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# *Mentha arvensis* oil exhibits repellent acute toxic and antioxidant activities in *Nauphoeta cinerea*

Carlos Alonso Leite dos Santos[1,3], Amanda Maria Tavares Moreira[1,3], Bárbara Rayanne da Silva Teles[2,3], Jean Paul Kamdem[3,4], Abdullah F. AlAsmari[3,5], Fawaz Alasmari[3,5], Momin Khan[3,6], Luiz Marivando Barros[2,3] & Mohammad Ibrahim[3,6]✉

***Mentha arvensis*** **is an herbaceous plant commonly known as peppermint or Japanese mint. This study investigated the toxic potential and repellent efficacy of *M. arvensis* essential oil (MaEO) at varying concentrations (15.625–250 mg/mL) in *Nauphoeta cinerea*, along with its impact on biochemical parameters in *N. cinerea*. The potential of the major compounds as a new analgesic target was investigated using molecular docking. The essential oil was analyzed by gas Chromatography–mass spectrometry (GC–MS) and the toxic potential, repellent property, and changes in lipid peroxidation (LPO) levels were evaluated as markers of oxidative stress. GC–MS results revealed that the main components were oxygenated monoterpenes such as menthol (71.31%), mentone (13.34%) and isomentone (5.35%). MaEO significantly reduced lipid peroxidation (LPO), the levels of non-protein thiols and iron(II) at the concentration of 125 mg/mL in *N. cinerea*. Furthermore, the major components, L-(−)-Menthol and menthone demonstrated high gastrointestinal absorption and high affinity with the target protein, suggesting possible links that contribute to the analgesic effect of MaEO.**

**Keywords** *Mentha arvensis*, Essential oil, Toxicity, Phytochemical prospecting, Oxidative stress

*Mentha arvensis* is an herbaceous plant originally perennial from the region of Europe and Asia, popularly known as peppermint or Japanese mint. It belongs to the genus *Mentha* and have been extensively used in folk medicine due to its pharmacological properties[1].

Essential oils are composed of mixtures of volatile substances, usually liquid and with distinct aromas, originating from various parts of plants, such as leaves, roots, barks and seeds. These oils are mainly known for their unique olfactory and taste characteristics[2,3]. The essential oil of *M. arvensis* obtained from different parts of the plant has been reported to contain menthol as its major phytochemical[4]. The leaves, in particular, are highly valued in national and international markets due to their use in food preparation or natural medicines[5]. The juice extracted from the leaves is administered in the treatment of problems such as diarrhea and dysentery[6]. In addition, the leaves are also employed to relieve stomach discomforts and allergies[7]. These therapeutic properties are attributed to several phytochemical compounds present in the *M. arvensis* Linn. plant, such as terpenoids, alcohols, rosmarinic receptors, and phenolics. One of the most prominent compounds is menthol, derived from the leaves of the plant, which finds applications in pharmaceuticals, perfumery and food[6].

*Nauphoeta cinerea* popularly known as the lobster cockroaches, native to West and Central Africa, are considered a nutritious food source for reptiles because they contain protein, vitamins, and essential minerals, and are also widely used in scientific research because of their ease of breeding and reproduction, being a more accessible and practical option[8]. The cockroach *N. cinerea*, along with other cockroach species, has been recognized in the scientific community as a valuable alternative model for toxicological studies[9,10].

[1]Laboratory of Biology and Toxicology, Regional University of Cariri (URCA), Crato, CE, Brazil. [2]Laboratory of Plant Ecophysiology, Regional University of Cariri (URCA), Crato, CE, Brazil. [3]Laboratory of Entomology, Federal University of Cariri (UFCA), Crato, CE, Brazil. [4]Department of Biochemistry, Microbiology and Immunology (BMI), College of Medicine, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK S7N 5E5, Canada. [5]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia. [6]Department of Chemistry, Abdul Wali Khan University Mardan (AWKUM) KPK, Mardan 23200, Pakistan. ✉email: dribrahim@awkum.edu.pk

Arthropods, like cockroaches, share a number of physiological and biochemical characteristics similar to those of mammals, including metabolism, nervous system, and immune response. This makes it possible to make reasonable inferences about the effects of toxic substances. Many arthropod species are relatively easy to breed under laboratory conditions, are generally smaller in size and easier to manage than most vertebrates, allowing tests to be performed on multiple individuals simultaneously[11,12].

Considering the aforementioned information, the present study was undertaken with the objective to evaluate the antioxidative and repellency properties of *M. arvensis* L. essential oil using *N. cinerea* as animal model. In addition, the majors' components of *M. arvensis* L. essential oil were investigated for their potential as a new analgesic target using molecular docking. Furthermore, ADME (Absorption, Distribution, Metabolism, and Excretion) analyses and in silico metabolic assays were conducted to evaluate the viability of the compounds as drug candidates. The results obtained may contribute to the development of new natural repellent formulations and to the understanding of the effects of *M. arvensis* on the human organism.

## Materials and methods

### Essential oil acquisition

The essential oil of *M. arvensis* was purchased from Terraflor Aromaterapia (https://terra-flor.com/loja/) in Brazil.

### Analysis of chemical composition of the essential oil of *M. arvensis* by GC–MS

The chemical composition of the essential oil was performed by Gas Chromatography Coupled to Mass Spectrometry (GC/MS) using a Shimidzu equipment, QP2010 Series, according to[13]. The capillary column used was Rtx-5MS type measuring 30 m in length by 0.25 mm in diameter and 0.25 mm film thickness. Helium gas was used as a carrier at a rate of 1.5 mL/min. The injector temperature was be 250 °C and the detector temperature was be 290 °C. The column temperature was initially varied from 60° to 180° increasing at 5 °C/min, then varied from 180 to 280 °C increasing 10 °C/min. The essential oil was diluted 1:200 in chloroform with 1 mL of sample injected. The mass spectrophotometer was set to an ionization energy of 70 eV. Identification of individual components was based on their mass spectrum fragmentation according to their NIST Mass 08 spectral library, retention rates, and comparison with published data[14].

### Creation and diet formulation of *N. cinerea*

The nymphs of the lobster cockroach *N. cinerea* used in this study were obtained from the Biology and Toxicology Laboratory (BIOTOX) of the Regional University of Cariri (URCA), Brazil. The animals were raised in plastic boxes with controlled temperature (23–25 °C) and relative humidity of 70%, on a 12 h:12 h (light/dark) cycle.

Cockroaches were reared in 2 L containers and grown in medium containing: 83% corn paste, 4% sugar, 4% freeze-dried milk, 4% soybean meal, 4% wheat bran, and 1% salt. When cooking the mixture, 1 g Nipagin (Methylparaben) was added.

### Repellency test

Nymphs aged between 3 and 4 months, kept in the same conditions as those described above were used for the experiment. Each group was composed of 5 nymphs and the experiment was repeated 3 times as suggested by Gomes et al.[15].

Initially, a filter paper was cut in half, half of which was impregnated only with the vehicle and the other half with a solution of the test substance at concentrations of 0.25, 0.5 and 1 mg/mL (w/v). This filter paper was placed at room temperature for 3 h in order to evaporate all the solvent and then was placed on the bottom of a Petri dish so as to cover its entire surface. The nymphs were then placed on the filter paper and four observations were made, the first one after 1 h of the experiment, and the others, every hour, until the fourth hour. During these observations the number of insects on the test side (containing the diluted substance) and on the control side (containing only the vehicle) was checked.

To determine the repellent effect, the following formula was applied, as suggested by Procópio et al.[16]: IP = (%IPT − %IPC)/(%IPT + %IPC), where IP is the preference index, %IPT is the percentage of insects on the test paper and %IPC is the percentage of insects on the control paper. For the interpretation of the results, the following score was adopted: IP between −1.00 and −0.10 means repellent substance, IP between −0.10 and +0.10 means neutral substance and IP between +0.10 and +1.00 means attractive substance.

### Evaluation of acute toxicity in *N. cinerea*

To perform the survival tests, ten cockroaches were placed inside the vials with the *M. arvensis* L. essential oil (MaEO) at various concentrations (15.625, 31.25, 62.5, 125, and 250 mg/mL). They were placed inside the freezer so that the cockroaches would be in a state of dormancy. The evaluations took place for 3 days, to evaluate how many cockroaches would survive to MaEO.

### Sample preparation for biochemical assays

After the toxicological tests, the heads of *N. cinerea* from the "control" and "exposed to *M. arvensis* essential oil (15.625, 31.25, 62.5, 125, and 250 mg/mL )" groups were transferred to eppendorf tubes (2 mL) and anesthetized on ice (3 min). They were homogenized by maceration in ice-cold 0.1 M phosphate buffer, pH 7.4 (ratio of 1 mg of precipitate: 40 µL of buffer) and centrifuged at 10,000 rpm for 10 min. The supernatant was separated from the pellet and used for the determination of antioxidant and oxidative stress-related parameters.

### Measurement of protein thiol and non-protein thiol (NPSH) levels

The levels of protein thiol and non-protein thiol were estimated as an endpoint of the oxidative changes in the sulfhydryl (–SH) groups of the proteins and peptides in the supernatant. The content of protein thiol and non-protein thiol was determined by the method described by Ellman[17].

### Determination of 2-thiobarbituric acid reactive species

The 2-thiobarbituric acid reactive substances (TBARS) were measured to determine the products of lipid peroxidation (LP) as a measure of oxidative stress, following the method described by Filho et al.[18]. with slight modifications. The results were expressed as MDA mol (Malondialdehyde)/g tissue.

### Determination of free $Fe^{2+}$ content

To check whether there was a change in the free iron(II) ion content in the supernatant of cockroach brains, the free iron II ($Fe^{2+}$) content was determined using a modified method of Kamdem et al.[19]. and the results were expressed as nmol of Fe(II)/g of tissue.

### Prediction of absorption, distribution, metabolism and excretion (ADME) of L-(—)-Menthol and menthone (the major components)

In silico prediction of these molecules on absorption, distribution, metabolism and excretion parameters was possible through SwissADME using its SMILES format[20]. It was employed to determine the physicochemical properties (molecular weight, number of hydrogen bond donors and acceptors, and topological polar surface area), lipophilicity (WLogP), water solubility (Ali LogS), pharmacokinetics (gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein substrate probability, and CYP enzyme inhibition), and synthetic accessibility (SA) of the ligands studied[21–25].

### Epoxidation

Epoxides are a common reactive metabolite, often formed by cytochromes P450 acting on aromatic or double bonds. The specific location on a molecule that undergoes epoxidation is its epoxidation site (SOE). This algorithm systematically and quantitatively summarizes knowledge of hundreds of epoxidation reactions, identifying SOES with 94.9% accuracy of the area under the curve and separating epoxidized and non-epoxidized molecules with 78.6% accuracy[26].

### Quinonation

Quinone species, including quinone-imines, quinone-methids, and imino-methides, are electrophilic Michael acceptors that are often highly reactive and comprise more than 40% of all known reactive metabolites. Quinone metabolites are created by cytochromes P450 and peroxidases. This is the first published method to predict quinone formation, including one- and two-step quinone formation. At the atom level, we predict quinone formation sites with an AUC accuracy of 97.6% and identify molecules that form quinones with 88.2% AUC[27].

### Reactivity

Despite significant investment of resources, about 40% of drug candidates are discontinued due to toxicity, often stemming from reactions between electrophilic drugs or drug metabolites and nucleophilic biological macromolecules such as DNA and proteins. A deep convolution neural network to predict both sites of reactivity (SOR) and molecular reactivity. Cross-validated predictions predicted with 89.8% AURIC DNA SOR and 94.4% AUC SOR, separating reactive molecules with DNA and protein from non-reactive molecules with cross-validated AUCs of 78.7% and 79.8%, respectively[28,29].

### Phase 1

Phase I enzymes, responsible for the metabolism of more than 90% of FDA-approved drugs, catalyze highly diverse types of reactions and produce metabolites with substantial structural variability. We proposed a system for simultaneously labeling metabolism sites and reaction types, classifying them into five main classes of reactions: stable and unstable oxidations, dehydrogenation, hydrolysis, and reduction. These classes unambiguously identify 21 types of phases I reactions, which cover 92.3% of the known reactions in our database. We used this labeling system to train a neural network on 20,736 human phase I metabolic reactions, capable of identifying specific sites of reaction-type metabolism with a cross-validation accuracy of 97.1% of the area under the receptor operator curve[30].

### *N*-dealkylation

Metabolic studies often neglect to report or investigate aldehydes, even though they may be toxic. They are supposed to be efficiently detoxified into carboxylic acids and alcohols. However, some aldehydes are reactive and escape detoxification pathways to cause adverse events, forming DNA and protein adducts. This model accurately predicted the location of *N*-dealkylation on the metabolized substrates (97% of the first two and 94% of the area under the ROC curve)[31].

### UGT conjugation

Uridines diphosphate glucuronosyltransferases (UGTs) metabolize 15% of FDA-approved drugs. Lead optimization efforts benefit from knowledge of how drug candidates are metabolized by UGTs. The XenoSite UGT model predicts sites of UGT-mediated metabolism in drug-like molecules. In the training data, the metabolism sites of 2839 UGT substrates are identified by our method with 86% (Top-1) and 97% (Top-2) accuracy[32].

### Recipient processing
The target of interest chosen from the literature review was submitted to molecular docking. The target protein (PDB ID: 8F0P) with its respective ligand was obtained from the Protein Data Bank. The PDB is a data repository of proteins and their three-dimensional structures. Several types of information are associated with each entry in the PDB file, including atomic coordinates in three-dimensional space, polymer sequence, and metadata[33]. The removal of the protein inhibitor and water molecules from the receptor structure was performed using the DISCOVERY STUDIO 2021 CLIENT software.

### Binder treatment
The compound Doxorubicin was selected for in silico evaluation by molecular docking. The ligand chosen for the study was drawn in 3D using ACD/ChemSketch software, and the 2D model was obtained from ChemSpider (L-(−)-Menthol ID: 15803 and Menthone ID: 24636). The compounds were subjected to 'flexible rigid protein ligand' coupling using the Autodock VINA system in the PyRx software[34]. After docking, the ligands in the most stable conformation were analyzed using the Discovery software.

### Grid and fit calculation
The grid calculation was processed with 100 conformations in the Autodock VINA system of the PyRx software. For the ligand–protein coupling procedure, the grid dimensions on the X, Y, and Z axes were defined as 50 × 50 × 50 Å, with grid spacing of 0.375 Å. The grid center on the X, Y, and Z axes was defined as 165.1, 167.778, and 172.45 Å for the 8F0P protein. The location of the binding site was defined based on known ligands previously co-crystallized with the protein and available in the Protein Data Bank. The calculation of the interaction energy between the ligands and the amino acids of the 8F0P protein was performed using the Discovery Studio software. This software provides free binding energy calculation based on its energetic components, including van der Waals, electrostatic bonding, and hydrogen bonding.

### Statistical analysis
The results were expressed as mean ± SD. Statistical differences between groups were performed using analysis of variance (ANOVA), Post hoc Bonferroni. The results were considered statistically significant when $p < 0.05$.

## Results
### Chemical composition
The chemical composition of *M. arvensis* essential oil revealed the presence of 10 chemical components, out of which the oxygenated monoterpene L-(−)-Menthol (71.31%) was the most abundant, followed by Menthone (13.34%) (Table 1). However, the hydrocarbon monoterpenes such as (−)-Isopulegol (0.50%) and 1-limonene (2.37%) were found in smaller amounts (Table 1).

### Repellent potential of *M. arvensis* essential oil against *N. cinerea*
Natural repellents represent an excellent alternative in reducing the use of chemicals harmful to man, environment and domestic animals. Because of this, the repellent potential of MaEO was investigated on the model *N. cinerea*. It was possible to observe a promising repellent action at all the concentrations and exposure time tested (Fig. 1).

### Toxicity and markers of oxidative stress
Acute toxicity is a parameter commonly related to rapid lethal effects. For this reason, the toxicity of MaEO at different concentrations was analyzed. In analyzing the toxic potential of *M. arvensis* essential oil, no toxicity was observed during the 72 h of exposure, with 100% survival of individuals in the control group and the different concentrations (Fig. 2).

To investigate the possible oxidative damage in *N. cinerea* exposed to different concentrations of MaEO, the levels of protein (Fig. 3A) and non-protein thiols (Fig. 3B), were analyzed in the cellular tissues of the lobster cockroach. As shown in Fig. 3A, the exposure of MaEO did not cause any significant change to the total levels of –SH in comparison with the control ($p > 0.05$). However, the level of non-protein thiols (NPSH) was

| Formula | Compound | RT (Min) | LRI | % | Terpenoid group |
|---|---|---|---|---|---|
| $C_{10}H_{16}$ | α-pinene | 12.51 | 1055.1 | 0.57 | Hydrocarbon monoterpene |
| $C_{10}H_{16}$ | β-pinene | 14.92 | 1098.9 | 0.60 | Hydrocarbon monoterpene |
| $C_{10}H_{16}$ | 1-limonene | 17.88 | 1143.2 | 2.37 | Hydrocarbon monoterpene |
| $C_{10}H_{18}O$ | (−)-Isopulegol | 25.00 | 1200 | 0.50 | Hydrocarbon monoterpenes |
| $C_{10}H_{18}O$ | Menthone | 25.52 | 1212.7 | 13.34 | Oxygenated monoterpenes |
| $C_{10}H_{18}O$ | Isomenthone | 26.16 | 1225.3 | 5.35 | Oxygenated monoterpenes |
| $C_{10}H_{20}O$ | L-(−)-Menthol | 26.70 | 1235.4 | 71.31 | Oxygenated monoterpenes |
| $C_{10}H_{16}O$ | Piperitone | 31.54 | 1332.8 | 1.24 | Oxygenated monoterpenes |
| $C_{12}H_{22}O_2$ | Menthil acetate | 33.67 | 1374.6 | 4.20 | Oxygenated monoterpenes |
| $C_{15}H_{24}$ | Trans-caryophyllene | 41.13 | 1423.5 | 0.53 | Hydrocarbon sesquiterpenes |

**Table 1**. Chemical composition (%) of the essential oil of *Mentha arvensis* L. *RT* retention times (minutes), *LRI* linear retention index.

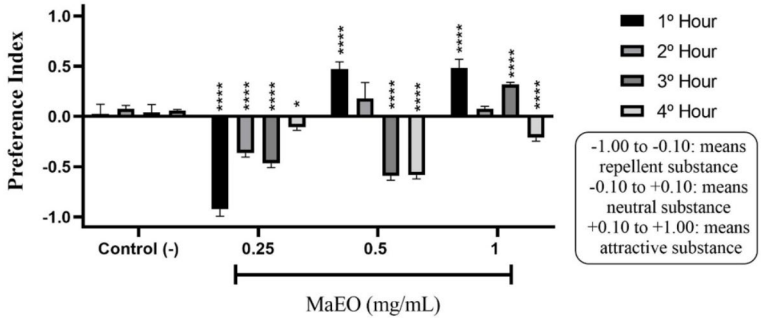


**Fig. 1**. The repellent activity of *Mentha arvensis* essential oil (MaEO) was evaluated in a confined space, using the *N. cinerea* model. Analyzing the effectiveness of MaEO in different concentrations over time, observing the behavioral response of exposed individuals. $N = 3$, *Indicates significant difference in relation to control (−) ($p < 0.05$).


**Fig. 2**. Toxicity evaluation of MaEO using *N. cinerea*.

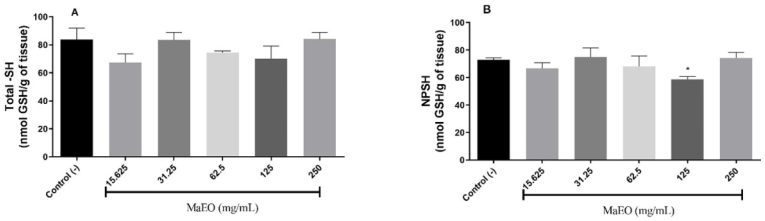


**Fig. 3**. Thiol content. An analysis of total thiol (**A**) and non-protein thiol (**B**) levels of *N. cinerea* after 3 days of supplementation with MaEO. $N = 3$, *Indicates significant difference in relation to control (−) ($p < 0.05$).

significantly reduced after MaEO exposure to concentration of 125 mg/mL when compared to control group (Fig. 3B, $p < 0.05$).

Malondialdehyde (MDA), one of the side products of lipid peroxidation (LPO), is used as an index of lipid damage. For this reason, the concentration of thiobarbituric acid reactive substances (TBARS) was observed for groups treated at different concentrations of MaEO. The concentration of 125 mg/mL was able to significantly reduce the MDA content in *N. cinerea* cell tissues when compared to the control group (Fig. 4).

As depicted in Fig. 5, MaEO caused a significant reduction in the level of iron(II) in *N. cinerea* only at 125 mg/mL compared to the control, while the other concentrations showed no significant differences in iron levels when compared to the control ($p < 0.05$).

## In silico assays

The two major compounds L-(−)-Menthol and Menthone showed similar results in the Physicochemical Properties tests (Table 2). Similar results were also observed in Lipophilicity (Table 3), where Log Po/w values demonstrated a significant similarity. In the Pharmacokinetic study, both compounds showed a high rate of gastrointestinal absorption and permeation through the blood–brain barrier. However, there was no inhibition

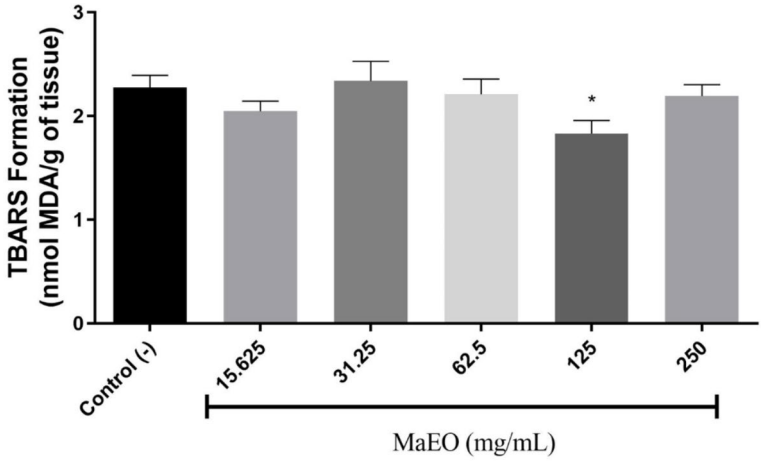


**Fig. 4**. Thiobarbituric acid reactive species (TBARS) formation in *N. cinerea* exposed to MaEO. $N = 3–4$, *Indicates significant difference in relation to control (−) ($p < 0.05$).

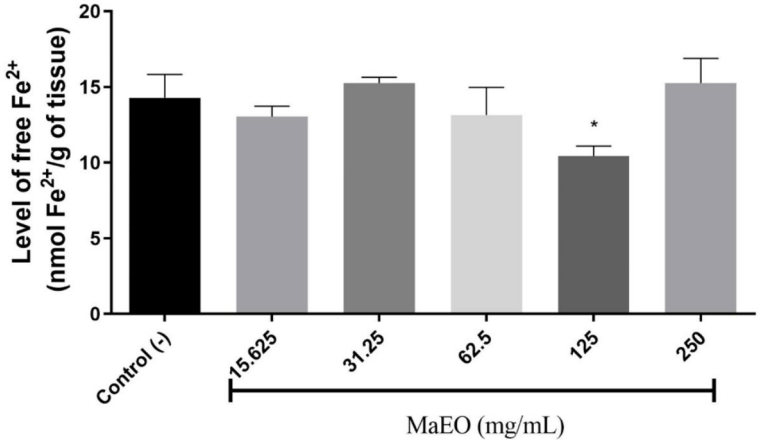


**Fig. 5**. Effect of MaEO after supplementation on iron(II) level in an alternative model of *N. cinerea* after 3 days of exposure. $N = 3$, *Indicates significant difference in relation to control (−) ($p < 0.05$).

| Physicochemical Properties | L-(−)-Menthol | Menthone |
|---|---|---|
| Formula | $C_{10}H_{20}O$ | $C_{10}H_{18}O$ |
| Molecular weight | 156.27 g/mol | 154.25 g/mol |
| Num. heavy atoms | 11 | 11 |
| Num. arom. heavy atoms | 0 | 0 |
| Fraction Csp3 | 1.00 | 0.90 |
| Num. rotatable bonds | 1 | 1 |
| Num. H-bond acceptors | 1 | 1 |
| Num. H-bond donors | 1 | 0 |
| Molar refractivity | 49.23 | 48.27 |
| TPSA | 20.23 Å² | 17.07 Å² |

**Table 2**. SwissADME numerical results. Physicochemical properties results.

of CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 enzymes. Skin permeability was similar, with values of approximately − 4.84 cm/s for L-(−)-Menthol and − 5.08 cm/s for Menthone (Table 4). Both compounds showed similar bioactivity to some diseases and a Bioavailability Score of 0.55 (Table 5). Furthermore, there were no alerts for PAINS and Brenk rules (Table 6).

| Lipophilicity | L-(−)-Menthol | Menthone |
|---|---|---|
| Log Po/w (iLOGP) | 2.63 | 2.40 |
| Log Po/w (XLOGP3) | 3.40 | 3.05 |
| Log Po/w (WLOGP) | 2.44 | 2.65 |
| Log Po/w (MLOGP) | 2.45 | 2.30 |
| Log Po/w (SILICOS-IT) | 2.06 | 2.64 |
| Consensus Log Po/w | 2.59 | 2.61 |

**Table 3**. SwissADME numerical results. Lipophilicity results.

| Pharmacokinetics | L-(−)-Menthol | Menthone |
|---|---|---|
| GI absorption | High | High |
| BBB permeant | Yes | Yes |
| P-gp substrate | No | No |
| CYP1A2 inhibitor | No | No |
| CYP2C19 inhibitor | No | No |
| CYP2C9 inhibitor | No | No |
| CYP2D6 inhibitor | No | No |
| CYP3A4 inhibitor | No | No |
| Log Kp (skin permeation) | − 4.84 cm/s | − 5.08 cm/s |

**Table 4**. SwissADME numerical results. Pharmacokinetics results.

| Druglikeness | L-(−)-Menthol | Menthone |
|---|---|---|
| Lipinski | Yes; 0 violation | Yes; 0 violation |
| Ghose | No; 1 violation: MW < 160 | No; 1 violation: MW < 160 |
| Veber | Yes | Yes |
| Egan | Yes | Yes |
| Muegge | No; 2 violations: MW < 200, Heteroatoms < 2 | No; 2 violations: MW < 200, Heteroatoms < 2 |
| Bioavailability score | 0.55 | 0.55 |

**Table 5**. SwissADME numerical results. Druglikeness results.

| Medicinal chemistry | L-(−)-Menthol | Menthone |
|---|---|---|
| PAINS | 0 alert | 0 alert |
| Brenk | 0 alert | 0 alert |
| Leadlikeness | No; 1 violation: MW < 250 | No; 1 violation: MW < 250 |
| Synthetic accessibility | 2.63 | 2.42 |

**Table 6**. SwissADME numerical results. Medicinal chemistry results.

L-(−)-Menthol showed several light reactions in the structure (Fig. 6A–F), both in stable oxygenation and dehydrogenation (Fig. 6D). Other assays, such as GSH, cyanide, and DNA, also showed mild reactions (Fig. 6C). However, when analyzed for UGT conjugation, L-(−)-Menthol exhibited high activity at the hydroxyl structure (Fig. 6F). Menthone (Fig. 7A–F), also showed mild reactions in GSH, cyanide and DNA (Fig. 7C), and slightly lighter reactions in stable oxygenation. However, Menthone demonstrated a medium intensity reaction at the double oxygen bond during reduction (Fig. 7D) and, in UGT conjugation, showed a reaction at the double bond oxygen (Fig. 7F).

In molecular docking (Fig. 8A–D), L-(−)-Menthol showed an affinity of -6.3 Kcal/mol, forming bonds at the alkyl and pi-alkyl sites with residues PHE A:1320, LEU A:1386, TYR A:1390, ALA A:1389, VAL A:1312 and PHE A:956, in addition to a pi-sigma bond at the TYR A:1313 site (Fig. 8B). On the other hand, Menthone showed an affinity of -6.4 Kcal/mol, interacting with the conventional hydrogen bonding sites in the SER A:1393 and TYR A:1313 regions, and with the alkyl and pi-alkyl sites in the residues PHE A:1320, LEU A:1386, PHE A:1316, TYR A:1390 and ALA A:1389 (Fig. 8D).

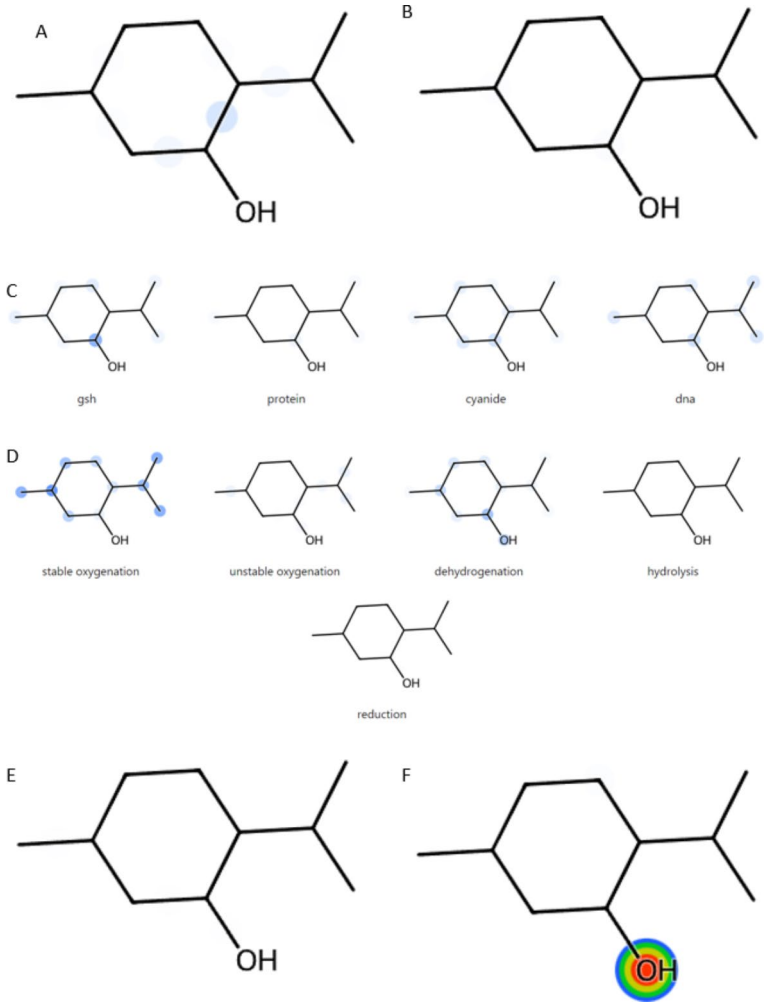


**Fig. 6**. **A–F** In silico assay of the monoterpene borneol (L-(−)-Menthol SMILES: CC(C)[C@@H]1CC[C@@H](C)C[C@H]1O) in the following processes: epoxidation (**A**), quinonation (**B**), reactivity (**C**), Phase 1 (**D**), *N*-dealkylation (**E**), and UGT conjugation (**F**).

## Discussion

There is a great diversity of plant constituents in nature, possessing great structural and functional variety. Essential oils (EOs), are defined as any non-polar volatile organic extract, responsible for a characteristic odor of plants in which are reported for therapeutic uses[35].

It has been reported that the essential oil of *M. arvensis*, shows variations of compounds according to recent literature[36,37]. In this context, the chemical composition of EOs varies depending on factors, from the time of harvest to the stage of plant development[38], as well as, the interaction with biotic and abiotic ecosystems, factors that contribute to the synthesis and production of metabolite compounds in plants[39].

Generally, the majority compound determines the biological property of the essential oil and in this study the main constituent was menthol (71.31%), an oxygenated monoterpene widely used in folk medicine (Table 1). This result is in agreement with previous studies, where menthol was also the most abundant constituent in *M. arvensis* L. essential oil[36,37,40].

Menthol acts as a strong antioxidant and plays an important role in the pharmaceutical, perfumery, cosmetics and food industries for its characteristic minty flavor and refreshing effect[41]. Biologically, this compound shows some biological properties with analgesic, antibacterial, antifungal, anesthetic, chemopreventive, immunomodulatory, and insecticidal effects in vitro and in vivo studies[42,43].

Limonene, on the other hand, is a compound with low acute toxicity in animal tests, found in many essential oils with antioxidant, anticancer, anti-inflammatory, and antimicrobial properties[44].

Many aromatic plants are used as insect repellents due to the presence of highly odorous and bioactive volatile organic compounds[45]. The use of bioactive plants has been consolidating as an excellent alternative in pest control[46].

Essential oils have been shown to be effective in controlling pests of stored products, compared to the use of synthetic insecticides, regarding their low environmental impact[47].

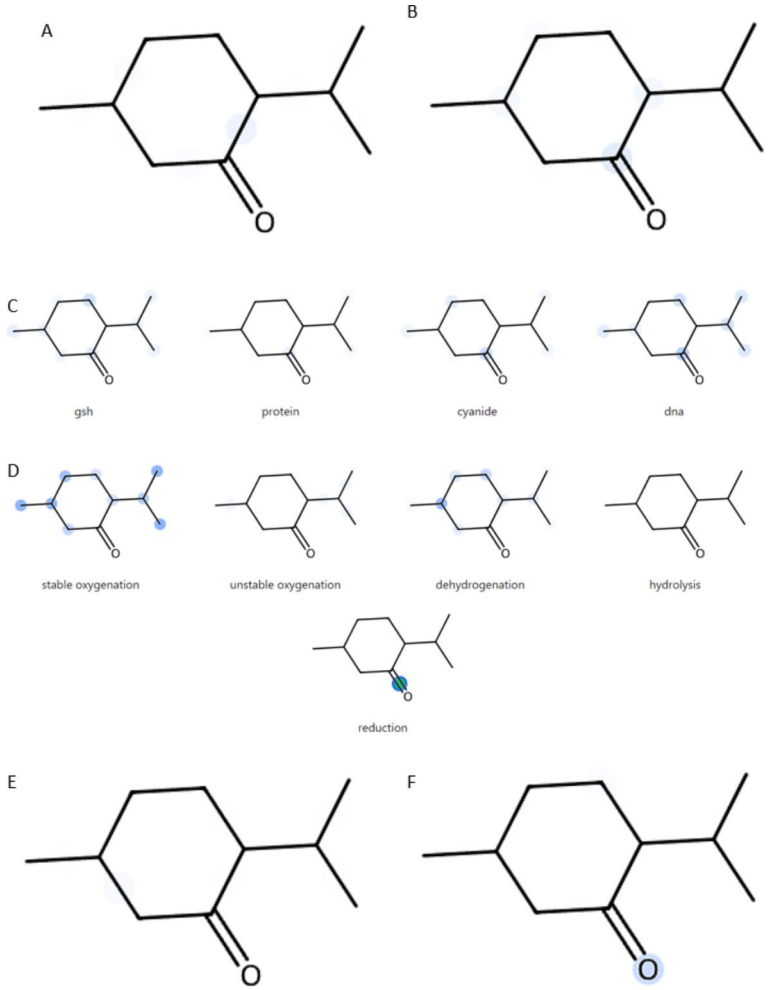


**Fig. 7**. **A–F** In silico assay of the monoterpene borneol (Menthone SMILES: CC(C)[C@@H]1CC[C@@H](C)CC1=O) in the following processes: epoxidation (**A**), quinonation (**B**), reactivity (**C**), Phase 1 (**D**), *N*-dealkylation (**E**), and UGT conjugation (**F**).

Bioinsecticides, have been used as alternatives to chemical insecticides, exhibiting fast action and effectiveness, besides, being biodegradable reducing damage to the environment[47]. In this study, Fig. 1 shows the repellency of the essential oil at all the concentrations and time of exposure, although it was reported that the repellency of the essential oil increased with concentration[37].

Toxicity on target pests is the first step for any kind of modern pesticide formulation[36]. Regarding the mortality rate of the individuals in the exposure time, MaEO showed a beneficial effect on the percentage of mortality (Fig. 2). In our results there was 100% survival of the individuals, indicating that MaEO was not toxic to the tested nymphs of *N. cinerea*. However, the toxicity of MaEO has already been pointed out for its activities against various pests of stored products[48].

Furthermore, a high mortality rate of MaEO was observed by on weevils *S. oryzae* and *S. zeamais*, possibly attributed to the major compounds menthol and mentone, which are extremely toxic compounds on stored grain pests[49]. The mode of action may be directly related to the presence of monoterpenes and sesquiterpenes present in the oil of *M. arvensis* that act by inhibiting the activity of the acetylcholinesterase enzyme (AChE) in the nervous system[50].

The use of synthetic products can lead to toxic side effects, thus the use of natural products that have antioxidant potential is increasingly growing[51]. Oxidative stress is known to be a disproportion between the production of reactive oxygen species (ROS) and its degradation by antioxidants. Electron transfer, one of the chemical processes essential to cell survival, can generate undesirable effects that is the production of free radicals and other ROS that can trigger oxidative damage process[52,53]. In the current study, exposure of *N. cinerea* to MaEO lead to a significant decrease in sulfhydryl (–SH) groups indicating the presence of oxidative stress[54–57].

Antioxidants are substances of any origin that has the role of inhibiting oxidative stress, reducing the number of free radicals such as peroxides[58–61]. The essential oils of plants of the genus *Mentha* are known to have antioxidant properties, a property that can be attributed due to the presence of its phenolic compounds[62–64]. In this study when observing the protein levels (Fig. 3) MaEO showed antioxidant effects at concentrations of

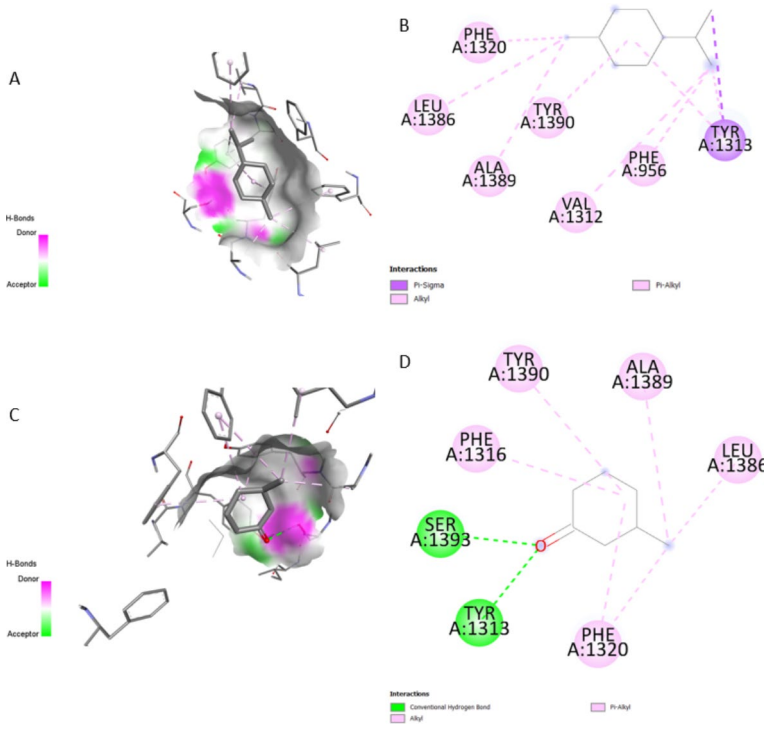


**Fig. 8**. In silico molecular docking assay. (**A**) 3D interaction of L-(−)-Menthol with target protein 8F0P; (**B**) 2D interaction of L-(−)-Menthol with target protein 8F0P; (**C**) 3D interaction of Menthone with target protein 8F0P; (**D**) 2D interaction of menthone with target protein 8F0P.

125 mg/ml. These results corroborate with the studies conducted by Benabdallah et al.[65] and by Almeida et al.[66]. that highlight the potential of MaEO as a potential for the elimination of free radicals.

The membrane is one of the cellular components most susceptible to oxidative stress due to lipid peroxidation. Malondialdehyde is a product of lipid peroxidation and can be used as an indicator of the presence of free radicals. Thiobarbituric acid reactive substances (TBARS) that are formed as a by-product of lipid peroxidation, thus its value only changes in cases of oxidative stress[54,67]. Analyzing the results in Fig. 3, it is possible to observe that the essential oil of *M. arvensis* managed to reduce the content of MDA in cell tissues of *N. cinerea* significantly. Boutakiout et al.[68]. described that the essential oil of *Mentha piperita* belonging to the same family as *M. arvensis* presents lipidic antiperoxidation effect.

The presence of Iron(II) is essential for the activity of several enzymes that help in the catalysis and several biochemical processes that occur inside the plant[69]. The levels of $Fe^{2+}$ was significantly reduced after exposure of *N. cinerea* to MaEO, which might be in accordance with the ability of MaEO to exhibit ferric reducing power potential[58].

According to Clemente, Robledo and Ravetti[70], ADME tests revealed that menthol has a topological polar surface area (TPSA) of 20.23 Å². This value is identical to that of L-(−)-menthol, indicating a similarity between the compounds. Furthermore, the voltage-gated sodium channel NaV1.7 has been identified as a potential analgesic target due to its role in human pain syndromes[70]. Both L-(−)-menthol and menthone were observed to exhibit high binding affinity with this target protein in molecular docking. These compounds also demonstrated high gastrointestinal absorption and skin permeability, with permeability values of − 4.84 cm/s for L-(−)-menthol and − 5.08 cm/s for menthone.

Corroborating these findings, the literature already establishes the analgesic action of menthol[42]. Behm et al.[71]. demonstrated similar reductions in pain sensitivity regardless of the site of application of a menthol-based topical analgesic, attributing these effects to the compound's high skin permeability. Thus, the data indicate that both L-(−)-menthol and menthone have the potential to be effective analgesic agents due to their high absorption and binding affinity with the NaV1.7 sodium channel.

## Conclusion

In summary, the essential oil of *M. arvensis* showed repellent and non-toxic action against *N. cinerea* nymphs. More studies on field applications, as well as new formulations of repellents based on the essential oil are needed. MaEO has been shown to possess antioxidant action, reducing lipid peroxidation of iron levels in *N. cinerea* and both L-(−)-menthol and menthone demonstrated substantial potential as effective analgesic agents, due to their high absorption rate and high binding affinity with the NaV1.7 sodium channel.

## Data availability

The authors declare that the data supporting the findings of this study are available within the paper.

Received: 27 February 2024; Accepted: 10 September 2024
Published online: 16 September 2024

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## Acknowledgements

Authors are thankful to researchers supporting Project Number (RSP2024R335), King Saud University, Riyadh, Saudi Arabia.The authors are also grateful to the Ceara Research Support Foundation (FUNCAP) for financial support of this research (concession BP3-0139-00018.02.00/18), and the bestowal of 3 scholarships.

## Author contributions

Each author participated sufficiently in taking public responsibility for appropriate portions of the content. Study conception and design: C.A.L.S. and A.M.T.M., conceived the idea and designed experiments and wrote manuscript. B.R.S.T., L.M.B. and J.P.K. analyse the data performed the experiments; M.I., A.F.A., F.A., and M.K., analyzed the data and revised the manuscript. All authors reviewed and approved the final version.

## Declarations

### Competing interests
The authors declare no competing interests.

### Additional information
**Correspondence** and requests for materials should be addressed to M.I.

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