Research Article

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# Unveiling the molecular composition and biological properties of essential oil derived from the leaves of wild *Mentha aquatica* L.: A comprehensive *in vitro* and *in silico* exploration

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**Abstract:** The purpose of the current study is to assess the chemical profile, antioxidant, antimicrobial, and insecticide efficacy of essential oil derived from the leaf of Moroccan *Mentha aquatica* L. (MA-EO) using *in vitro* and *in silico* analysis. Using GC-MS/MS analysis, 18 components of MA-EO were identified, including linalool (42.42%), α-elemol (10.45%), α-terpineol (8.07%), linalyl acetate (7.37%), and caryophyllene (4.05%). Additionally, MA-EO has a strong antioxidant capacity with $IC_{50}$ values of 0.64 ± 0.01 µg/mL using the DPPH assay and 0.167 ± 0.13 µg/mL using the ABTS test. Total antioxidant capacity activities were found to be 188.21 ± 0.31 mg EAA/g, while RP activities were 1.95 ± 0.023. The powerful antibacterial properties of MA-EO were proven to be effective against *Escherichia coli* and *Candida albicans*. MA-EO showed insecticidal potential using the fumigation experiment, with an $LC_{50}$ of 3.33 µL/L in the air after 24 h of exposure. At a dose of 20 µL/mL, MA-EO reduced fertility, fecundity, and emergence of adult *C. maculatus*. MA-EO had 95% mortality at the same dosage. *In silico* analysis revealed that the antioxidant activity of MA-EO is linked to γ-eudesmol, while its antibacterial efficacy is associated with phenol, 2,4-di-tert-butyl-, and its antifungal capacity with phenol, 2,4-di-tert-butyl-. MA-EO demonstrates potent bactericidal, fungicidal, and bioinsecticide properties, making it effective for controlling bacteria, fungi, and insect pests in stored grains.

**Keywords:** *Mentha aquatica* L, essential oil, Antioxidant activity, antimicrobial activity, insecticidal activity, *in silico* analysis

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# 1 Introduction

Currently, herbal therapy has seen a significant increase in the use of medicinal and aromatic plants due to their dense chemical composition, notably those that fall under the Lamiaceae family. The *Mentha* species is one of the most prevalent species and has a large spectrum of biological properties, including insecticidal, antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, antiseptic, antispasmodic, and analgesic [1].

*Mentha aquatica* L. is a herb native to the Lamiaceae family. It is distributed extensively across all continents (except in South America and Antarctica). In folk medicine, in the traditions of several nations, such as Vietnam, Algeria, South Africa, and Arab countries, the plant is commonly used as a natural treatment for various diseases. In addition, the plant has been reported to treat cold and respiratory problems, such as cough and ulcerative colitis [2]. It is used as a tonic, used as a stimulant, a digestive stomachic, carminative, relaxing, antispasmodic, sedative, and analgesic [3]. Numerous pharmacological characteristics of *M. aquatica* have been confirmed by strong scientific evidence, notably insecticidal [4], antihemolytic [5], anti-inflammatory [6] antimicrobial [7], hepatoprotective [8], anticancer [9], gastroprotective [10], and antiemetic effects [3]. Indeed, previous studies have shown that the *M. aquatica* essential oil (EO) has a powerful capacity to scavenge free radicals and is an efficient antimicrobial agent against various pathogenic bacteria [11,12]. The investigation of the phytochemicals of *M. aquatica* discovered the presence of various bioactive compounds, namely rosmarinic acid, cinnamic acid, ferulic acid, gallic acid, ellagic acid, catechin, chlorogenic acid, quercetin, naringenin, rutin, and hesperidin [8]. Andro and coworkers showed that menthone, δ-cadinene, viridiflorol, viridiflorol, caryophyllene oxide, α-cadinol, β-bisabolenol, epoxide II, borneol, *p*-cymene, -myrcene, sabinene, geranyl acetate, α-*trans*-bergamotene 1,8-cineole, and linalyl acetate were distinctive bioactive chemicals of *M. aquatic*a EO [13]. The chemical constituents of plants are strongly affected by a variety of geographical and environmental factors, most notably geological and ecological ones. However, little is acknowledged about the biological characteristics of Moroccan *M. aquatica*. Despite the greatest antimicrobial, antioxidant, and insecticidal ability of *M. aquatica,* EOs had never previously been evaluated for these bioactivities.

To our best insight, there are currently no reports that investigate the impact of Moroccan *M. aquatica* EO on antibacterial, antifungal, antioxidant, and insecticidal capacities, and its potential toxic on the fecundity, fertility, and adult emergence of *C. maculatus* pest. The goals of the present study are to examine the chemical compounds of the EO that were isolated from Moroccan *M. aquatica*, as well as evaluate its activities as an antioxidant, antimicrobial, and insecticide using an *in vitro* and *in silico* approach. The chemical components of EO were identified using GC/MS/MS analysis, the DPPH scavenging capacity using 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), reducing power (RP) and total antioxidant assays (TAC) were used to assess the *in vitro* antioxidant activities, as well as antibacterial and antifungal activities were also evaluated against the pathogenic strain. Furthermore, the insecticidal properties of MA-EO were assessed against *C. maculatus*, a cowpea grain pest.

# 2 Materials and methods

## 2.1 Collection of vegetable matter

The aerial parts were gathered from Merja Zerga (or Moulay Bousselham lagoon) in June 2021 and subsequently processed into herbarium specimens. These specimens were then analyzed by botanist Professor Amina BARI from the Faculty of Biology at Sidi Mohamed Ben Abdellah University. The reference sample has been registered in the herbarium of the faculty under the number 002MAMZ2121.

## 2.2 EO extraction

About 100 g of *M. aquatica* leaves and flowers were dried, ground, and hydrodistilled for 3 h with 1 L of distilled water using Clevenger equipment [11]. The extracted EO was isolated from water using anhydrous sodium sulfate. It was then transferred to a sealed bottle and stored in a dark place at 4°C until use [14].

## 2.3 GC-MS/MS chemical analysis of EOs

Gas chromatography was used to perform the GC-MS analysis using a Trace GC ULTRA (S/N 20062969) coupled with a Polaris Q mass spectrometer (Thermo Fischer, France) fitted with an iron trap mass spectrometer. The chromatographic separations were conducted using an HP-5MS capillary column (60 m in length, with an internal diameter of 0.32 mm and a stationary phase thickness of 0.25 μm). The transition line and the ionic source had temperatures of 300 and 200°C,

respectively. The scan range was 40–650AmU at 3.9 scans per second. The temperature was adjusted between 40 and 280°C with a ramp of 5°C/min; the temperature of the injector was 260°C; the gas carrier was helium at 1 mL/min; the volume injected was 1 µL (solution of 10% cyclohexane); and the splitting ratio was 1:30. The components were determined by comparing retention times with original samples and assessing the linear retention indices related to the series (C8–C29 alkanes). Computer matching is used to compare commercial NISTMS and laboratory-developed library mass spectral data, which were constructed from pure chemicals, components of recognized oils, and MS research data. [15].

## 2.4 Evaluation of the antioxidant potential

The antioxidant potential of the EO *in vitro* was investigated by four different assays: ABTS assay, DPPH assay, power-reducing assay, and tests of total antioxidant potential.

### 2.4.1 DPPH radical scavenging ability

The potential of EOs for scavenging free radicals was assessed utilizing the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging technique, according to Burits and Bucar [16]. In brief, 50 µL of EO with varying concentrations were dissolved in ethanol. In each dilution, 825 µL of the DPPH reagent (given as 60 µM with 0.7 absorbance at 517 nm) was included. After incubation at room temperature for 60 min, the absorbance at 517 nm was read. The graph was used to determine the $IC_{50}$ values by calculating the percentage of inhibition using the following equation:

$$PI\ (\%) = [(Abs_{co} - Abs_{sa}|Abs_{co}) \times 100], \quad (1)$$

where co represents the control and sa represents the sample. BHT was used as the standard, and the concentration of $IC_{50}$ was obtained by removing the inhibition curve percentage.

### 2.4.2 Radical cation decolorization (ABTS assay)

The extracts were decolorized by cationic radicals (ABTS+) in accordance with the technique designed by Re et al. [17]. For this, the ABTS+ radical was prepared by adding a 7 mM aqueous solution of ABTS to 2.45 mM K2S2O8 and reacting for 16 h in the dark. The absorbance at 734 nm was adjusted to 0.7 at room temperature. About 50 µL of EO was combined with 825 µL of ABTS solution. Following a 30-min incubation period in darkness, absorbances were recorded at 734 nm using a Perkin Elmer Lambda 40 UV/Vis (BARCELONA, SPAIN) spectrophotometer. The $IC_{50}$ was determined by calculating the percentage of ABTS inhibition using the following formula:

$$\%inhibition = [(Ac - As|Ac) \times 100]. \quad (2)$$

Results were run in triplicate and expressed as mg/mL.

The positive control was Trolox, and the $IC_{50}$ was determined by calculating the percentage of the inhibition curve.

### 2.4.3 RP

The assessment of the RP of EOs was conducted following the methodology outlined by Parki et al. [18]. With minor modification, 50 µL of *M. aquatica* EO was combined with 250.0 µL of 0.2 M Na + phosphate buffer (pH 6.6) and 250.0 µL of 1% $K_3Fe(CN)_6$. The mixture was incubated for 20 min at 50°C. Next, 250 µL of a 10% trichloroacetic acid solution was added and mixed with 250.0 µL of distilled water and 60.0 µL of 0.1% $FeCl_3$. Absorbance values were measured at 700 nm using a PerkinElmer Lambda 40 spectrophotometer (Barcelona, Spain).

The reference standard was ascorbic acid. The results achieved comprised the concentration of the EO that contributed 0.5 of the absorbance ($EC_{50}$). The $EC_{50}$ results are expressed in µg/mL.

### 2.4.4 Total antioxidant capacity (TAC)

The TAC was assessed using the ammonium phosphomolybdate test according to Prieto et al. [19]: 25.0 µL of EO was added to 1 mL of the reagent solution ($H_2SO_4$ at 6 M, $Na_3PO_4$ at 28 mM, and ammonium molybdate at 4 mM). The sample absorbance was measured after 90 min at 95°C in a water bath at 700 nm vs blank. The results are presented in mg AAE/g EO (milligrams of ascorbic acid equivalent per gram of EO).

## 2.5 Assessing the antimicrobial activity

### 2.5.1 Antibacterial activities of EOs

The antimicrobial potency of the EOs was tested against different bacterial and fungal strains, including Gram-negative bacteria, Gram-positive bacteria, and fungal

**Table 1:** Bacterial and fungal strains tested

| Gram-negative bacteria | Gram-positive bacteria | Fungal strains |
|---|---|---|
| *Escherichia coli*, K12 | *Staphylococcus aureus*, ATCC 6633 | *Candida albicans*, ATCC 10231 |
| *Pseudomonas aeruginosa*, CIP A22 | *Bacillus subtilis*, DSM 6333 | *Aspergillus niger*, MTCC 282 |

strains (Table 1). These strains were found to be highly resistant to several drugs. All isolates tested were clinically isolated at the Fez University Hospital Complex, Morocco.

#### 2.5.2 Assessment of the antimicrobial capacity

The disc diffusion technique was used to evaluate the antimicrobial efficacy of *Mentha aquatica* EO [20,21]. Briefly, 15–20 mL of Mueller–Hinton (MH) agar and Sabouraud dextrose agar (SDA) were poured into Petri dishes. Spots were spotted on agar plates with culture medium, and each isolate was then cultured for 7 days at 30°C. After pre-inoculation with 100 µL of an overnight culture of the microorganism under test (OD 600 nm ≈ 0.1 for bacteria; OD 625 nm ≈ 0.1 for fungi), these plates were covered with 5 mL of soft agar (0.5% agar) of MH medium for bacteria or SDA for the growth of *C. albicans* and *A. niger*. These cultures were carefully placed on the surfaces of the plates and incubated for 24 h at 30°C for the yeast and 37°C for the tested bacteria. Next, Whatman paper discs with a diameter of 6 mm were impregnated with 10 µL of pure EO and then placed in a Petri plate that had previously been inoculated with bacteria (between $10^6$ and $10^8$ CFU/mL) and yeast. Petri dishes were inoculated with bacteria and yeast at 37 and 30°C. Inhibition diameters were measured for bacterial, fungal, and yeast strains after 24 and 48 h. Each trial was conducted in triplicate. This activity involves the use of streptomycin (STR), ampicillin (AMP), gentamycin (GEN), and fluconazole (FCZ) as antibiotics.

#### 2.5.3 Minimum inhibitory concentration (MIC)

To determine the MIC of EO derived from *Mentha aquatica* for different microbiological strains, the microdilution technique is utilized. The following was achieved using a dilution series of *M. aquatica* EO. Mixing 100 µL of the oil with a dilution of 1:10 (v/v) dimethyl sulfoxide (DMSO) (10%) in a microplate pot containing sterile MH broth. Subsequently, 50.0 µL of microbial inoculum, adjusted to a final concentration of ($10^6$–$10^8$ CFU/mL), was added to each well. Following an incubation period lasting between 48 h and 1 week for fungal strains as well as 24 h for bacteria, maintained at 37°C [22], the MIC value was determined by the colorimetric technique with 0.015% resazurin [23].

## 2.6 Insecticidal activity

#### 2.6.1 Breeding of insects

Following the scientific method, the glass must be positioned under controlled conditions, maintaining a $T$ of 27 ± 1°C, HR% of 75%, and subjected to photoperiod of 14 h of light followed by 10 h of darkness [24]. This environment glass is the natural habitat where *Callosobruchus maculatus* typically thrives, fostering their growth and reproduction.

#### 2.6.2 Efficacy of EOs against *Callosobruchus maculatus* by fumigation test

The fumigation assay was conducted to assess the insecticidal efficacy of the EO when vaporized. The assay was performed in jars of 1 L, which should prevent direct touch with insects. First, pieces of Whatman paper (3 $cm^2$) were soaked with different concentrations of essential from 4 to 20 µL/L of air and fastened on the underside of each jar's lid. Second, every container received 20 individual *C. maculatus* insects (10 males and 10 females), aged 0–48 h, introduced individually, while the control group did not receive any treatment. Each test was conducted with three replicates. The number of dead individuals was collected every day for each dosage for 1 day. The ability of females of *C. maculatus* to lay eggs was estimated using a magnifying binocular. The culture chamber was used to keep the jars (controls and treated) at $T$: 27 ± 1°C, HR%: 75%, and a photoperiod: 14 h (light)/10 h (darkness), up to the adult emergence stage [25].

By using the Abbott formula, the observed mortality rate was corrected [26].

#### 2.6.3 Repellency bioassay of EOs

The repellency of EO against adult *C. maculatus* was tested by the preference zone flow-through method using filter

paper according to the method described by McDonald et al. [27]. Discs of Whatman filter paper with a diameter of 8 cm were cut into segments. One segment was soaked with EO being tested at varying doses from 4 to 20 µL, made up of 0.5 mL of acetone. The remaining segment was saturated solely with a similar amount of acetone as a non-treated group (negative control). Both sides of the filter paper were air-dried before being taped together. The boxes were filled with ten insects with *C. maculatus*, with five males and five females. Three separate experiments are done for each dosage. The number of individuals on both portions was tallied after half an hour after each treatment.

The percentage of repulsion (PR) of adult *C. maculatus* individuals was determined using the formula of McDonald et al. [27].

## 2.7 Molecular docking

Molecular docking is a computer approach that predicts the degree of binding and direction of a small molecule (ligand) to a target protein or receptor. Molecular docking was conducted to investigate the possible antioxidant and antibacterial efficiency of the identified EO in *Mentha aquatica*.

### 2.7.1 Preparation of ligands

For the preparation of the ligands, the compounds present in the EO were obtained from the PubChem database as SDF files. The ligands were then prepared using the LigPrep tool from the Schrödinger software program (version 11.5) with the OPLS3 force field. Ionization state selection was performed on each ligand, resulting in 32 stereoisomers for each molecule at a pH of 7.0 ± 2.0 [28].

### 2.7.2 Preparation of proteins

The direct structures of human NADPH oxidase (PDB ID: 2CDU) were provided by the Protein Data Bank [29]: ß-ketoacyltransferase from *Escherichia coli* (PDB ID: 1FJ4) [30], *Staphylococcus aureus* nucleoside diphosphate kinase (PDB ID: 3Q8U), a beta-1,4-endoglucanase from *Aspergillus niger* (PDB ID: 5I77), and sterol 14-alpha demethylase (CYP51) from the pathogenic yeast *Candida albicans* (PDB ID: 5FSA) [31]. The optimization method was carried out by the introduction of hydrogen atoms, the finalization of the bond order, the elimination of water molecules, the assignment of hydrogen bonds, the adjustment of the potential of the receptor atoms, and the energy reduction using the OPLS3 force field [32,33].

## 2.8 Statistical analysis

The mean standard deviation of the data was calculated using GraphPad Prism 8.0.1. Data were analyzed using analysis of variance (ANOVA). Variation was considered significant at $p < 0.05$. For the fumigation experiments, probit analysis was used to calculate lethal concentrations ($LC_{50}$ and $LC_{90}$) and chi-squared values for each regression coefficient.

# 3 Results and discussion

## 3.1 Phytochemical profile of EO

The molecular makeup of *M. aquatica* EO was determined using gas chromatography-mass-mass spectrometry (GC-MS/MS), and the result is detailed in Figure 1 and Table 2. The analysis identified a total of 18 components, collectively constituting 100% of the detected constituents in the EO. The major constituents within the MA-EO include linalool (42.42%), α-elmol (10.58%), α-terpineol (8.07%), linalyl acetate (7.37%), caryophyllene (4.05%), geranyl acetate (3.02%), γ-eudesmol (2.90%), β-myrcene (2.56%), and 1,8-cineol (2.55%) (Figure 2). Oxygenated monoterpenes were the dominant class, accounting for a significant 71.03% of the EO composition, while sesquiterpenes account for 22.54% of the EO. Furthermore, other compounds made up 6.44% of the constituents detected in the MA-EO.

Several bioactive compounds with biological effects were discovered in the identified EO, notably linalool, linalyl acetate, caryophyllene, and geranyl acetate [34,35]. Linalool, the main active constituent in *M. aquatica* EO, is well-documented and has a wide range of biological properties, namely antimicrobial, antioxidant, anticancer, anti-inflammatory, and insecticide properties [36].

## 3.2 Antioxidant activities

The *in vitro* antioxidant capability of *M. aquatica* EO was performed using the DPPH scavenging test, $ABTS^{\cdot+}$

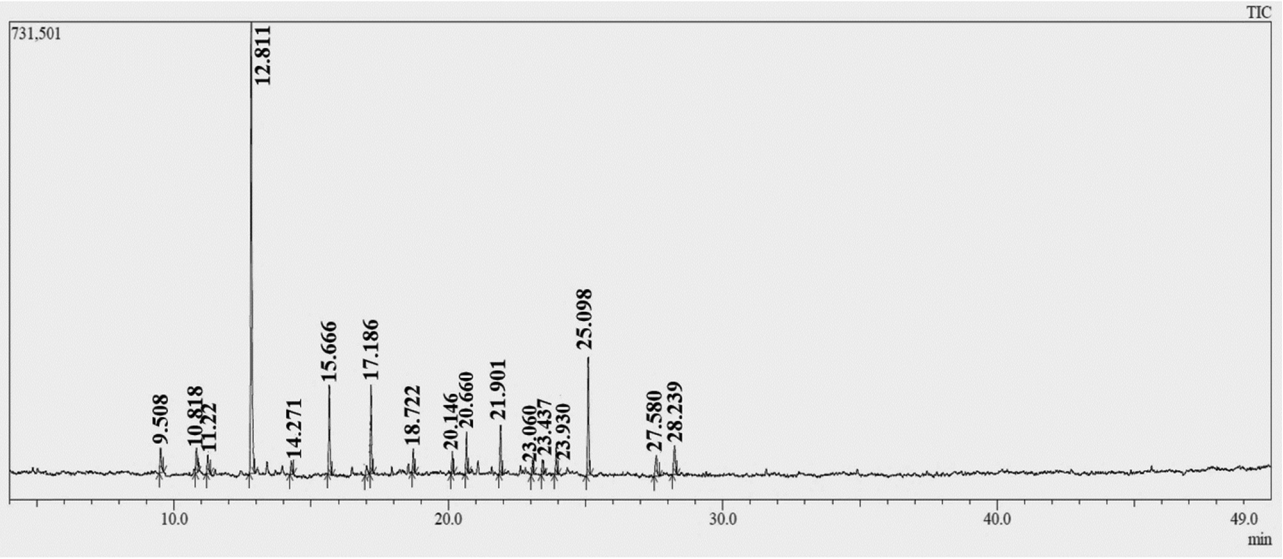


**Figure 1:** Chromatogram of the EO derived from *M. aquatica* leaves using GC-MS/MS analysis.

reduction assay, RP, and TAC. Powerful antioxidant activity was found to scavenge DPPH and the ABTS˙+ radical cation, as shown in Figure 3a and b; the reduction power of the EO had an activity profile that was concentration-dependent, and the inhibition percentage of DPPH was increased due to an increase in EO concentration, and ABTS test. The $IC_{50}$ values were 0.64 ± 0.01 µg/mL for the DPPH assay and 0.167 ± 0.13 µg/mL for the ABTS assay, which are lower than the synthetic antioxidants used as a standard (BHT and Trolox) (Figure 3c and d). The results obtained using *M. aquatica*

**Table 2:** Phytochemical components found in the EO extracted from the leaves of *M. aquatica*

| Peak | RT (min) | Compounds | Chemical classes | RI | | Formula | Area% |
|---|---|---|---|---|---|---|---|
| | | | | Cal | Lit | | |
| 1 | 9.508 | β-Myrcene | Monoterpene | 958 | 987 | $C_{10}H_{16}$ | 2.56 |
| 2 | 10.818 | 1,8-Cineole | MO | 1,059 | 1,042 | $C_{10}H_{18}O$ | 2.55 |
| 3 | 11.226 | Cis-β-Ocimene | MO | 976 | 1,027 | $C_{10}H_{16}$ | 1.99 |
| 4 | 12.811 | Linalool | MO | 1,082 | 1,082 | $C_{10}H_{18}O$ | 42.42 |
| 5 | 14.271 | (+)-2-Bornanone | MO | 1,121 | 1,120 | $C_{10}H_{16}O$ | 1.38 |
| 6 | 15.666 | α-Terpineol | MO | 1,143 | 1,169 | $C_{10}H_{18}O$ | 8.07 |
| 7 | 17.015 | Benzaldehyde, 4-(1-methylethyl)- | Other | 1,230 | 1,242 | $C_{10}H_{12}O$ | 1.12 |
| 8 | 17.186 | Linalyl acetate | MO | 1,272 | 1,258 | $C_{12}H_{20}O_2$ | 7.37 |
| 9 | 18.722 | Cyclohexasiloxane, dodecamethyl- | O | 1,240 | 1,330 | $C_{12}H_{36}O_6Si_6$ | 1.96 |
| 10 | 20.146 | Neryl acetate | MO | 1,352 | 1,363 | $C_{12}H_{20}O_2$ | 1.67 |
| 11 | 20.660 | Geranyl acetate | MO | 1,352 | 1,380 | $C_{12}H_{20}O_2$ | 3.02 |
| 12 | 21.901 | Caryophyllene | Sesquiterpene | 1,494 | 1,434 | $C_{15}H_{24}$ | 4.05 |
| 13 | 23.060 | Cycloheptasiloxane, tetradecamethyl- | O | 1,447 | 1,494 | $C_{14}H_{42}O_7Si_7$ | 1.18 |
| 14 | 23.437 | (−)-Germacrene D | ST | 1,515 | 1,499 | $C_{15}H_{24}$ | 1.42 |
| 15 | 23.930 | Phenol, 2,4-di-tert-butyl- | O | 1,555 | 1,515 | $C_{14}H_{22}O$ | 2.18 |
| 16 | 25.098 | α-Elemol | ST | 1,522 | 1,544 | $C_{15}H_{26}O$ | 10.54 |
| 17 | 27.580 | γ_Eudesmol | ST | 1,626 | 1,630 | $C_{15}H_{26}O$ | 2.90 |
| 18 | 28.239 | Proximadiol | ST | 1,738 | 1,780 | $C_{15}H_{28}O_2$ | 3.63 |
| | | | Chemical classes<br>Monoterpene (MO)<br>Sesquiterpene (ST)<br>Others (O) | | | | |
| Total (%) | | | | | | | 100% |

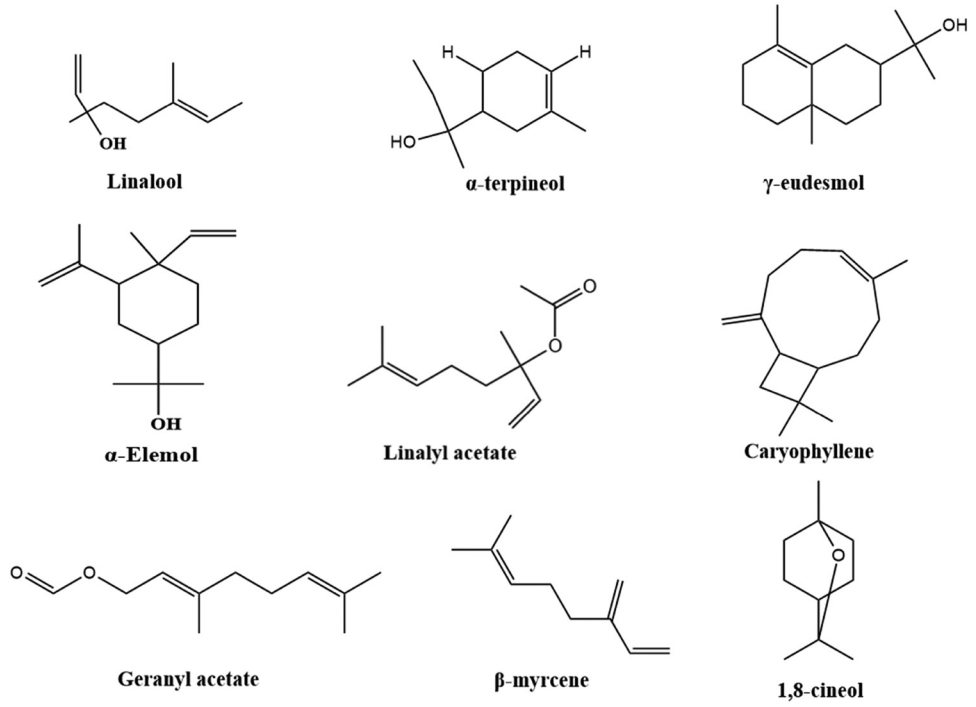


**Figure 2:** Molecular structure of the volatile oil extracted from *M. aquatica* leaves.

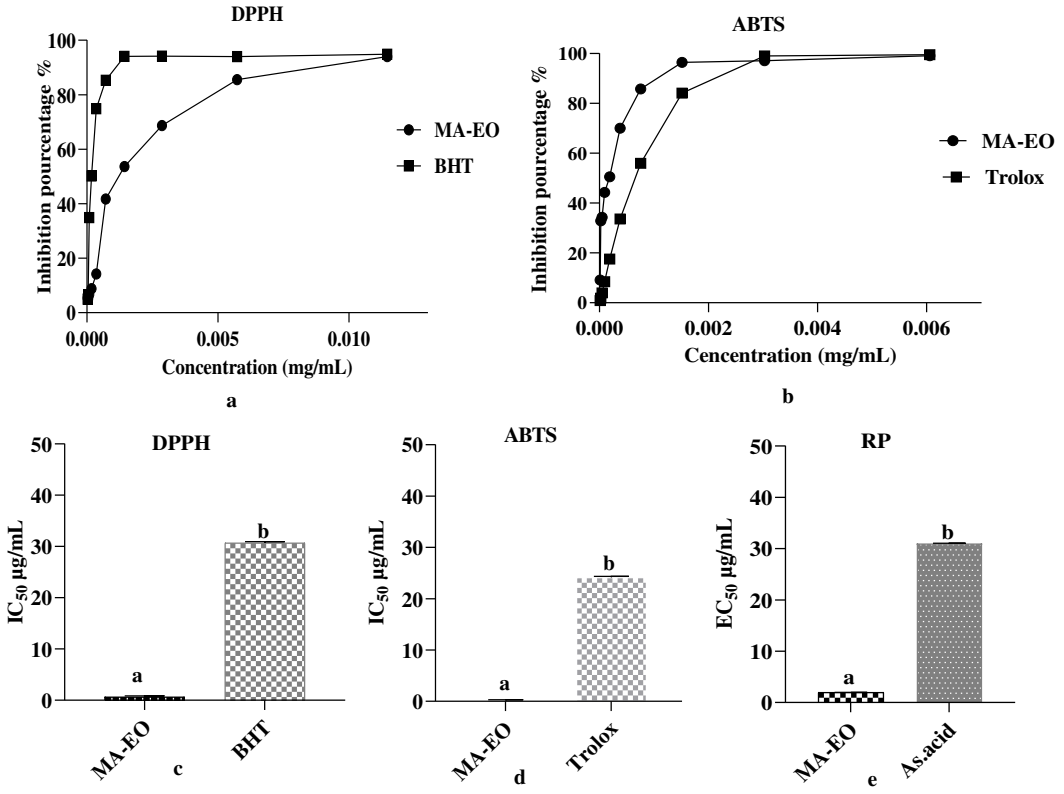


**Figure 3:** (a) Antioxidant power utilizing DPPH scavenging activity (BHT: butylated hydroxytoluene), (b) antioxidant activity using ABTS assay (Trolox standard), (c) $IC_{50}$ values of *M. aquatica* EO of DPPH, and BHT as a standard, (d) $IC_{50}$ values of *M. aquatica* EO of ABTS, and Trolox as a standard, (e) $EC_{50}$ value of *M. aquatica* EO and As.acide: Ascorbic acid as a standard. The variation between bars with identical letters is not significant ($p < 0.05$).

EO are better than those obtained from south Tunisia *M. aquatica* EO with an $IC_{50}$ value of 10 µg/mL for the DPPH test and 50 µg/mL for the ABTS assay [37]. Similarly, Getam and coworkers reported that the *M. aquatica* EO growing in Ethiopia exhibited an antiradical capacity with an $IC_{50}$ value of 11.2 µg/mL using the DPPH method [38]. The RP findings of the examined EO show a low $EC_{50}$ level of 1.95 ± 0.023 µg/mL in contrast to standard ascorbic acid (31 ± 0.07 µg/mL) (Figure 3e). Our results are much less favorable than others signaled by Brahmi et al. and Ahmed et al. for *Mentha spicata* growing in Algeria with an $EC_{50}$ of 452. 3 ± 0.4 µg/mL and an $EC_{50}$ of 22.68 ± 0.87 mg/mL for Moroccan *Mentha pulegium* EO [39,40].

The TAC of *M. aquatica* EO was assessed using the ammonium phosphomolybdate assay. Table 3 displays the results and indicates that our sample possessed a TAC value of 188.21 ± 0.31 mg/mL. These findings are superior to the previous ones signaled by Baali et al. for Algerian *Mentha pulegium*, in which the TAC for rats was 109.52 ± 0.910 µg EAA/mg [41]. Many studies have pointed out that the variations in antioxidant capabilities are attributed to the *in vitro* synergy between phytochemical components. Ciesla and coworkers underlined the synergistic antioxidant effect of several monoterpenes [42]. Indeed, monoterpenes found in EOs were shown to possess potent antioxidant properties, support protecting cells against damage caused by oxidation, and reduce the risk of numerous chronic illnesses [43].

**Table 3:** TAC of *M. aquatica* EO (mg/mL)

| | TAC (mg/mL) |
|---|---|
| MA-EO | 188.21 ± 0.31 |

## 3.3 Antibacterial activities of *M. aquatica* EO

The findings of the antibacterial capacity of *M. aquatica* EO against *P. aeruginosa*, *E. coli, S. aureus,* and *B. subtilis* are summarized in Table 4 and Figure 4, which show that the *M. aquatica* EO has a powerful inhibitory effect on all examined bacteria, either of them Gram-negative or Gram-positive bacteria with various diameters of inhibition. *E. coli* were the most sensitive bacteria to *M. aquatica* EO with a zone of inhibition of 15.50 ± 0.71 mm and MIC of 0.010 ± 0.00 and to S. *aureus* that showed an inhibition diameter of 13.00 ± 0.00 mm and an MIC of 0.011 ± 0.00, compared to *B. subtilis* that displayed an area of inhibition of 11.00 ± 0.00 mm and MIC of 0.011 ± 0.00. However, *P. aeruginosa* showed an inhibition zone of 7.50 ± 1.41 mm and MIC of 0.011 ± 0.00. The data showed that all strains were found to be sensitive to antibiotics tested (STR, AMP, and GEN) with different diameters of inhibition (Table 3 and Figure 4).

Likewise, our data stated that the tested volatile oil of *M. aquatica* has a potent antibacterial capacity against Gram (−), and Gram (+), which was in line with the result discovered by Tourabi et al., who demonstrated that the EO of *M. longifolia* had the seam efficiency against every tested pathogen [31]. In the seam context, the tested EO of *M. aquatica* demonstrated a potent inhibitory efficiency against *E. coli* with an inhibition zone of 15.50 ± 0.71 mm. This result was higher than those carried by Getahun and co-workers, who discovered that their *M. aquatica* EO gathered in Ethiopia showed no antibacterial effect against *Escherichia coli* (ATCC 10536) [38]. Another study showed that the Korean *M. aquatica* EO has the seam effect carried out by our EO into *E. coli* (KF 918342) with an inhibition zone of 16.00 ± 0.00 mm [44]. Furthermore, the volatile oil of *M. aquatica* significantly inhibited the growth of Gram-

**Table 4:** MIC and diameter of the inhibition zone (mm) of *M. aquatica* EO compared with antibiotics

| Simple | | Gram− Bact | | Gram+ Bact | |
|---|---|---|---|---|---|
| | | *P. aeruginosa* | *E. coli* | *S. aureus* | *B. subtilis* |
| EO | Antiba activity (mm) | 7.50 ± 1.41 | 15.50 ± 0.71 | 13.00 ± 0.00 | 11.00 ± 0.00 |
| | MIC (µg/mL) | 0.011 ± 0.00 | 0.010 ± 0.00 | 0.011 ± 0.00 | 0.011 ± 0.00 |
| STR (5 mg/mL) | Antiba activity (mm) | 20 ± 0.00 | — | — | — |
| | MIC (mg/mL) | 0.625 ± 0.00 | — | — | — |
| AMP (5 mg/mL) | Antiba activity (mm) | — | 30 ± 0.00 | 13 ± 0.00 | 6 ± 0.00 (Re) |
| | MIC (mg/mL) | — | 0.625 ± 0.00 | 0.625 ± 0.00 | 0.312 ± 0.00 |
| GEN (5 mg/mL) | Antiba activity (mm) | — | — | — | 30 |
| | MIC (mg/mL) | — | — | — | 0.078 ± 0.00 |
| DMSO (10%) | Antiba activity (mm) | 6 ± 0.00 (Re) | 6 ± 0.00 (Re) | 6 ± 0.00 (Re) | 6 ± 0.00 (Re) |

Antiba activity: antibacterial activity; Re: resistant; MIC: minimum inhibitory concentration; Gram− Bact: Gram-negative bacteria; and Gram+ Bact: Gram-positive bacteria.

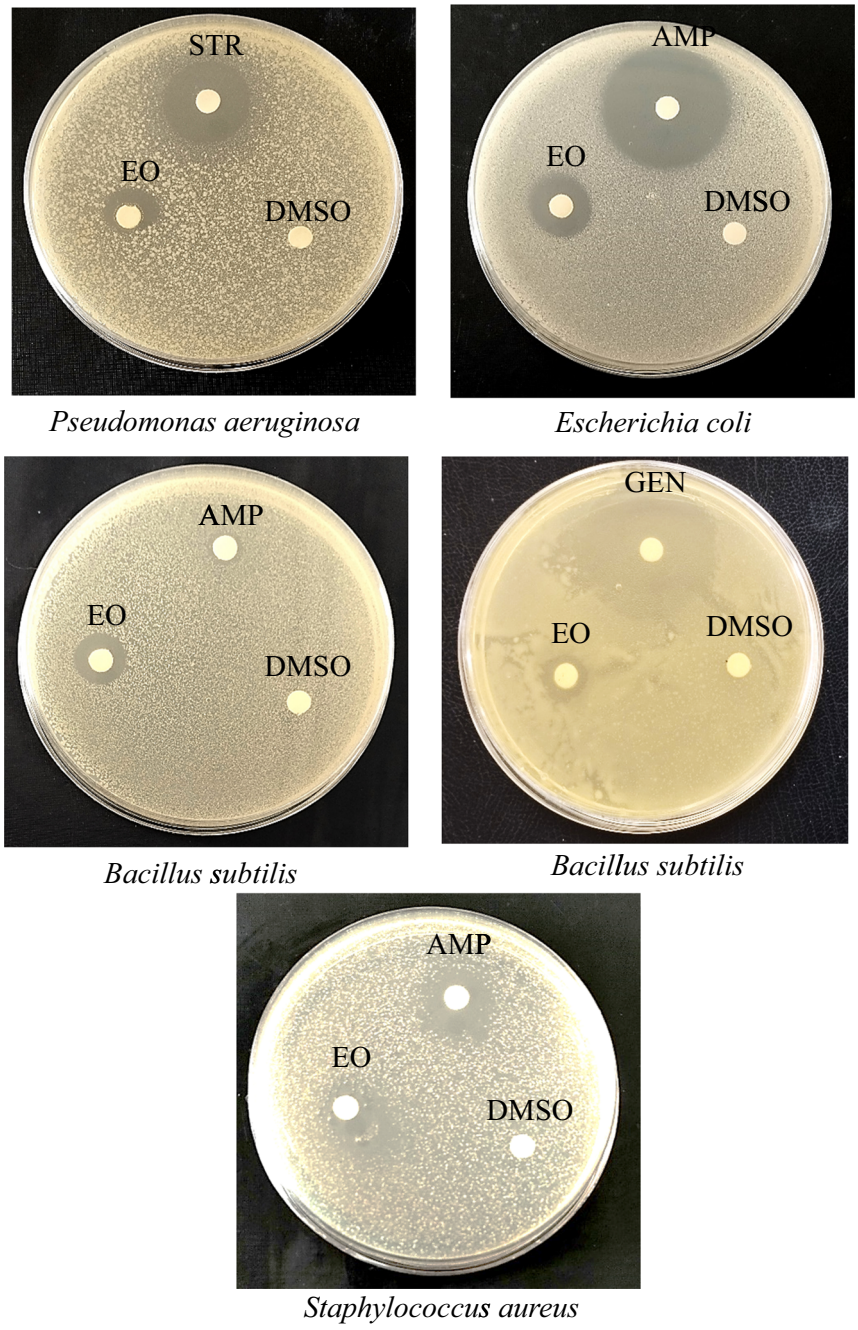


**Figure 4:** Antibacterial efficiency of *M. aquatica* EO against *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus*. Essential oil (EO), ampicillin (AMP), dimethyl sulfoxide (DMSO), streptomycin (STR), and gentamycin (GEN).

positive bacteria, notably *S. aureus* and *B. subtilis*, with inhibition diameters of 13.00 ± 0.00 mm and 11.00 ± 0.00 mm, respectively. Our findings were contrasted with those discovered by Getahun et al., who indicated a light inhibition of 11.5 mm and 10.50 mm against *Staphylococcus aureus* (29737) and *B. subtilis* (ATCC) growth, respectively, by *M. aquatica* EO [38].

Antibiotic resistance has grown in importance and become a danger to human health in recent years. Multidrug-resistant bacteria-caused chronic diseases remain one of the main causes of death in both industrialized and developing nations nowadays. To combat this, new approaches and solutions must be developed [45]. In the same concept, EO can serve as a perfect matrix for the search for novel, secure, and potent antibacterial molecules that can either supplement or improve the potency of traditional antibacterial treatments [46]. Several studies have reported the

mechanisms of the antibacterial effect of monoterpenes via various pathways, notably membrane potential decrease, infiltrating the cell wall of bacteria, and cytoplasmic protein membranes have been attributed to their lipophilic character, causing the breakdown of the proton pump, producing ion loss and overexploitation of the ATP reserve. Consequently, they cause bacterial cell destruction and the evacuation of their contents by causing the separation of lipids between cell membranes and mitochondria.

Furthermore, the mode of action of EO molecules depends on their concentrations, chemical composition, placement of functional groups, and structures. The volatile oil of *M. aquatica* is rich in terpene molecules, especially oxygenated monoterpenes that are the dominant class, including linalool, linalyl acetate, α-terpineol, and 1,8-cineole. They are now well-recognized for their strong antibacterial properties against a range of pathogens [34,47,48].

Linalool is the major oxygenated monoterpene found in our EO and presents about 50%. Similarly, a great deal of research is now being conducted to identify how linalool functions against photogenic bacteria. Additionally, Herman and co-workers have studied the antimicrobial efficiency of linalool against several types of bacteria, and the results have shown that the above molecule has a potent inhibitory growth in *Pseudomonas aeruginosa* (NCTC 12924) with inhibitory zones of 8 and 21 mm against *E. coli* (NCTC 12923) [49]. Another research carried out by Liu et al. stated that linalool exhibited remarkable antibacterial activity against *Pseudomonas aeruginosa* (ATCC9027) with MIC and MBC of 431 and 862 µg/mL, respectively. According to Liu et al., the high concentration of linalool caused the cell's normal structure to be disrupted by the leakage of nucleic acids, and the decrease in membrane potential revealed that *P. aeruginosa* membrane integrity had been damaged [34]. The distinctive hydrophobic nature of EOs and their components is a key element that enables them to interact with the fats in bacterial cell walls of mitochondria. This interaction disrupts cell morphology and increases membrane permeability [50]. Consequently, the inhibitory power of EO components arises from synergistic effects among several molecular groups rather than being solely attributed to a single potent inhibitor effect.

## 3.4 Antifungal activity of *M. aquatica* EO

The antifungal effectiveness of *M. aquatica* EO was evaluated using the disc diffusion technique against *C. albicans* and *A. niger*. The results are displayed in Table 5 and Figure 5 and show that the EO of *M. aquatica* has an appreciable antifungal effect, especially against *C. albicans* with an inhibitory zone of 21 mm. The antibiotics used as a standard or positive control (FCZ) were more effective against both strains when compared to the tested EO with an inhibition zone of 30 mm. The obtained findings revealed that our tested EO exhibited a potent efficacy against both yeast *C. albicans* and *A. niger* in comparison to the positive control with MIC values of 33.01 ± 0.00 and 0.011 ± 0.00 µg/mL, respectively.

Recently, antifungal resistance has been an important defiance to clinicians in treating invasive fungal infections since the number of systemically accessible antifungal drugs is limited. However, *C. albicans* is the most often isolated species of *Candida* from patients who suffer from candidiasis. Furthermore, *Aspergillus* is a genus of mold fungi that encompasses a diverse group of airborne spore-forming molds. While many species of *Aspergillus* are harmless and play essential roles in natural processes, certain strains pose health risks to humans [51]. Additionally, major side effects or toxicities that prevent continuous usage or dose increases may restrict the effectiveness of existing drugs (e.g., FCZ). In addition, the development of future strategies to combat this resistance is urgent. Additionally, a wide range of plant extracts and EOs have been used to treat the most complex infections, including those caused by resistant bacteria and yeasts.

Indeed, the findings obtained demonstrated that the tested EO has an appreciable antifungal potential, especially toward *C. albicans,* with a zone of inhibition of 21.00 ± 0.00 mm. This result was more important than those found by Dhifi and colleagues, indicating that the EO of *M. aquatica* gathered in Tunisia unregistered no antifungal potency toward *C. albicans* with a zone of inhibition of 1.7 ± 0.5 mm [37]. Similarly, the EO of *M. aquatica* tested by Mimica-Dukic et al. revealed moderate antifungal ability against *C. albicans* with an MIC of 4 µL/mL [52]. In

**Table 5:** Antifungal capability of *M. aquatica* EO, the diameter of the inhibition zone (mm), and MIC (µg/mL)

| Sample | | Antifungal activity | |
|---|---|---|---|
| | | *C. albicans* | *A. niger* |
| EO | Antifungal activity (mm) | 21.00 ± 0.00 | 33.01 ± 0.00 |
| | MIC (µg/mL) | 0.010 ± 0.00 | 0.011 ± 0.00 |
| FCZ (5 mg/mL) | Antifungal activity (mm) | 30 ± 0.00 | 00.00 ± 0.00 |
| | MIC (mg/mL) | 1.25 ± 0.00 | 1.25 ± 0.00 |
| DMSO | Antifungal activity (mm) | 6 ± 0.00 (Re) | 6 ± 0.00 (Re) |

*C. albicans*: *Candida albicans* and *A. niger*: *Aspergillus niger*.

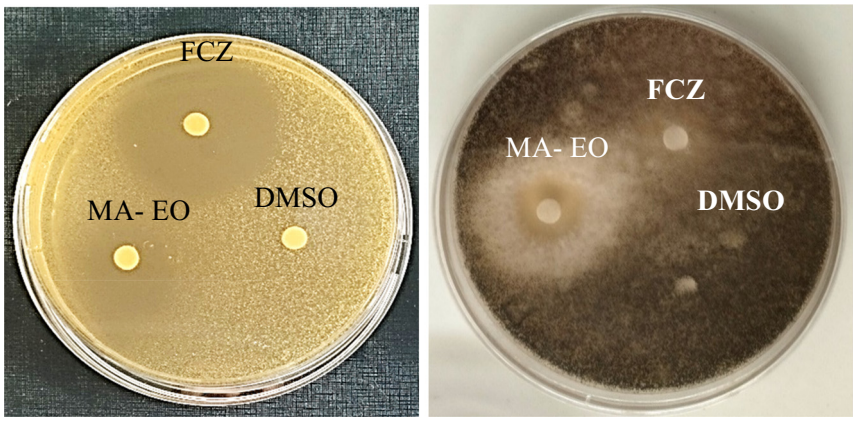


**Figure 5:** Antifungal activity of *M. aquatica* EO against *C. albicans* and *A. niger*: Essential oil (EO), dimethyl sulfoxide (DMSO), Fluconazole (FCZ).

addition, the antifungal capacity of the tested volatile oil is contributed to its richness of a wide range of bioactive molecules, notably linalool, which is well known to have potent antimicrobial properties [49,53].

Several current reports have described the toxicity and mechanism of action of linalool, using both acute and long-term studies [53,54]. According to experiments on long-term toxicity, exposure to linalool induces changes in the fatty acid composition of cell membranes, particularly increasing levels of polyunsaturated and unsaturated fatty acids. These changes disrupt the normal structure of the fungal plasma membrane, ultimately activating cellular signaling pathways that lead to the programmed cell death process known as apoptosis [55]. The cytotoxic effects of this monoterpene on fungal cells arise from the build-up of reactive oxygen species, such as superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($^{\cdot}OH$).

## 3.5 Insecticidal activity

The toxicity of EO of *M. aquatica* against *C. maculatus* was the subject of our current study; over 96 h at different doses were used to perform fumigation and repellency tests. The $LC_{50}$ value was calculated for every EO dose at appropriate treatment durations.

## 3.6 Fumigation bioassay of MA-EO

The fumigation bioassay was used to assess the toxicity of the volatile oil of *M. aquatica* against the *C. maculatus* pest of cowpea grains. Because of their extreme volatility and slight toxicity to animals with warm blood, EO has displayed intriguing fumigant capabilities.

### 3.6.1 Effect on adult mortality

The biocide efficacy of MA-EO is illustrated in Table 6. Our results indicate that the variability in the fumigation test results can be linked to factors such as the concentration of oil, the type of plant species used, and the duration of exposure. MA-EO is harmful in all dosages tested, with a complete mortality rate of 100% on *C. maculatus* at concentrations from 12.0 µL/L in air of MA-EO in 48 h of treatment. In contrast, under 24 h, the lethal concentration ($LC_{50}$) for *M. aquatica* EO was 3.33 µL/L in air, with a 95% confidence interval (0.613–5.506), and the $LC_{90}$ was 15.08 µL/L in air with a 95% confidence interval (10.011–42.485) after the same exposure time (Table 7). The highest dose (16.00 µL/L

**Table 6:** MA-EO effect on *C. maculatus* mortality rate in a fumigation bioassay depends on both the concentration and duration of exposure

| | EO doses (µL/L) | Exposure time | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| MA-EO | Control | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| | 4 | 58.33 ± 5.77 | 75 ± 5 | 95 ± 5 | 100 ± 0 |
| | 12 | 80 ± 18.02 | 95 ± 8.66 | 100 ± 0 | 100 ± 0 |
| | 16 | 91.66 ± 7.64 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| | 20 | 96.66 ± 2.88 | 100 ± 0 | 100 ± 0 | 100 ± 0 |

**Table 7:** *M. aquatica* EO fumigation test against *C. maculatus*: LC50 and chi-square ($\chi^2$) values

| | Treatment (h) | $LC_{50}$(µL/L) | 95% CI | $LC_{90}$ (µL/L) | 95% CI | df | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| MA-EO | 24 | 3.33 | 0.613–5.506 | 15.08 | 10.011–42.485 | 2 | 1.001 |
| | 48 | 2.32 | 0.251–3.86 | 6.821 | 4.236–13.701 | 2 | 0.821 |
| | 72 | 1.76 | — | 3.33 | — | 2 | 0.001 |
| | 96 | — | — | — | — | — | — |

(–): No data are available because the insects were killed the first time of the test.

in air) of MA-EO indicates the strongest fumigant effect with 100% mortality of insects after a whole day of exposure.

To the best of our knowledge, this is the first report on the efficiency of MA-EO fumigant against *C. maculatus*. Nevertheless, the fumigation activity of EO of the *Mentha* species has been examined against a range of insects such as the efficiency of Moroccan *M. pulegium* L. against the chickpea crop pest, *C. maculatus*, has been studied [56]. Fumigant efficacy of EOs from *Mentha piperita* L. (peppermint) leaves was studied against *Musca domestica* L. [57]. *Mentha rotundifolia* L. showed remarkable fumigant activity against *Rhyzopertha dominica* [58].

Other toxicity studies of EO from different wild mint timija populations against *Tribolium castaneum* adults displayed activity to a different level with the *M. suaveolens* EO. Insecticidal activity corresponding with their chemical [59], as well as *M. aquatica* EO rich in linalool, α-terpineol, and linalyl acetate, exhibited potent insecticidal activity against insect vectors and agricultural pests [60].

The robust insecticidal effect of the EO that was tested can be attributed to the substantial existence of major monoterpene and sesquiterpene composites, notably linalool, linalyl acetate, α-terpineol, 1,8-cineole, and caryophyllene.

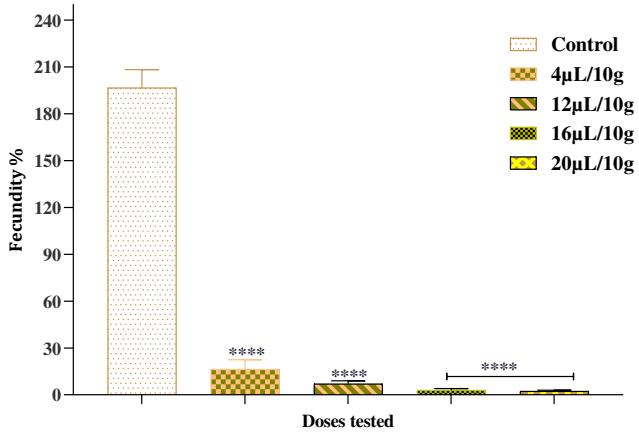


**Figure 6:** Effects of MA-EO on the fecundity of *C. maculatus* (mean ± SD). The findings are expressed as mean ± SD. (*) Control group compared to all other concentrations (significance at ****$p < 0.0001$).

This is consistent with prior reports, indicating the robust insecticidal efficacy of these compounds [60,61].

### 3.6.2 Effect of MA-EO on fecundity

The examined EO significantly affects *C. maculatus* females' fecundity by its insecticidal activity. The effect of MA-EO on fecundity results showed a marked diminution in the eggs laid by females in comparison to the non-treated group after exposure to MA-EO vapor (Figure 6 and Table 8). The MA-EO significantly reduces fecundity as the dose tested increases when compared to the control of 196.67 ± 11.55 at the superior test doses. According to statistical analysis, the MA-EO-induced toxic effect on *C. maculatus* fecundity was very significant for various doses (ANOVA: $F = 614.8$; df = 4, 10; $p < 0.0001$). Prior reports affirmed that different plant products, including EOs and their constituents, reduced insect fertility [25,62]. According to the current literature, this study describes the first exploration of MA-EO's effectiveness on the fecundity of *C. maculatus*, a common pest of cowpea grains. However, the effects of EOs from *Mentha* species on fertility have been investigated against several pests, notably the impact of M × *rotundifolia* (L.) Huds. EO reduces female fecundity by 64–90% of *C. maculates* at the volatile oil dose of 0.25–1% [63]. Indeed, *M. piperita* L. EO decreases 52% of fecundity from *Anopheles stephensi* [64]. In the same regard, Saxena and Mathur proposed that the decrease in fertility caused by plant extracts could be linked to the disturbance of regulation mechanisms, not directly to the ovarian tissue [65].

**Table 8:** Influence of MA-EO on multiple biological parameters of *C. maculatus* (mean + SD)

| Dose (µL/L) | Fecundity | Fertility (%) | Adult emergence (%) |
|---|---|---|---|
| 4 µL | 16.33 ± 6.11 | 67.32 ± 16.15 | 29.09 ± 10.12 |
| 12 µL | 7 ± 2 | 0 ± 0 | 0 ± 0 |
| 16 µL | 3 ± 11 | 0 ± 0 | 0 ± 0 |
| 20 µL | 2.33 ± 0.57 | 0 ± 0 | 0 ± 0 |
| Control | 196.67 ± 11.55 | 94.02 ± 11.55 | 93.35 ± 5.20 |

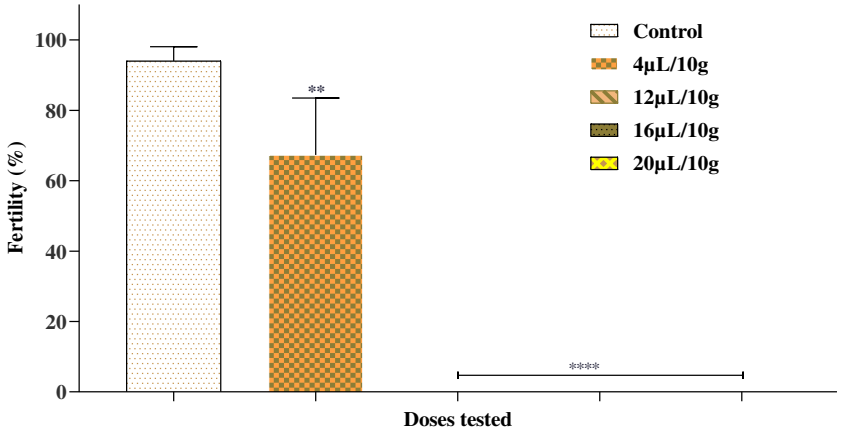


**Figure 7:** Effects of MA-EO on the fertility of *C. maculatus* (mean ± SD). The findings are expressed as mean ± SD. (*) Control group compared to all other concentrations (significance at ****$p < 0.0001$).

### 3.6.3 Effect on fertility

Additionally, our results demonstrated that the examined EO exhibited inhibitory effects on the fertility of *C. maculatus*. The data indicated that MA-EO was dependent on both dose and time in reducing egg production, which had a significant difference from the control group (Figure 7 and Table 8). Furthermore, MA-EO demonstrated a significant inhibitory impact on the hatching of eggs (fertility) at a dose of 12 µL/10 g compared to the control. The EO's power to repel insects was tested, and the results indicated that varied concentrations significantly decreased the fertility of *C. maculatus* females ($F = 110.3$; df = 4, 10; $p < 0.0001$) (Figure 7, Table 5). In our experiment, the female *C. maculatus* eggs were protected from hatching at the lowest dosage of MA-EO applied. Our data were stronger than those reported by Ansari and colleagues, particularly at a concentration of 3 mL/m$^2$ of EO derived from *M. piperita* L., which completely suppressed the fertility of *Ae. aegypti*, and *Cx. quinquefasciatus* [64]. In addition, the EO of *M.* × *piperita* L. was found to reduce 96.9% of the hatching eggs of *An. Stephensi* at a dose of 2 mL/m$^2$ [64].

## 3.7 Effect on adult emergence

The obtained results suggested that there was no emergence of *C. maculatus* females in cowpea seed that received preliminary treatment with MA-EO, initiation at 12 µL/10 g (Figure 8 and Table 8). Prior studies indicated that *Mentha species* EO had the most powerful impact on a variety of insect pests [60,65,66]. On the other hand, when the EO of *M. pulegium* L. was tested through inhalation against *C. maculatus*, it significantly decreased larval emergence,

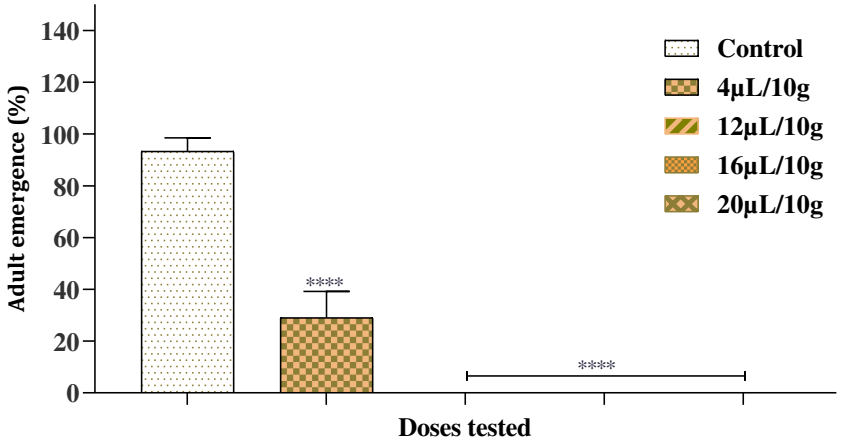


**Figure 8:** Effects of MA-EO on the adult emergence of *C. maculatus* (mean ± SD). The findings are expressed as mean ± SD. (*) Control group compared to all other concentrations (significance at ****$p < 0.0001$).

**Table 9:** Repellent efficiency of MA-EO against *C. maculatus*

| Dose (μL/cm$^2$) | Repellency class | | |
|---|---|---|---|
| | MA-EO | | MA-EO |
| 4 | 45 ± 5.14 | Repellent | 68.75 |
| 12 | 60 ± 9.33 | Repellent | |
| 16 | 75 ± 10 | Repellent | |
| 20 | 95 ± 10 | Repellent | |

resulting in 100% mortality within 24 h of application at a concentration of 20.0 μL/L air [56]. Similarly, Kumar et al. stated that the *M. arvensis* L. EO inhibits totally (100%) *C. chinensis* adult emergence at a concentration of 200 μL/L using contact assay [67].

## 3.8 Repellency bioassay

The effectiveness of *Mentha* species in repelling agricultural insect pests was evaluated through multiple experiences [64,67,68]. The repelling effect in the current study depends on the concentration utilized. Indeed, MA-EO demonstrated a powerful repellent effect against *C. maculatus* insect. In the test using filter paper discs, within 24 h of treatment, the repulsion power against *C. maculatus* was greater than 50% for all doses of MA-EO (Table 9). According to McDonald's 1970 classification, at a high dose of 20 μL/cm$^2$, MA-EO exhibited a robust repellent effect of 80%, which had an average repulsion rate of 68.75%. This places EO in the repellent category. The obtained results are in line with those signaled by Kumar and their collaborators who discovered that *M. arvensis* EO shows 85% repelled adult against *C. chinensis* [67].

Multiple investigations have been conducted on plant extracts, specifically focusing on the EOs derived from *Mentha* species to assess their effectiveness in combating pest insects that infest stored crops. Our results indicate that MA-EO showed high efficacy against the insect pest *Callosobruchus maculatus*, aligning with previously reported findings [4].

Similarly, multiple EOs derived from plants are rich in volatile chemicals possessing biocidal properties comprised terpenoids (monoterpenoids), alkanes, alcohols, and aldehydes [69]. Volatile oil and its components influence metabolic processes via multiple mechanisms, especially affecting the endocrinological functioning of insects [70]. Monoterpenoids and sesquiterpenoids, highly volatile compounds, have a characteristic that protects plants from insect invasion. Monoterpenoids, being lipophilic compounds, have been extensively examined due to their neurotoxic properties, namely features such as convulsions, agitation, and convulsions followed by immobility are comparable to organophosphate and carbamate insecticides [61,71]. Its operation through various mechanisms of action, namely via GABA,

**Table 10:** Docking results of ligands in the active sites

| Title | Glide score (kcal/mol) | | | | |
|---|---|---|---|---|---|
| | Antioxidant activity | Antibacterial activity | | Antifungal activity | |
| | 2CDU | 1FJ4 | 3Q8U | 5I77 | 5FSA |
| (−)-Germacrene D | −3.08 | −5.993 | −3.202 | −3.889 | −6.825 |
| (+)-2-Bornanone | −3.081 | −5.676 | −3.953 | −4.415 | −5.38 |
| 1,8-Cineole | −3.312 | −5.703 | −3.275 | −4.451 | −4.739 |
| α-Elemol | −3.402 | −4.577 | −4.348 | −3.374 | −6.188 |
| α-Terpineol | −4.055 | −6.102 | −4.874 | −4.397 | −5.921 |
| Benzaldehyde,4-(1-methylethyl)- | −4.599 | −5.722 | −5.558 | −3.945 | −5.83 |
| β-Myrcene | −1.606 | −3.247 | −1.866 | −1.6 | −3.004 |
| Caryophyllene | −3.94 | −5.056 | −3.298 | −3.869 | −7.025 |
| *Cis*-beta-Ocimene | −1.948 | −3.769 | −2.106 | −2.012 | −3.452 |
| Cycloheptasiloxane, tetradecamethyl- | −1.692 | — | −2.103 | −2.44 | — |
| Cyclohexasiloxane, dodecamethyl- | −1.803 | — | −1.23 | −2.148 | — |
| γ_Eudesmol | −4.991 | −4.96 | −4.816 | −4.351 | −6.49 |
| Geranyl acetate | −2.06 | −4.474 | −4.864 | −2.51 | −3.717 |
| Linalool | −2.325 | −3.99 | −3.158 | −2.652 | −3.673 |
| Linalyl acetate | −2.731 | −5.067 | −3.604 | −2.459 | −4.636 |
| Neryl acetate | −3.032 | −5.364 | −4.419 | −2.489 | −4.48 |
| Phenol, 2,4-di-tert-butyl- | −4.379 | −6.402 | −4.478 | −5.094 | −7.964 |

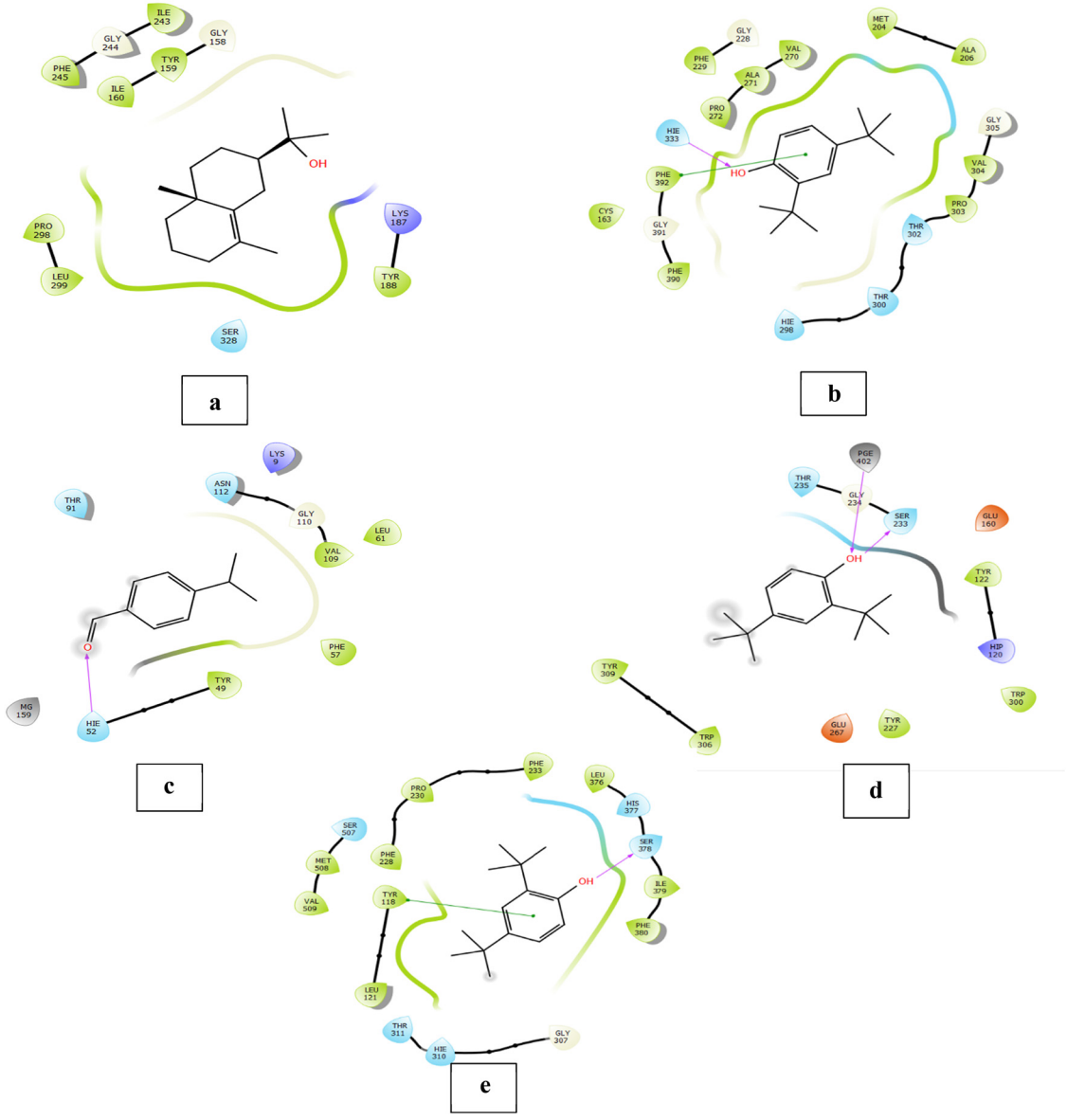


**Figure 9:** The ligand's interaction with the active site is shown in the 2D viewer. (a) γ_Eudesmol interacts with NADPH oxidase's active site. (b, d, and e) Phenol and 2,4-di-tert-butyl interactions with the active site of *Aspergillus niger's* beta-1,4-endoglucanase, *Escherichia coli's* beta-ketoacyl-[acyl carrier protein] synthase, and *Candida albicans'* pathogenic yeast sterol 14-alpha demethylase (CYP51). (c) *Staphylococcus aureus* nucleoside diphosphate kinase's active site interacts with benzaldehyde, 4-(1-methylethyl)-.

blocking neurotransmitters including octopamine synapses [72] as well as acetylcholinesterase [73]. Acetylcholine is a neurotransmitter that exists inside synaptic gaps and can be blocked by AChE inhibition; it is a particular enzyme utilized by pest control agents [73].

The EO of *M. aquatica* is abundant in numerous molecular components; nevertheless, certain components are more abundant, such as linalool, linalyl acetate, 1,8-cineole, β-myrcene, caryophyllene, germacrene D, α-elemol, and α.-terpineol. Antecedent reports have exhibited the phyto-insecticidal efficiency of these major compounds [74–80]. Unfortunately, the mechanism of action from *M. aquatica* EO has not been proposed, except for the mode of action from the earlier cited individual compounds. Thus, linalool was previously reported to be insect repellent, but it is not completely known what the mode of action of this molecule. It is mostly known that monoterpenes can influence a variety of insect pests through several pathways, notably at the nervous system level, starting to act particularly on acetylcholine esterase, gamma-aminobutyric acid (GABA) induced activation of chloride and sodium channels, tyramine nicotinic acetylcholine (nAChR) receptors, octopamine, and other targets [73,81,82].

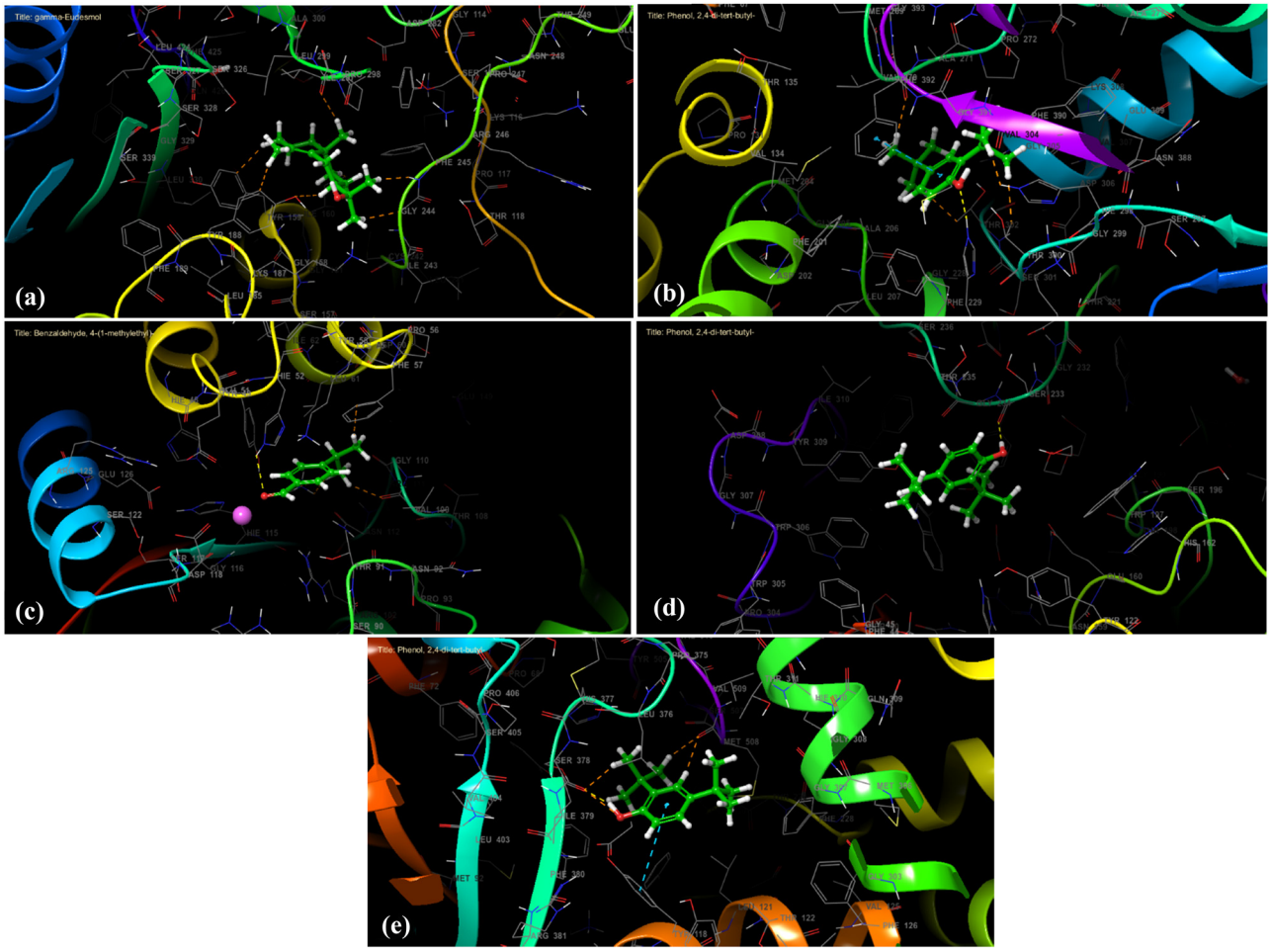


**Figure 10:** The 3D viewer of ligands interactions with the active site. a: Eudesmol interactions with the active site of NADPH oxidase. (b, d, and e): Phenol, 2,4-di-tert-butyl- interactions with the active site of beta-ketoacyl-[acyl carrier protein] synthase from *Escherichia coli*, a beta-1,4-endoglucanase from *Aspergillus niger*, and sterol 14-alpha demethylase (CYP51) from the pathogenic yeast *Candida albicans*. c: benzaldehyde, 4-(1-methylethyl)- interactions with active site of *Staphylococcus aureus* nucleoside diphosphate kinase.

Thus, the inhibitory impact of EO components occurred due to synergistic interactions among different chemical groups rather than relying solely on one potent inhibitor [83].

Overall, this examination indicates that EO extracted from Moroccan *Mentha aquatica* has a potent repellency capacity and can be applied as a biocidal to control *C. maculatus* invasion in stored cowpea grain. In an appropriate control of pests setting, it is necessary to conduct additional research to discover the ideal dosage and strategy for using this EO.

## 3.9 Molecular docking

NADPH (nicotinamide adenine dinucleotide phosphate) plays a critical function in antioxidant activity inside cells. It functions as a reducing agent, supplying electrons to antioxidant enzymes like glutathione reductase and thioredoxin reductase, which in turn replenish antioxidants like glutathione and thioredoxin, respectively. NADPH inhibition may drastically alter antioxidant function, adding to oxidative stress and related clinical disorders.

Throughout the *in silico* study, all the compounds discovered in the *M. aquatica* EO displayed a significant antioxidant capacity reflected by binding energy between −4.991 and −1.565 kcal/mol. γ_Eudesmol and benzaldehyde, 4-(1-methylethyl-) demonstrated substantial inhibition energy against NADPH oxidase with glide scores of −4.991 and −4.599 kcal/mol (Table 9).

In the antimicrobial efficacy, phenol 2,4-di-tert-butyl- showed the strongest activity against *E. coli*, with a glide score of −6.402 kcal/mol. In contrast, benzaldehyde, 4-(1-methylethyl)- demonstrated higher effectiveness against *S. aureus*, with a glide score of −5.558 kcal/mol. Regarding

the antifungal activity, phenol 2,4-di-tert-butyl- displayed remarkable efficiency against *Candida albicans*, with a glide score of −7.964 kcal/mol. Similarly, this molecule emerged as the most powerful compound versus *Aspergillus niger*, with a glide score of −5.094 kcal/mol (Table 10).

2D and 3D imaging of the interaction between ligands and the functional site of beta-ketoacyl-[acyl carrier protein] synthase from *Escherichia coli* showed that phenol, 2,4-di-tert-butyl- established a single hydrogen bond with residue HIE 333 and another pi–pi stacking bond with residue PHE 392.

In the energetic site of *Staphylococcus aureus* nucleoside diphosphate kinase, benzaldehyde, 4-(1-methylethyl)- formed a sole hydrogen bond with HIE 52 residue. Additionally, phenol 2,4-di-tert-butyl- formed two hydrogen bonds with residues SER 233 and PGE 402 in the active site of a beta-1,4-endoglucanase from *Aspergillus niger*. This same molecule, phenol 2,4-di-tert-butyl-, established a single hydrogen bond with the SER 378 residue and a single pi–pi stacking bond with the TYR118 residue in the active site of sterol 14-alpha demethylase (CYP51) generated by pathogenic fungi *Candida albicans* (Figures 9 and 10).

# 4 Conclusion

Our research indicates that the EO derived from *M. aquatica* leaves is distinguished by a broad category of bioactive compounds, notably terpenoids, which are known by a wide spectrum of bioactivities, such as antioxidant, antimicrobial, and insecticidal activities. The investigated volatile oil demonstrated powerful antioxidant capacity. The antimicrobial test showed that the tested oil revealed a powerful toxic effect against all strains tested in the current study, with a better effect against Gram-negative bacterial strains. Regarding the insecticidal activity, the EO extracted from *M. aquatica* showed a more effective effect against the biological parameters of the *C. maculatus* insect pest of legume seeds. The findings show that MA-EO is a rich source for developing innovative drugs to combat bacterial infections and insect infestations in the food sector.

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