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Chemical Characterization, Antioxidant, Insecticidal and Anti-Cholinesterase Activity of Essential Oils Extracted from *Cinnamomum verum* L.

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Abstract: This study is aimed at evaluating the potential of the essential oil of *Cinnamomum verum* (EOCV) as an antioxidant, as an insecticide against *Callosobruchus maculatus* and for its anti-acetylcholinesterase activity. To this end, EOCV was extracted via hydrodistillation from this plant, and the identification of the phytochemicals was performed using gas chromatography–mass spectrometry (GC–MS). The antioxidant power was determined via in vitro tests, the insecticidal ability was tested via exposing *C. maculatus* to EOCV, and molecular docking was used to evaluate the anti-cholinesterase ability. The results of these GC–MS analyses show that the main composition of EOCV comprises Cinnamaldehyde dimethyl acetal (64.50%), cinnamicaldehyde (35.04%) and α-Copaene (0.11%). The insecticidal potential of the studied OEs, determined by using the inhalation test, and expressed as the concentration of EOs required for the death of 50% of the insects (LC50) and that required the death of 95% of adults (LC95) after 96 h of exposure, was 3.99 $\pm$ 0.40 and 14.91 $\pm$ 0.10 µL/L of air, respectively. In the contact test, 96 h of exposure gave an LC50 and LC95 of 3.17 $\pm$ 0.28 and 8.09 $\pm$ 0.05 µL/L of air, respectively. A comparison of the antioxidant activity of EOCV to that of ascorbic acid via DPPH free radical scavenging ability and Ferric Reducing Antioxidant Power (FRAP) revealed the IC50 and EC50 values of EOCV to be much higher than that obtained for ascorbic acid, and the molecular docking simulation revealed Coumarin, Piperonal, Cinnamaldehyde dimethyl and alpha-Copaene as possessing potential inhibitory activities against human acetylcholinesterase. However, further experimental validation is needed to enhance the prospects of this study.

Keywords: *Cinnamomum verum*; chickpea; *Callosobruchus maculatus*; molecular docking; FRAP; DPPH

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1. Introduction

The utilization of medicinal plants in combating diseases facing humanity dates back thousands of years in an empirical approach, and advances in science have enabled the identification of the components responsible for these health benefits [1]. Notably, some plants including *Cinnamomum verum* L. have also been identified as possessing both medicinal and culinary value [2]. Hence, they are widely explored for both their medicinal and culinary species. In the context of this, the essential oils (EOs) derived from *Cinnamomum verum* L. plants are often reputed for their numerous pharmacological properties. EOs of *C. verum* L. have been reported to possess a panoply of pharmacological activities, including antimicrobial, insecticidal, antioxidant and cytotoxic activity, among many others; hence, it could potentially be utilized in combating several diseases militating against normal human living [2]. The antioxidant properties of EOs have been reported in studies [2,3], and this is of particular interest due to the central driving role that oxidative stress plays in various human diseases. Specifically, the generation of reactive oxygen species (ROS) that is concomitant with many biological processes, including cellular respiration and the reactions of the electron transport chain, has been reported to promote oxidative stress, and it culminates in modifications at the cellular and molecular levels, which drive diseases including cancer, cardiovascular diseases, diabetes and obesity, among many others [1,4,5]. Although there are pharmacological products utilized in combating oxidative stress, the panoply of phytochemicals such as the polyphenols contained in plants, including EOCVs, have rendered as a cynosure for use as antioxidants in place of synthetic antioxidants, which can pose deleterious effects to organisms [6]. Hence, the evaluation of the antioxidant potential of EOCVs remains of interest to researchers.

In tandem with the multi-pathogenic and oxidative ability, Alzheimer's disease (AD) has also been identified to be multi-factorial, and the search for treatment remains elusive due to this reason [7]. Intriguingly, non-pharmacological approaches, including aromatherapeutics, have been considered viable, and EOCVs have been reported to possess anticholinesterase activity [8]. However, studies exploring the precise phytochemical constituents of EOCVs as potent inhibitors of acetylcholinesterase are lacking, and their exploration could give rise to the development of a newer set of therapeutics against AD. Similarly, a nascent body of evidence has also found EOCV worthy of exploration as an alternative food preservative [9–11]. Notably, insect infestations are one of the main factors that cause a decrease in the economic value of stored foods, such as legumes, and corresponding efforts in terms of advancements in storage methods and production of insecticides have been employed to combat them [12]. However, treatment success is often low, as seen in bruchids, even in countries where contact pesticides and fumigants are often deployed against them [13]. Hence, efforts aimed at tackling them remain unabated. Noteworthy, of all the bruchid species, *Callosobruchus maculatus* F (Coleoptera: Bruchidae) is often considered the most destructive and notorious bruchid species in terms of their impact on stored pulses in tropical and subtropical regions due to significant losses in pulse yields, which have been previously reported to be as high as 90% in black gram [14–16]. Hence, farmers tend to resort to abusive use of insecticides against *Callosobruchus maculatus* F, resulting in increased ecotoxicological and toxicological risks, such as environmental degradation and the recrudescence of disease vectors [17]. However, these insects often evolve resistance mechanisms against these insecticides, and the effects of spraying insecticides on consumables have been worrisome [17]. Hence, there is still a need for alternative and safer methods of combating this phenomenon. Intriguingly, the insecticidal activity of EOCVs against *Callosobruchus chinensis* (L.), which belong to the same specie as *C. maculatus* F, has been reported in a study by Kalita et al., in which they reported that leaf and bark oil of two variants of *C. verum* L. have a repellant effect toward, reduce the fecundity rate of and decrease the egg hatchability of *Callosobruchus chinensis* (L.). Consequently, the investigation of EOCVs against *C. maculatus* F can be deemed a viable research area.

In furtherance of research aimed at exploring the plethora of biological properties of EOCVs, this study is aimed at characterizing the phytochemical constituents of EOCV

using the hydrodistillation method and gas chromatography–mass spectrometry (GC-MS), to evaluate its antioxidant potential using bioanalytical methods, to evaluate its insecticidal activity and to evaluate the anti-cholinesterase activity of the identified phytochemicals using the molecular docking approach.

2. Materials and Methods

2.1. Chemicals and Reagents

Butylated hydroxytoluene (BHT), ammonium molybdate, 2,2 diphenylpicrylhydrazyl (DPPH), ascorbic acid, sodium phosphate, iron III chloride ($FeCl_3$), potassium ferricyanide ($K_3Fe(CN)_6$) and acetone were purchased from Sigma-Aldrich (Munich, Germany).

2.2. Preparation of Essential Oils

The plant was collected in Fez, Morocco, identified under 20/22-CV/G13 before the EOs were extracted via hydrodistillation. Briefly, 100 g of the *C. verum* L. powder was mixed with 700 mL of water before being heated at 120 °C for 180 min. The EOs were stored in small opaque bottles and kept in a refrigerator at 4 °C until their use.

2.3. GC-MS Analysis

The phytochemical compositions of EOGP were identified using gas chromatography–mass spectrometry in vitro (GC-MS-Thermo Fischer; GC/ULTRAS/N-20062969; Polaris QS/N-210729), equipped with a HP5MS non-polar fused silica capillary column (60 m × 320 µm, film thickness 0.25 µm), as detailed in previous works [18].

2.4. Insecticidal Activity of EOCV

2.4.1. Test Insects

Adults of *C. maculatus* were collected in Morocco before being acclimatized via the use of a temperature of about 25 ± 2 °C with a humidity of about 65% ± 5% and a photoperiodic cycle of 14 h (light)/10 h (dark).

2.4.2. Effect of EOCV on *C. maculatus* Adults Using Contact Test

To investigate the contact toxicity of EOCV against adults of *C. maculatus,* five pairs of insects were released on 100 g of chickpea seeds. Plastic containers (0.25 L volume) with perforated lids and thin transparent cloth were used for conducting experiments. Based on the results obtained in the preliminary tests, a range of concentrations was determined (0, 1, 5, 10 and 124 20 µL/100 g). The tests were repeated three times for each concentration. The insecticidal power of the EOs was assessed by counting the number of dead adults of *C. maculatus* under a stereoscopic microscope on a daily basis (Equation (1)) [19,20]. This test was performed for 3 replications.

$$Pc(\%) = \frac{Po - Pt}{100 - Pt} \times 100 \quad (1)$$

Pc: corrected mortality in %; *Po*: observed mortality in the trial assay; and *Pt*: mortality in the negative control.

Insect eggs were counted 12 days after the beginning of the experiment, and emergent individuals were counted 30 days after the beginning of the experiment. The decrease in fecundity of emerging females and adults for each concentration of EOCV was calculated via the use of the below equation.

$$Pr(\%) = (1 - \frac{Nt}{Nc}) \times 100 \quad (2)$$

Pr: rate of reduction compared to the control; *Nc*: number of eggs or insects in the control; and *Nt*: egg number in the trial assay.

2.4.3. Effect of EOCV on the Survival of *C. maculatus* Adults by Inhalation Test for 96 h

The toxicity of EOCVs on *C. maculatus* adults was determined after inhalation exposure. For this purpose, 10 pairs of *C. maculatus* adults aged 0–48 h were placed in one-liter glass vials. A small piece of cotton was suspended with a thread attached to the inside of the lid. Concentrations of 0, 1, 5, 10 or 20 µL of EOCV/L were applied to the cotton with a ProPette™. Three replicates were performed for each dose. The mortality rate was calculated according to Abbott's formula (Equation (3)) [19,20]. This test was performed for 3 replications.

$$Pc(\%) = \frac{Po - Pt}{100 - Pt} \times 100 \quad (3)$$

Pc: corrected % mortality; *Po*: mortality in the trial; and *Pt*: mortality in the negative control.

2.4.4. Repellent Potential of EOCV against *C. maculatus*

The repellent effect of EOCVs against adult *C. maculatus* is reported elsewhere [21]. Briefly, the discs used for experiment were divided into two halves, each having a surface area of 31.80 cm^2. Next, a volume of 500 µL of each concentration of EOCVs previously prepared in acetone (0, 1, 5, 10, and 20 µL/mL) was evenly distributed on one of the two halves, resulting in doses of 0.0160, 0.0790, 0.1570 and 0.3150 µL/cm^2 per disc. The other half received only 500 µL of solvent (negative control). After that, the Petri dishes were covered with parafilm for 30 min. Thereafter, the bruchid population on the EOCV-treated area of the disc was compared to the untreated area. A formula (Equation (4)) reported in earlier studies was used to calculate the level of repulsion [22,23]. This test was performed in triplicate.

$$Pr(\%) = \frac{Nc - Nt}{Nc + Nt} \times 100 \quad (4)$$

Pr (%): repulsion percentage; *Nc*: number of insects in the untreated area; and *Nt*: number of insects in the treated area with EOCVs.

2.5. Antioxidant Activity

2.5.1. DPPH Test

In the present study, DPPH radical scavenging activity was determined by combining 1 mL of DPPH solution (0.004%) with 100 µL of various concentrations of EOCV. The absorbance was measured at 517 nm after 30 min of incubation at room temperature. The reduction in DPPH free radical scavenging by ascorbic acid was also analyzed for comparison [24]. The reaction kinetics and the parameters for calculating the antioxidant activity of ascorbic acid and the EOCV were calculated. The determination of the percentage inhibition of free radicals (%) was calculated using the following formula [25]:

$$\% = (1 - \frac{\text{Abs test}}{\text{Abs control}}) \times 100 \quad (5)$$

Abs test: absorbance of the sample; Abs control: absorbance negative control.

The concentrations of EO and ascorbic acid were plotted as a function of the percentages of DPPH inhibited to obtain the IC_{50} index.

2.5.2. FRAP Test

Briefly, tubes were filled with 200 µL of various EOCV concentrations, 500 µL of 0.2 M phosphate buffer with pH = 6.6 and 500 µL of 1% $K_3Fe(CN)_6$. Consequently, the mixture was heated to 50 °C for 20 min. Next, 500 mL of 10% $C_2HCl_3O_2$ was pipetted into the solution before being centrifuged at 3000 rpm for 10 min. The supernatant was recorded and mixed with 500 µL of double-distilled water and 100 µL of 1% $FeCl_3$. The absorbance was read at 594 nm against the blank (negative control). Standard antioxidants, either BHT or quercetin, were used as positive controls [26,27].

2.5.3. TAC Test

The phosphomolybdenum technique was used to calculate the total antioxidant capacity (TAC) of EOCVs. Briefly, 100 µL of various EOCV concentrations were added to 1000 µL of a reagent mixture of H_2SO_4, Na_2PO_4 and ammonium molybdate, resulting in concentrations of 0.6 M, 28 mM and 4 mM, respectively. For 90 min, the tubes were kept at a temperature of roughly 95 °C. The absorbance was measured at 695 nm after cooling. The control included 1000 µL of reagent combination and 100 µL of methanol. The conditions for incubating the samples and controls were the same. The results are expressed as mg equivalents of ascorbic acid per gram (mg EAA/g) [28,29].

2.6. In Silico Human Acetylcholinesterase Inhibition Studies

The SDF files containing compounds in EOs in *Cinnamomum verum* L. were taken from the PubChem database before being prepared via the use of the LigPrep tool on the Maestro-Schrödinger Software program using the OPLS3 force field. After the ionization states were carried out at a pH of 7.00 a maximum of 32 stereoisomers were generated for each ligand. Using the PDB ID 4EY7, the three-dimensional crystal structure of human acetylcholinesterase was obtained in the PDB format from the protein data bank. Schrodinger-Maestro v11.5's Protein Preparation Wizard was utilized to assist in the preparation and refinement of the structure. The OPLS3 force field was utilized in order to carry out the minimizing of the construction. When the volumetric spacing was conducted, a 20 × 20 × 20 grid was used for the receptors. The following coordinates were used to set the receptor grid: X = −14.024, Y = −43.956 and Z = 27.949. Docking of SP flexible ligands was performed in Glide of Schrodinger-Maestro version 11.5

2.7. Statistical Analysis

Results are presented as the mean of three replicates with standard deviations. An analysis of variance was performed followed by Tukey's multiple comparisons test to handle multiple comparisons. Differences were regarded as statistically significant at $p < 0.05$.

3. Results

3.1. Chromatographic Analysis of EOCV by GC-MS

The extraction of terpenic compounds from *Cinnamomum verum* L. revealed that the yield was in the order of 2.11 ± 0.15%, with a yellow color and an aromatic odor. The analysis of the chemical composition of EOCV via GC–MS (Table 1, Figure 1) revealed that this studied plant was rich in terpenic compounds, such that 11 compounds were identified with a total of about 99.98%. The majority of phytochemical compounds were Cinnamaldehyde dimethyl acetal (64.50%), followed by cinnamicaldehyde (35.04%), but the other compounds comprised less than 1%.

Table 1. Chemical composition of EO extracted from *C. verum* L.

Peak	RT	Compounds	Molecular Formula	Chemical Class	Area (%)
1	5.11	Heptanal	C7H14O	Other	0.05
2	8.36	Hexyl acetate	C8H16O2	Other	0.02
3	10.91	Acetophenone	C8H8O	Other	0.07
4	16.86	Verbenone	C10H14O	Monterpene	0.03
5	19.38	Cinnamicaldehyde	C10H10O2	Monterpene	35.04
6	20.95	Piperonal	C8H16O3	Other	0.01
7	22.21	α-Copaene	C15H24	Sesquiterpene	0.11
8	23.85	Cinnamaldehyde dimethyl acetal	C11H14O2	Other	64.50
9	26.41	Isoeugenol	C10H12O2	Monterpene	0.01
10	27.19	Thujopsene	C15H24	Sesquiterpene	0.04
11	31.23	Coumarin	C9H6O2	Other	0.01
				Total	99.98

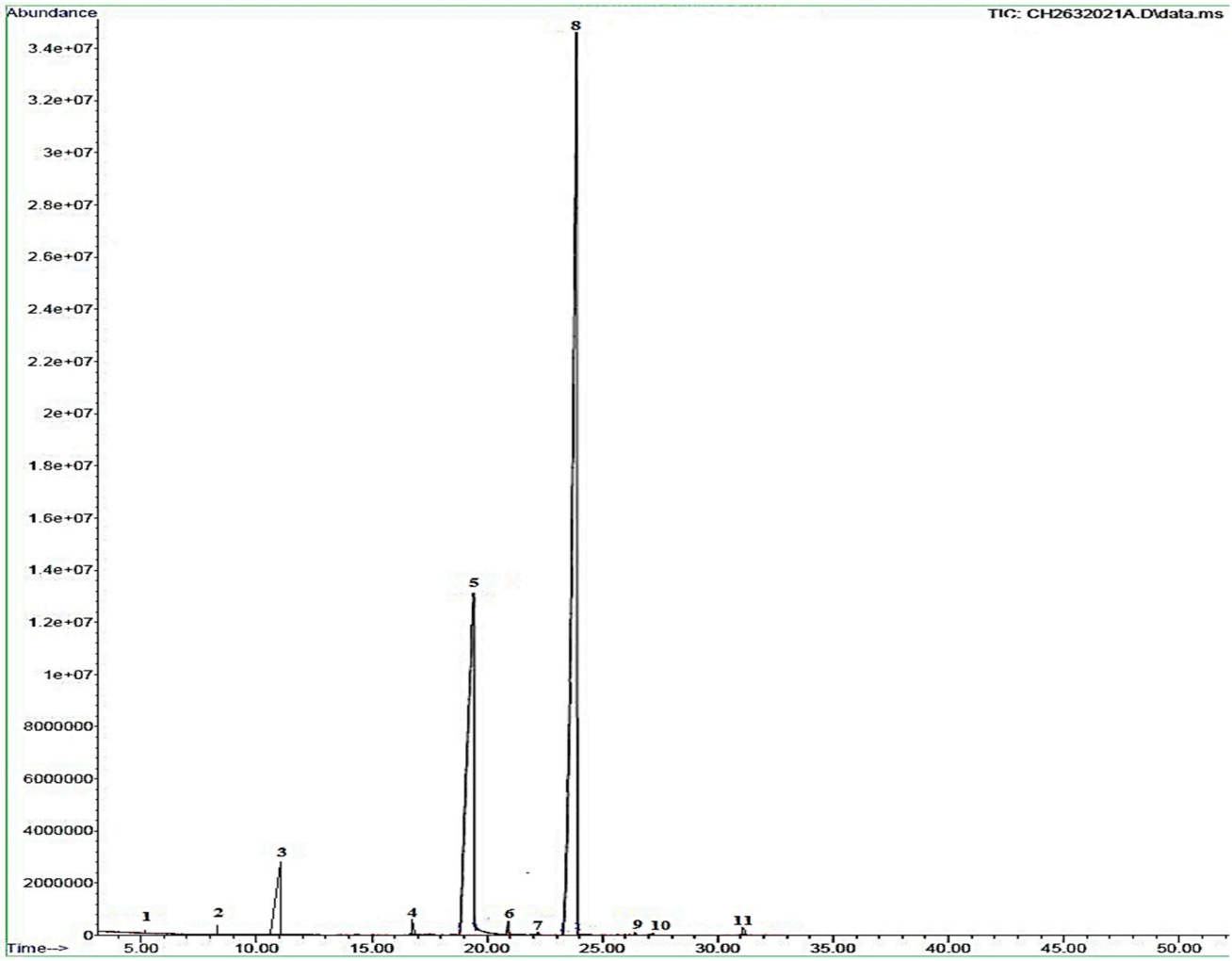

Figure 1. GC–MS chromatogram of EO extracted from *C. verum* (EOCV).

3.2. Insecticidal Activity of EOCV

The analysis of Figure 2a showed that the mortality rate of *C. maculatus* adults exposed via inhalation was dependent on the dose and duration of exposure to the tested essential oils. The tested EOCV showed important insecticidal activity toward adults of *C. maculatus*. Mortality rates at the low dose of 1 µL of EOCV/L of air during the observation periods (24, 48, 72 and 96 h) were 0.0 ± 0.0, 8.66 ± 1.52, 27.67 ± 2.08 and 42.0 ± 2.64%, respectively. Mortalities at the high dose of EOCV (20 µL/L air) during the observation periods were 48.3 ± 2.10, 71.33 ± 7.50, 87.33 ± 3.05 and 98.3 ± 2.89%, respectively. During the four exposure periods via contact (24, 48, 72 and 96 h), the minimum concentration of EOCV (1 µL/L air) caused mortalities in the order of 12.33 ± 2.52, 19.0 ± 3.61, 32.0 ± 3.46 and 44.0 ± 4.0%, respectively. However, for the maximum concentration (20 µL EOCV/L air), for the four exposure periods, mortality was 59.66 ± 5.51, 81.33 ± 7.09, 95.33 ± 4.16 and 100.0 ± 0.0%, respectively (Figure 2b).

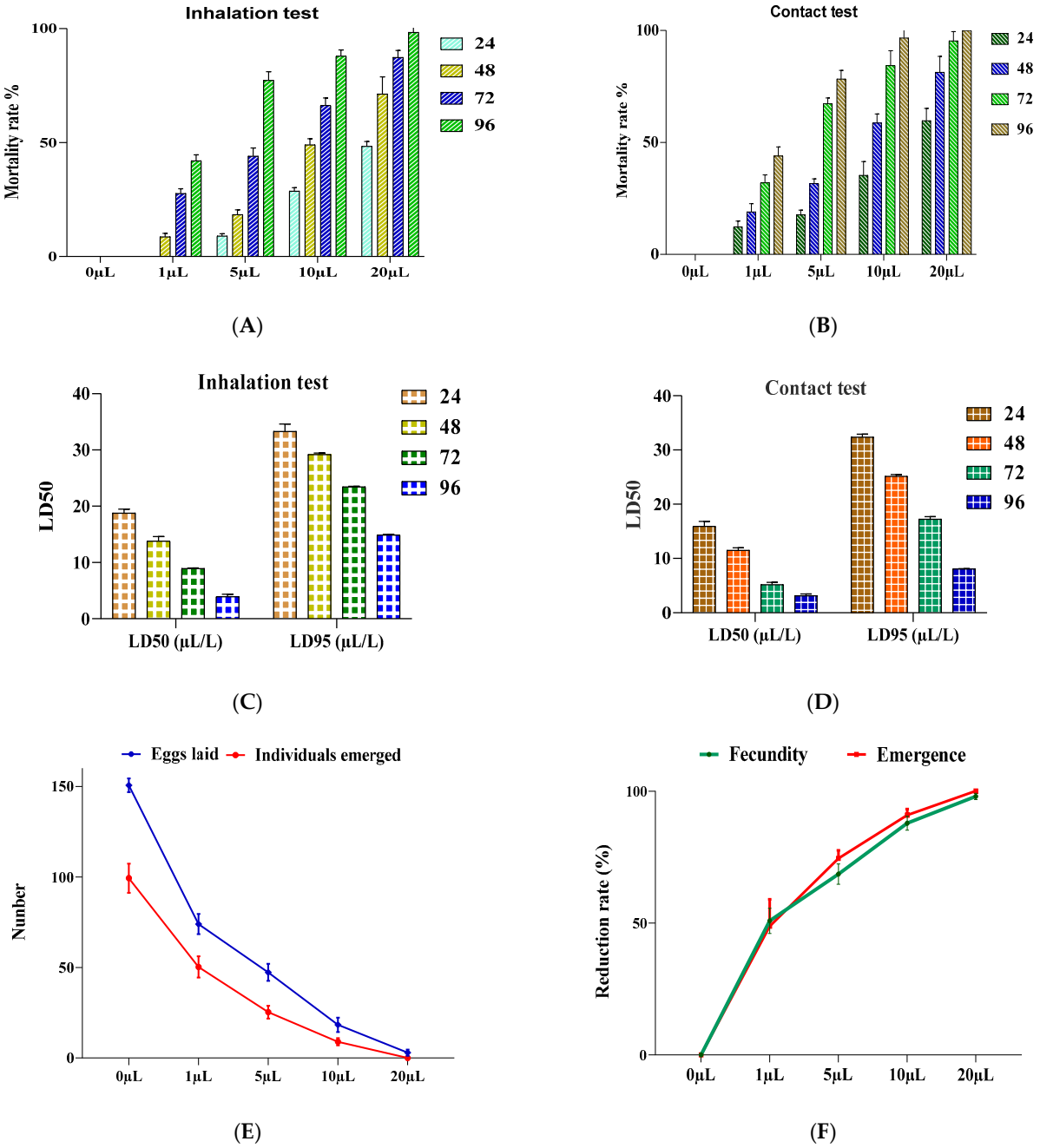

Figure 2. Mortality rate (%) of *C. maculatus* adults from the inhalation test (**A**) and contact test (**B**). LC_{50} and LC_{95} obtained from the inhalation test (**C**), LC_{50} and LC_{95} obtained from the contact test (**D**). Fecundity and emergence of new individuals after direct contact with 5 doses of EOCV (**E**). Reduction rate of fecundity and emergence after direct contact with 5 doses of EOCV (**F**).

The differences were considered statistically significant at the (p) 0.05 level. In *C. maculatus*, the EO of *C. verum* L. eliminated 98.3 ± 2.89% of the adults at a dose of 20 µL/L. The LC_{50} and LC_{95} obtained with this essential oil were 3.99 ± 0.40 and 14.91 ± 0.10 µg/L, respectively (Figure 2c). With *C. verum* EO, the highest tested dose (20 µL/L) caused 100% mortality in *C. maculatus* adults after the 96 h treatment. The obtained LC_{50} and LC_{95} obtained were 3.17 ± 0.28 and 8.09 ± 0.05 µg/L, respectively (Figure 2d). The fertility of *C. maculatus* eggs, laid on seeds fumigated with EOCV, was lower than that obtained with the control, and it was null with the 20 µL/L air concentration. The EOCV had ovocidal properties (Figure 2e). The fecundity of *C. maculatus* on chickpea seeds fumigated

with the different concentrations of EOCV was strongly affected, and the number of eggs released on fumigated seeds was significantly lower than on untreated seeds. The decrease in fecundity of *C. maculatus* was related to the early death of the females, which did not exceed three days in the treated batches (Figure 2f).

3.3. Antioxidant Activity of EOCV

The results of the antioxidant activity of EOCV are represented in Table 2 and Figure 3. The results obtained by the two tests (DPPH and FRAP) showed remarkable antioxidant activity. The IC_{50} value (obtained by DPPH assay) and EC_{50} value (obtained by FRAP assay) were 39.80 ± 1.34 mg/mL and 68.38 ± 2.51 mg/mL, respectively. These values are higher than those obtained for ascorbic acid (IC_{50} = 0.0027 mg/mL and EC_{50} = 0.0029 mg/mL). On the other hand, cinnamon oil had a very high total antioxidant capacity
(229.15 ± 29.54 mg EAA/g *C. verum* EO; y = 1.5033x + 0.0936 R^2 = 0.9931).

Table 2. Results of antioxidant activity of EOCV from the three methods (DPPH, FRAP and TAC).

Sample	DPPH (IC_{50} mg/mL)	FRAP (EC_{50} mg/mL)	TAC (mg AAE/g EOCV)
C. verum	39.80 ±1.34	68.38 ± 2.51	229.15 ± 29.54
Ascorbic acid	0.0027	0.0029	

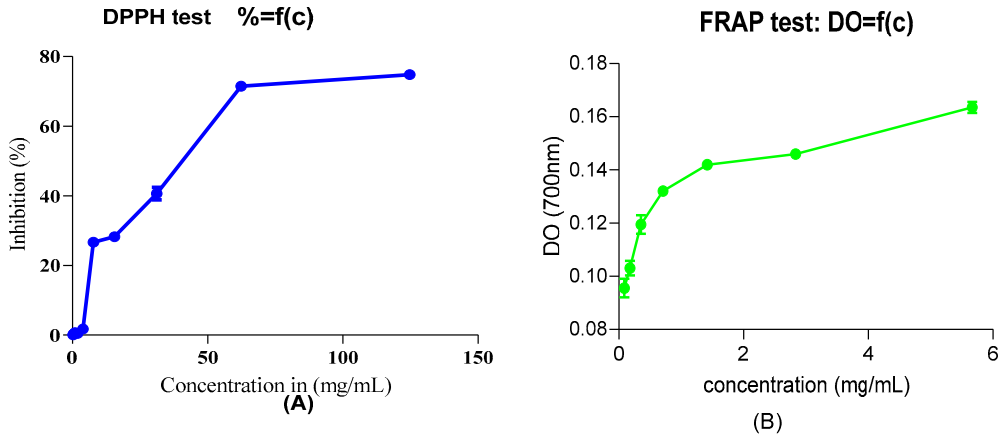

Figure 3. Antioxidant effects of EOCV by use of DPPH (**A**) and FRAP assay (**B**).

3.4. In Silico Human Acetylcholinesterase Inhibition Studies

The in silico approach showed that coumarin, piperonal, cinnamaldehyde dimethyl and alpha-copaene were the most active compounds against human acetylcholinesterase, with a Glide score of −7.665, −7.207, −6.878 and −6.68 Kcal/mol, respectively (Table 3). Figures 4 and 5 show the 2D and 3D structures of coumarin and piperonal interactions with the active site of human acetylcholinesterase (PDB: 4EY7). Importantly, each one of them revealed the formation of one hydrogen bond with PHE 295 residue and 2 Pi-Pi stacking bonds with TYR 341 residue. The cinnamaldehyde dimethyl interactions with the active site of human acetylcholinesterase showed the formation of one hydrogen bond with PHE 295 residue and one Pi-Pi stacking bond with TYR 341 residue.

Table 3. Docking results with *cinnamomum verum* EO in the active site of human acetylcholinesterase (PDB: 4EY7).

	Human Acetylcholinesterase (PDB: 4EY7)		
	Glide Gscore (Kcal/mol)	**Glide Emodel (Kcal/mol)**	**Glide Energy (Kcal/mol)**
Coumarin	−7.665	−39.64	−26.212
Piperonal	−7.207	−36.878	−24.88
Cinnamaldehyde dimethyl	−6.878	−38.909	−28.743
Alpha-Copaene	−6.68	−31.517	−23.203
Thujopsene	−6.455	−30.441	−22.661
Cinnamaldehyde	−5.954	−38.092	−28.04
Isoeugenol	−5.951	−39.713	−28.46
Verbenone	−5.862	−31.121	−22.721
Acetophenone	−5.617	−33.031	−24.488
Heptanal	−3.909	−25.405	−20.508
Hexyl acetate	−3.836	−28.588	−23.732

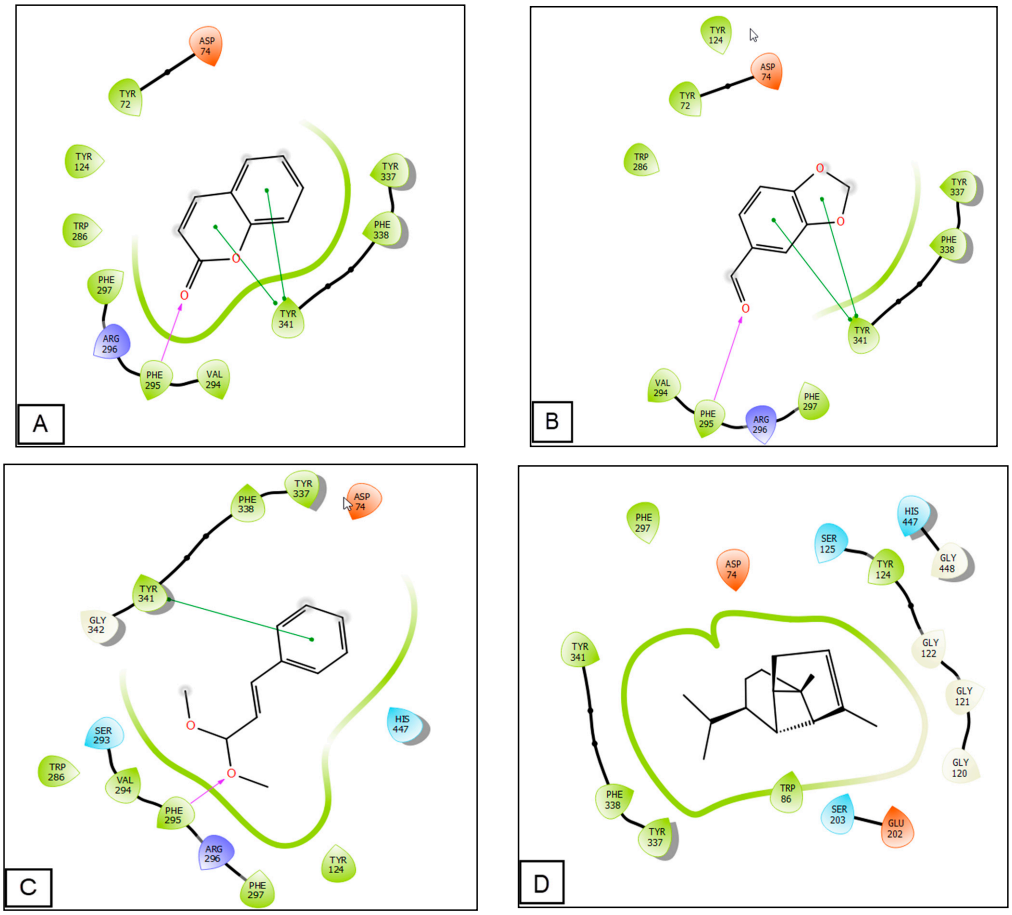

Figure 4. Two-dimensional diagrams of ligands interactions with the active site of human acetylcholinesterase (PDB: 4EY7): (**A**) coumarin, (**B**): piperonal, (**C**) cinnamaldehyde dimethyl and (**D**) alpha-copaene.

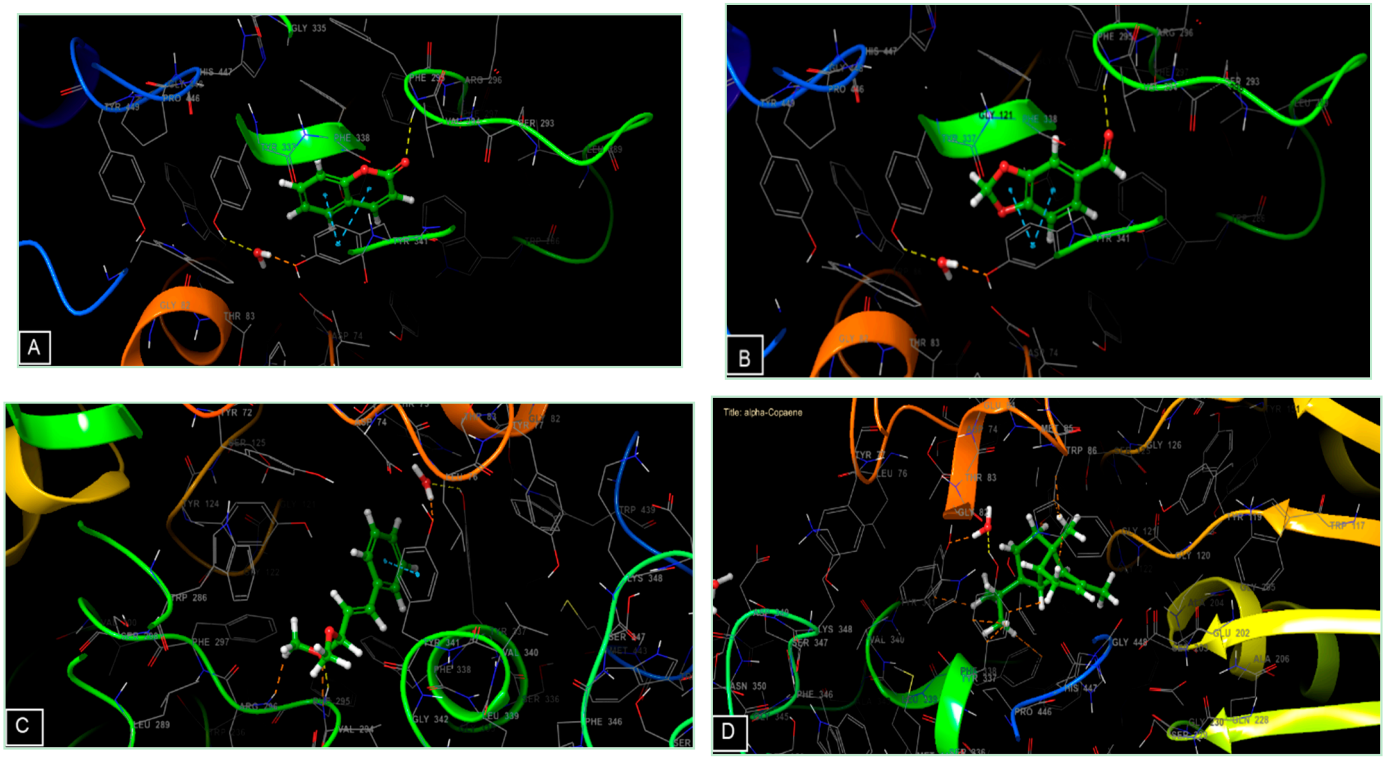

Figure 5. Three-dimensional diagrams of ligands interactions with the active site of human acetylcholinesterase (PDB: 4EY7): (**A**) coumarin, (**B**): piperonal, (**C**) cinnamaldehyde dimethyl and (**D**) alpha-copaene.

4. Discussion

In this study, we sought to identify the compounds present in EOCV and to evaluate their antioxidant, insecticidal and anti-cholinesterase activity. To this end, the EOs were extracted via the hydrodistillation method and subjected to GC–MS analysis. It is worth noting that there exist several methods that are utilized in the extraction of EOs from plants, and the used methods influence the obtained results. Notably, the methods are mainly of two types, which are the conventional and non-conventional methods of EO extraction. The conventional methods of extraction include steam distillation, hydrodistillation, soxhlet extraction and percolation, among others [30]. Conversely, the non-conventional methods include microwave-assisted extraction, microwave-assisted hydrodistillation, microwave steam distillation, microwave hydro diffusion and gravity [30]. The efficacy and effectiveness of both methods vary and can be dependent on the plants of interest. However, non-conventional methods are more advantageous over conventional methods due to factors including reduced energy consumption, improved extraction efficiency and reduced solvent usage. Despite these factors, hydrodistillation, which is the oldest and simplest method, has also retained its reliability as a good and environmentally friendly method of extracting EOs from plants [31]. Exemplifying this is its utilization of water as a key component in place of techniques that require chemicals [31], as well as its ability to give a more accurate representation of the phytochemical profile of the essential oil [32]. Furthermore, its availability and accessibility, as well as its ability to retain the therapeutic properties of the EO via minimizing exposure to high temperatures and harsh conditions, have rendered it a suitable method despite advances in other methods [31,33]. Consequently, this method was utilized in this study.

The yield of the essential oils of cinnamon is 2.11 $\pm$ 0.15%, and that found by another study was of the order of 3.98 $\pm$ 0.25% [34]. This can be caused by different factors (the method of extraction, the time of distillation, the quality of the dried cinnamon, noting that the quality improves from year to year and that the aged cinnamon has a higher

content of essential oils, as well as the geographical origin and the time of harvesting and preservation, etc.).

Studies on EO extracted from *C. verum* bark using various extraction techniques have produced EO yields of 2.32%, 1.42% and 1.05%. These methods include n-hexane solvent, water distillation and ultrasonic aided extraction, respectively. The effect of certain parameters on the yield of essential oil can be highlighted. It has been shown that the microwave solventless extraction method provides a higher yield and a faster extraction time when compared to the microwave hydrodistillation method [35]. The findings of this investigation show that organic solvents have a greater extraction efficiency than that of water distillation or ultrasonic aided extraction. However, compared to EOs derived using ultrasonic or organic solvent-assisted extraction, those obtained through water distillation have a wider range of chemical components. In addition, compared to the other two methods, the major chemical components of the EOs obtained via water distillation, such as (E)-cinnamaldehyde, cinnamyl acetate and trans- and cis-cinnamic acid, were present at a comparatively high level of 61.531%, 15.512%, 0.437% and 6.401%, respectively [36]. Contrary to earlier research, the target chemicals were selectively extracted using an ultrasound-assisted technique through a liquid medium, which required less energy, solvent and processing time [37]. However, the analysis of chemical properties showed that the essential oils obtained via microwave air-hydrodistillation are of better quality (flavor) than the oils obtained by use of hydrodistillation alone [35]. One study suggests that the solventless microwave extraction method is a fast and efficient alternative for the extraction of essential oil from dried (*Pogostemon cablin* Benth) leaves, with great potential for industrial applications [38]. A comparison of the compounds obtained following GC–MS identification with that obtained from other study that utilized a different extraction method revealed the presence of eugenol and cinnamaldehyde in higher concentrations in EOCV extracted using superheated water extraction [39].

By monitoring the post-treatment effect of EOCVs on treated *C. maculatus* adults observed over the four periods, we found that the adulticidal activity of the tested EOs persisted during the post-treatment exposure periods. This EOCVs effect was more important when the dose used for treatment was 20 µL/L. On the other hand, at doses below 20 µL/L, the evolution of the mortality rate was dose-dependent regardless of the dose. At 96 h, the cumulative mortality obtained in the different tests (inhalation and contact), regardless of the dose, was more than 90% of the adults of *C. maculatus*.

The number of eggs laid by female *C. maculatus* during EOCV treatment was very low. This reduction in oviposition was dose dependent over the range of tested doses. However, the highest reduction rates were obtained with EOCV, which caused a reduction of about 80% in the number of eggs laid at 10 µL/L and above. Under our experimental conditions, the tested EOs showed effective effects on the mortality of *C. maculatus* adults.

Cinnamomum is a genus of plant that is well known for its essential oil production capabilities and applications in the control of various insect pests/vectors, and the efficacy of this oil on *C. maculatus* has been reported by [40]. However, EOCVs are less explored for such activities using experimental studies.

The oil we used contained a cinnamaldehyde compound, and the effectiveness of essential oils is generally linked to the nature of their major components. These authors have indeed shown that the terpenes emitted by essential oils are toxic to *C. maculatus* adults. The adulticidal activity of essential oils extracted from other species of the Cinnamomum genus has been reported. For example, essential oils from *Cinnamomum osmophloeum* leaves, whose insecticidal properties are due to cinnamaldehyde of the cinnamaldehyde/cinnamyl acetate type, have been shown to have an excellent inhibitory effect on larvae [41,42]. The reduction in oviposition observed in both the bruchid species in the presence of the essential oils is likely related to the early deaths of some females or to the morbidity observed in the adults. The observed reduction could be due to a direct interaction between EO and AChE or indirectly through action on neural cells [43–45].

The reproductive potential of these bruchid species, their ability to develop on other substrates and the high level of damage caused to legume stocks necessitate the establishment of a control programmer for these pests. However, the difficulty of controlling bruchid populations under storage conditions is also reinforced by the limitations and consequences of using conventional pesticides under storage conditions. The search for alternative solutions through the use of plant-based products such as essential oils has yielded satisfactory results. Indeed, the evaluation of the insecticidal properties of the studied EOs showed them to be effective in controlling the different developmental stages of bruchids (*C. maculatus*).

The antioxidant activity of the essential oils of cinnamon depends on chemical composition. Various studies have reported that the antioxidant activity of cinnamon is correlated with the presence of higher amounts of E-cinnamaldehyde and eugenol [46]. The presence of higher amounts of eugenol in the EOCV chemotype result in significant antioxidant activity against DPPH at 10 µL/mL [47]. On the other hand, another study reported that cinnamon EOs are devoid of antioxidant potential in DPPH and ABTS assays [48]. In addition, EOCV was found to have the highest antioxidant activity compared to other essential oils [49].

EOCV at concentrations of 125, 250, 500 and 1000 µg/mL significantly increased the percentage inhibition of DPPH by 8.22 ± 0.70, 12.74 ± 2.48, 20.10 ± 1.71 and 28.74 ± 1.61%. At the same concentrations, the percentage inhibition of ABTS was 8.44 ± 1.19, 28.08 ± 2.19, 30.70 ± 1.33 and 50.17 ± 3.95%, respectively [50]. In addition, EOCVs showed inhibitory power against DPPH with a percentage inhibition of 94.42% at 8.0 µg/mL, and the IC50 was 0.245 µg/mL. For eugenol, the concentration of 20 µg/mL was required to achieve 88.67% inhibition, with an IC50 of 1.258 µg/mL. With a concentration of 0.1 µg/mL allowing 90.0% hydroxyl radical scavenging, it has been reported that the topical administration of C. verum increases antioxidant activity and reduces the content of malondialdehyde levels compared to control animals.

Other plant extracts from this family also possess antioxidant properties in addition to cinnamon essential oils. Regarding the findings of [49], significant antioxidant activity was found employing a cinnamon methanolic extract with an IC50 value of 11.9 g/mL. Regarding antioxidant enzymes, cinnamon powder includes glutathione, catalase, peroxidase and superoxide dismutase [51].

Molecular docking simulations of the interaction of the identified compounds with human acetylcholinesterase revealed that the compounds possessed a high affinity for the protein, as revealed by their docking scores, which ranged from −3.836 to −7.665 kcal/mol. Notably, compounds including coumarin, piperonal, cinnamaldehyde dimethyl, alpha-Copaene showed high binding affinities for this protein, interacting with the protein via their scaffolds to form hydrogen bonds, hydrophobic bonds, polar interactions and charged interactions. These compounds can be further explored for the development of therapeutics to combat AD via the optimization of their scaffolds to increase selectivity and maximize interactions.

5. Conclusions

In summary, this study was aimed at evaluating the antioxidant, insecticidal and anti-acetylcholinesterase activity of EOCVs. Following an extensive analysis, two compounds, namely cinnamaldehyde dimethyl acetal (64.50%) and cinnamicaldehyde (35.34%), were identified as the most abundant compounds in EOCV, and EOCVs also showed appreciable antioxidant capacity. Furthermore, the potential of EOCV to act as a biocide against *C. maculatus* was established, and the anti-acetylcholinesterase activity was also demonstrated. Conclusively, this study contributes to the nascent body of literature that has demonstrated the antioxidant activity of EOCV, and it provides a novel research direction toward the potential usage of EOCV as an insecticide against *C. maculatus*, while also providing further evidence of its neuroprotective potential.

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