

Research Article

Scolymus hispanicus L. From Algerian Flora: Phytochemical Profiling, Biological Assessment, and Molecular Docking Insights

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Scolymus hispanicus L. extracts exhibited significant bioactive properties. The dichloromethane extract was rich in polyphenols and flavonoids, while phytochemical screening revealed coumarins, sterols, triterpenes, tannins, and carbohydrates. Volatile profiling by HS-SPME-GC-MS identified 27 compounds, with α -pinene (30.9%) as the dominant component, followed by 2(4H)-benzofuranone, dodecane derivatives, octanoic acid, lauric acid, and limonene. Antidenaturation assays showed that the *n*-hexane extract effectively inhibited protein denaturation, surpassing aspirin, likely due to α -pinene and limonene. Dichloromethane extract displayed strong antibacterial activity against five pathogenic bacterial strains, including three Gram-positive species. Antifungal tests indicated *Candida albicans* was sensitive, while *Saccharomyces cerevisiae* was resistant; *Fusarium* sp. and *Botrytis* sp. showed reduced growth at higher extract concentrations. Molecular docking of 32 volatile compounds against bacterial dihydrofolate reductase (DHFR) and fungal lanosterol 14 α -demethylase (CYP51) revealed strong binding affinities for compounds such as hexadecene, 1,2-epoxy, and hexahydrofarnesyl acetone, consistent with the observed antimicrobial activity. These results demonstrate the potential of *S. hispanicus* L. extracts as promising sources of novel antimicrobial agents.

Keywords: antidenaturation activity; antimicrobial activity; bacterial DHFR enzyme; fungal CYP51 enzyme; molecular docking; polyphenol compounds; *Scolymus hispanicus* L.; volatile compounds

1. Introduction

In Algeria, wild and traditional plants play a crucial role in the daily lives of local communities, especially in rural and semirural areas, where they have been used for centuries as sources of both nutrition and traditional medicine [1, 2]. The country's rich ecological and climatic diversity has fostered the growth of a wide range of valuable plant species [3, 4].

These plants are highly regarded for their nutritional properties, being rich in dietary fiber, essential vitamins, minerals, and antioxidant compounds that support immune function and contribute to the prevention of chronic diseases. Their dual function as food and medicine reflects a deep body of indigenous knowledge and highlights the importance of scientific research into these natural assets [5, 6].

In this context, *Scolymus hispanicus* L., locally known in Algeria as “Qarnoun” or “Sqala,” is considered one of the most important wild edible plants. Traditionally consumed in spring, its tender shoots are foraged and prepared in soups, stews, or salads. In addition to its culinary uses, the plant has long been employed in folk medicine to aid digestion, purify the blood, and support liver function [7–9].

Recent phytochemical investigations have revealed that *S. hispanicus* L. is rich in bioactive compounds such as flavonoids, phenolic acids, and sesquiterpene lactones. These not only contribute to its nutritional value but also exhibit notable antimicrobial properties, positioning the plant as a promising candidate for natural drug development, especially in the face of escalating antimicrobial resistance [10, 11]. Furthermore, the volatile compounds present in *S. hispanicus* L. have attracted growing scientific interest due to their potential bioactivities. These aromatic molecules are known to play key roles in plant defense and may possess antimicrobial effects through their interactions with microbial cell membranes or metabolic enzymes [12].

To gain deeper insight into the mode of action of these natural molecules, computational tools such as *in silico* methods—particularly molecular docking—have become essential. These techniques allow for the prediction and visualization of interactions between phytochemicals and microbial targets, thus accelerating the discovery of effective natural antimicrobial agents. However, the growing antimicrobial resistance poses a serious global health threat, highlighting the urgent need for new, natural, and effective bioactive compounds.

Molecular docking was employed as a complementary *in silico* strategy to investigate the potential antimicrobial properties of volatile compounds isolated from *S. hispanicus* L. The primary objective of this computational analysis was to predict and evaluate the interactions between these bioactive molecules and key microbial proteins from selected bacterial and fungal pathogens. This approach aimed not only to assess the theoretical binding affinities of the tested compounds but also to provide valuable insights into their potential mechanisms of antimicrobial action at the molecular level. Ultimately, the findings contribute to the growing body of research supporting the use of plant-derived compounds as promising leads in the development of novel antimicrobial agents, particularly amid rising resistance to conventional antibiotics and antifungal drugs.

Given its traditional medicinal use and promising phytochemical profile, *S. hispanicus* L. represents a valuable candidate for such investigation.

Accordingly, the present study aims to (1) investigate the phytochemical profile of *S. hispanicus* L., (2) evaluate its antinutritional and antimicrobial activity through *in vitro* assays, and (3) explore the interaction of its major volatile compounds with microbial enzymes using *in silico* molecular docking techniques.

2. Materials and Methods

2.1. Plant Material Collection. *S. hispanicus* L. plants were collected during the flowering period in April 2022 by Dr. Roukia Zatout in the Larhat region of Tipaza, Algeria

(36°34′59.99″N, 2°25′0.01″E; Figure 1, panels A and B). The plant was taxonomically identified by Dr. Djamel Sarri, a botanist from the Department of Biology at the University of M'sila, Algeria, and its identity was further confirmed by molecular analysis under the reference code PQ112635. The entire aerial parts of the plant were thoroughly rinsed with running water to remove any traces of dirt, then left to air dry for 10 days. After drying, the plant material was ground into a fine powder using an electric mixer and stored in an airtight container for future use.

2.2. Extraction of Phytochemicals From Aerial Parts of *Scolymus hispanicus* L. Aerial parts of *Scolymus hispanicus* L. (50 g, powdered) were macerated in a hydro-methanolic solution (1:1, v/v) under continuous stirring at room temperature for 24 h, following the method of Masi et al. [13]. The extraction was repeated twice with fresh solvent, and the combined filtrates were concentrated under reduced pressure at 40°C. The resulting aqueous fraction was partitioned successively with *n*-hexane (2 × 200 mL) and dichloromethane (DCM) (2 × 200 mL) to remove lipophilic constituents. Organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated at 40°C. Extraction yield was calculated as the ratio of dry extract to initial plant material and expressed per 100 g of dry weight.

2.3. Phytochemical Analysis. Preliminary qualitative phytochemical tests were carried out according to Harborne [14] and Zatout et al. [15] to detect major classes such as tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids. The presence of compounds was indicated by characteristic color changes or precipitate formation.

2.3.1. Total Phenolic Content (TPC). TPC was determined using the Folin–Ciocalteu colorimetric method, as described by Makhoul et al. [16], with slight modifications. In brief, 200 μ L of appropriately diluted plant extract was mixed with 100 μ L of Folin–Ciocalteu reagent and allowed to stand for 4 min. Then, 800 μ L of 5% Na₂CO₃ solution was added, and the mixture was incubated at 40°C for 30 min in a water bath. The absorbance was measured at 750 nm using a microplate reader. TPC was calculated from a gallic acid calibration curve ($R^2 = 0.987$) made in the same conditions and expressed as mg of gallic acid equivalents per g of dry matter (mg GAE/g d.m.).

2.3.2. Total Flavonoid Content (TFC). TFC was assessed following the aluminum chloride colorimetric method described by Makhoul et al. [17]. Briefly, 500 μ L of extract was mixed with 2 mL of distilled water and 150 μ L of 5% NaNO₂ solution. After 6 min, 150 μ L of 10% AlCl₃ solution was added, followed by the addition of 2 mL of 1 M NaOH after another 6 min. The final volume was adjusted to 5 mL with distilled water. After 15 min of incubation at room temperature, absorbance was measured at 510 nm. Quercetin was used as the standard, and results were expressed as mg of quercetin equivalent per g dry matter (mg QE/g d.m.), based on a calibration curve prepared under the same conditions ($R^2 = 0.989$).



FIGURE 1: (a) The geographic location of Larhat region. (b) The plant studied by R. Zatout.

2.3.3. HS-SPME-GC-MS Analysis of *Scolymus hispanicus* L. Dry Powder. The volatile chemical composition of *S. hispanicus* L. dry powder was performed by HS-SPME sampling technique followed by GC-MS analysis [18–21]. About 0.5 g of the powder was placed inside a 4-mL glass vial with PTFE-coated silicone septum. The extraction process of volatiles was carried out on an SPME device from Supelco (Bellefonte, PA) with 1-cm fiber coated with 50/30 μm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane). Before use, the fiber was conditioned at 270°C for 30 min. The equilibration time was obtained heating to 60°C for 10 min. After this time, the fiber was exposed to the headspace of the samples for 10 min at 60°C to capture and concentrate the volatiles. Lastly, the analytes were desorbed thermally in the GC injector maintained at 250°C for 2 min in splitless mode. The analysis was carried out on a Clarus 500 model PerkinElmer (Waltham, MA, USA) gas chromatograph coupled with a mass spectrometer equipped. The capillary column was a Varian Factor Four VF-5. To characterize the volatile composition of the sample, the operative conditions were set as follows: from 50°C to 220°C at 6°/min and finally held for 15 min. Helium was used as carrier gas at a constant rate of 1 mL/min. MS scans were recorded within the range 35–500 m/z using EI ionization (energy 70 eV). Identification of compounds was based on the comparison of the mass spectra of pure components stored in the Wiley 2.2 and Nist 11 libraries database and on the

comparison of the linear retention indices (LRIs) calculated using a series of alkane standards (C_8 – C_{25} *n*-alkanes) with that available retention data reported in the literature. The relative amounts of the constituents were expressed as percentages (mean of three replicates) without the use of an internal standard and any factor correction.

2.3.4. Antidenaturation Activity. The protective effect of the extracts against protein denaturation was determined using a bovine serum albumin (BSA) model, adapted from Bailey-Shaw et al. [22]. A 0.4% (w/v) BSA solution was freshly prepared in Tris-buffered saline (TBS, pH 6.4, adjusted with glacial acetic acid when necessary). Each extract (*n*-hexane and DCM) was dissolved in distilled water and tested at concentrations ranging from 0.125 to 4.0 mg/mL. For each assay, 1 mL of BSA solution was mixed with the appropriate volume of extract and distilled water to reach a final volume of 2.5 mL. Aspirin, prepared at equivalent concentrations, served as a positive reference, while a mixture of BSA and distilled water was used as the negative control. The mixtures were incubated at 37°C for 15 min, followed by heating at 70°C for 10 min to induce protein denaturation. After cooling to room temperature, turbidity was measured spectrophotometrically at 660 nm. Inhibition of protein denaturation was expressed as a percentage relative to the untreated control, calculated as follows:

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100. \quad (1)$$

2.4. Antimicrobial Activity

2.4.1. Antibacterial Assay. The antibacterial activity of *S. hispanicus* L. extracts was assessed using the agar disk diffusion method as described by Balouiri et al. [23] with minor modifications. Test organisms included three Gram-positive strains, *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), and *Listeria monocytogenes* (CIP 82110), and two Gram-negative strains, *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027). Extracts (*n*-hexane and DCM) were diluted in dimethylsulfoxide (DMSO) to obtain concentrations of 5%, 10%, 25%, 50%, and 100% (v/v). Bacterial suspensions from 24-h cultures were evenly spread on Mueller–Hinton agar (MHA) plates. Sterile discs (6 mm diameter) were impregnated with 10 μ L of each extract and placed onto the inoculated agar surface. After a 2-h prediffusion at 4°C, the plates were incubated at 37°C for 24 h. Antibacterial activity was expressed as the diameter (mm) of the inhibition zone surrounding the discs.

2.5. Antifungal Assay

2.5.1. Well Diffusion Method. The antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans* (ATCC 10231) was tested using the well diffusion method on Sabouraud Dextrose Agar (SDA), as described by Balouiri et al. [23] with slight adjustments. A suspension of fungal cells (10^4 CFU/mL) was evenly spread over the agar surface. Wells (6 mm diameter) were aseptically created and filled with 20 μ L of extract solutions at different concentrations. DMSO served as the negative control. Plates were kept at 4°C for 2 h to allow diffusion and then incubated at 28°C for 72 h. Activity was determined by measuring the diameter of inhibition zones around the wells.

2.5.2. Direct Contact Technique. The antifungal efficacy against phytopathogenic fungi (*Fusarium* sp. and *Botrytis cinerea*) was assessed using the direct contact method, as adapted from Abdel-Motaal et al. [24], with slight modifications. A 6-mm mycelial disc of actively growing fungus was placed at one end of a fresh SDA plate. Opposite to it, at 50 mm, a 9-mm sterile disc was loaded with 80 μ L of the test extract (at 10% or 100% concentration).

The activity against phytopathogenic fungi (*Fusarium* sp. and *Botrytis cinerea*) was evaluated by the direct contact method, adapted from Abdel-Motaal et al. [24]. A 6-mm fungal mycelial disc was placed at one end of an SDA plate, while at the opposite end (50 mm away), a sterile 9-mm disc impregnated with 80 μ L of the extract (10% or 100%) was placed. Control plates contained only fungal discs without extract. All plates were incubated at 25°C for 7 days, after which radial mycelial growth was recorded. Growth inhibition was calculated using the equation proposed by Ting et al. [25]:

$$\% \text{ inhibition} = \frac{R_1 - R_2}{R_1} \times 100, \quad (2)$$

where R_1 is the radial growth in the control plate and R_2 is the radial growth in the treatment plate.

2.6. Statistical Analysis. All experiments were carried out in triplicate, and data are presented as mean $\pm$ standard deviation (SD). The effects of solvent type and extract concentration induced by *n*-hexane and DCM extracts were evaluated using two-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons. Statistical analyses were performed using XLSTAT software (Addinsoft SARL, New York, NY, USA). Differences were considered statistically significant at $p \leq 0.05$.

2.7. Molecular Docking Study. The molecular docking study was performed using the Molecular Operating Environment (MOE, 2020.09) software to predict the interaction between volatile compounds identified from *S. hispanicus* L. and microbial target proteins [26], following a previously described procedure [27]. The chemical structures of the identified compounds were retrieved in SMILES format from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). These SMILES were imported into MOE, where the molecules were prepared by standardizing protonation states, adding hydrogen atoms, assigning Gasteiger partial charges, and correcting any structural issues to ensure accurate molecular modeling. Energy minimization of the ligands was then performed using the MMFF94x force field to generate low-energy conformers suitable for docking simulations.

For the bacterial targets, dihydrofolate reductase (DHFR) enzymes from *S. aureus* (PDB ID: 3FRA), *E. coli* (PDB ID: 6CQA), and *B. subtilis* (PDB ID: 8UVZ) were downloaded from the Protein Data Bank (PDB). The structures were prepared in MOE by removing water molecules and nonrelevant chains, correcting bond orders, adding hydrogens, and energy minimizing the system using the AMBER10:EHT force field [28, 29]. For these targets, the native cocrystallized ligands were redocked into their respective binding pockets to validate the docking protocol by comparing the predicted binding poses with the experimental ones using RMSD values [30, 31].

For species with no available 3D structures in the PDB, such as *L. monocytogenes* (UniProt ID: L7VTF4) and *P. aeruginosa* (UniProt ID: A0A5P9WCV3), the DHFR protein models were retrieved from the AlphaFold Protein Structure Database. Binding sites on these predicted structures were identified using the Site Finder tool in MOE, which detects potential active pockets based on the geometry and physicochemical properties of the surface.

Regarding fungal pathogens, the sterol 14- α demethylase (CYP51) enzyme structures of *C. albicans* (PDB ID: 5TZ1) and *S. cerevisiae* (PDB ID: 5EAF) were obtained from the PDB and prepared similarly as described above.

TABLE 1: Phytochemical screening of *Scolymus hispanicus* L. extracts.

Alkaloids	Reducing compounds	Coumarins	Tannins	Flavonoids	Sterols/triterpenes	Saponin
–	++	++	++	+	+++	+

Note: +++, Fairly positive: ++, Weakly positive: +, Negative test: –.

TABLE 2: Total phenolic and flavonoid contents of *Scolymus hispanicus* L. extracts.

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
HEX	23.2 ± 1.1 ^b	10.9 ± 0.8 ^b
DCM	55.0 ± 1.6 ^a	42.5 ± 1.2 ^a

Note: Values are expressed as mean ± standard deviation ($n = 3$). Different letters within the same column indicate significant differences ($p \leq 0.05$, one-way ANOVA followed by Tukey's HSD test).

TABLE 3: Chemical composition of *Scolymus hispanicus* L. (percentage mean values).

N.	Component	LRI ^{calc}	LRI ^{lit}	%
1	α -Pinene	938	941	30.9
2	Camphene	941	943	0.8
3	β -Pinene	978	980	1.9
4	p-Cymene	1015	1022	1.7
5	Limonene	1021	1029	4.0
6	1,8-Cineole	1028	1033	1.6
7	trans- β -Ocimene	1051	1048	1.3
8	Decane, 4-methyl-	1056	1054	0.6
9	4-Oxoisophorone	1113	1108	0.6
10	Undecane	1117	1115	1.9
11	3,4-Dimethylcyclohexanol	1124	1126	0.5
12	Octanoic acid	1168	1170	5.0
14	Safranal	1184	1181	1.1
13	n-Octyl acetate	1190	1191	0.7
16	Dodecane, 2,6,11-trimethyl-	1270	1275	6.8
15	Dodecan, 4,6-dimethyl-	1279	1285	1.9
17	Tridecane	1289	1291	3.1
18	Farnesane	1375	1381	2.6
19	Tetradecane	1408	1413	0.9
20	Megastigmatrienone	1465	1473	1.1
22	2,4-di-tert-Butylphenol	1510	1512	2.4
24	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	1518	1525	10.1
21	1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	1538	1541	3.7
23	Lauric acid	1546	1550	4.2
25	Caryophyllene oxide	1575	1580	1.9
26	Hexadecane, 1,2-epoxy	1672	1676	1.9
27	Hexahydrofarnesyl acetone	1852	1846	6.9
	Total			99.7

Note: The components are reported according to their elution order on a polar column. Linear retention indices measured on a polar column. Linear retention indices from the literature.

The docking protocol was validated through redocking of the cocrystallized ligands. For *F. graminearum* (UniProt ID: I1RBR4) and *B. cinerea* (UniProt ID: Q9HDR2), the CYP51 protein models were obtained from AlphaFoldDB, and their binding sites were also predicted using the MOE Site Finder tool. All docking simulations were carried out using the default docking protocol in MOE. The triangle matcher placement method was used to position ligands in the binding sites, and the London dG scoring function was applied for initial scoring. Final poses were refined using the force-field-based refinement, and the binding energies were

rescored with the GBVI/WSA dG scoring function to estimate the binding free energy [32, 33]. The top-ranked docking poses for each ligand–receptor pair were analyzed for hydrogen bonding, hydrophobic contacts, and π – π stacking interactions using Discovery Studio Visualizer (version 21.1.0.20298).

3. Results and Discussion

In this study, the aerial parts of *S. hispanicus* L. were extracted using *n*-hexane and DCM. Initial sample weight

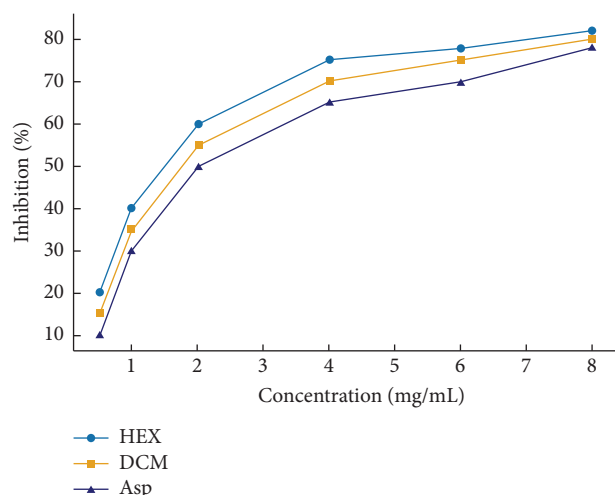


FIGURE 2: Effect of *n*-hexane and dichloromethane extracts of *Scolymus hispanicus* L. on the inhibition of protein (BSA) denaturation.

was 50 g. The highest percentage yield of solid residue was obtained with DCM extract at 2.404%, while the *n*-hexane extract yielded a lower percentage with 0.33%.

3.1. Phytochemical Screening. The phytochemical screening of *S. hispanicus* L. demonstrated positive results for tannin, flavonoids, sterols, terpenoids, coumarin, and glycosides except for alkaloids and saponins as shown in Table 1.

3.1.1. TPC and TFC. TPC and TFC of the *Scolymus hispanicus* extracts were quantified using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. The results are expressed as mg GAE/g extract for TPC and mg QE/g extract for TFC, as shown in Table 2.

Significant differences ($p \leq 0.05$) were observed between the two extraction solvents. The DCM extract exhibited significantly higher levels of both TPC and TFC compared to the HEX extract. Specifically, the TPC in the DCM extract was approximately 2.4 times higher, while the TFC was nearly four times greater. These differences can be attributed to the differences in solvent polarity [34]. DCM, being more polar than *n*-hexane, is more effective at extracting semipolar phenolics and flavonoids molecules. In contrast, HEX, a nonpolar solvent, is less efficient at solubilizing such compounds.

This observation is consistent with previous studies. For instance, Sultana et al. [35] found that ethyl acetate and acetone extracts yielded significantly higher polyphenolic contents than *n*-hexane extracts in various medicinal plants. Similarly, Do et al. [36] highlighted the critical role of solvent polarity in optimizing the extraction of phenolics, with polar and semipolar solvents such as methanol, ethanol, and DCM demonstrating greater efficiency. Furthermore, Aboukhalaf et al. [10] reported comparably high TPC levels (~58 mg GAE/g extract) in methanolic extracts of *S. hispanicus* L., reinforcing the phenolic richness of the species when extracted with semipolar solvents.

3.2. Volatile Chemical Composition of *Scolymus hispanicus* L. Dry Powder. The GC–MS analyses allowed to identify 27 volatile components emitted from headspace of *S. hispanicus* powder listed in Table 3. Monoterpenes, fatty acids, and other chemical classes were found. The volatile fraction showed α -pinene (30.9%) as the major component followed by 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (10.1%), dodecane, 2,6,11-trimethyl (6.8%), octanoic acid (5.0%), lauric acid (4.2%), and limonene (4.0%).

The major volatile constituent was α -pinene (30.9%), a monoterpene hydrocarbon known for its pine-like aroma and well-documented antimicrobial, anti-inflammatory, and antioxidant properties [37, 38]. The high proportion of α -pinene suggests it may play a critical role in the characteristic aroma and potential biological activity of *S. hispanicus*. The second most abundant compound, 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (10.1%), is an oxygenated heterocyclic compound possibly contributing to the sweet, woody notes in the plant's aroma profile. Although less common in plant headspaces, such furanone derivatives can exhibit potent bioactivities, including antioxidant and enzyme-inhibitory effects [39, 40]. Significant levels of aliphatic hydrocarbons were also observed, notably dodecane, 2,6,11-trimethyl (6.8%) and hexahydrofarnesyl acetone (6.9%), both of which are often linked to wax components, possibly influencing the plant's texture or surface chemistry [41]. Fatty acids like octanoic acid (5.0%) and lauric acid (4.2%) further enrich the volatile profile. These medium-chain fatty acids are known for their antimicrobial and emulsifying properties, potentially contributing to the preservation of dried plant material or its interaction with microbes [42, 43]. Among other notable terpenoids, limonene (4.0%) and β -pinene (1.9%) also contribute to the aromatic complexity, imparting citrus and resinous notes, respectively [44, 45]. Their presence reinforces the plant's aromatic appeal and suggests functional roles in plant defense or pollinator attraction. Compounds such as safranal (1.1%), megastigmatrienone (1.1%), and caryophyllene oxide (1.9%), though present in relatively low concentrations, are notable for their well-established sensory and pharmacological activities. Safranal, a major aromatic constituent of saffron, exhibits potent antioxidant and anticonvulsant properties, while caryophyllene oxide is widely recognized for its antifungal activity [46].

The detection of 2,4-di-tert-butylphenol (2.4%) is particularly noteworthy. Although typically considered a synthetic compound or a degradation byproduct, it has occasionally been reported in natural matrices [47]. Its presence in *S. hispanicus* L. may be either endogenous or the result of external factors, and thus merits further investigation to determine its origin.

The dominance of monoterpenes, particularly α -pinene and limonene, suggests that *S. hispanicus* L. follows biosynthetic pathways characteristic of the Asteraceae family. The co-occurrence of volatile terpenoids and fatty acid derivatives indicates a dual functional strategy, potentially serving both defensive roles and functions related to pollinator attraction or seed dispersal [48, 49].

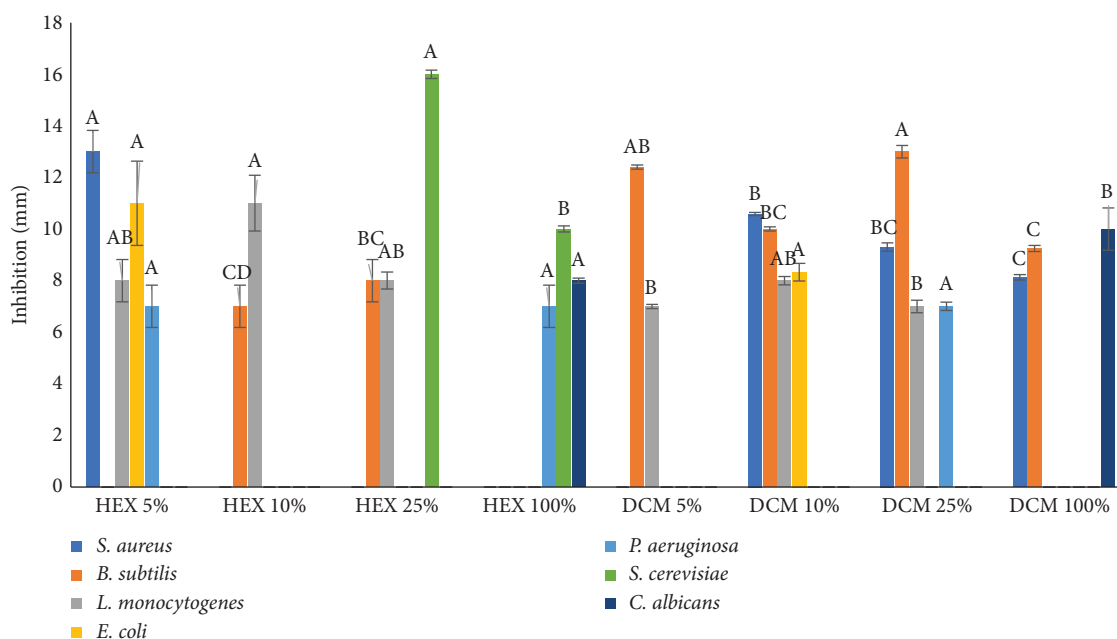


FIGURE 3: Diameters of the inhibition zones of *Scolymus hispanicus* extracts at different concentrations against selected microorganisms. Vertical bars represent standard error ($n = 3$). Different letters indicate significant differences at $p \leq 0.05$ according to two-way ANOVA followed by Tukey's HSD test.

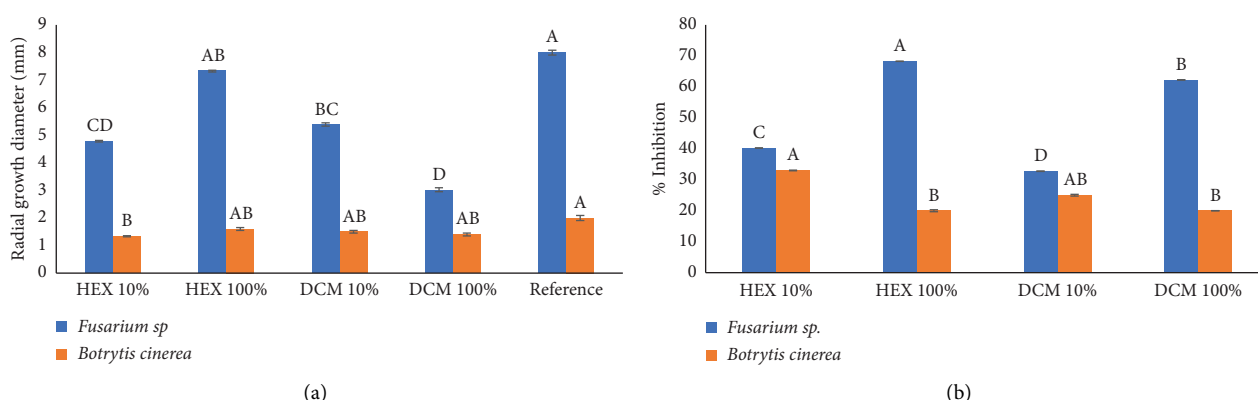


FIGURE 4: (a) Radial growth diameters and (b) percentage inhibition of *Fusarium sp.* and *Botrytis cinerea* treated with *S. hispanicus* L. extracts. Vertical bars represent standard error ($n = 3$). Different letters indicate significant differences at $p \leq 0.05$ according to two-way ANOVA followed by Tukey's HSD test.

Overall, the volatile chemical profile of *S. hispanicus* L. aligns with its traditional use in Mediterranean ethnobotany, especially for digestive and anti-inflammatory applications. The abundance of bioactive volatiles may significantly contribute to both its medicinal efficacy and its organoleptic qualities.

3.3. Antidenaturation Activity. The results of the bovine serum albumin (BSA) antidenaturation assay revealed a significant anti-inflammatory activity of the nonpolar extracts of *S. hispanicus* L. (Figure 2). The *n*-hexane extract (HEX) exhibited the highest inhibition percentage, exceeding 70% even at low concentrations, surpassing the reference drug aspirin. This strong activity suggests the presence of bioactive anti-inflammatory compounds, which

is supported by the volatile analysis showing a high content of α -pinene (27.1%) and limonene (3.5%), both known for their anti-inflammatory properties. In contrast, the DCM extract showed moderate activity, with inhibition ranging between 10% and 40%, indicating the presence of moderately polar bioactive compounds. These findings highlight the potential role of volatile constituents in the anti-inflammatory activity of *S. hispanicus* L. and validate its traditional use in folk medicine.

3.4. Antimicrobial Activity. Figure 3 illustrates the antimicrobial activity of *S. hispanicus* L. extracts prepared using hexane (HEX) and DCM at different concentrations (5%, 10%, 25%, and 100%) against a range of bacterial and fungal

TABLE 4: Binding energies (kcal/mol) of major volatile compounds from *Scolymus hispanicus* L. with dihydrofolate reductase (DHFR) from the tested bacterial strains.

Bacterial strain (PDB ID)	Ligand type	Compound name	Binding energy (kcal/mol)	Hydrogen interactions (distance Å)	Hydrophobic interactions
<i>S. aureus</i> (3FRA)	Cocrystallized ligand	Iclaprim	-8.83	Asp27 (2.53), Phe92 (2.59), Phe92 (2.95), Leu5 (2.63), Tyr98 (3.24)	Leu20 (2), Ala7, Phe92 (2), Leu5, Val31 (2), Ile50, Leu28 (2)
	Best docked compound	2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	-6.72	Ala7 (2.36), Val6 (2.85)	Leu20 (2), Phe92 (3), Val31 (2)
		Lauric acid	-6.42	Ala7 (2.45), Val6 (2.60)	Leu20, Ile50
		Propanedioic acid, propyl-	-6.26	Val6 (2.84), Ala7 (1.86)	Val31
<i>E. coli</i> (6CQA)	Cocrystallized ligand	PQD	-8.02	Ile5 (2.36), Ile94 (2.95), Ala6 (2.65), Asp27 (2.48)	Ala7, Phe31 (3), Ile5, Met16, Leu28, Ile50
	Best docked compound	Hexadecene, 1,2-epoxy	-7.42	Ala7 (3.16), Gly15 (2.54)	Leu28, Ile50, Phe31 (2), Met16 (2), Ala7, Ala6
		Hexahydrofarnesyl acetone	-7.33	Ala6 (2.82), Ala7 (2.53)	Met16 (2), Phe31 (2), Leu28, Ala7, Ile50
		(Z)-7-Hexadecenal	-7.15	Ala6 (2.60), Ala7 (3.15)	Phe31, Ile94, Met16 (2), Ile50, Leu28, Pro55, Leu54
<i>B. subtilis</i> (8UVZ)	Cocrystallized ligand	Folic acid	-8.52	Ala29 (2.95), Arg57 (1.99), Lys32 (1.94), Asp27 (2.29), Ile95 (2.62)	Ile50, Leu28, Phe31 (2), Ala7, Phe6
	Best docked compound	Hexadecene, 1,2-epoxy	-7.85	Phe6 (2.33, 2.62), Ala7 (1.95, 2.55), Ile14 (2.36)	Ala29, Lys32, Leu54 (2), Phe31 (2), Leu20, Ile50, Leu28 (2)
		Propanedioic acid, propyl-	-7.12	Ala7 (2.71), Phe6 (3.25), Tyr101 (2.82), Ile95 (2.39)	Phe31, Ile5, Ile95
		2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	-6.75	Phe6 (3.40), Ala7 (2.66)	Ile95, Ile50, Phe31, Leu28 (2), Leu20 (3)
<i>L. monocytogenes</i> (L7VTF4)	Best docked compound	Octyl acetate	-7.56	Ala8 (3.12), Ala7 (2.65), Phe96 (2.33), Tyr102 (3.05)	Lys46
		Hexadecene, 1,2-epoxy	-6.52	Ala7 (2.74), Ala8 (2.51)	Arg45, Lys45, Ile21, Ile15 (2)
		Lauric acid	-6.21	Ala7 (2.36), Ala8 (2.49)	Lys46, Ile15
<i>P. aeruginosa</i> (A0A5P9WCV3)	Best docked compound	Hexadecene, 1,2-epoxy	-7.32	Ala9 (2.63), Ile7 (2.48), Asp29 (3.45, 2.66)	Met56 (2), Met53, Ile116, Phe33 (2)
		Octyl acetate	-7.06	Ala8 (2.70), Ala9 (2.84), Tyr122 (2.37)	Phe16, Ile22
		Lauric acid	-6.72	Val8 (2.66), Ala9 (2.93)	Met56, Ile22, Met53, P

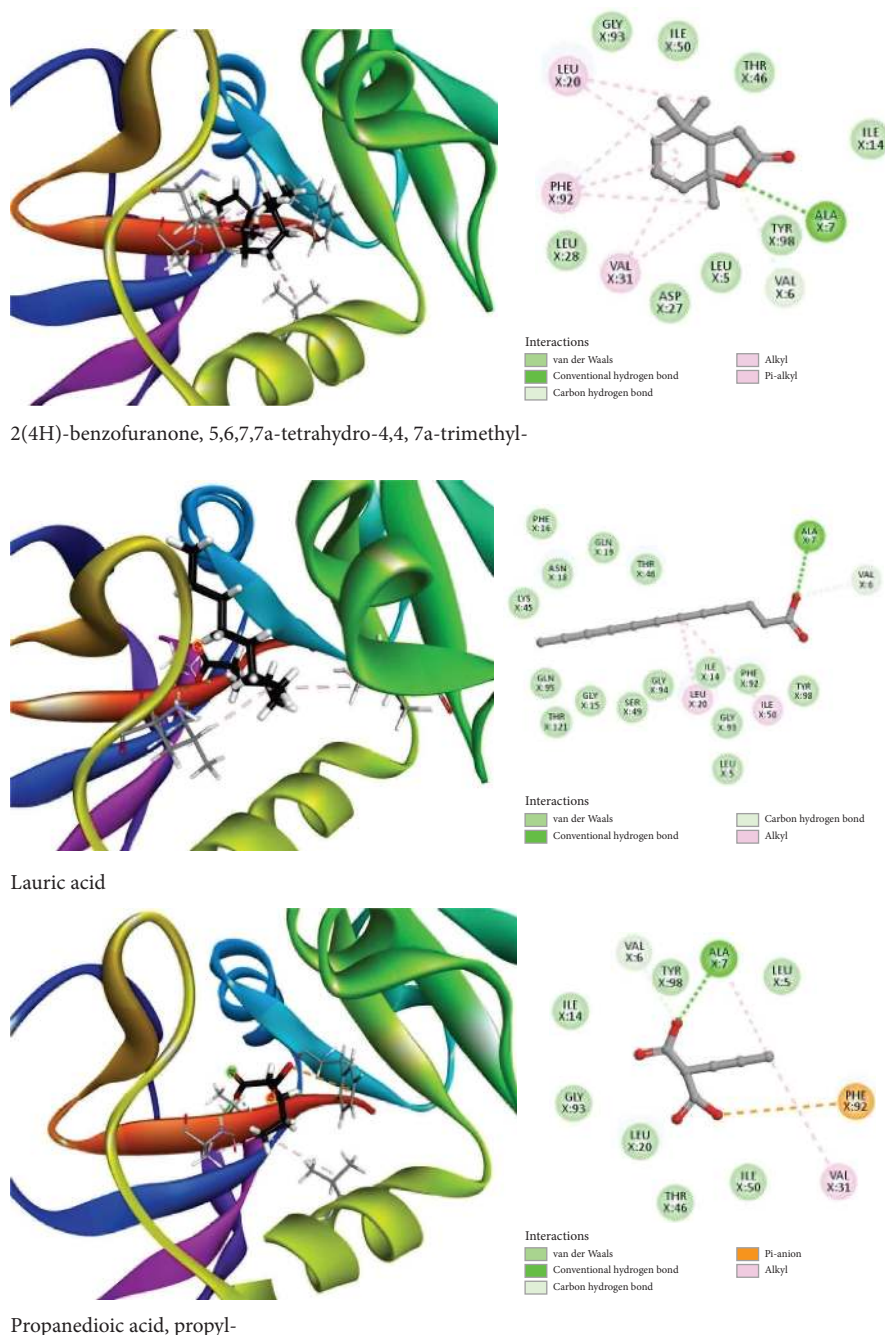


FIGURE 5: 2D and 3D molecular docking interaction diagrams of the top-ranked compound with *S. aureus* DHFR (PDB ID: 3FRA).

strains. The activity was assessed by measuring the diameters of the inhibition zones (in mm), with statistical significance was evaluated using a two-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$).

The antimicrobial activity of *S. hispanicus* L. extracts varied significantly depending on the solvent type, concentration, and microbial strain. A strictly dose-dependent relationship was not observed. Instead, intermediate concentrations (notably 10% and 25%) often showed the strongest inhibitory effects, while the 100% extracts were frequently less effective or inactive. Overall, DCM extracts

exhibited broader-spectrum antimicrobial activity, particularly at intermediate concentrations. In contrast, HEX extracts were more selective and often required higher concentrations to be effective, especially against fungal strains. Among the bacterial strains, *S. aureus* was most sensitive to 5% HEX extract (13.00 ± 0.82 mm), significantly outperforming all other treatments, while moderate inhibition was observed with 10% DCM (10.58 ± 0.06 mm). *Bacillus subtilis* exhibited the highest inhibition with 25% DCM (13.00 ± 0.24 mm), followed by a moderate effect from 25% *n*-hexane (8.00 ± 0.82 mm). *Listeria monocytogenes*

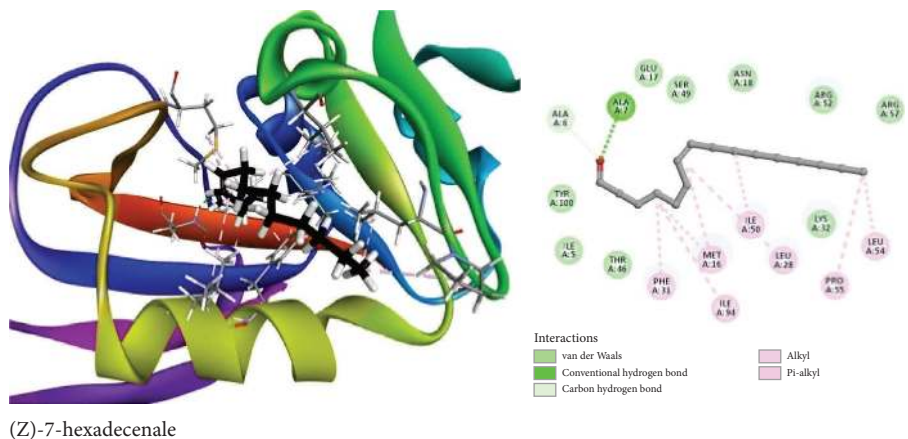
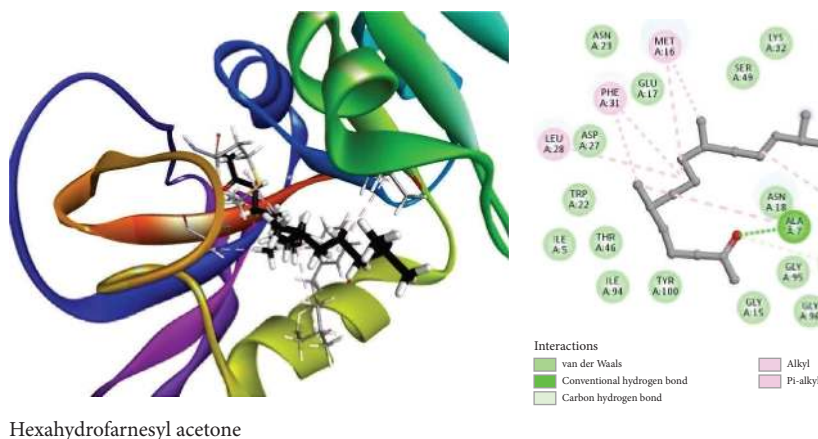


FIGURE 6: 2D and 3D molecular docking interaction diagrams of the top-ranked compound with *E. coli* DHFR (PDB ID: 6CQA).

Regarding the fungal strains, *S. cerevisiae* was inhibited only at higher HEX concentrations (25% and 100%), whereas *C. albicans* showed sensitivity exclusively to 100% extracts of both solvents.

These findings highlight that the antimicrobial activity of *S. hispanicus* L. extracts is influenced by a complex interplay of solvent polarity, extract concentration, and microbial sensitivity as previously reported [50, 51]. The frequent observation of greater inhibition at intermediate concentrations suggests that increased concentration does not necessarily enhance bioactivity, which may be attributed to factors such as compound aggregation, reduced solubility, or limited diffusion through the agar medium [23, 52]. These results are in line with previous studies showing that the antimicrobial effectiveness of plant-derived compounds does not always scale linearly with concentration, and

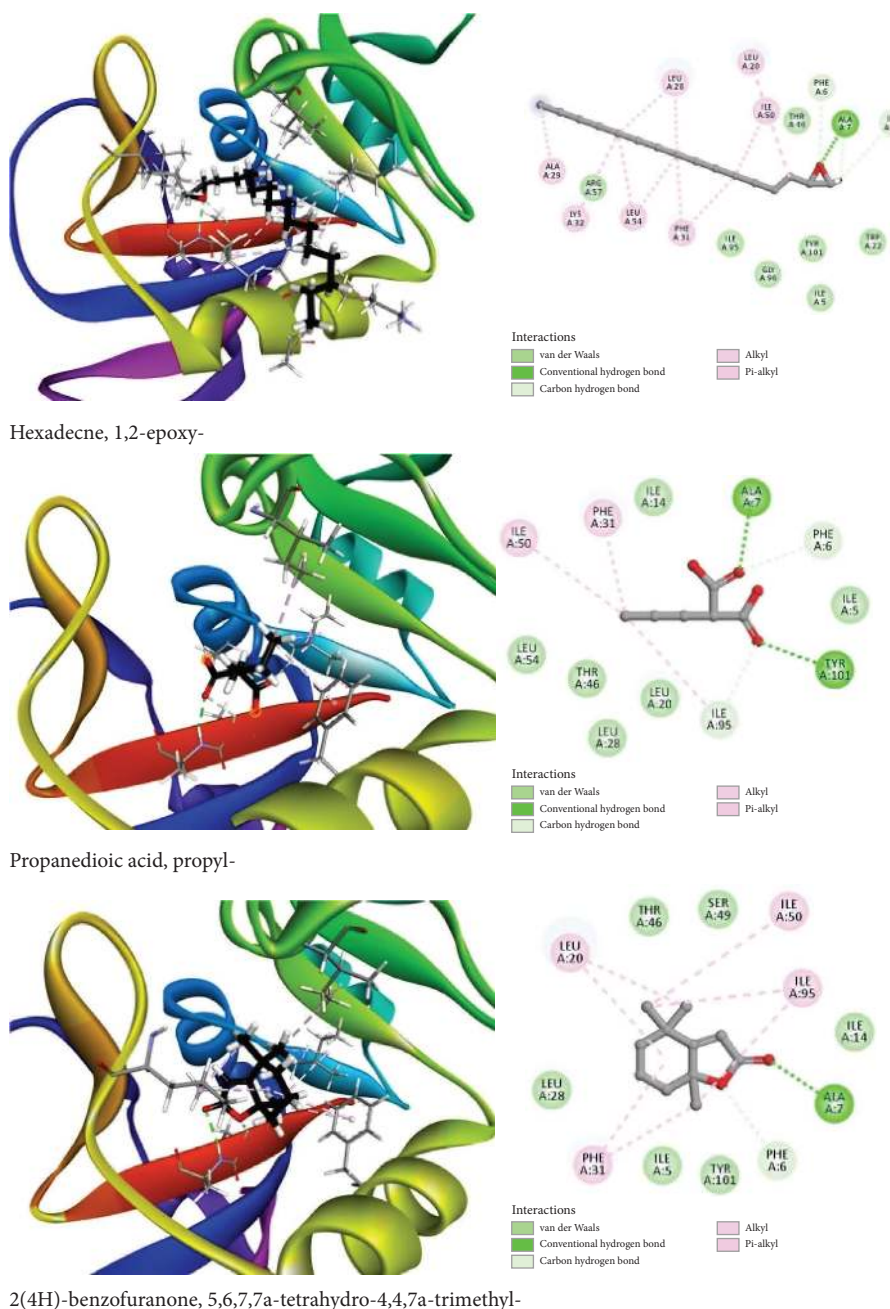


FIGURE 7: 2D and 3D molecular docking interaction diagrams of the top-ranked compound with *B. subtilis* DHFR (PDB ID: 8UVZ).

optimal bioactivity often occurs at intermediate levels [50, 52]. Additionally, the choice of extraction solvent played a pivotal role; DCM extracts were more potent against bacterial strains, while HEX extracts demonstrated stronger antifungal effects, particularly at higher concentrations. Such solvent-dependent variation has been well documented and underscores the importance of selecting appropriate solvents to target specific microbial groups [50, 51]. Furthermore, our use of the agar-based diffusion technique, known for its sensitivity to compound infusibility and media interactions, may explain some of the observed variability and have been extensively validated in previous antimicrobial screening studies [23, 53].

To further investigate the antifungal potential of *S. hispanicus* L. extracts, radial growth diameters and inhibition percentages were assessed for two phytopathogenic fungi, *Fusarium* sp. and *Botrytis cinerea*, using the disc diffusion method and direct contact technique, respectively. The results are presented in Figure 4.

The antifungal activity of *S. hispanicus* L. extracts revealed a significant difference in pathogen sensitivity based on both solvent type and extract concentration. For *Fusarium* sp., the 100% HEX extract showed the highest radial growth, indicating weak antifungal effect, while 100% DCM extract significantly reduced fungal growth, suggesting potent activity. Conversely, *Botrytis cinerea* exhibited relatively

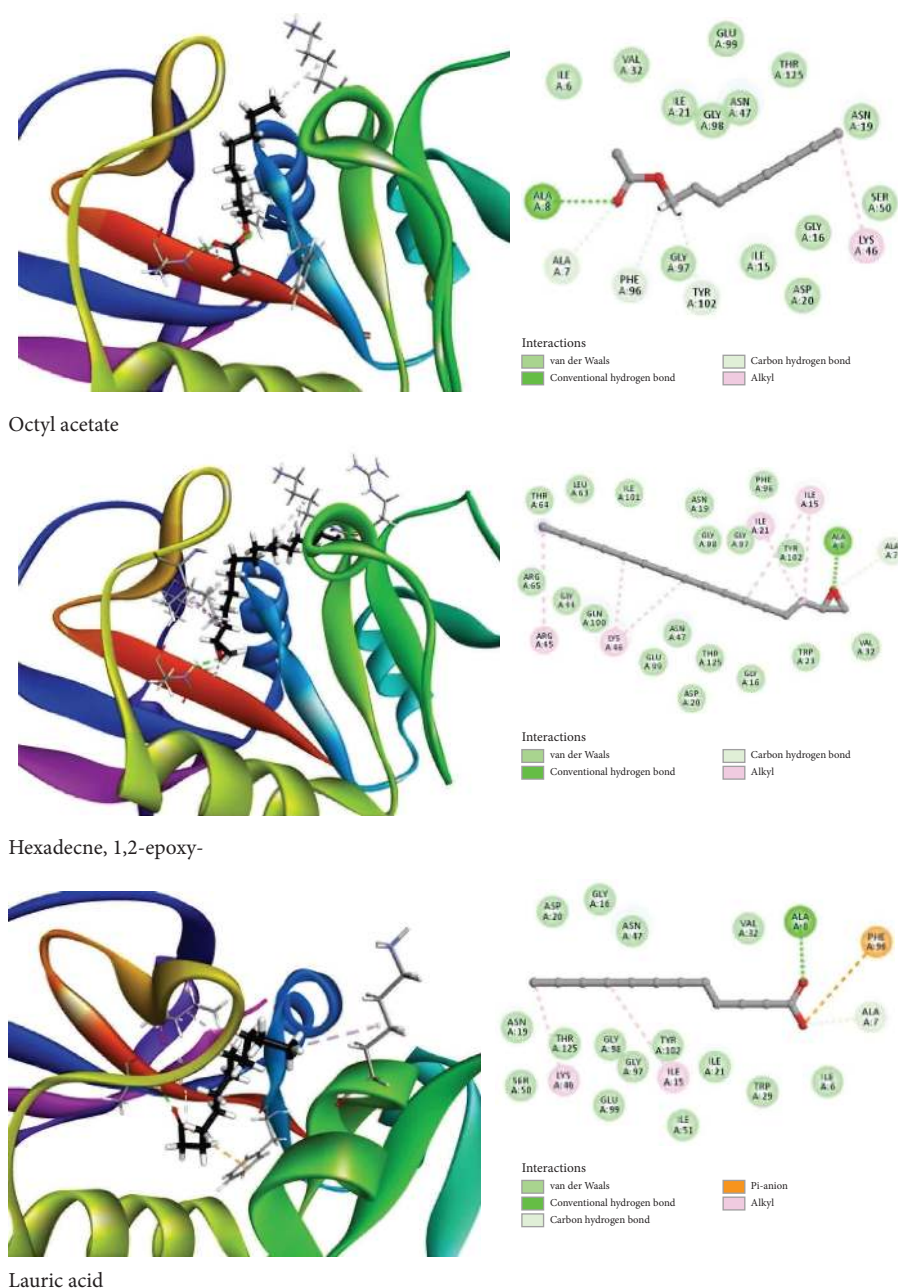


FIGURE 8: 2D and 3D molecular docking interaction diagrams of the top-ranked compound with *L. monocytogenes* DHFR (UniProt ID: L7VTF4).

consistent sensitivity across treatments, with minor differences among extracts. Notably, DCM 100% maintained strong efficacy against both fungi. These results reinforce that *S. hispanicus* L. extracts exhibit pathogen-specific antifungal activity influenced by solvent type and concentration [34, 54]. Importantly, maximal inhibition did not always correspond to higher concentrations, suggesting that both extracts polarity and compound bioavailability play critical roles in determining antifungal potency [55, 56].

These findings further emphasize the importance of solvent selection and concentration optimization when developing plant-derived antimicrobial agents [23, 50].

3.5. Molecular Docking. In our *in silico* study, we selected DHFR as the enzymatic target for all the tested bacterial strains. This choice was motivated by the enzyme's essential role in the folate biosynthetic pathway, which is crucial for DNA synthesis and cell proliferation in bacteria [57, 58]. DHFR is a well-conserved enzyme across Gram-positive and Gram-negative bacteria, and it represents a validated antimicrobial target for several clinically used antibiotics, such as trimethoprim and iclaprim [59, 60]. By targeting a single conserved enzyme, our aim was to evaluate the broad-spectrum antimicrobial potential of the volatile compounds identified from *S. hispanicus* L.

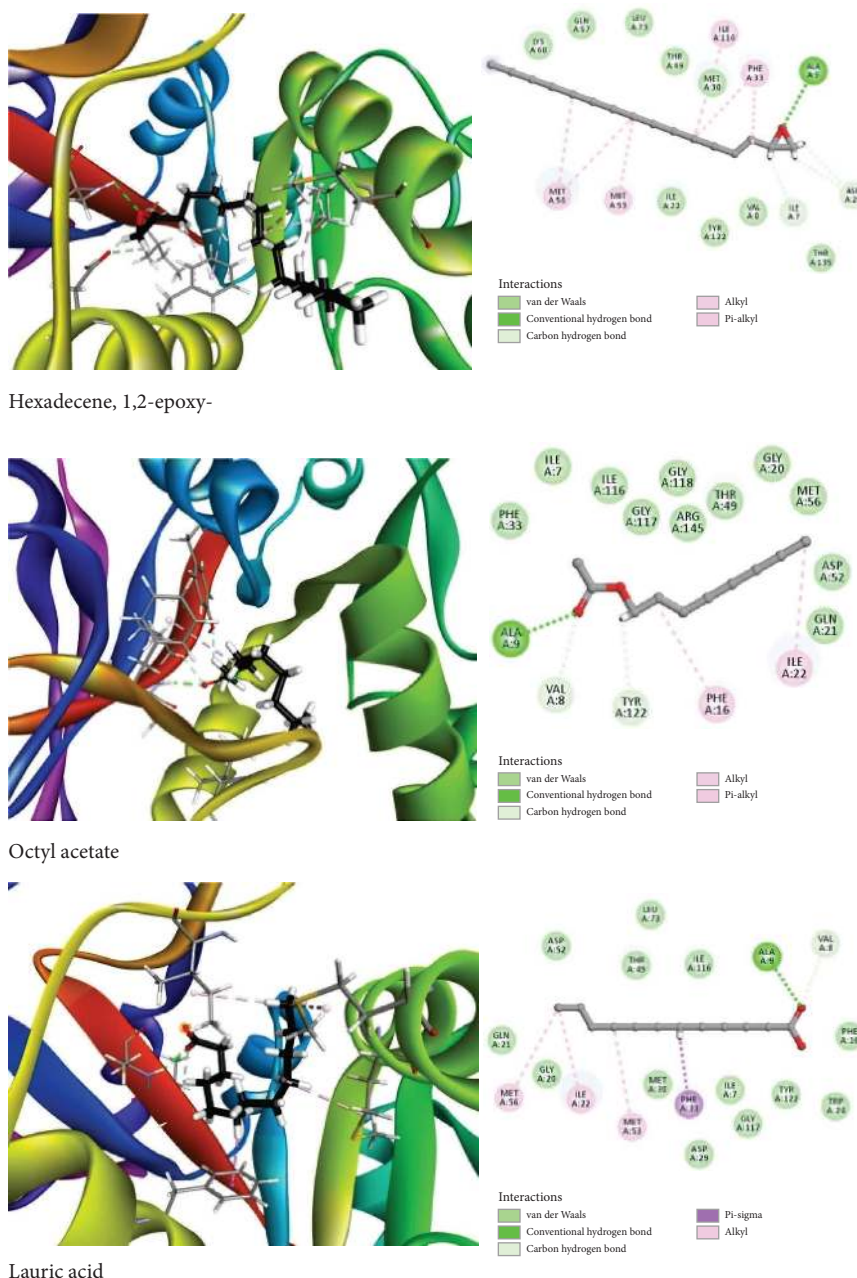


FIGURE 9: 2D and 3D molecular docking interaction diagrams of the top-ranked compound with *P. aeruginosa* DHFR (UniProt ID: A0A5P9WCV3).

A total of 27 compounds, identified via SPME-GC-MS analysis, were docked against the DHFR structures of five pathogenic bacteria: *S. aureus*, *Escherichia coli*, *B. subtilis*, *L. monocytogenes*, and *P. aeruginosa*. The three top-performing ligands for each bacterial strain were selected for detailed analysis, based on their binding energies and interaction profiles.

As shown in Table 4 and illustrated in Figures 5, 6, 7, 8, and 9, the selected ligands displayed stable binding within the active site of DHFR, forming both hydrogen bonds and hydrophobic interactions with key catalytic residues.

For *S. aureus* (Figure 5), the compound 2(4H)-benzo-furanone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl showed a binding energy of -6.72 kcal/mol and established hydrogen bonds with Ala7 and Val6, as well as hydrophobic contacts with Leu20, Phe92, and Val31. Although less potent than the reference ligand iclaprim (-8.83 kcal/mol), this interaction profile suggests a strong binding affinity to the active site.

For *E. coli* (Figure 6), hexadecene and 1,2-epoxy emerged as the best binder (-7.42 kcal/mol), forming hydrogen bonds with Ala7 and Gly15, and hydrophobic interactions involving Phe31, Leu28, and Ile50. A similar trend was

TABLE 5: Binding energies (kcal/mol) of major volatile compounds from *Scolymus hispanicus* with fungal CYP51 enzymes.

Fungal strain (PDB ID)	Ligand type	Compound name	Binding energy (kcal/mol)	Hydrogen interactions (distance Å)	Hydrophobic interactions
<i>C. albicans</i> (5TZ1)	Cocrystallized ligand	VT1161	-7.96	His377 (3.10), Met508 (3.54), Gly307 (3.32)	Pro230, Tyr64, His377, Met508, Leu376 (2), Tyr118
	Best docked compound	Hexahydrofarnesyl acetone	-7.62	His377 (2.75)	Phe233, Leu376 (3), Val509, His310, Phe228 (2), Tyr132, Tyr118 (2), Met508 (2), Leu121 (2)
		Propanedioic acid, propyl-	-6.84	Leu376 (2.89, 2.65), Ser378 (3.52), His377 (3.25, 2.52, 3.18)	Leu121, Phe233, Met508
		Octyl acetate	-6.52	His377 (2.65), Ser378 (3.45, 2.30, 2.66)	Leu376 (2), Phe233, Phe380, Tyr118, Leu121
<i>S. cerevisiae</i> (5EAF)	Cocrystallized ligand	Fluquinconazole	-7.58	Gly314 (3.24)	Leu380 (2), Ile139
	Best docked compound	Hexahydrofarnesyl acetone	-7.33	Tyr126 (2.35), Arg385 (2.40)	His378, Leu383, Leu380 (2), Cys470 (2), Leu158, Ile471, Val154, Val311
		(Z)-7-Hexadecenal	-6.84	Gly314 (2.65)	Val311, Cys470, Tyr140, Ile139, Tyr126
		Hexadecene, 1,2-epoxy	-6.60	Arg385 (3.22), His468 (2.84)	Val154, Ile139, Leu307, Lys151, Ile471, Val311, Leu380, Cys470, Leu383
<i>F. graminearum</i> (11RBR4)	Best docked compound	Hexahydrofarnesyl acetone	-8.11	Lys95 (3.42)	Lys95, Leu92 (2), Met378 (2), Phe235 (4), Leu93, Pro232, Leu510 (3), Ile374, His375
		(Z)-7-Hexadecenal	-7.64	Tyr123 (2.95)	Tyr69, Leu510, His375 (2), Leu93 (2), Phe235 (3), Leu92 (2), Met378, Lys95, Pro232
		Hexadecene, 1,2-epoxy	-7.25	Lys95 (3.44), Glu121 (3.19)	Ala508, Pro232, Leu92 (2), Phe235 (3), Ile122, Lys95
<i>B. cinerea</i> (Q9HDDR2)	Best docked compound	Hexahydrofarnesyl acetone	-8.30	Tyr113 (2.62)	Tyr113 (2), Met141, Ile459 (2), Lys138 (2), Val126 (2), Ala295, Ala299, Phe121 (2), Phe220, Leu498, Leu116
		(Z)-7-Hexadecenal	-7.30	Arg370 (2.95)	Phe499, Phe121, Ala299 (2), Val126, Met141, Leu296, Ile459, Ile365, Tyr113
		Hexadecene, 1,2-epoxy	-7.10	Tyr127 (3.25)	Ala464, Cys458 (3), Ile365, Ile368 (2), Tyr113

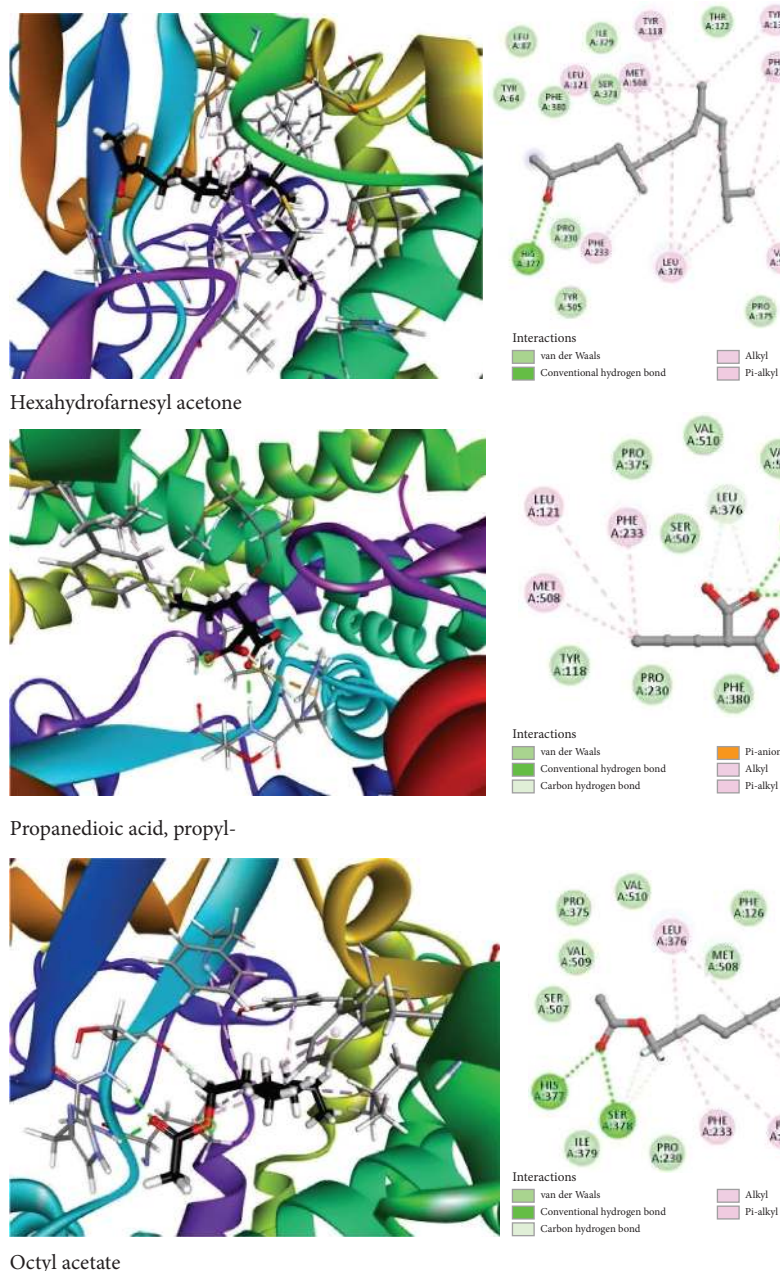


FIGURE 10: 2D and 3D molecular docking interaction diagrams of hexahydrofarnesyl acetone with *C. albicans* sterol 14- α demethylase (CYP51; PDB ID: 5TZ1).

observed in *B. subtilis* (Figure 3), where the same compound achieved the highest binding energy (-7.85 kcal/mol) and interacted with Phe6, Ala7, and Ile14 through hydrogen bonding, while engaging multiple hydrophobic residues in the catalytic pocket.

In *L. monocytogenes* (Figure 8), octyl acetate showed the best affinity (-7.56 kcal/mol), forming hydrogen bonds with Ala7, Ala8, Phe96, and Tyr102, along with interactions with Lys46. For *P. aeruginosa* (Figure 5), the top ligand was again hexadecane, 1,2-epoxy (-7.32 kcal/mol), which formed hydrogen bonds with Ala9, Ile7, and Asp29, and interacted hydrophobically with Met56, Phe33, and Ile116.

Importantly, several key residues such as Ala7, Phe31, and Leu28 were recurrently involved in the binding of multiple ligands across different bacterial DHFR enzymes. This pattern indicates a conserved binding mode, which supports the hypothesis of a shared mechanism of action.

These in silico findings are consistent with the in vitro antibacterial activities previously observed. Notably, *S. aureus*, *B. subtilis*, and *E. coli* exhibited moderate to strong inhibition zones with *Scolymus hispanicus* extracts, particularly with the CH_2Cl_2 fraction, which was rich in volatile compounds. The strong binding affinities of specific compounds, such as hexadecene, 1,2-epoxy, and octyl acetate,

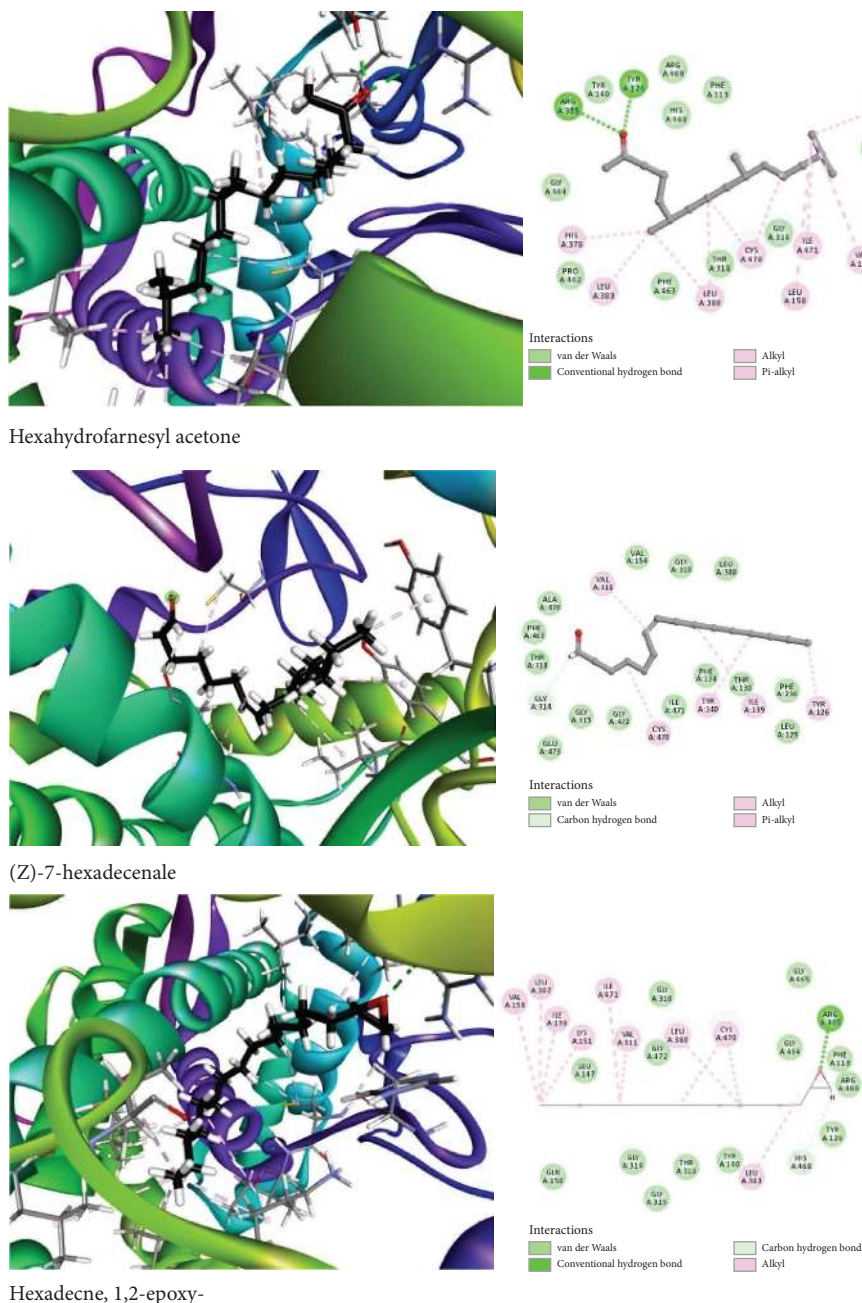


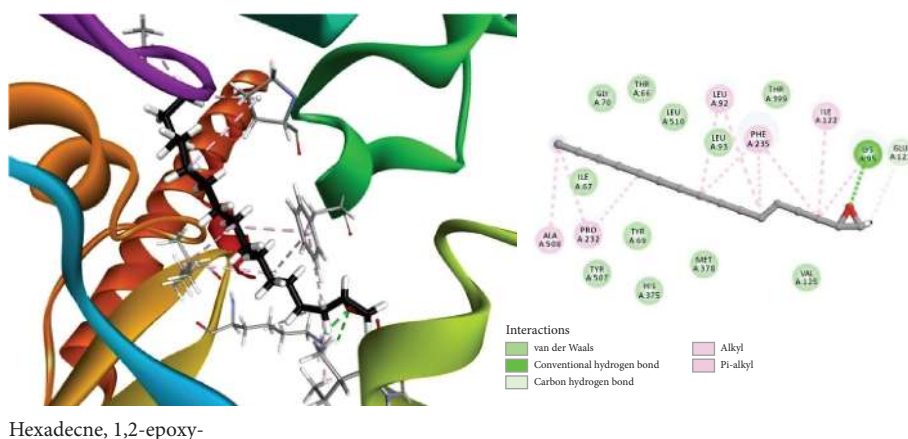
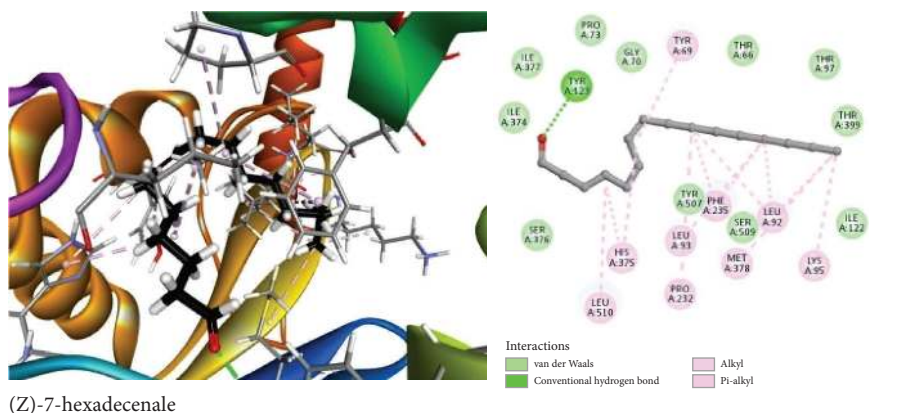
FIGURE 11: 2D and 3D molecular docking interaction diagrams of hexahydrofarnesyl acetone with *S. cerevisiae* sterol 14- α demethylase (CYP51; PDB ID: 5EAF).

may partially explain the observed growth inhibition, suggesting that DHFR inhibition is one of the mechanisms contributing to the antimicrobial effects of the extract.

Following the in silico investigation of bacterial targets, we extended our docking analysis to fungal pathogens, aiming to explore the potential antifungal properties of the identified bioactive molecules. For this purpose, we selected the CYP51 enzyme (sterol 14- α -demethylase) as a molecular target across all fungal species tested. CYP51 is a well-known and highly conserved enzyme involved in the ergosterol biosynthesis pathway, catalyzing the removal of the 14 α -methyl group from lanosterol, an essential step for

fungal membrane integrity [61]. Its clinical relevance is well established, as it is the primary target of azole antifungals, including fluconazole and itraconazole [62]. Targeting CYP51 offers both biological significance and translational value, making it an appropriate focus for evaluating antifungal activity [63, 64].

As with the bacterial targets, all 27 molecules identified were docked into the active site of CYP51 for each fungal species. Only the top three ligands for each species were selected for detailed interaction analysis. These results are summarized in Table 5 and visually represented in Figures 10, 11, 12, and 13.



For *C. albicans* (PDB ID: 5TZ1) (Figure 10), hexahydrofarnesyl acetone exhibited the most favorable binding affinity, with a docking score of -7.62 kcal/mol. The compound established significant interactions within the active site of CYP51, notably with His377 and Met508, two residues known to play a central role in heme coordination and substrate recognition. These interactions suggest a potential inhibitory mechanism that could interfere with ergosterol biosynthesis.

contributed to the stabilization of the ligand–enzyme complex, supporting the high binding affinity observed.

For *F. graminearum* (Figure 12), hexahydrofarnesyl acetone achieved the highest binding score among all fungal species tested (-8.11 kcal/mol). The compound formed a hydrogen bond with Lys95, while also engaging in multiple hydrophobic and π - π interactions with key residues such as Phe235, Leu510, and Met378. These findings highlight a particularly strong interaction with the fungal CYP51 enzyme, suggesting potential specificity and effectiveness.

Finally, in the case of *B. cinerea* (UniProt ID: Q9HDR2) (Figure 13), hexahydrofarnesyl acetone once again emerged as the top-scoring ligand (-8.30 kcal/mol). The binding



conformation was stabilized through interactions with Tyr113, Met141, and Ile459, which are residues likely involved in the structural integrity of the active site. These consistent and favorable docking scores across different fungi reinforce the potential of hexahydrofarnesyl acetone as a broad-spectrum antifungal agent targeting sterol 14 α -demethylase (CYP51).

reinforcing its potential as a core pharmacophore for antifungal lead optimization.

4. Conclusions

The present study highlights the significant pharmacological potential of *Scolymus hispanicus* L., a wild Algerian plant traditionally used for food and medicinal purposes. Through a comprehensive analysis of its hydro-methanolic aerial part extracts and their solvent-based fractions, the plant was shown to be rich in bioactive compounds, notably polyphenols, flavonoids, and a diverse array of volatile constituents.

The chemical profiling revealed α -pinene, limonene, and various fatty acid derivatives as dominant volatile compounds, many of which are known for their antimicrobial and anti-inflammatory properties. The DCM extract, in particular, demonstrated strong antibacterial effects, while the n-hexane extract showed notable antidenaturation activity, even surpassing aspirin in some instances. Antifungal assays further confirmed selective activity, especially against *Candida albicans*, *Fusarium* sp., and *Botrytis* sp.

Moreover, molecular docking studies validated the biological potential of several volatile compounds, with hexadecene, 1,2-epoxy, and hexahydrofarnesyl acetone showing strong binding to microbial target enzymes, correlating with in vitro results.

Collectively, these findings confirm that *S. hispanicus* L. possesses considerable antimicrobial and anti-inflammatory properties, making it a promising candidate for the development of natural therapeutic agents. Further in vivo studies and formulation work are recommended to explore its potential applications in pharmaceutical or nutraceutical products.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

R.Z.: conceptualization, resources, methodology, investigation, and writing—original draft. O.B.: methodology and data curation. F.Z.M.: total phenolic and flavonoid quantification, and statistical analysis. M.M. and A.C.: methodology. S.R.: formal analysis and methodology. S.G.: formal analysis, data curation, methodology, supervision, and writing—review and editing. All authors reviewed the manuscript.

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