ANTIBACTERIAL EFFECTS AND MOLECULAR DOCKING INTERACTIONS OF PLANT ESSENTIAL OILS AGAINST DRUG-RESISTANT BACTERIAL PATHOGENS: A COMPARATIVE STUDY

HUMA NASEEM[1]*, HIRA ARIF[1], KIRAN RAFIQ[2], FATIMA KHURRAM[1], AYISHA KAMAL[1], MUHAMMAD[1]

*[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ziauddin University, Karachi-75600, Pakistan*
[2] Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi-75510, Pakistan
Corresponding Author's E-mail: huma.naseem@hotmail.com,

خلاصہ

ادویات کے خلاف مزاحمت کرنے والے بیکٹیریل پیتھوجینز کا اضافہ صحت کی ایک بڑی عالمی تشویش ہے، کیونکہ یہ ادویات موجودہ اینٹی بائیوٹکس کی تاثیر کو محدود کرتا ہے۔ پودوں کے ضروری تیل ایک امید افزا حل پیش کرتے ہیں، کیونکہ ان کی اینٹی مائکروبیل ایجنٹ ہونے کی تاریخ ہے۔ اس تحقیقی مطالعے کا مقصد منشیات کے خلاف مزاحم بیکٹیریل پیتھوجینز کے خلاف پانچ پودوں کے ضروری تیلوں کے اینٹی بیکٹیریل اثرات کی تحقیقات کرنا اور اہم بیکٹیریل پروٹینز ،اور انزائمز کے ساتھ ان کے فعال اجزاء کے مالیکیولر ڈاکنگ تعاملات کو تلاش کرنا تھا۔ روزمیری کا تیل، لیموں کا تیل لیوینڈر کا تیل، تلسی کا تیل، اور صندل کی لکڑی کے تیل کو پانی کے عرقوں اور مائیکلز کی شکل میں Staphylococcus aureus، Escherichia coli، اور Bacillus کے خلاف آزمایا گیا۔ ڈسک کے پھیلاؤ کا طریقہ ابتدائی اینٹی بیکٹیریل کا استعمال کرتے ہوئے انجام دی گئی تھیں PYRX اسکریننگ کے لیے استعمال کیا گیا تھا، اور مالیکیولر ڈاکنگ اسٹڈیز کے ذریعے تصور کیا گیا تھا۔ نتائج سے یہ بات سامنے آئی کہ ضروری تیلوں نے BIOVIA Discovery Studio اور اینٹی بیکٹیریل اثرات دکھائے، لیوینڈر اور تلسی کے تیل بیکٹیریل ایجنٹوں کے خلاف زیادہ سرگرمی دکھاتے ہیں۔ مالیکیولر انزائم کی فعال سائٹ کے ساتھ اہم ضروری تیل کے مرکبات کے مضبوط DNA gyrase ڈاکنگ اسٹڈیز نے بیکٹیریل تعامل کا اشارہ کیا، جو کہ اینٹی بیکٹیریل اثرات کے لیے ممکنہ طریقہ کار کی تجویز کرتا ہے۔ آخر میں، یہ مطالعہ ضروری تیلوں کی اینٹی بیکٹیریل خصوصیات اور اینٹی بیکٹیریل ایجنٹوں کے ذرائع کے طور پر ان کی صلاحیت کو نمایاں کرتا ہے، خاص طور پر اہم بیکٹیریل انزائمز کے ساتھ تعامل کے ذریعے۔

Abstract

The rise of drug-resistant bacterial pathogens is a major global health concern, as it limits the effectiveness of existing antibiotics. Plant essential oils offer a promising solution, as they have a history of being antimicrobial agents.This research study aimed to investigate the antibacterial effects of five plant essential oils against drug-resistant bacterial pathogens and to explore the molecular docking interactions of their active components with key bacterial proteins and enzymes. Rosemary oil, lemon oil, lavender oil, basil oil, and sandalwood oil were tested against Staphylococcus aureus, Escherichia coli, and Bacillus in the form of aqueous extracts and micelles. The disc diffusion method was used for the initial antibacterial screening, and molecular docking studies were performed using PYRX and visualized by BIOVIA Discovery Studio. The results revealed that the essential oils exhibited antibacterial effects, with lavender and basil oils showing high activity against bacterial agents. Molecular docking studies indicated strong interactions of major essential oil compounds with the active site of the bacterial DNA gyrase enzyme, suggesting a potential mode of action for the antibacterial effects. In conclusion, this study highlights the antibacterial properties of essential oils and their potential as sources of antibacterial agents, particularly through interactions with crucial bacterial enzymes.

Keywords: Essential oils; Antibacterial activity**;** Molecular docking; Disc-diffusion method

Introduction

The emergence of drug-resistant bacterial pathogens poses a significant threat to public health worldwide. In the face of dwindling antibiotic options, exploring alternative approaches becomes imperative. One promising avenue is the use of plant essential oils, which have long been recognized for their antimicrobial properties. Essential oils hold significant importance as they serve as a valuable source of biomolecules. It is essential to comprehend the chemical composition of essential oils, as the concentrations of major compounds directly impact their biological activities. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties(Burt, 2004),(Kordali et al., 2005).

Numerous studies have reported the antimicrobial properties of essential oils (EOs) against various microorganisms, including pathogenic Gram-negative and Gram-positive bacteria. For instance, research has shown that EOs derived from plants such as oregano, thyme, and tea tree exhibit potent antimicrobial activity against both Gram-negative bacteria, such as Escherichia coli and Pseudomonas aeruginosa, and Gram-positive bacteria, such as Staphylococcus aureus and Streptococcus pneumoniae. These EOs have demonstrated inhibitory effects on bacterial growth, as well as the ability to disrupt bacterial cell membranes and inhibit essential enzyme activities. Additionally, studies have highlighted the efficacy of EOs from other plant sources, such as lavender, peppermint, and cinnamon, in combating a wide range of pathogenic bacteria. The reported antimicrobial activities of EOs against various microorganisms underscore their potential as natural alternatives to conventional antibiotics in the battle against drug-resistant bacterial pathogens(Chouhan, Sharma, & Guleria, 2017).

Rosemary herbs belong to the plant Rosmarinus officinalis extensively used in traditional medicines and cosmetics. Itis a part of the Lamiaceae family (Yang et al., 2016). Rosmarinus officinalis essential oil is used for many therapeutic applications such as potent cytotoxic,antibacterial, antioxidant,antimutagenic, antiphlogistic, and chemopreventive(Bousbia et al., 2009), (Celiktas et al., 2007),(Koschier & Sedy, 2003). Typical growth of food bacteria that causes food poisoning is prevented by using Rosemary oil(Ohno et al., 2003). Antibacterial activity of Rosemary essential oil's against E. coli and S. aureus, Bacillus cereus was also discussed by Burt (Cox & Markham, 2007). Growth of Enterobacteriaceae and Brochothrixthermosphactais also suppressed byRosemary Essential oil(Sirocchi et al., 2013).

Citrus limon (Lemon) from Rutaceae family is a flowering plant. It is an important source of essential oil that is commonly used in food and medications(EM Mustafa & agriculture, 2015). It is a significant medicinal plant that is used primarily for its alkaloids, which have anticancer properties. It has also been reported that the crude extracts of this plant can fight off clinically significant bacterial strains of different bacteria. Vitamin C is also present in plant and its oil is incorporated in a variety of skin-nourishing preparations to lessen skin itchiness. Citrus limon acetone extract results in remarkable antibacterial activity against Bacillus subtilis and Enterococcus faecalis. As per the studies, the mean inhibition diameters reported among all the bacteria, the gram-positive B. subtilis and E. faecalis, the gram-negative Salmonella typhimurium, Shigella sonnei, were the most susceptible ones(Otang & Afolayan, 2016).

Lavender (*Lavandula angustifolia*) is a perennial evergreen shrub that is one of the most valued aromatic and therapeutic plants. Lavender (Lavandula angustifolia) has been used for centuries to cure cramps, muscle spasms, burns, parasite infections, and insect bites(Denner, 2009).Scientific studies have shown that the L. angustifolia essential oil has anti-inflammatory, analgesic(Hajhashemi, Ghannadi, & Sharif, 2003), antioxidant (Nurzynska-Wierdak & Zawislak, 2016),sedative(Prusinowska & Śmigielski, 2014), antimicrobial (Adaszyńska-Skwirzyńska, Swarcewicz, & Dobrowolska, 2014) and anticonvulsant properties(YAMADA, MIMAKI, SASHIDA, & Bulletin, 1994).

Various bacterial species, including antibiotic-resistant ones such as vancomycin-resistant enterococcus (VRE) and MRSA, have been discovered to be susceptible to lavender oil(Nelson, 1997). Recent studies supporting the lavender oils as antibacterial agents shown that some oils, including L. heterophylla, exhibitedexcellent antibacterial activity which had not previously been studiedagainst number of bacterial strains, including, Enterobacter aerogenes, Streptococcus pyogenes, S. aureus, Pseudomonas aeruginosa, MRSA, Citrobacter freundii, Proteus vulgaris(Moon, Cavanagh, & Wilkinson, 2004).

Sandalwood (*Santalum album* L.), is world's second-most important wood. Pharmaceuticals, cosmetics, aromatherapy, and perfumes all use sandalwood essential oil(Kumar, Joshi, & Ram, 2012). α-santalolthe main component of sandalwood, was useful as a skin cancer chemopreventive agent(Kumar et al., 2012). The plant was useful for illness treatment like jaundice, dysentery, and gastric irritability in various medical systems in previous times. Sandalwood oil is beneficial as a liver tonic, heart tonic, fever reducer, memory stimulator, anti-poison, and blood purifier. It is also used as astringent, sedative, expectorant, diuretic, stimulant, cooling, and astringentin Ayurveda. In addition, the plant has been linked to medicinal and pharmacological activities like antioxidant,anticancer,antiviral, antifungal,anti-inflammatory, hepatoprotective, antibacterial, and cardio-protective effects(Karossi, Agustina, & Sutedja, 1993).Studies shave discovered that sandalwood oil obtained from sandalwood sawdust has antibacterial effects against Bacillus cereus and Staphylococcus aureus(Kaur et al., 2005).

Basil, also known as sweet basil belongs to the Lamiaceae family. Basil has previously been used for treating renal problems, warts, worns, diarrhea, migraines, and coughs as a medicinal plant(Simon et al., 1999). Basil essential oils are discovered to be active against a variety of Gram-positive and Gram-negative bacteria, yeast, and molds and their main ingredients also shows antibacterial activity(Suppakul, Miltz, Sonneveld, Bigger, & chemistry, 2003). Study have been shown that the common antibiotics used in clinical settings possesses antimicrobial effects when mixing with O. basilicum essential oil against S. aureus and P. aeruginosa. Studies have also demonstrated that O. basilicum essential oil either individually or with imipenem (resulting in synergistic effect) against S. aureus show antibacterial activity. The oil and imipenem and ciprofloxacin combinations were effective against the P. aeruginosa strains that were examined(Araújo Silva et al., 2016). Linalool may be associated with O. basilicum essential oil's antibacterial properties(Ravid, Putievsky, Katzir, Lewinsohn, & journal, 1997).

In order to investigate the interactions at atomic level between bioactive constituents present in plant essential oils and target receptors, molecular docking approach is used. It gives insight about potential therapeutic effects and determines

the binding capability of different ligands to see their affinity towards target binding sites present in receptor proteins.Different strains of bacteria have different structure of bacterial cell wall which makes the difference in their Susceptibility to Antimicrobial compounds. Three different bacterial strains (S. aureus, Bacillus anthracis and Escherichia coli) have been subjected in this study to evaluate the antibacterial properties of aforementioned essential oils.

This research study aims to investigate the potential of various plant essential oils in combating drug-resistant bacteria. Through a comparative analysis, this study seeks to elucidate the antibacterial effects of these oils and explore their molecular docking interactions with drug-resistant bacterial pathogens. By shedding light on the mechanisms underlying these interactions, this research contributes to the development of effective strategies to combat antibiotic resistance and opens new avenues for the utilization of plant-based therapeutics in the fight against drug-resistant bacterial infections.

Materials and Methods

Essential Oils: Five different essential oils (rosemary oil, lemon oil, lavender oil, basil oil, and sandalwood oil) were bought from the retail shop which were of good quality and have no other synthetic compounds and their comparative antibacterial studies were performed by disc-diffusion method using three different bacterial strains.

Bacterial Strains: A selection of three clinical isolates from the Ziauddin hospital (Staphylococcus aureus, Escherichia coli, and bacillus aureus) were collected and kept at body temperature. All these bacterial strains were checked for their susceptibility against five selected essential oils.

Working Solution: For homogenous micelles, water and essential oil were mixed with the water-based liquid culture medium by mixing 2ml of sterile water and essential oil in a microcentrifuge tube. For this, the micelles were obtained by sonication at 50 K HZ for 25 min at 30°C in a sonicator water bath using sweep mode, and then by using a sterile pipette the bottom homogeneous opalescent phase was separated(Man, Santacroce, Iacob, Mare, & Man, 2019).

Disk Diffusion Method: Inhibition zone diameter (cm) formed by essential oils around bacterial species were measured by using disk diffusion method. Whatman paper (1 number) disk having 0.6cm diameter is placed with essential oil (10 µL). In petri dish Muller Hinton medium is poured and the bacterial strains were inoculated in it. Disk were placed on the surface of medium and after 24 hours incubation (37 ^{0}C) anti-bacterial activity of essential oils waschecked(Man et al., 2019).

Docking Studies

Preparation of Ligands and Receptors: Before the emergence of reports to anti-bacterial activity, a search of potential natural molecules in essential oils having antibacterial activity against target proteins was performed. The 32 selected structural ligands were downloaded from the PubChem database in mol2 format (Table 1). Subsequently, BIOVIA Discovery Studio version 4.5 was utilized for tasks such as structural correction, geometric optimization, hydrogen addition, charge arrangement, and handling of ionizable groups. Along with 32 ligands, an antibacterial agent (ciprofloxacin) was downloaded and used as standard drug molecule. In contrast, Staphylococcus aureus nucleoside diphosphate kinase and Escherichia coli beta-ketoacyl-[acyl carrier protein] synthase were selected for the evaluation of

antibacterial activity. These proteins were obtained in PDB format from the protein data bank, with the respective PDB IDs being 1FJ4 and 3Q8U. Afterwards, the proteins were prepared in PDBQT format by adding hydrogen atoms, removing solvent (water), and eliminating ligands using pyrx software. The preparation process also involved utilizing the MMFF94 force field.

Molecular Docking*:* The PyRx 0.8 software graphical interface was utilized to conduct molecular docking. A virtual screening approach was employed to identify molecules with the highest structural affinity against the antibacterial proteins 1FJ4 and 3Q8U. To achieve this, the ligands underwent energetic minimization using the mmff94 force field, employing conjugated gradients in 200 steps facilitated by Open Babel tools(O'Boyle et al., 2011).Blind docking was performed, followed by simulation to generate conformations categorized based on affinity energy value and RMSD. The Discovery Studio software (Ver. 21.1.0.20298)(Jejurikar & Rohane, 2021)was used to visualize the interacting profiles of the best docked complexes. The top five candidates for each receptor protein were selected based on their best S-scores and the binding patterns observed between the ligands and the targeted proteins.

Toxicity, Pharmacokinetic and Drug-Likeness Prediction: Using the molecules exhibiting the highest affinity for proteases as a basis, a predictive search was conducted using SwissADME.16 to assess their pharmacokinetic, toxicological, and drug-likeness properties(Ranjith, Ravikumar, & Phytochemistry, 2019).

Results and Discussion

Anti-bacterial Activity**:** All five essential oils (aqueous extract and micelles forms) were tested for the inhibitory and bactericidal effects on three strains of bacteria: Escherichia coli, Staphylococcus aureus and, Bacilli. After incubation, zone of Inhibition was observed and calculated in cms of all five essential oils against above mentioned pathogenic microorganisms as follow:

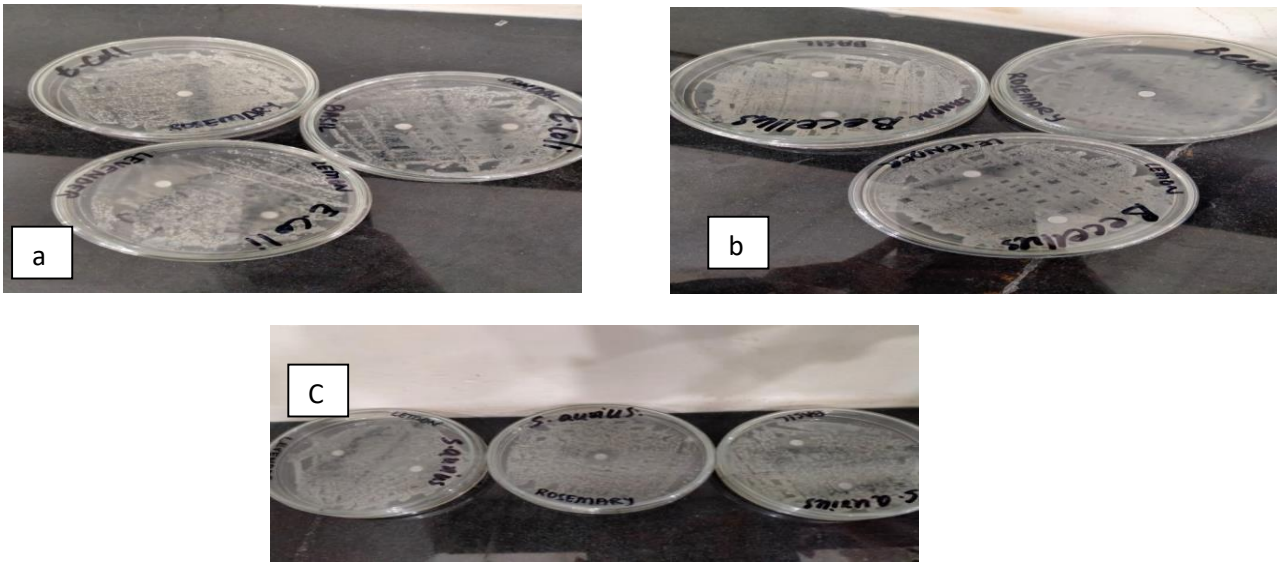


Fig. 1: Disc Diffusion test for *E. Coli* (a), Disc Diffusion test for Bacilli (b), Disc Diffusion test for *S. aureus.*

Table-1: Zone of Inhibition of bacterial strains of different essential oils

Zone of inhibitions				
S. No.	Essential Oils	*E. coli* (cm)	*Bacillus* (cm)	*S. aureus* (cm)
1.	Rosemary	1	0.5	2
2.	Sandalwood	3	0.7	1
3.	Basil	1.4	2.5	2
4.	Lemon	2	1	2.5
5.	Lavender	3	2.8	0.5

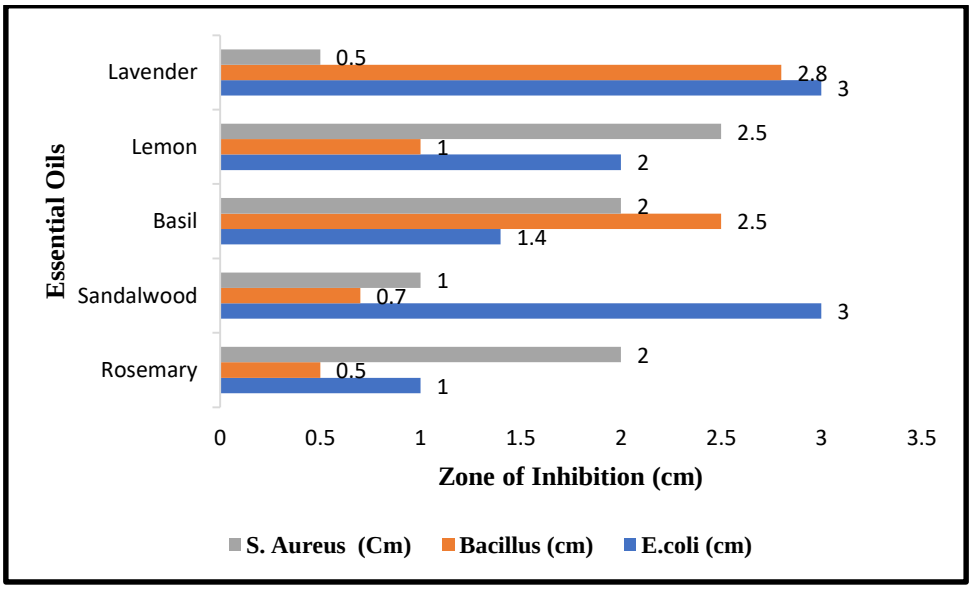


Fig. 2: Comparative analysis of zone of inhibition of different essential oils for anti-bacterial activity.

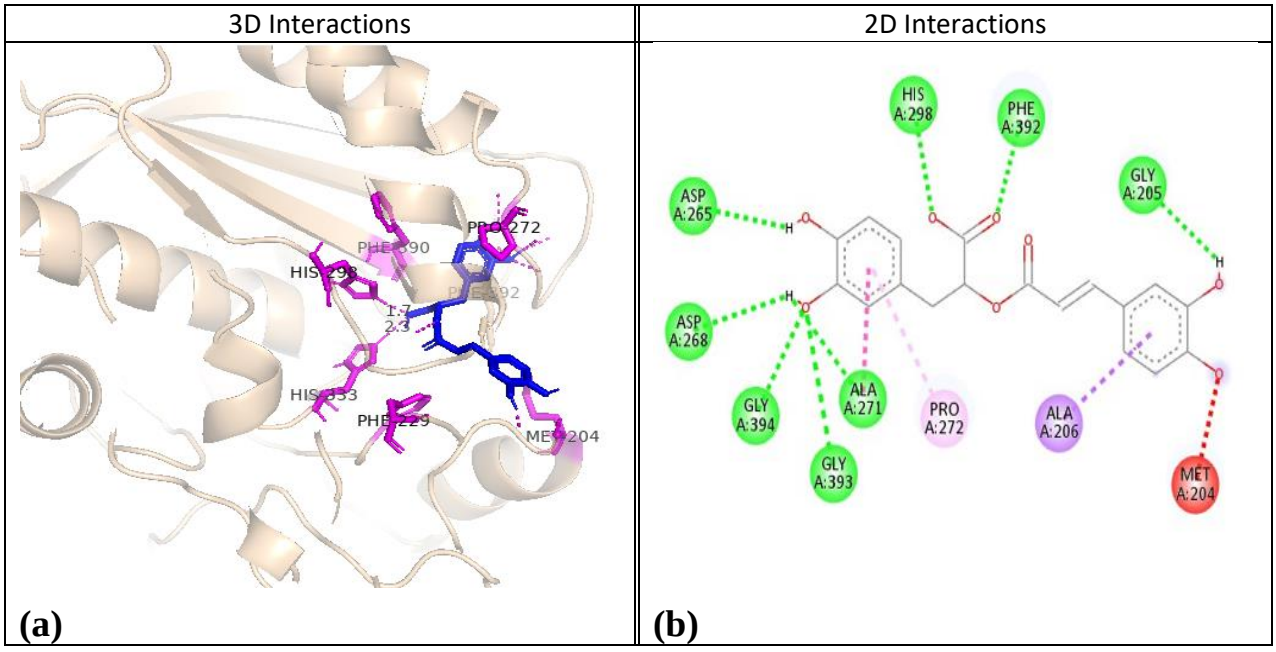
Docking Studies

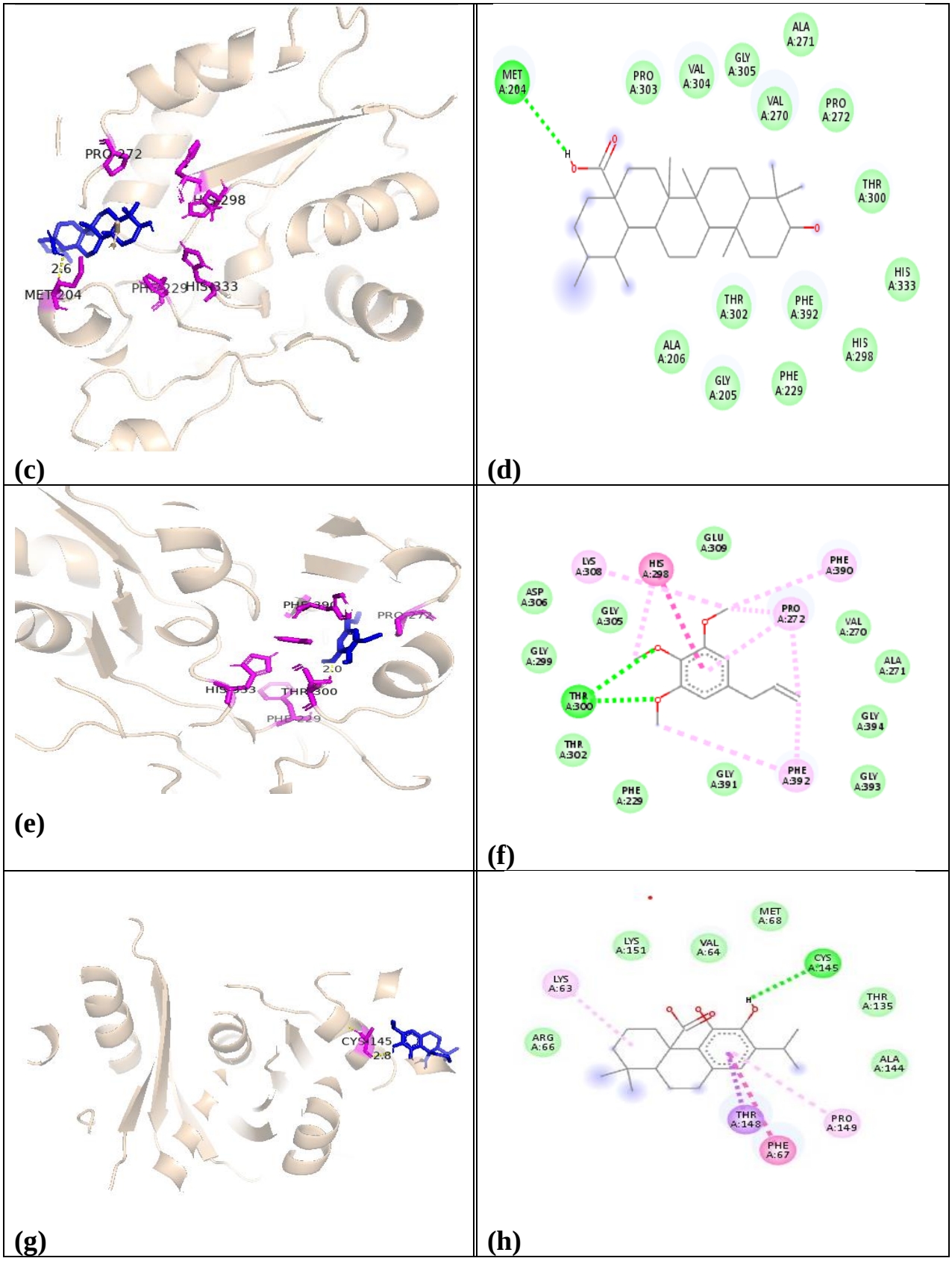
The molecular interactions between bioactive molecules with the highest binding energy present in essential oils and proteins are revealed in Table 2 and 3.

Table-2: Docking score of potent molecules of essential oils, with binding interaction residues against E. coli beta-ketoacyl-[acyl carrier protein] synthase 1FJ4.

Name	Binding Energy	Hydrogen bonding residues	Hydrophobic, Electrostatic and Van der Waal interactions
Rosmarinic Acid	-9.00	ASP265, GLY394,	ASP268, HIS333, THR300, GLY391, PHE390, GLY393, CYS163, THR302, PHE229, THR395,

		ALA271, HIS298, PHE392, GLY205	GLY228, MET269, SER 273, PRO272, ALA206, MET204
Urosilic Acid	-8.4	MET204	PRO303, VAL304, GLY305, ALA271, PRO272, THR300, HIS333, PHE392, THR302, HIS298, PHE229, GLY205, ALA206
Elemicin	-7.1	THR300	GLY299, GLY305, ASP306, GLU309, VAL270, ALA271, GLY394, GLY393, GLY391, PHE229, THR302, LYS308, HIS298, PHE390, PRO272, PHE392
Carnosic acid	-6.9	CYS145	ARG66, LYS151, VAL64, MET68, THR135, ALA144, LYS63, THR148, PHE67, PRO149
Methyl Isoeugenol	-6.5	THR300, THR302	THR395, GLY391, ASN396, ASP265, GLY394, PHE390, PHE392, HIS333, GLY305, HIS298, PRO272, PHE229
Cipro	-6.5	CYS145	LYS63, VAL64, MET138, THR135, PHE67, ALA144, THR148, THR149




PRO 272
HIS 298
2.6
MET 204
PHE 229
HIS 333
(c)
MET A:204
PRO A:303
VAL A:304
GLY A:305
ALA A:271
VAL A:270
PRO A:272
THR A:300
HIS A:333
THR A:302
PHE A:392
ALA A:206
HIS A:298
GLY A:205
PHE A:229
(d)
PHE 390
PRO 272
2.0
HIS 333
THR 300
PHE 229
(e)
GLU A:309
LYS A:308
HIS A:298
PHE A:390
ASP A:306
GLY A:305
PRO A:272
VAL A:270
GLY A:299
ALA A:271
THR A:300
GLY A:394
THR A:302
GLY A:391
PHE A:392
GLY A:393
PHE A:229
(f)
CYS 145
2.8
(g)
MET A:68
LYS A:151
VAL A:64
CYS A:145
LYS A:63
THR A:135
ARG A:66
ALA A:144
THR A:148
PRO A:149
PHE A:67
(h)

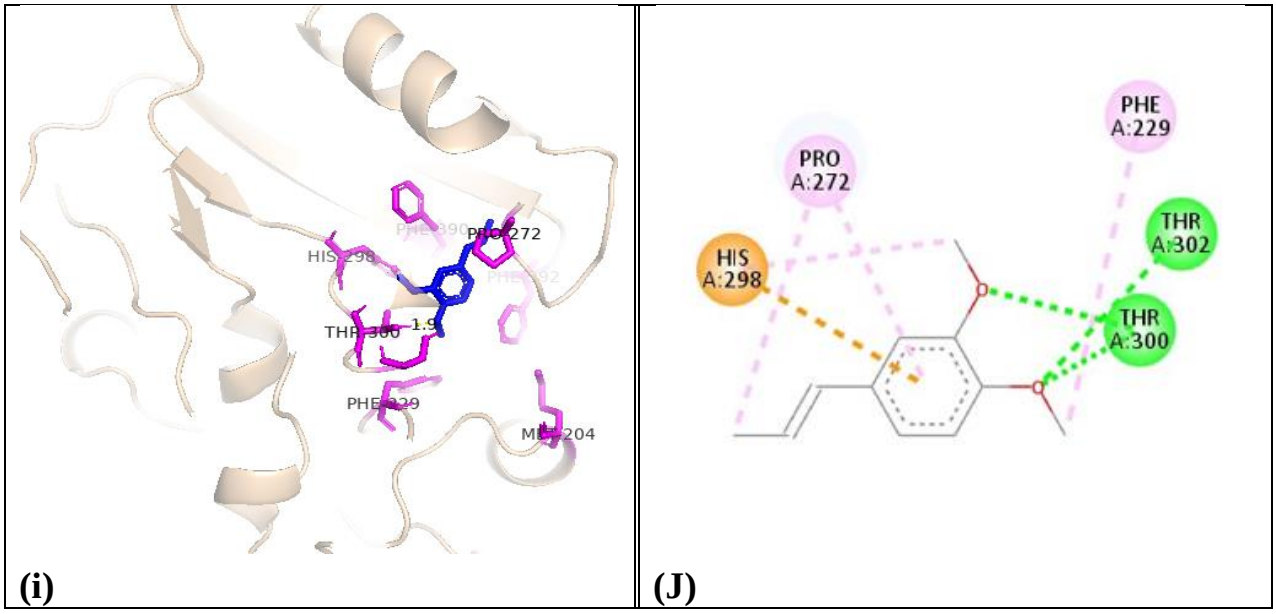
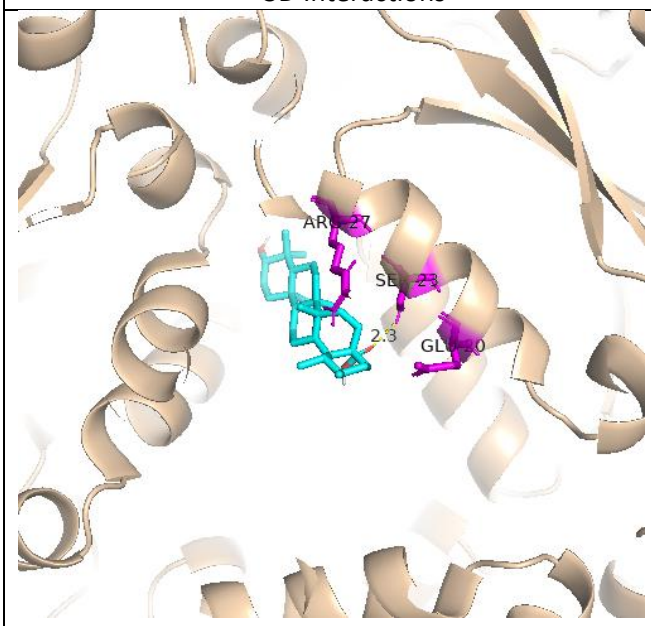
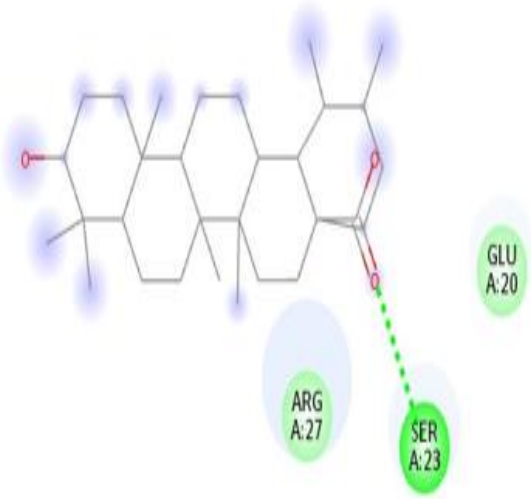
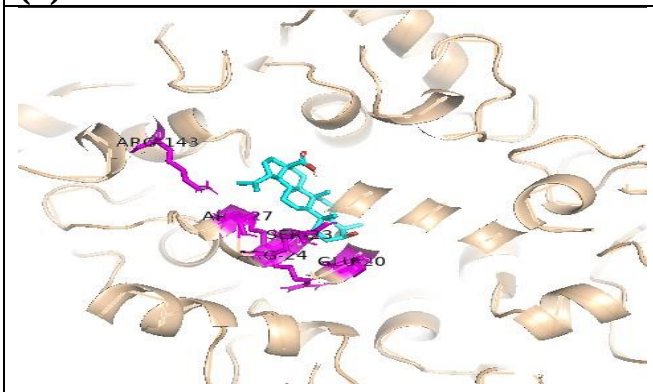
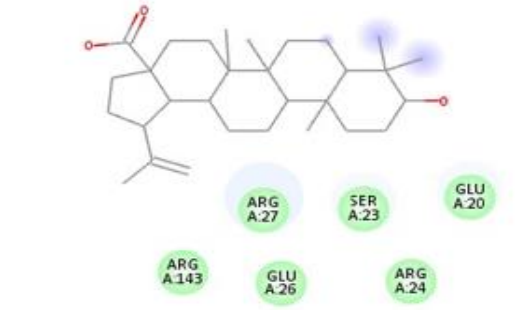
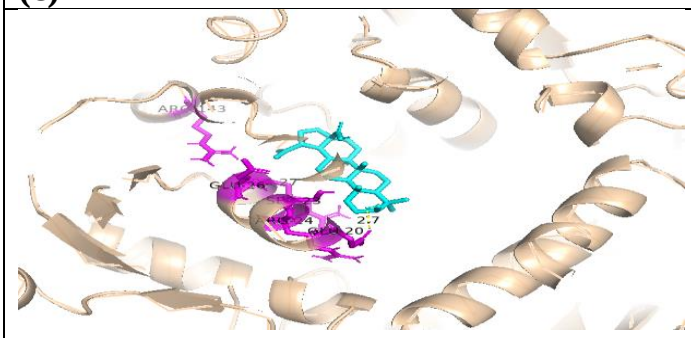
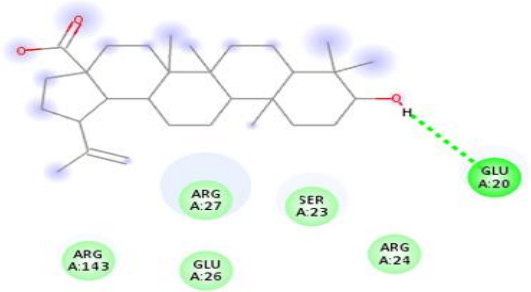
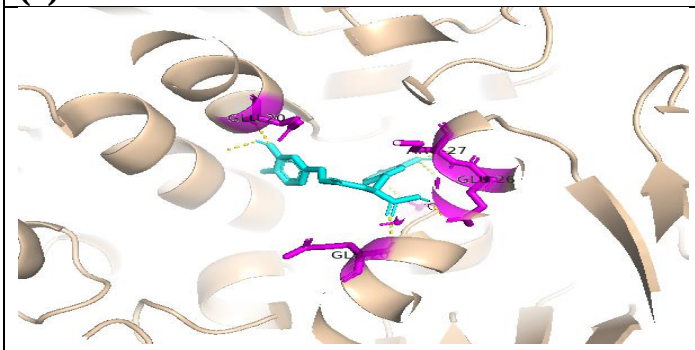
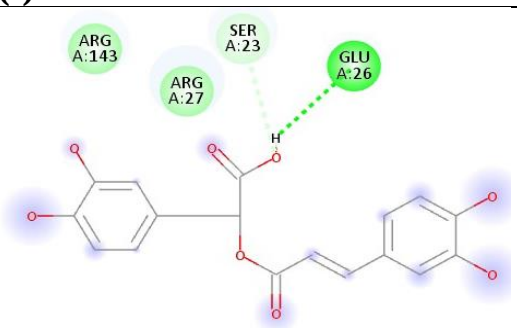


Fig. 3: 3D and 2D interaction studies of predominant ligands of essential oils with target protein 1FJ4. (a & b) Rosmarinic acid, (c & d) Urosilic acid, (e & f) Elemicin, (g & h) Carnosic acid, (I & j) Methyl isoeugenol.

Table-3: Docking score of potent molecules of essential oils, with binding interaction residues against *S. aureus* nucleoside diphosphate kinase 3Q8U.

Name	Binding Energy	Hydrogen bonding residues	Hydrophobic, Electrostatic and Van der Waal interactions
Urosilic Acid	-8.5	SER23	ARG27, GLU20
Beta Pinene	-8.3	-	ARG27, SER23, GLU20, ARG143, GLU26, ARG24
Betulinic Acid	-8.3	GLU20	ARG27, SER23, ARG24, ARG143, GLU26
Rosmarinic Acid	-8.1	GLU26	ARG143, ARG27, SER23
Carnosic Acid	-7.9	GLU20	ARG27, SER23, ARG24, ARG143, GLU26, ASN16, LEU17, ARG15, LEU105,
Cipro	-7.9	-	ARG143, ARG27, SER23

3D Interactions	2D Interactions
 (a)	 (b)
 (c)	 (d)
 (e)	 (f)
 (g)	 (h)

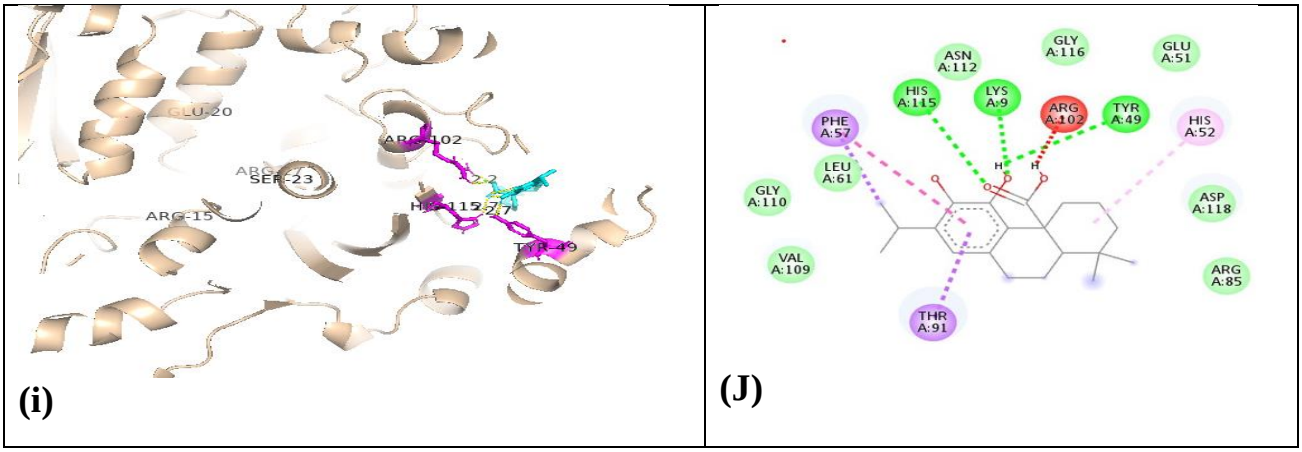


Fig. 4: 3D and 2D interaction studies of predominant ligands of essential oils with target protein 1FJ4. (a & b) Urosilic acid, (c & d) Beta pinene, (e & f) Betulinic acid, (g & h) Rosmarinic acid, (I & j) Carnosic acid.

***Anti-bacterial Activity*:** In present study, after incubation for specific period of time by disc diffusion method results were calculated in terms of zone of inhibition in cms and it was observed that Escherichia coli was most susceptible bacteria as Sandalwood, Lavender, and Lemon were very effective over it. The highest resistance was shown by Bacillus against most of the essential oils, except for the Basil and Lavender. S. Aureus shows high susceptibility against Lemon and Rosemary oil, however, shows resistance against Lavender oil. Rosemary and Sandal wood oils were observed as least active and only little effect is shown on the Bacillus strain. However, Rosemary oil shows some susceptibility against S. aureus.Among all five essential oils, Lavender oil was proven to be an efficient anti-bacterial agent as compared to other oils and Sandalwood is less effective among others except for E. coli.

Docking Studies

The specified binding energies of the predominant ligands in essential oils indicate affinity values ranging from -9.0 to -6.5 Kcal/mol for the target protein 1FJ4. The beta-ketoacyl synthase enzyme is responsible for facilitating the production of lipopolysaccharides, lipoproteins and phospholipids, which are crucial for the growth and survival of bacteria. (Goodall et al., 2021). Due to their significant involvement in bacterial virulence, these enzymes are considered potential targets for drugs andbioactive substances. Among the molecules tested, Rosmarinic acid exhibited the highest activity against E. coli beta-ketoacyl-[acyl carrier protein] synthase (1FJ4) with a binding energy of -9.00 kcal/mol (Table-2). The 2D and 3D viewer analysis revealed that Rosmarinic acid formed eight hydrogen bonds with specific residues ASP265, ASP268, GLY394, GLY393, ALA271, HIS298, PHE392, GLY205 in the active site of 1FJ4 (Fig-3). On the other hand, common amino acids located in the binding site are shown as HIS298, HIS333, PHE229, PHE390, PRO 272 and PHE392. The highest affinity with 1FJ4 was demonstrated with rosmarinic acid, urosilic acid, elemicin, carnosic acid, methyl isoeugenol and ciprofloxacin (standard drug) which has shown that these structures interact with HIS298, HIS333, PHE229, PHE390, PRO 272 and PHE392 (Fig-3). These findings indicate a high level of hydrophobicity and significant electron acceptor and donor properties, with the presence of alkyl, π-alkyl, and C-H bond interactions, as observed in the 2D and 3D viewer.

Among the evaluated molecules, Urosilic acid exhibited the highest activity against S. aureus nucleoside diphosphate kinase (3Q8U) (Abou-Dobara et al., 2019)with a binding energy of -8.5 kcal/mol (Table-3). This interaction was facilitated by a hydrogen bond formed with the SER23 residue in the active site (Fig-4).3Q8U showed that the most active molecules were urosilic acid, beta pinene, betulinic acid, rosmarinic acid, carnosic acid and ciprofloxacin (standard drug) which interact with the amino acids located in its binding site ARG27, SER23, GLU20, ARG143, GLU26 and ARG24 (Table-3). These results showed hydrophobicity along with the presence of Vander wall interactions and C-H bonds.

In the in-silico assessment of antibacterial activity, rosmarinic acid, urosilic acid, and carnosic acid emerged as highly active compounds against both target proteins. Additionally, a standard antibacterial drug Ciprofloxacin was also docked, showed less binding affinity among top five bioactive molecules towards both proteins. Therefore, through molecular docking in in silico studies, various natural molecules demonstrated representative binding to proteins 1FJ4 and 3Q8U.

Conclusion

The findings from the research indicate that the studied essential oils, including rosemary, lemon, lavender, basil, and sandalwood oils, possess significant antibacterial activity, as evidenced by their inhibitory effects on bacterial growth. Additionally, molecular docking studies using PYRX provided insights into the potential mechanisms of action at a molecular level by elucidating the interactions of the active components of these essential oils with key bacterial proteins and enzymes. These results contribute to a better understanding of the antibacterial effects of essential oils, which may have implications for developing natural and effective antibacterial agents against drug-resistant bacterial pathogens. Further studies can explore the in vivo efficacy and potential applications of these essential oils in clinical or public health settings.

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References

Abou-Dobara, M. I., Omar, N. F., Diab, M. A., El-Sonbati, A. Z., Morgan, S. M., & El-Mogazy, M. A. J. J. o. C. B. (2019). Allyl rhodanine azo dye derivatives: Potential antimicrobials target d-alanyl carrier protein ligase and nucleoside diphosphate kinase. *120*(2), 1667-1678.

Adaszyńska-Skwirzyńska, M., Swarcewicz, M., & Dobrowolska, A. J. M. C. (2014). The potential of use lavender from vegetable waste as effective antibacterial and sedative agents. *4*(11), 734-737.

Araújo Silva, V., Pereira da Sousa, J., de Luna Freire Pessôa, H., Fernanda Ramos de Freitas, A., Douglas Melo Coutinho, H., Beuttenmuller Nogueira Alves, L., & Oliveira Lima, E. J. P. b. (2016). Ocimum basilicum: Antibacterial activity and association study with antibiotics against bacteria of clinical importance. *54*(5), 863-867.

Bousbia, N., Vian, M. A., Ferhat, M. A., Petitcolas, E., Meklati, B. Y., & Chemat, F. J. F. c. (2009). Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *114*(1), 355-362.

Burt, S. J. I. j. o. f. m. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *94*(3), 223-253.

Celiktas, O. Y., Kocabas, E. H., Bedir, E., Sukan, F. V., Ozek, T., & Baser, K. J. F. C. (2007). Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. *100*(2), 553-559.

Chouhan, S., Sharma, K., & Guleria, S. J. M. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *4*(3), 58.

Cox, S. D., & Markham, J. J. J. o. A. M. (2007). Susceptibility and intrinsic tolerance of Pseudomonas aeruginosa to selected plant volatile compounds. *103*(4), 930-936.

Denner, S. S. J. H. N. P. (2009). Lavandula angustifolia miller: english lavender. *23*(1), 57-64.

EM Mustafa, N. J. R. p. o. f., nutrition, & agriculture. (2015). Citrus essential oils: current and prospective uses in the food industry. *7*(2), 115-127.

Goodall, E. C., Isom, G. L., Rooke, J. L., Pullela, K., Icke, C., Yang, Z., . . . Da Costa, R. J. P. g. (2021). Loss of YhcB results in dysregulation of coordinated peptidoglycan, LPS and phospholipid synthesis during Escherichia coli cell growth. *17*(12), e1009586.

Hajhashemi, V., Ghannadi, A., & Sharif, B. J. J. o. e. (2003). Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill. *89*(1), 67-71.

Jejurikar, B. L., & Rohane, S. H. (2021). Drug designing in discovery studio.

Karossi, A., Agustina, H., & Sutedja, L. J. J. K. T. I. (1993). Antibacterial Activity of Fractionated Sandalwood Oils. *3*(1).

Kaur, M., Agarwal, C., Singh, R. P., Guan, X., Dwivedi, C., & Agarwal, R. J. C. (2005). Skin cancer chemopreventive agent, α-santalol, induces apoptotic death of human epidermoid carcinoma A431 cells via caspase activation together with dissipation of mitochondrial membrane potential and cytochrome c release. *26*(2), 369-380.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., Yildirim, A. J. J. o. a., & chemistry, f. (2005). Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera essential oils. *53*(24), 9452-9458.

Koschier, E. H., & Sedy, K. A. J. C. p. (2003). Labiate essential oils affecting host selection and acceptance of Thrips tabaci Lindeman. *22*(7), 929-934.

Kumar, A. A., Joshi, G., & Ram, H. M. J. C. S. (2012). Sandalwood: history, uses, present status and the future. 1408-1416.

Man, A., Santacroce, L., Iacob, R., Mare, A., & Man, L. J. P. (2019). Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. *8*(1), 15.

Moon, T., Cavanagh, H., & Wilkinson, J. (2004). *Lavender as an antibacterial essential oil—are all lavenders equal.* Paper presented at the AICA national conference.

Nelson, R. J. T. J. o. a. c. (1997). In-vitro activities of five plant essential oils against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium. *40*(2), 305-306.

Nurzynska-Wierdak, R., & Zawislak, G. J. A. S. P. H. C. (2016). Chemical composition and antioxidant activity of lavender (Lavandula angustifolia Mill.) aboveground parts. *15*(5).

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. J. J. o. c. (2011). Open Babel: An open chemical toolbox. *3*(1), 1-14.

Ohno, T., Kita, M., Yamaoka, Y., Imamura, S., Yamamoto, T., Mitsufuji, S., . . . Imanishi, J. J. H. (2003). Antimicrobial activity of essential oils against Helicobacter pylori. *8*(3), 207-215.

Otang, W., & Afolayan, A. J. S. A. J. o. B. (2016). Antimicrobial and antioxidant efficacy of Citrus limon L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa. *102*, 46-49.

Prusinowska, R., & Śmigielski, K. B. J. H. p. (2014). Composition, biological properties and therapeutic effects of lavender L). A review. *60*(2), 56-66.

Ranjith, D., Ravikumar, C. J. J. o. P., & Phytochemistry. (2019). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Ipomoea mauritiana Jacq. *8*(5), 2063-2073.

Ravid, U., Putievsky, E., Katzir, I., Lewinsohn, E. J. F., & journal, f. (1997). Enantiomeric composition of linalol in the essential oils of Ocimum species and in commercial basil oils. *12*(4), 293-296.

Simon, J. E., Morales, M. R., Phippen, W. B., Vieira, R. F., Hao, Z. J. P. o. n. c., & uses, n. (1999). Basil: A source of aroma compounds and a popular culinary and ornamental herb. *16*, 499-505.

Sirocchi, V., Caprioli, G., Cecchini, C., Coman, M. M., Cresci, A., Maggi, F., . . . nutrition. (2013). Biogenic amines as freshness index of meat wrapped in a new active packaging system formulated with essential oils of Rosmarinus officinalis. *64*(8), 921-928.

Suppakul, P., Miltz, J., Sonneveld, K., Bigger, S. W. J. J. o. a., & chemistry, f. (2003). Antimicrobial properties of basil and its possible application in food packaging. *51*(11), 3197-3207.

YAMADA, K., Mimaki, Y., Sashida, Y. J. B., & Bulletin, P. (1994). Anticonvulsive effects of inhaling lavender oil vapour. *17*(2), 359-360.

Yang, Y., Song, X., Sui, X., Qi, B., Wang, Z., Li, Y., . . . Products. (2016). Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. *80*, 141-147.