

Molecular characterization, GC-MS analysis, and biological activities of *Matricaria chamomilla* var. *recutita* L. essential oils from Taounate, Morocco: *In vitro* and *in silico* investigations of antioxidant, antimicrobial, and insecticidal effects

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Abstract

Chamomile (*Matricaria chamomilla* L.), a widely recognized medicinal plant, was investigated for its chemical composition, functional properties, and molecular characterization, focusing on samples cultivated in the Taounate region of Morocco. Essential oil (EO) was extracted using a Clevenger apparatus, and GC/MS analysis identified 26 compounds, with germacrene (19.46%), α -curcumene (19.00%), and capric acid (15.81%) as the major components. The EO exhibited significant antioxidant activity, with an IC_{50} of 456.57 μ g/mL against the DPPH radical. It demonstrated strong antibacterial effects, particularly against *E. coli*, with an inhibition zone of 21.50 ± 0.50 mm and a minimum inhibitory concentration (MIC) of 20.00 μ g/mL. Antifungal activity was also notable, inhibiting *Aspergillus niger* by 31.19 ± 0.00 mm. In fumigation tests, the EO caused 100% insect mortality at 16 μ L/L after 72 hours, with an LC_{50} of 1.86 μ L/L of air, and showed a 55% repellency rate at 12 μ L/cm². DNA sequencing confirmed a 99.22% similarity with *Matricaria chamomilla* var. *recutita* (L.). These results highlight the EO's multifaceted biological activities, including antioxidant, antimicrobial, and insecticidal properties, underscoring its potential for applications in healthcare, agriculture, and food production.

1. Introduction

Rising global population is increasing the demand for cereals, prompting the expansion and intensification of large-scale cereal production. However, this agricultural intensification has led to a surge in cereal pest populations, contributing to approximately one-third of global cereal losses (Murugesan et al. 2021). *Callosobruchus maculatus* is identified as a significant pest that poses a serious threat to stored grains. It is found globally and is particularly damaging to crops such as chickpeas, green beans, black beans, kidney beans, and cowpeas, especially in tropical and subtropical regions (Tuda et al. 2005). Damage caused by seed weevils, including weight loss and reduced nutritional quality, can lead to substantial economic and health-related impacts (Alves et al. 2019). In Africa, losses due to this pest can lead to food shortages and malnutrition (Banga et al. 2019). The widespread use of synthetic pesticides has contributed to the development of pesticide resistance among numerous insect populations, driving the search for natural alternatives that pose fewer risks to both the environment and human health (Abdelli et al. 2016). Essential oils demonstrate potential as an eco-friendly solution for managing stored grain pests. Notably, they exhibit higher efficacy even at lower concentrations, minimizing the risk of environmental harm (Ayslane et al. 2019; Oyedele and Afolayan 2005).

The rise of microbial pathogen resistance poses a significant threat to global health, as microorganisms are increasingly developing resistance mechanisms against antibiotics, antimicrobials, and even newly developed drugs (Abduljabbar and Aljanaby 2018; Noshad, Hojjati, and Alizadeh 2018). According to a simulation model, microbial resistance is projected to cause more than ten million deaths each year by 2050 (Soriano et al. 2020). The primary driver behind microbial pathogen resistance is the improper utilization of antibiotics and synthetic antimicrobials. Scientists are exploring novel compounds sourced from various outlets, such as medicinal plants, in the pursuit of infection treatments. (Abirami et al. 2021; Sharma et al. 2016). In recent years, there has been a renewed interest in using medicinal plants, rooted in tradition, to treat and cure ailments. (Jaadan et al. 2020). This increase in popularity is mainly attributable to the growing demand for these herbs, especially in emerging markets, where they are more affordable than conventional medicines (El-Assri et al. 2021; Hilah et al. 2016).

In Africa, traditional medicine is used by more than 80% of the population. The continent is rich in plant diversity, with a significant number of species being harnessed for their medicinal properties. Globally, there are approximately 300,000 plant species, of which over 200,000 are found in the African tropics and are valued for their health benefits (Emmanuel and Didier 2012; Komoreng et al. 2017).

Morocco is home to a substantial portion of its population that depends on medicinal plants for self-care and treatment (Bouyahya et al. 2016). Morocco's unique geographical location, diverse geological formations, varied topography, and favorable climate make it a vital center of plant biodiversity in North Africa (Eddouks, Ajebli, and Hebi 2016). Due to its diverse and varied environment, it exhibits exceptional plant diversity, with approximately 4,800 species (Verlaquea, Médail, and Aboucaya 2001), distributed among 981 genera and 155 families (Fennane and Rejdali 2018). Among these, roughly 800 are indigenous (Dobignard and Chatelain 2010), and 1,600 taxa are classified as scarce (Fennane and Tattou 1999). Moreover, approximately 600 species are employed in herbal medicine (Hmamouchi 2005). Moroccan flora contributes to over half of the endemic species found in North African nations (Bakha et al. 2017).

German chamomile (*Matricaria recutita*, syn. *Matricaria chamomilla*) is an annual herb belonging to the Asteraceae family (Mehmood et al. 2015). It is originally from Europe, Western Asia, North and East Africa (Lim 2014), now widely distributed worldwide (Singh et al. 2011). This plant is among the most widely used medicinal herbs globally, traditionally employed in the treatment of various conditions, including digestive disorders (Menale et al. 2021), sleep and hepatic disorders (Zivkovic et al. 2020), also used against pain and infections (Mikou, Rachiq, and Oulidi 2015), colds (Güzel, Mehmet and Miski 2015). *M. chamomilla* (L.) contains a variety of bioactive compounds, such as flavonoids, tannins, and coumarins, which contribute to its therapeutic properties. These constituents are responsible for its medicinal effects (Höferl et al. 2020). Studies have shown that German chamomile exhibits anti-inflammatory effects (Göger et al. 2018), antioxidant (Formisano et al. 2015), antifungal (Tolouee et al. 2010), antimicrobial (Romha et al. 2018), antispasmodic, sedative, and analgesic properties, among others (Tolouee et al. 2010). As a medicinal plant, German chamomile can be consumed in various forms, such as herbal tea, infusions, or extracts (Mehmood et al. 2015). Chamomile essential oil is widely utilized across various industries, such as pharmaceuticals, cosmetics, agri-food, and more, due to its versatile properties and benefits (Singh et al. 2011). To our knowledge, no prior studies have explored the molecular characterization and biological activities of the essential oil derived from the flowers of *Matricaria chamomilla* (L.). In this study, we aimed to address this gap by investigating the molecular characteristics, chemical composition, and biological properties of this essential oil. Specifically, we evaluated its antibacterial, antioxidant, and antifungal activities, with a focus on its efficacy against antibiotic-resistant bacteria. Additionally, we examined its insecticidal potential against *Callosobruchus maculatus*.

2. Materials and Methods

2.1. Plant material and extraction of essential oil

M. chamomilla (L.) flowers were harvested on the morning of April 1, 2022, during the peak flowering season, in the Tahar Souk region, located approximately 50 km from the town of Taounate, Morocco (geographical coordinates: 35°1'22" N, 4°8'27" W, altitude: 592 m). The region experiences a Mediterranean climate, with maximum temperatures soaring up to 40°C. The collected plant material was identified by botanist Amina Bari, a Professor in the Biology Department of the Faculty of Sciences Moulay Driss (FSMD), located in Fez, Morocco, and was assigned the specimen reference number 05-21-TT0015. The flowers of *Matricaria chamomilla* were air-dried in the laboratory under controlled conditions, protected from light and humidity, at room temperature. The essential oil of *M. chamomilla* (EO-MC) was extracted using a Clevenger apparatus in a 2-liter flask, where 100 grams of dried flowers were combined with 1 liter of distilled water. Following extraction, the EO-MC was dehydrated using anhydrous sodium sulfate, filtered to remove impurities, and stored in sealed flasks at 4°C for subsequent use (El-Assri et al. 2021; El Moussaoui et al. 2021).

All experimental research and field studies on *Matricaria chamomilla* (L.) complied with institutional, national, and international guidelines and legislation. The plant material was collected with appropriate authorization, and its identification was verified by a qualified botanist. No endangered or protected species were involved in this study.

2.2. Extraction and PCR of genomic DNA and sequencing

The protocol described by Jawhari et al. (2023) was used for DNA extraction from randomly selected fresh *M. chamomilla* flowers of the same age (Jawhari et al. 2023). The RbcL (Ribulose-1,5-Bisphosphate Carboxylase) region was amplified by PCR with the universal primers rbcL a-f (5'-ATGTCACCACAAACAGAGACTAAAGC3') and rbcL a-r (5'-GTAAAATCAAGTCCACCGCG3') (Parvathy et al. 2018). Amplification conditions were as follows: 35 cycles at 95°C for 4 min, 94°C for 30 seconds, 55°C for 1 min, 72°C for 1 min. PCR results were visualized by 1.5% agarose gel electrophoresis. Sequences were aligned and processed using ChromasPro sequence analysis software (version 2.1.10.1). They were then analyzed using Blast search to identify sequences homologous to each other in the GenBank databases. They were then deposited in the previous database. A phylogenetic tree was created from the sequences obtained. The *M. chamomilla* (L.) isolate was used to form a main group using MEGA 5.0 software. The sequence of *Artemisia girdii* (OK128342) was selected as outgroup. The maximum likelihood (ML) approach was employed to compute phylogenetic connections. To evaluate the support for each branch in the resulting tree, 1000 bootstrap replications were conducted.

2.3. Oil of *M. chamomilla* (L.) identified by GC-MS

Gas chromatography-mass spectrometry (GC-MS) analysis was utilized to determine the chemical composition of the essential oil (EO). Two capillary columns were employed for the analysis: an HP-5MS column with a non-polar stationary phase and a DB-HeavyWAX column with a polar stationary phase. Mass spectrometry was conducted in electron impact (EI) mode. The retention indices of the sample components were compared with those documented in the NIST-MS Search Version 2.0 library to accurately identify the compounds present in the EO (Atki et al. 2020; Mssillou et al. 2022).

2.4. Antioxidant activity in vitro

The DPPH assay was conducted following the procedure suggested by El Moussaoui et al. (El Moussaoui et al. 2021). A 0.004% DPPH solution was prepared, and 100 µl of *M. chamomilla* (L.) essential oil extract, diluted to various concentrations in methanol, was mixed with 750 µl of the DPPH solution. The same procedure was applied to butylated hydroxytoluene (BHT), a synthetic antioxidant, also diluted to different concentrations in methanol. After incubating for 30 minutes at room temperature, absorbance at 517 nm was measured using a UV-Vis spectrophotometer (JENWAY 85617). The percentage inhibition of DPPH (PI%) was computed using the following equation:

$$PI(\%) = 100 \times (A_0 - A/A_0)$$

PI stands for the percentage of inhibition. A₀ indicates the absorbance of the negative control (DPPH without the sample), while A represents the absorbance of the sample with DPPH.

2.5. The antibacterial activity of EO-MC

The antibacterial effects of EOMC were evaluated against four bacterial strains: *Proteus mirabilis* ATCC29906, *Escherichia coli* K12, *Klebsiella pneumoniae* CIP A22, and *Staphylococcus aureus* ATCC6633. These strains were obtained from the CHU Hassan II in Fez. The zone of inhibition was determined using the disk diffusion technique. Bacterial strains were introduced onto Petri dishes filled with Mueller-Hinton agar at a concentration of 10⁶ to 10⁸ CFU/mL (0.5 McFarland). Following this, 6 mm-diameter filter paper discs were soaked with 20 µL of EOMC, and a positive control was conducted using streptomycin antibiotic (25 µg/disc). The plates were incubated for 24 hours at 37°C. After incubation, the diameter of growth inhibition zones was assessed. The results, expressed in millimeters, were used to evaluate the antibacterial activity of EOMC (El Barnossi, Moussaid, and Housseini 2020; El-Assri et al. 2021; Lafraxo, Barnossi, et al. 2022).

2.6. Antifungal activity

The study aimed to evaluate the antifungal activity of OE-MC against four fungal species: *Candida albicans* ATCC, *Aspergillus flavus*, *Fusarium oxysporum* MTCC9913, and *Aspergillus niger* MTCC282. To perform the test, fungi were inoculated onto Petri dishes containing malt agar extract medium. Then, 6 mm Whatman paper discs were soaked with 20µL of OE-MC. The plates were incubated for 7 days at 30°C for *A. niger*, *A. flavus* and *F. oxysporum*, while *C. albicans* was incubated at 37°C for 24–48 hours. Positive controls were included using the antibiotic Fluconazole (15mg/mL). After the incubation, the inhibition diameter (in mm) was measured for *C. albicans*, while growth in mm was assessed for both the negative and positive tests. This data was then used to calculate the percentage of inhibition for strains of filamentous fungi using the following formula (Moussaid et al. 2019):

$$\frac{\text{Negative test} - \text{Positive test}}{\text{Negative test}}$$

2.7. Measurement of minimum inhibitory concentration

The MICs of OE-MC against the four fungal and four bacterial strains were determined using the micro-dilution method, following the protocol described by Sarker *et al.* (Sarker, Nahar, and Kumarasamy 2007). Briefly, a sterile 96-well microplate was employed. For bacterial and fungal strains, 50 µl of Mueller-Hinton (M-H) medium and malt extract (ME), respectively, were added to each well. EO-MC was diluted at a ratio of 1/10 (v/v) in 10% DMSO, and 100 µl of this solution was dispensed into the first column of the microplate. Subsequently, microbial strains (30 µl) were added following a 1:2 dilution series up to column 11. Plates were placed in incubators at 37°C or 30°C for 24 h, 48 h or 7 days, respectively for bacteria, *C. albicans* and filamentous fungi. Following the incubation period, 20 µl of a 0.2% solution of 2,3,5-triphenyl-tetrazolium-chloride was added to each well to facilitate the visualization of microbial growth (El Abdali et al. 2023). The MIC was defined as the minimum concentration that did not lead to red coloration, and the outcomes were expressed in mg/ml.

2.8. Insecticidal action against *C. maculatus*

2.8.1. Fumigation test

The fumigation experiment evaluated the efficacy of essential oil vapors against *C. maculatus* using sealed 1L containers. Whatman No. 1 paper squares (3 x 3 cm) were saturated with varying concentrations of essential oil (4–20 µl/L of air) and attached to the inner surface of the container lids to prevent direct contact with the insects. Ten pairs of *C. maculatus*, aged 0–48 hours, were introduced into each container. The experiment included repeated treatments and an untreated control group. Mortality rates were recorded daily for five days under controlled environmental conditions (temperature 27 ± 1°C, relative humidity 70 ± 5%, and a 14:10 light/dark photoperiod). The process continued until complete mortality of bruchid insects was observed in all treated groups (Baghouz et al. 2022).

Total adult mortality was calculated using Abbott's formula (Abbott 1925):

$$P_v = \frac{\text{varvecP}\text{varveca} - \text{varvecP}\text{varveci}}{100 - \text{varvecP}\text{varveci}} \times 100$$

Where: **Pc**: percentage mortality, **Pi**: mortality observed in the negative control and **Pa**: mortality observed in the test.

2.8.2. Repellent effect of EO-MR

The repellency of EO-MC against adults *C. maculatus* was evaluated using the preferential zone methodology on filter paper, following the method described by (McDonald, Guy, and Speirs 1970). Half-discs of Whatman N°1 paper (8 cm) were treated with different doses of EO-MC (4–20 µl/0.5 ml acetone) or with pure acetone as a control. After allowing the treated halves to dry, they were reassembled into full discs, and five pairs of adult insects were placed at the center. Repellency was assessed 30 minutes later based on the distribution of the insects, using the formula by McDonald et al. (McDonald, Guy, and Speirs 1970):

$$PR (\%) = \frac{N - NT}{N + NT} \times 100$$

Repellency percentage (RP) is determined by comparing the number of *C. maculatus* adults found on the acetone-treated (control) side (N) to those on the essential oil-treated side (NT).

2.9. Molecular Docking

The study explored the effects of OE-MC on insecticidal, antioxidant, and antimicrobial activities through molecular docking techniques.

2.9.1. Preparation of the ligand

We retrieved all GC/MS-identified molecules of EOMC from the PubChem database in SDF format. Subsequently, Schrödinger's Maestro 11.5 software was used to prepare the molecular structures. The OPLS3 force field was applied, and the LigPrep tool was employed to generate 32 stereoisomers for each ligand, considering ionization states at pH 7.0 ± 2.0 (Lafraxo, Moussaoui, et al. 2022).

2.9.2. Protein preparation

Proteins targeting NADPH oxidase (2CDU.pdb) (Herrera-calderon et al. 2021), β-ketoacyl synthase from *E. coli* K12 (PDB ID: 1FJ4), nucleoside diphosphate kinase from *Staphylococcus aureus* (PDB ID: 3Q8U), β-1,4 endoglucanase from *A. niger* (PDB ID: 5I77), sterol 14-alpha demethylase (CYP51) from *C. albicans* (PDB ID: 5FSA) (Tourabi et al. 2023), and the crystal structure of an insecticide-resistant acetylcholinesterase (PDB ID: 6ARY) were obtained from the RCSB protein database (pdb) (Venugopala et al. 2020). These proteins were then prepared according to the standard protocol by removing water molecules and all corresponding co-crystallized ligands while adding Gasteiger fillers.

The prepared proteins were docked to the main compound of the plant under study using Autodock software. Finally, all the interactions produced were visualized using Discovery Studio software (Amrati et al. 2023).

2.10. Statistical Analysis

Mean standard deviation values were acquired using GraphPad Prism 8.0.1. Statistical analysis was conducted employing ANOVA, followed by Tukey's test. Significance was determined by a p-value of less than 0.05.

3. Results and discussion

3.1. Molecular identification of *M. chamomilla* (L.)

PCR amplification of the *rbcL* gene yielded products approximately 600 bp in size. Sample A (*M. chamomilla* (L.)) demonstrated strong amplification of the *rbcL* primer. BLAST analysis, conducted using the NCBI GenBank, revealed that sequence A exhibited 99.29% identity with *M. chamomilla* var. *recutita*. The sequence was subsequently deposited in the GenBank database under the accession number OR838665 and identified as *M. chamomilla* var. *recutita*.

Using the Neighbor-Joining (NJ) method for phylogenetic analysis, the *rbcL* gene was shown to be a reliable tool for species identification and classification. Genetic similarities among species were utilized to construct phylogenetic trees, facilitating a deeper understanding of evolutionary relationships (Sundari et al. 2019). In this study, the analyzed plant sample clustered within a single clade of *M. chamomilla* (Fig. 1), indicating a very close genetic relationship.

3.2. Volatile profile of essential oil

The yield of MCEO, obtained by Clevenger, was approximately $0.45 \pm 0.26\%$ (w/w), with the identification of 26 chemical compounds in the EO-MC representing 99.96% of the overall EO (Fig. 2 and Table 1). Analysis via GC/MS indicated that sesquiterpenes comprised the predominant chemical class in EO-MC, accounting for 56.57%, followed by monoterpenes at 30.15%. The main components were germacrene (19.46%), followed by alpha-curcumene (19.00%) and caprylic acid (15.81%). It's worth mentioning that the yield rate achieved in this research is inferior to that reported in earlier studies by Neelav et al., and Mahdavi et al., who documented rates of 1.27% and 0.65%, respectively (Mahdavi et al. 2019; Sarma et al. 2023). However, it exceeds the rate reported by Hajjaj et al., who documented a rate of 0.4% (Hajjaj et al. 2014). The findings align with previous studies, indicating that the EO-MC from Romania is predominantly comprised of sesquiterpenes (91.65%), with oxyde de bisabolol A (70.2%) being the most abundant component (Berechet et al. 2017). Similarly, Neelav et al. (2023) demonstrated that *M. chamomilla* in India has a predominance of sesquiterpenes, representing 92.75% of the identified compounds (Sarma et al. 2023). Research carried out in Brazil by Demarque et al. (2012) revealed that OE-MC was composed by 18 elements, of which alpha-bisabolol oxide B (26.08%), beta-farnesene (16.35%) and bisabolol oxide A (14.7%) are the main constituents (Demarque et al. 2012). In contrast, Fadel et al. (2020) discovered 16 compounds, constituting 96.0% of the essential oil. The principal constituents found were alpha-Bisabolol oxide A (45.5%), alpha-bisabolol oxide B (14.7%), cis-beta-farnesene (5.7%), alpha-bisabolone oxide A (12.9%) and cis-beta-farnesene (5.7%) (Hanem et al. 2020). Further research by Stanojevic et al. (2016) revealed the presence of 52 different compounds in OE-MC, with (E)-β-farnesene (29.8%) being the main constituent, followed by α-farnesene (9.3%) and alpha-bisabolol oxide A (7%) (Stanojevic et al. 2016). The content and chemical composition of plant essences can fluctuate due to a variety of environmental factors. These include the specific part of the plant used, the plant's stage of development, its maturity, the time of harvest and even the plant's genetic heritage (Tourabi et al. 2023).

Table 1
Phytochemical composition of *M. chamomilla* identified by GC/MS

Peak	Retention time	Compound	Retention index	Chemical formula	Terpene class	Area (%)
1	10.771	p-Menthane	979	C ₁₀ H ₂₀	MNT	1.97
2	10.834	Eucalyptol	1014	C ₁₀ H ₁₈ O	MNT	1.25
3	11.475	Prenyl isobutyrate	1054	C ₉ H ₁₆ O ₂	O	1.41
4	12.309	Germacrene	1485	C ₁₅ H ₂₄	MNQ	19.46
5	13.684	Phenyl-tert-butanol	1158	C ₁₀ H ₁₄ O	MNT	3.34
6	14.099	Limonen-10-ol	1289	C ₁₀ H ₁₆ O	MNT	2.21
7	16.909	Butanoate	1141	C ₁₀ H ₁₈ O ₂	MNT	1.54
8	20.346	Caprinic acid	1201	C ₁₀ H ₂₀ O	MNT	15.81
9	22.646	β-Farnesene	1442	C ₁₅ H ₂₄	MNQ	1.24
10	22.730	Prenyl isobutyrate	1054	C ₉ H ₁₆ O ₂	O	0.97
11	23.315	β-Himachalene	1500	C ₁₅ H ₂₄	MNQ	7.67
12	23.384	alpha-Curcumene	1515	C ₁₅ H ₂₄	MNQ	19.00
13	24.271	Prenyl cyclopentanone	1227	C ₁₀ H ₁₆ O	MNT	1.08
14	24.783	Piperitenone oxide	1368	C ₁₀ H ₁₄ O ₂	MNT	1.52
15	25.921	curcumene	1480	C ₁₅ H ₂₂	MNQ	1.08
16	26.004	Spathulenol	1578	C ₁₅ H ₂₄ O	MNQ	2.15
17	26.456	Globulol	1590	C ₁₅ H ₂₆ O	MNQ	0.95
18	27.481	Pinene	1099	C ₁₀ H ₁₆ O	MNT	1.43
19	30.052	α-Santalene	1417	C ₁₅ H ₂₄	MNQ	1.43
20	31.594	α-Patchoulene	1456	C ₁₅ H ₂₄	MNQ	1.21
21	35.451	Bisabolene	1515	C ₁₅ H ₂₄	MNQ	1.21
22	38.334	Eicosane	2000	C ₂₀ H ₄₂	MND	3.09
23	39.119	Methyl linoleate	2085	C ₁₉ H ₃₄ O ₂	O	1.37
24	41.942	Tetradecanal	1612	C ₁₄ H ₂₈ O	O	2.69
25	45.220	Triacontane	3000	C ₃₀ H ₆₂	MTT	3.74
26	48.230	Bisabolone oxide	1685	C ₁₅ H ₂₄ O ₂	MNQ	1.17
Terpene class						
Monoterpenes (MNT)						30.15
Sesquiterpenes (MNQ)						56.57
Diterpenes (MND)						3.06
Triterpenes (MTT)						3.74
Other (O)						6.44
Total						99.96

3.3. Scavenging of the Free Radical DPPH[•]

The DPPH assay is a widely used method for evaluating the antioxidant activity of substances in laboratory settings. The test is based on the transfer of hydrogen (H) atoms or electrons (E) from antioxidant compounds to DPPH radicals dissolved in a solution. This interaction causes the DPPH radicals to change color from purple to yellow, signifying the formation of a stable diamagnetic molecule as a result of their reaction with reducing substances (Nchez 2002). The alteration in color is quantified spectrophotometrically at a wavelength of 517 nm (Magalhaes et al. 2008). This method is highly effective and

useful for assessing antioxidant activity. Lower IC₅₀ values indicate a higher antioxidant capacity of the sample (Beniaich et al. 2022; Moussaoui et al. 2019). The antioxidant capacity of EO-MC was assessed using the DPPH test. The results obtained are summarized in Table 2. EO-MC demonstrated PDPH inhibition with an IC₅₀ value of approximately 456.57 µg/mL. For BHT, a standard antioxidant, the IC₅₀ value was 17.83 µg/mL (Fig. 3).

Table 2
Antibacterial activity (mm) and MIC in (µg/mL) of EO-MC against the bacterial strains tested.

		Essential oil	Streptomycin
<i>E. coli</i> ATCC29213	Diameter of inhibition (mm)	21.5 ± 0.5 ^a	20.9 ± 0.36 ^a
	MIC (µg/mL)	20.0 ± 0.00 ^a	1.56 ± 0.00 ^b
<i>S. aureus</i> ATCC6633	Diameter of inhibition (mm)	11.67 ± 0.58 ^a	22.5 ± 0.2 ^b
	MIC (µg/mL)	2.5 ± 0.0 ^a	1.56 ± 0.0 ^a
<i>K. pneumoniae</i> CIP A22	Diameter of inhibition (mm)	12.5 ± 1.5 ^a	17.6 ± 0.36 ^b
	MIC (µg/mL)	5.00 ± 0.0 ^a	3.125 ± 0.0 ^b
<i>P. mirabilis</i> ATCC 29906	Diameter of inhibition (mm)	15.5 ± 1.03 ^a	16.73 ± 0.25 ^a
	MIC (µg/mL)	6.25 ± 0.0 ^a	3.125 ± 0.0 ^b
Mean values (± SD, n = 3) labeled with distinct letters within the same row denote a significant contrast (ANOVA II, Tukey tests at p < 0.05).			

A literature review highlighted several studies on the antioxidant activity of EO-MC. One study conducted in Bosnia-Herzegovina revealed that essential oils derived from this species exhibit an IC₅₀ concentration of 2.07 mg/mL (Stanojevic et al. 2016). Another study, conducted in northern Iran, reported an IC₅₀ concentration of 5.63 mg/mL for EO-MC, further supporting its antioxidant properties (Ayoughi et al. 2011). These findings align with those reported by Chouia et al. (2018) and Qasem et al. (2022), who documented IC₅₀ values of 416.57 µg/mL and 533.89 ± 15.05 µg/mL, respectively, further corroborating the antioxidant potential of EO-MC (Chouia et al. 2018; Qasem et al. 2022). Conversely, these values are lower than those reported by Mahdavi et al. (2019) and Zeynep Demirci et al. (2018), who recorded IC₅₀ values of 793.89 ± 15.45 µg/mL and 2.20 mg/mL, respectively. This variability may reflect differences in experimental conditions, plant origin, or extraction methods (Mahdavi et al. 2019; Zeynep, Demirci, and Demirci 2018). Furthermore, our results surpass those published by Sarma et al. (2023) and Abdoul-Latif et al. (2011), who recorded IC₅₀ values of 21.95 and 4.18 µg/mL, respectively. (Abdoul-Latif et al. 2011; Sarma et al. 2023).

3.4. Bacterial activity of EO-MC

The EO-MC compound showed considerable anti-bacterial activity versus all four types of bacteria tested, in particular Gram-negative bacteria such as *Proteus mirabilis* ATCC29906, *Escherichia coli* K12 and *Klebsiella pneumoniae* CIP A22 and Gram-positive bacteria such as *Staphylococcus aureus* ATCC6633. The results of the disk diffusion method (Table 2) indicate that EO-MC blocked the growth of all examined species. Among the bacterial strains tested, the largest diameter of inhibition was found for *E. coli* (21.5 ± 0.5 mm), followed by *P. mirabilis* (15.5 ± 1.03 mm) and *K. pneumoniae* (12.5 ± 1.5 mm). The smallest inhibition diameter was found for *S. aureus* (11.667 ± 0.577 mm). These findings suggest that all the bacteria tested showed sensitivity to EO-MC. MIC results for EO-MC indicate that the lowest value was observed for *S. aureus* (2.5 ± 0.02 µg/mL), while the highest value was observed for *E. coli* (20.0 µg/mL). Essential oils of *M. recutita*, collected in Algeria, showed significant efficacy versus *K. pneumoniae*, *E. coli* and *P. aeruginosa* bacteria, with zones of inhibition varying from 10.67 mm to 18.33 mm (Chouia et al. 2018). In a Moroccan study by El-Assri et al. (2021), essential oil derived from *M. recutita* (L.) was shown to have considerable antibacterial efficacy versus *B. subtilis*, with a 15.2 mm diameter of inhibition and a MIC of 6.25 µL/mL, followed by *S. aureus* (14.13 mm) and an MIC of 8.33 µL/mL, then *E. coli* (13.28 mm) and an MIC of 8.33 µL/mL, and finally *P. aeruginosa* (13.07 mm) and an MIC of 8.33 µL/mL (El-Assri et al. 2021). In Iran, Kazemi et al (2015) demonstrated that EO-MC possessed significant anti-bacterial activity versus *S. aureus*, *B. subtilis* and *P. aeruginosa*, with zones of inhibition reaching up to 32 mm (Kazemi 2015). Conversely, oil extracted from *M. recutita* from the Horn of Africa (Djibouti) showed very encouraging antibacterial effects against eleven bacterial strains, with inhibition diameters of between 14 mm and 30 mm, while MICs vary from 1 to 4 µg/mL (Abdoul-Latif et al. 2011). In addition, Soković et al. (2010) demonstrated that the MIC of *M. recutita* essential oil was 7 µg/mL (*B. subtilis*), 8 µg/mL (*S. aureus*) and 10 µg/mL (*E. coli* and *P. aeruginosa*). (Soković et al. 2010). Other publications have shown that EO-MC was capable of destroying *S.aureus*, followed by *E. coli* and *Salmonella enterica*, with diameter of inhibition of 40, 31 and 25 mm, resp (Shakya et al. 2019). Mahdavi et al. (2019) demonstrated that *M. recutita* essential oil was effective against *P. aeruginosa* ATCC27853 (108.77%), *Enterococcus faecalis* ATCC 14506 (106.7%), *E. coli* ATCC (99.66%) and *K. pneumoniae* ATCC 13883 (75.04%) (Mahdavi et al. 2019). Numerous previous studies have shown that positive Gram bacteria are more susceptible than negative Gram bacteria (Barbosa et al. 2015; Kazemi 2015). However, this study is contrary to the previous one, as Gram-negative bacteria, such as *E. coli*, were found to be more sensitive to EO-MC.

3.5. Antifungal activity of EO-MC

The percentages of inhibition and MIC values of EO-MC versus the 4 fungal strains tested are shown in Table 3. The findings show that EO-MC exerts significant antifungal activity, with higher percentages of inhibition against *A. niger* (31.19%) than against *A. flavus* (17%). MIC values for EO-MC varied between 0.02 ± 0.0 to 0.04 ± 0.0 µg/ml. The results of this investigation align with previous scientific research demonstrating the antifungal efficacy of OE-MC versus various fungal strains. Al-snafi (2016), Göger et al. (2018), Roby et al. (2012), and Tolouee et al. (2010) have all documented similar findings in their respective studies (Al-snafi 2016; Göger et al. 2018; Roby et al. 2012; Tolouee et al. 2010). The results obtained concerning the antifungal activity of EOMCs

are consistent with results published in 2012 in Egypt by Roby et al (2012) (Roby et al. 2012), who showed antifungal activity of EOMCs against *C. albicans* ATCC10231 a 14 mm inhibition diameter and a MIC of 10 µg/mL, while the inhibition diameter of *A. flavus* ATCC 16875 was 18 mm and the MIC 12.5 µg/mL. Abdoul-Iatif et al (2011) conducted a study in Djibouti, which demonstrated notable antifungal effects versus *C. albicans* ATCC10231, with a 20 mm zone of inhibition and a MIC of 1 µg/mL. In addition, they observed activity against *A. niger*, with an inhibition zone of 17 mm and a MIC of 2 µg/MI (Abdoul-Iatif et al. 2011). In parallel, results obtained in another study revealed significant antifungal efficacy, notably against *A. flavus* AFI375, *A. niger* FC24771, *F. culmorum* CBS 128537 with percentage inhibition of 10.66–52.33%, 89.66–100%, 91-86.66%, respectively (EL-Hefny et al. 2019). Moreover, Prabodh Satyal et al. (2015) indicated that the MIC of EOMC against *C. albicans* ATCC10231 was 313 µg/mL, and for *A. niger* ATCC 16888, it was 625 µg/mL. (Satyal, Shrestha, and Setzer 2015).

Table 3
Antifungal activity (% inhibition) and MIC (mg/mL) of EO-MC against fungal strains tested.

		Essential oil	Fluconazole
<i>C. albicans</i> ATCC10231	Diameter of inhibition (mm)	28.33 ± 0.58 ^a	41.33 ± 1.15 ^b
	MIC (mg/mL)	0.04 ± 0.00 ^a	0.0125 ± 0.00 ^b
<i>A. niger</i> MTCC282	Percentage of inhibition (%)	31.19 ± 0.00 ^a	47.67 ± 1.53 ^b
	MIC (mg/mL)	0.02 ± 0.00 ^a	0.0062 ± 0.00 ^b
<i>A. flavus</i> MTCC9606	Percentage of inhibition (%)	17.00 ± 0.00 ^a	43.67 ± 1.53 ^b
	MIC (mg/mL)	0.04 ± 0.00 ^a	0.0125 ± 0.00 ^b ^a
<i>F. oxysporum</i> MTCC9913	Percentage of inhibition (%)	23.47 ± 0.7 ^a	59.17 ± 0.76 ^b
	MIC (mg/mL)	0.02 ± 0.00 ^a	0.0062 ± 0.00 ^b
Mean values (± SD, n = 3) labeled with distinct letters within the same row denote a significant contrast (ANOVA II, Tukey tests at p < 0.05).			

3.6. Insecticide activity test

3.6.1. Fumigation test

The results of the study, illustrated in Fig. 4 and Table 4, show a direct correlation between the applied dose, the duration of exposure, and the mortality of adult *C. maculatus*. Statistical testing revealed a significant correlation between *C. maculatus* adult mortality and the dose applied, as well as the duration of exposure (F = 271.73; df = 4, 40; P < 0.0001; F = 129.76; df = 3, 40; P < 0.0001). After a 72-hour exposure period, EO-MC showed increased toxicity at higher concentrations of 20 and 16 µL/L of air. In fact, EO-MC caused 100% absolute mortality in insects at concentrations of 16 µL/L and above after 72 hours' exposure (Table 3). The median lethal concentration (LC₅₀) of EO-MC was 20.64 and 1.86 µL/L of air after 24 and 72 hours, respectively. These findings suggest that EO-MC compounds are relatively more toxic to *C. maculatus* adults. The findings of this study align closely with those reported by Tandorost *et al.* (2012), El-Khyat *et al.* (2017), and Arannilewa *et al.* (2006), demonstrating a positive relationship between mortality rates and the duration and dosage of exposure (Arannilewa, Ekrakene, and Akinneye 2006; El-Khyat *et al.* 2017; Tandorost and Karimpour 2012). Similarly, the results are consistent with previous research conducted by Abouellata *et al.* (2016), indicating that MC essential oil exhibits strong insecticidal activity against *C. maculatus* adults, with a lethal concentration (LC₅₀ = 2.058 mg/L) observed after 24 hours of fumigation (Abouellata *et al.* 2016). Moreover, OE-MC displayed potent insecticidal effects against *E. cautella* adults at concentrations of 62.5, 125, and 250 mg/L of air over a 72-hour exposure period. Additionally, Mousavi *et al.* (2020) found that chamomile essential oil was more efficacious against this pest compared to other essential oils (Mousavi, Ghosta, and Maroofpour 2020). Furthermore, several studies have highlighted the effectiveness of essential oils rich in sesquiterpenes and monoterpenes against insect pests due to their potent volatile nature (Karabörklü, Ayvaz, and Semih Yilmaz 2011; Tourabi *et al.* 2023; Tunc *et al.* 2000).

Table 4. LC₅₀ and χ² of EO-MC against *C. maculatus* adults.

	Time (H)	LC ₅₀ (µl/L)	95% CI	LC ₉₀ (µl/L)	95%CI	Df	χ ²
EO-MC	24	20.64	---	361.09	---	2	0.57
	48	4.70	0.028-8.35	50.73	22.02-836489.15	2	3.85
	72	1.86	0.004-4.12	11.43	6.40-53.94	2	2.30
	96	---	---	---	---	---	---

3.6.2. Repellency test

For generations, aromatic plants have served as highly efficient natural insect repellents in traditional herbal medicine (Pavela 2016). Several studies have evaluated the repellent properties of chamomile species against agricultural pests (Mousavi and Maroof 2020). This study assessed the repellent potential

of OE-MC versus *C. maculatus* insects using the surface preference technique on filter paper. The results presented in Fig. 5 indicate that the repellent effect of OE-MC correlates with dosage. Specifically, at the smallest concentration examined (4 $\mu\text{L}/\text{cm}^2$), a repellent effect of $30 \pm 1.54\%$ was observed. In contrast, at the highest concentration (20 $\mu\text{L}/\text{cm}^2$), a repellent effect of $55 \pm 19.14\%$ was observed against *C. maculatus* adults. Based on a concentration of 12 $\mu\text{L}/\text{cm}^2$, OE-MC was found to have an average repellency of 55%, using the calculation method of McDonald et al (1970) (McDonald, Guy, and Speirs 1970). The findings of this study accord with those of Allali et al. (2022), who also observed an average repellent activity (50.83%) of OE-MC against *C. maculatus* adults at a concentration of 20 $\mu\text{L}/\text{cm}^2$ (Allali et al. 2022). Similarly, El-Khyat et al. (2017) showed that EO-MC presented moderate repellent activity ($49.33 \pm 1.33\%$) at the concentration of 62.5 mg/L of air against *E. cautella* (El-Khyat et al. 2017). These data indicate that EO-MC could be used as a natural repellent against harmful agricultural insects. Essential oils comprise intricate blends of volatile organic compounds, encompassing monoterpenoids and sesquiterpenes. These constituents exhibit insecticidal attributes, particularly by perturbing the hormonal equilibrium of insects (Ahn et al. 1998; Rattan and Summt 2010).

3.7. Molecular Docking

During the assessment of antioxidant activity, three molecules namely butanoate, bisabolone oxide, and piperitenone oxide were found to be the most potent ones targeting the NAD(P)H oxidase active site. Butanoate was found to have the highest value of -5.313 kcal/mol, followed by bisabolone oxide with a value of -5.301 kcal/mol and piperitenone oxide with a value of -4.997 kcal/mol (Table 5). Moreover, butanoate showed interactions with two residues, TYR 159 and ILE 160, in the NAD(P)H oxidase active site, forming two H-bonds (Fig. 6A and 7A).

Table 5
Docking results of ligand in the active sites.

	Glide Gscore (kcal/mol)					
	Antioxidant Activity	Insecticide-resistant	Antibacterial Activity		Antifungal Activity	
Title	2CDU	6ARY	1FJ4	3Q8U	5FSA	5I77
α -Curcumene	-3.029	-5.197	-4.722	-4.278	-5.841	-2.813
β -Bisabolene	-	-5.314	-5.411	-4.056	-4.896	-2.776
β -Himachalene	-3.82	-6.415	-3.808	-3.112	-6.854	-3.749
Bisabolone oxide	-5.301	-6.988	-4.216	-5.252	-6.079	-3.87
Butanoate	-5.313	-4.408	-4.846	-7.903	-4.312	-3.411
Eucalyptol	-3.312	-4.665	-5.703	-3.275	-4.739	-4.451
Germacrene	-3.481	-5.53	-4.382	-3.72	-6.19	-3.243
Globulol	-3.762	-6.916	-4.493	-4.279	-6.909	-4.506
Limonen-10-ol	-3.785	-5.175	-5.694	-4.223	-5.672	-4.039
Phenyl-tert-butanol	-4.639	-4.843	-5.595	-4.384	-6.806	-5.06
Piperitenone oxide	-4.997	-6.052	-7.098	-4.276	-5.606	-4.629
p-Menthane	-4.173	-5.874	-5.81	-4.49	-5.82	-3.753
Spathulenol	-3.806	-5.978	-5.981	-4.126	-7.015	-4.86

Inhibition of acetylcholinesterase (AChE) is a common mechanism of action for many insecticides. Acetylcholinesterase is an enzyme that degrades the neurotransmitter acetylcholine in the nervous system. In insects, acetylcholine plays a crucial role in transmitting nerve impulses at synapses. Inhibiting acetylcholinesterase leads to an accumulation of acetylcholine, disrupting normal nerve function, and ultimately causing the insect's death (Fulton and Key 2001; Mirjana B.Colovic et al. 2013). In our *in silico* study, bisabolone oxide, Globulol and Prenyl cyclopentanone exhibited the greatest inhibitory impact on acetylcholinesterase, Glide Gscore values for these compounds were – 6.988, -6.916 and – 6.626 kcal/mol respectively (Table 5). In addition, bisabolone oxide established a unique H-bond with residue TYR 282 in the acetylcholinesterase active site, as illustrated in Fig. 6B and 7B.

In addition, the antibacterial effectiveness of a substance was tested against *Escherichia coli* K12 and *Staphylococcus aureus* ATCC6633 bacteria. The results showed that EO-MC had glide Gscores of -7.098, -6.545, and – 6.510 kcal/mol against *E. coli* β -ketoacyl-[acyl carrier protein] synthase, while butanoate, bisabolone oxide, and p-menthane were most effective against *S. aureus* nucleoside diphosphate kinase, with glide Gscores of -7.903, -5.252, and – 4.490 kcal/mol, respectively (Table 5). Furthermore, Figs. 6C and 7C show that piperitenone oxide formed two H-bonds with residues THR 300 and THR 302 in the active site of *E. coli* beta-ketoacyl-[acyl carrier protein] synthase. Meanwhile, Butanoate formed a single hydrogen bond with residue HIE 52 and a salt bridge with residue MG 159 in the active site of *S. aureus* nucleoside diphosphate kinase, as shown in Fig. 6D and 7D. In the evaluation of antifungal activity against *C. albicans* and *A. niger*, certain molecules were identified as active in the active site of sterol 14-alpha demethylase (CYP51) of the pathogenic yeast *C. albicans*. Spathulenol, globulol, and beta-himachalene were found to be active with glide Gscores of -7.015, -6.909, and – 6.854 kcal/mol, respectively (Table 5). In the active site of a beta-1,4-endoglucanase from *A niger*, researchers identified phenyl-tert-butanol, spathulenol, and piperitenone oxide as the most effective molecules. These molecules had glide gscore values of -5.060, -4.860, and – 4.629 kcal/mol, respectively. Moreover, among these three, phenyl-tert-butanol established a single hydrogen bond with residue TYR 227 in the same active site (Table 5, Fig. 6F and 7F).

4. Conclusion

The research findings reveal that EO-MC, extracted from Taounate chamomile, exhibits a wide spectrum of biological activities, including antioxidant, antimicrobial, and insecticidal properties. Rich in bioactive compounds such as sesquiterpenes and monoterpenes, EO-MC is known for its ability to shield cells from oxidative stress. Additionally, it has demonstrated significant antibacterial, antifungal, and insecticidal effects against a variety of microbes and insects, including antibiotic-resistant strains and the cowpea bruchid (*C. maculatus*). These findings highlight the potential of EO-MC as a therapeutic and preventive agent for combating oxidative damage, bacterial and fungal infections, and insect infestations.

Declarations

Author contribution:

Conceptualization, E.E., and A.B.; methodology, E.E., A.H., Y.E., A.B.; software, A.H., R.B., and M.C.; validation, E.E., A.B.; formal analysis, E.E., Y.A., and A.B.; investigation, A.M., and N.R.; resources, A.B., E.E.; data curation, A.H., and A.L.; writing—original draft preparation, E.E., A.H., and Y.E.; writing—review and editing, A.El., M.B., and N.E.; visualization, A.B., and A.S.A.; supervision, A.B.; funding acquisition, A.S.A. Project administration, A.B., All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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Figures

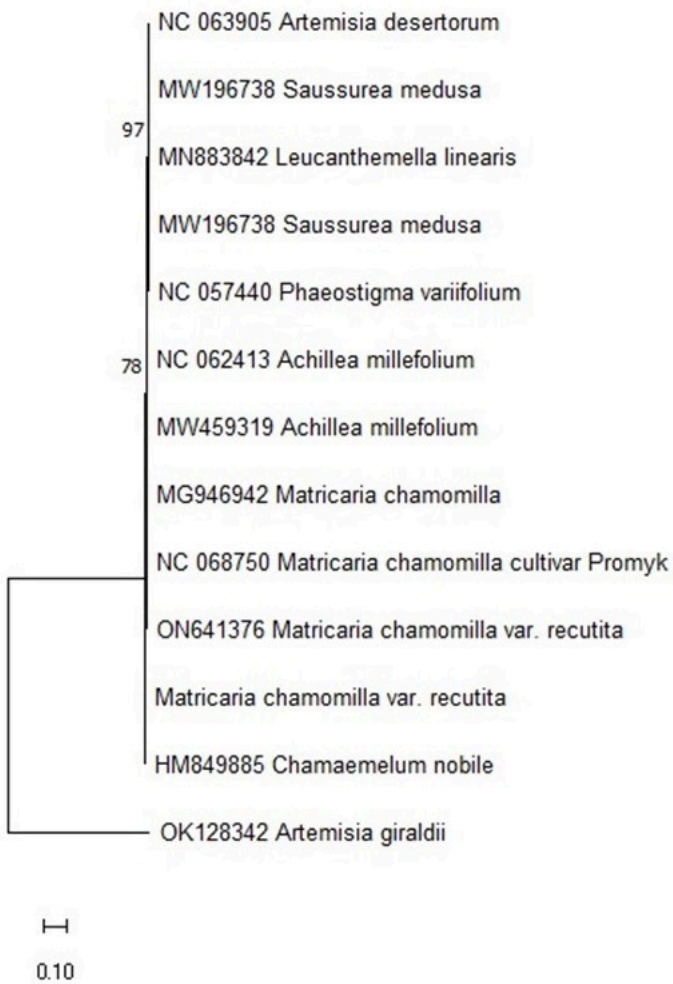


Figure 1

Phylogenetic analysis of *Matricaria chamomilla* OR838665 samples.

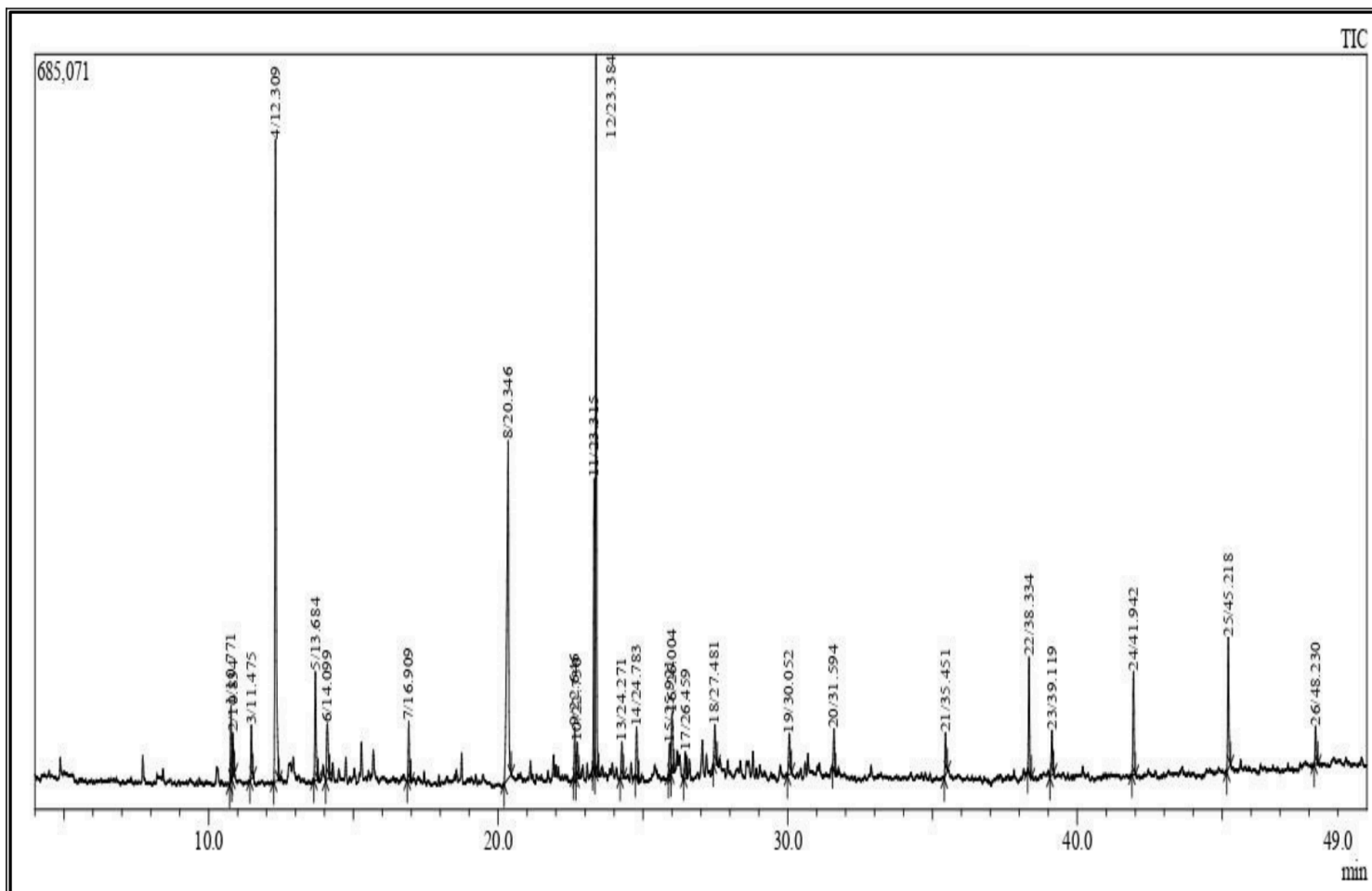


Figure 2

GC-MS chromatogram of *Matricaria chamomilla* (L.) EO.

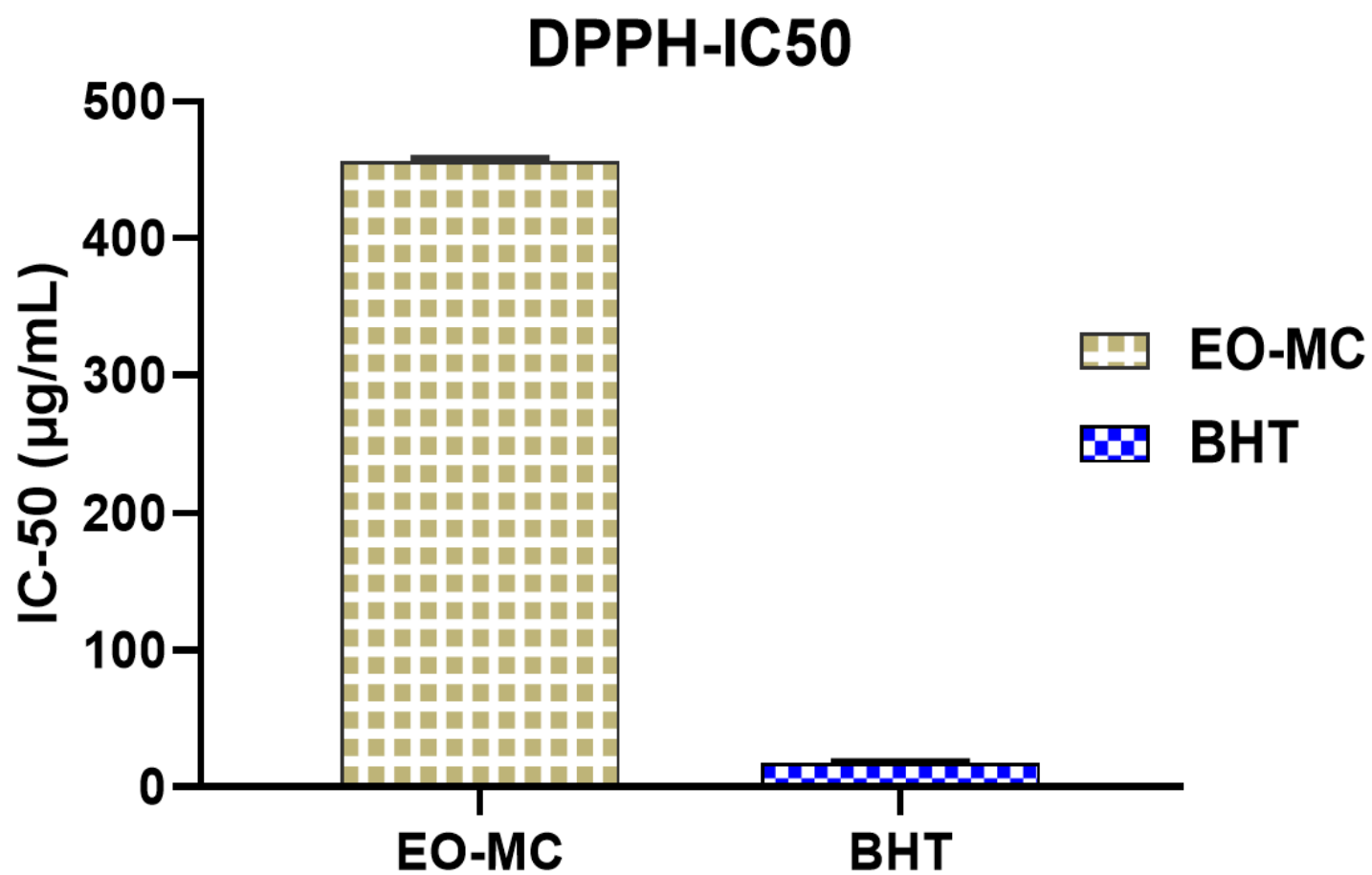


Figure 3

DPPH test for antioxidant activity of essential oil and BHT (µg/mL).

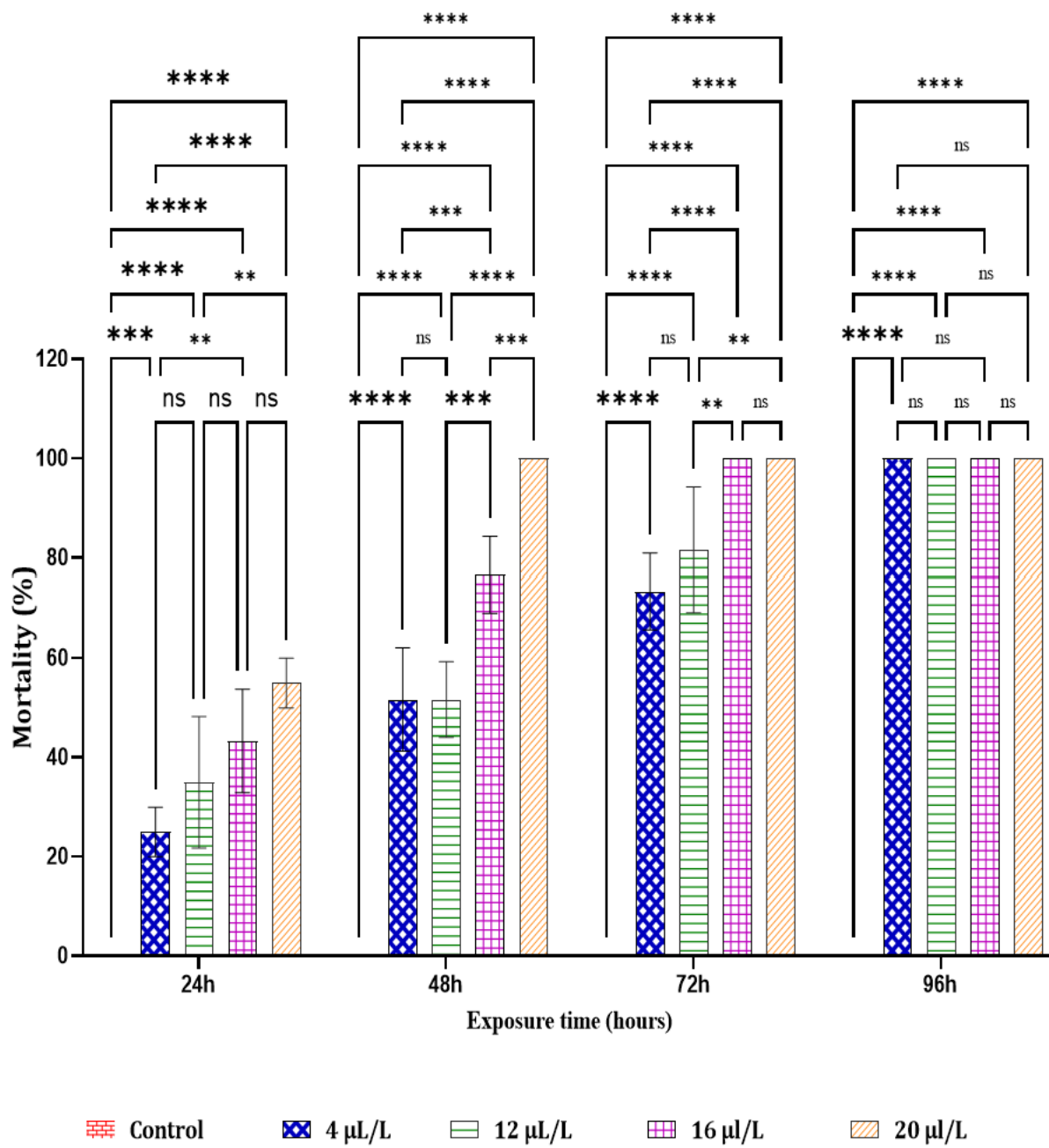


Figure 4

Percentage mortality of *C. maculatus* adults as a function of concentration and duration of exposure to EO-MC. Means ($\pm$ SD, n = 3) marked with a star indicate a significant difference according to two-way ANOVA and Tukey's multiple interval tests at $p < 0.05$.

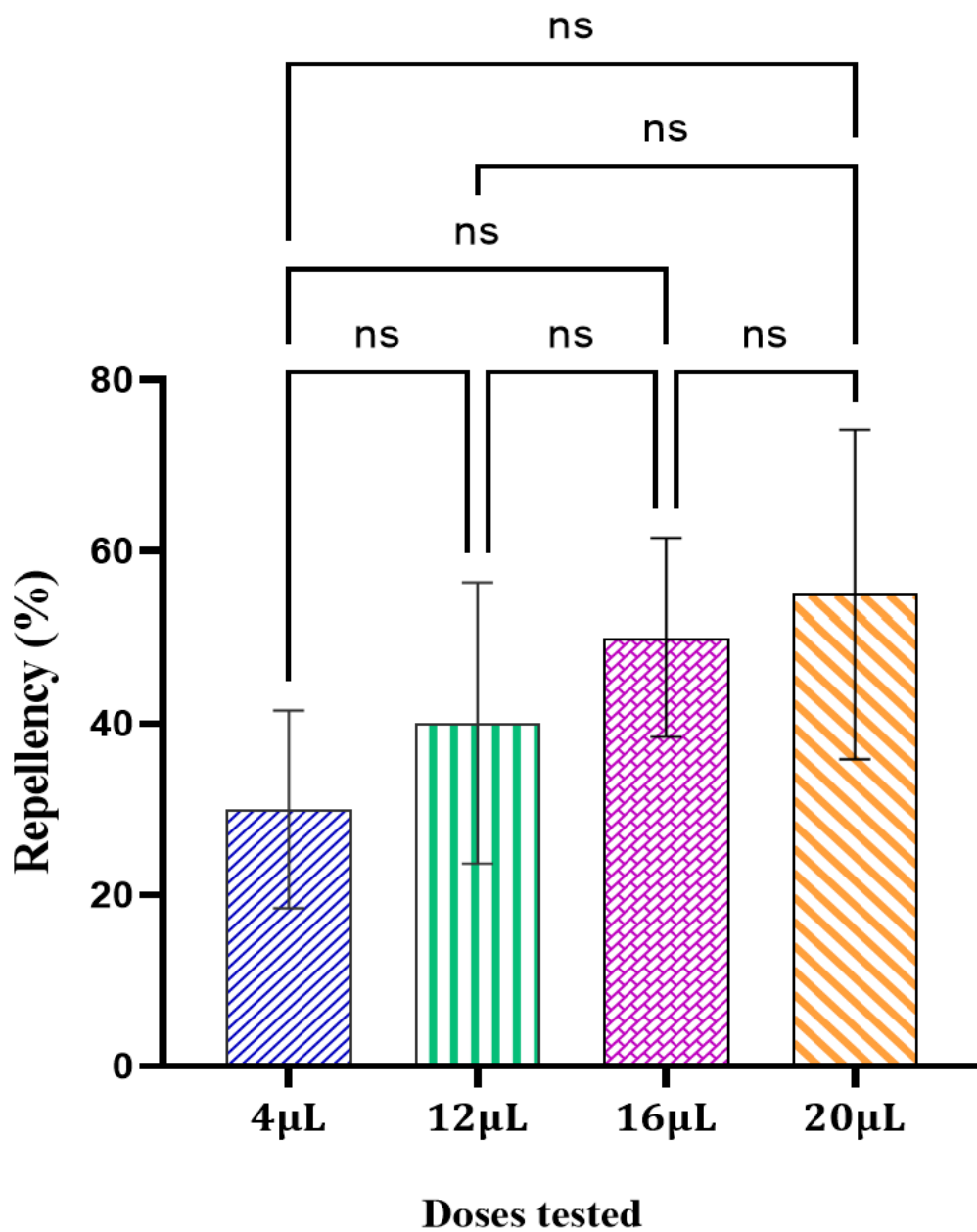


Figure 5

Repellent index of MC-EO against *C. maculatus*. Means ($\pm$ SD, $n = 3$) marked with a star indicate a significant difference according to two-way ANOVA and Tukey's multiple interval tests at $p < 0.05$.

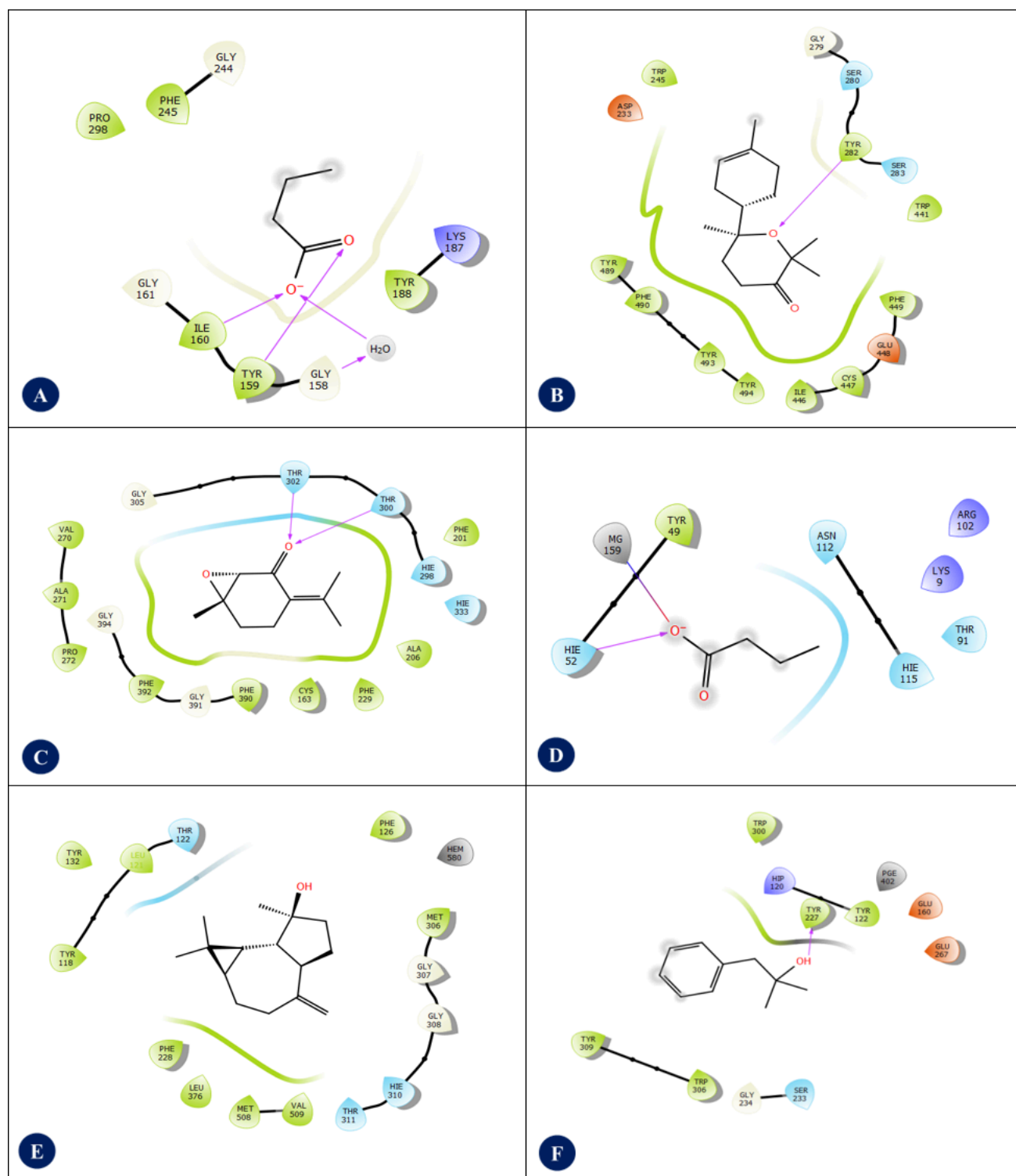


Figure 6

The following is a list of ligands and their interactions with various active sites, as viewed in 2D: **(A and D)**: Butanoate interacts with the active sites of NADPH oxidase and Staphylococcus aureus nucleoside diphosphate kinase. **(B)**: Bisabolone oxide interacts with the active site of acetylcholinesterase. **(C)**: Piperitenone oxide interacts with the active site of beta-ketoacyl-[acyl carrier protein] synthase from *E. coli*. **(E)**: Spathulenol interacts with the energetic site of a beta-1,4-endoglucanase from *A. niger*. **(F)**: Phenyl-tert-butanol interacts with the active site of sterol 14 alpha demethylase (CYP51) from a pathogenic yeast *C. albicans*.

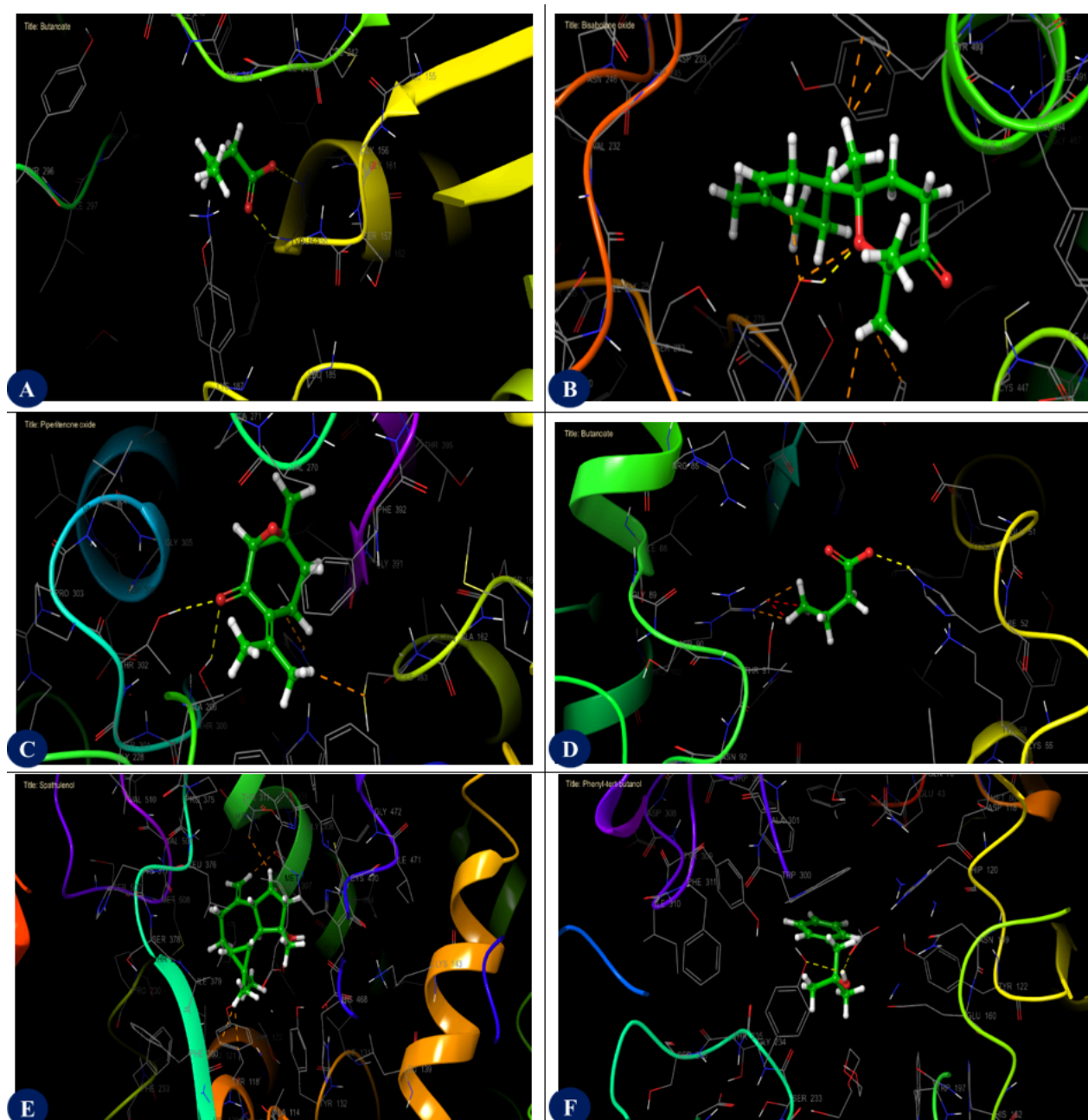


Figure 7

The list of ligands and their interactions with different active sites is visualized using a 3D viewer. **A and D:** interactions of butanoate with the active sites of NADPH oxidase and nucleoside diphosphate kinase from *S. aureus*. **B:** interactions of bisabolone oxide with the active site of acetylcholinesterase. **C:** Interactions of peritenone oxide with the active site of Escherichia coli beta-ketoacyl-[acyl carrier protein] synthase. **E:** Interactions of spathulenol with the energetic site of a beta-1,4-endoglucanase from *A. niger*. **F:** Interactions between phenyl-tert-butanol and the active site of sterol 14-alpha demethylase (CYP51) from the pathogenic yeast *C. albicans*.