antioxidant, helps aphids significantly reduce harmful H_2O_2. According to Łukasik, et al.[78] it falls as oxidative stress rises. These enzymes facilitate the conversion of superoxide to hydrogen peroxide and oxygen. SOD enzymes control the amounts of reactive oxygen species (ROS). Together with the different dosages, mosquito larvae's ascorbic acid and SOD activity decreased. Additionally, compared to a control, the *Spodoptera exigua* larvae exposed to high humidity showed significantly lower levels of SOD, peroxidase, and CAT activity[67]. These findings highlight the various ways that rising insect mortality adds to the depletion of the antioxidant system. Additionally, it supports the use of other oxidative stress markers or the antioxidant response of mosquito larvae as a signal for the material of plant extracts. Additionally, it was found to be a novel method for possibly upsetting mosquito larvae enzymes with *ASF extract*.

Environmental contaminants, including certain organic compounds, typically cause oxidative stress and all its negative effects on animals by raising the ROS level in the cells of those exposed[79,80]. Many elements of environmental pollution should be regarded as a nonbiological component of oxidative stress in invertebrates, according to Ahmad[81] and Chaitanya, et al.[82]. By assessing the enzyme activity (SOD, CAT, DPPH, and TAC) of *Cx. pipiens* fourth-instar larvae under the influence of *ASF extract*, the current study indirectly evaluated the level of enzymatic and non-enzymatic antioxidant capability of the mosquito. According to widely recognized expertise, the parameters were chosen[83].

Researchers frequently use a variety of indicators to determine the degree of oxidative stress. Two biomarkers that are frequently utilized are: Superoxide Anion Radicals: These extremely reactive oxygen species can cause oxidative stress and harm cellular constituents. Their concentration can be measured to determine the degree of oxidative stress. Lipid Peroxides: Lipid hydroperoxides are the result of a chain reaction called lipid peroxidation, which is started by ROS. These goods have the potential to harm cell membranes and cause several illnesses. Lipid peroxide levels are a good way to measure oxidative stress[84].

A biological system's power to neutralize ROS and stop oxidative damage is reflected in its antioxidant capacity. There are several ways to evaluate this capacity: Total Antioxidant Capacity: This measurement considers both enzymatic and non-enzymatic antioxidants to provide a comprehensive evaluation of a sample's antioxidant potential. Non-Enzymatic Antioxidant Capacity: This metric measures the antioxidant capacity of tiny molecules that can directly scavenge reactive oxygen species (ROS), such as vitamins C and E. The activity of antioxidant enzymes, such as superoxide dismutase (SOD), which are essential for shielding cells from oxidative damage, is measured by the Enzymatic Antioxidant Capacity. One important antioxidant enzyme that catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen is superoxide dismutase (SOD). SOD is essential for shielding cells from oxidative damage because it lowers the concentrations of superoxide radicals. SOD activity measurement offers important insights into the effectiveness of the antioxidant defense system. A broad class of secondary metabolites found in plants; phenolic compounds have strong antioxidant qualities. By chelating metal ions and directly scavenging ROS, they can stop oxidative damage. A sample's antioxidant potential can be inferred by measuring the concentration of phenolic chemicals in it[85,86].

The phenolic levels in the current study were significantly impacted by both time and dose. The phenolic concentration progressively rose from 0 ppm to 1000 ppm of ASF extract at 12 h after treatment. The phenolic concentration increased progressively from 0 to 250 ppm at 24 h after treatment, then declined noticeably at 500 ppm before showing a spectacular increase once again at 1000 ppm of ASF extract. At most ASF extract

concentrations, time after treatment had no discernible impact on the lipid peroxide concentration. Lipid peroxide levels were much higher at all ASF extract doses throughout the 24 h after treatment than at 0 ppm. Lipids can also be harmed by free radicals, which are produced in cells because of different chemical interactions[87].

Changes in lipid peroxidation have the potential to disrupt every bodily function[88]. In this investigation, lipid peroxide levels in larval tissues exposed to the *ASF* extract impact were significantly higher than those in the control group (Figs. 5, Table 5). Our results are in line with those of Lushchak[89] who concluded that lipid peroxides can be useful indicators of oxidative stress. The lipid peroxides and protein carbonyls in adult male and female *A. thalassinus* were investigated in a prior study[90]. High metabolism of larvae during their intensive growth, and high consumption of oxygen during development, may be the reasons of such results. Plant extracts have the potential to directly or indirectly induce oxidative stress and speed up the generation of ROS. The elimination of oxygen radicals is carried out by important antioxidant enzymes[91,92]. According to Abdelfattah[93] the malathion exposure of insect food container can induce oxidative stress in the larval and adult male stages of *H. illucens*. The levels of ROS, macromolecules damage, enzymatic and nonenzymatic antioxidants. In response to different *ASF extract* concentration may be used as a possible mechanism of plant oil toxicity. The biochemical analysis of insects could be used as a novel strategy for assessing the risk of pesticides accumulation in insects. Antioxidant enzymes like superoxide dismutase (SOD) become more active when oxidative stress levels are high. Except for the negligible effect of time at 0 and 1000 ppm of *ASF extract*, the current investigation showed that both time and dose had a considerable impact on SOD activities at 125, 500, and 1000 ppm was noticeably greater than the control after one hour after treatment. The maximum SOD activity was recorded at 250 ppm of *ASF extract* after 6 h of treatment. However, after 12 and 24 h of treatment, the highest SOD activity was seen at 500 ppm of *ASF extract* SOD transforms superoxide radicals into H_2O_2, while CAT stops H_2O_2 from building up inside the cell. It has been shown that a variety of inhibitors can cause cells to produce SOD activity[90,94].

It is also feasible to suppress the action of antioxidant enzymes. Except for the negligible change at 0 ppm, time had a considerable impact on total antioxidants at all *ASF extract* doses. After one hour and six hours of post-treatment, respectively, the highest total antioxidant levels were found at 250 and 125 ppm.

The total antioxidant content was significantly higher at 125 ppm and 1000 ppm 12 h after treatment than it was at the other *ASF extract* dosages. The overall antioxidant content peaked at 125 ppm of *ASF extract* during a 24-hour treatment period (Table 6). Figure 8 *ASF extract* impact on *Cx. pipiens*' total antioxidant content (mg/mg protein). Researchers hypothesize that a reduction in the production of antioxidant enzymes is what caused the suppression of antioxidant enzyme activity[95].

The antioxidant enzyme activities in *Cx. pipiens* larvae in their fourth instar were lower than those of the enzymes recommended for *Aiolopus thalasinuss* nymphs[90]. New enzyme molecule synthesis may be restricted during an individual's rapid development and expansion. It is necessary to strike a balance between growth and antioxidant protection.

However, Woodring and Sparks[96] proposed that the activity of antioxidant enzymes can vary depending on the stage of life, reflecting the expression of several isoenzymes. The LC50 value of methidathion pesticide could affect the malondialdehyde level and antioxidant enzyme activities in the gut tissues of *Lymantria dispar* (Lepidoptera) larvae[69].

Similarly, our results recorded the highest concentration of lipid peroxides concentration at 12 post treatment was 4.01 at 1000 ppm (Table 5). This may be due to the action of Lipid peroxidation which can disrupt the membrane of the polyunsaturated phospholipids bilayer structure and function[72]. Also, products of lipid peroxidation are capable of disrupting conformations of many cellular proteins, including enzymes, by forming cross links with these proteins, inactivating their functions[72].

Lipid peroxidation is considered as a chain reaction; it produces lipid radical, lipid peroxyl radical, and then lipid hydroperoxide. This reaction can be stopped by termination reactions, such as the recombination of lipid peroxyl radicals and by a reaction with glutathione catalyzed by peroxidase[73].

Therefore, the pattern of fluctuation of lipid peroxides in *H. illucens*, formed post treatment with different concentration of Malathion, may be due to unbalanced levels of lipid peroxides production and their repairing mechanisms that may include antioxidant enzymes.

The results showed that the *ASF extract* led to elevation in the activities of the key antioxidant enzymes, SOD over the constitutive levels except for different cases of *ASF extract* concentration (Fig. 3). The maximum observed elevation seemed to occur in 500 ppm at 12, 24 post treatment concomitance with the oxidative damages of the macromolecules; and may be in response to the formation of ROS consequently to the treated stressor as agreed with Renault, et al.[97] under the effect of plant compounds on desert locust.

SOD and CAT have a primary role in the oxidative stress defense through ROS elimination[73]. The DPPH results showed gradual increase elevation through 24 post treatment Fig. 6. Similarly, the malathion applications caused a serious risk to *Saccharomyces cerevisiae* (fungus). This in-vitro study showed that a flavonoid compound called naringin can inhibit some enzymes and can detoxify the DPPH radical[98].

Briefly, the tested hypothesis in this research has proved the ability of using oxidative stress parameters as bioindicator of plant oil extract impact on the physiological changes in insects. The current study demonstrates that the larvae's oxidative stress markers were disrupted and found notable variations in the environmental stress markers' levels in *Cx. pipiens* larvae. The different mechanisms that excessive insect mortality leads to depleted antioxidant systems are clarified by these findings. The possibility of utilizing alternative oxidative stress markers or the antioxidant response of mosquito larvae as a marker of the *ASF extract* was investigated assessed. Furthermore, a novel strategy for the potential use of the *ASF extract* as an enzyme disruptor for mosquito larvae was introduced.

The *ASF extract* was tested against some positive, negative microbial strains and some violent fungi of *B. subtilis*, *S. aureus*, *E. coli*, *K pneumonia*, *C. albicans* and *(A) Niger*. The antimicrobial results confirmed that the extract has greet activity against the first two pathogens of *(B) subtilis*, and *S. aureus* bacteria (the same

as the positive control, gentamicin) where modest results recorded with the other strains. Also, the minimal inhibitory concentration (MIC) revealed relatively low MIC's values near 31.25 µg/mL. Similarly, the minimal bactericidal concentration against the first two bacterial stains near 31.25 and 62.5 µg/mL with MIC/MBC index not exceeding 2 that confirming the bactericidal effect of *ASF extract*. According to [99], *ASF extract*, which has a pungent, persistent, sulfurous odor and oleo-gum resin, plays a crucial protagonist in both medicine and nutrition. Recent study has shown relaxing, neuroprotective, memory-enhancing, digestive enzyme, antioxidant, antispasmodic, hypotensive, hepatoprotective, antibacterial, anticarcinogenic, anticytotoxicity, anti-obesity, anthelmintic, and antagonistic properties. Its reliance on the emergence of the extract of the *ASF extract* plant, which has effective properties to combat Culex insects, as well as its antioxidant properties, in addition to its pungent smell and sulfur components, made it an anti-insect agent and beneficial to aquatic life when applied on a wide scale in fresh water.

Conclusion

Mosquito-transmitted diseases are a global concern. Due to its unlimited properties, *ASF extract*, of the antioxidant properties, in addition to its pungent smell and sulfur components, made it an anti-insect agent and beneficial to aquatic life when applied on a wide scale in fresh water. Our results show that the *ASF extract* has the potential to be one of the most effective larvicides since it has a solid understanding of many essential oils. EOs could be employed as larvicides in integrated mosquito control programs. After disclosing their ecotoxicological side views, more research is required to create nanoformulations that maximize efficacy and reduce uses.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conceptualization, methodology, investigation, data curation, formal analysis, resources, writing-original draft preparation, I.T.R, A.S., H.S.G. M.M.B., M.H.A., A.M.M., N.A.G., E.A.A.; editing and writing-review, I.T.R, A.S., H.S.G. M.M.B., M.H.A.,A.M.M., N.A.G., E.A.A.; project administration, A.S.; funding achievement, I.T.R, A.S., H.S.G. M.M.B., M.H.A., N.A.G., A.M.M., E.A.A. All authors have read and approved the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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