RESEARCH ARTICLE

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Bio-active components of *Cymbopogon martinii* essential oil as therapeutics targeting bacterial Penicillin-Binding-Proteins (PBPs): Aroma profile, in silico-docking, pharmacokinetics, and wet-lab validation

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ABSTRACT

Cymbopogon martinii, also known as Palmarosa, is an underutilized plant in the tropical region. Due to its outstanding antioxidant potential, it has been used as a part of conventional medicine and beauty product. The objective of the present study was aromatic profiling of Palmarosa essential oil and molecular docking of Palmarosa essential oil bioactive components (Geraniol, Geranial, Linalool, Fenchyl alcohol, 6-methylhept-5-en-2-one, Borneol, Elemol, δ-cadinol) against six bacterial Penicillin-binding Proteins (PBP1a, PBP2a, PBP3, PBP4, PBP5, and PBP 6) and in-vitro support. GC-FID was used to find out aromatic profiling. For docking Cb-dock2 tool was used. Ligand-Protein 3D interactions were also studied. *In-silico* ADMET pharmacoinformatics aspects (Physicochemical, Lipophilicity, Medicinal Chemistry, Druglikeness, Absorption, Water Solubility, Distribution, Metabolism, Pharmacokinetics, Excretion, Environmental Toxicity, Tox21 Pathway and Toxicophore Rules) with PASS prediction of all the ligands have been bio-prospected. Wet lab validation was performed by Gram-positive and Gram-negative bacterial strains. GC-FID profiling revealed the presence of various major and minor components. Docking analysis indicated effective binding of all the ligands with all the six PBPs (PBP1a, PBP2a, PBP3, PBP4, PBP5, and PBP 6). The interaction results indicate that the PBP-Ligand complexes form hydrogen and hydrophobic interactions. *in-silico* ADMET study revealed that all the ligand molecules have no toxic effect and good absorption as well. Wet lab validation was performed by Gram-positive and Gram-negative bacterial strains. *In-vitro* results revealed that the Palmarosa oil was able to inhibit the growth of the bacterial strains thus signifying its role as a potent anti-bacterial drug. These ligands can be used as a basic structure, and various structural modifications can ultimately yield stronger molecules. The Palmarosa essential oil could be a promising antibacterial agent against various strains.

Key words: Penicillin binding protein, GC-FID, ADMET, *Cymbopogon martinii*, Geraniol, *in-silico* studies

Introduction

Bacterial cell division and daughter cell formation is a complex mechanism the details of which are coordinated by at least 12 different proteins. Penicillin-binding proteins (PBPs), membrane-bound macromolecules that play a key role in the cell wall synthesis process, have been used as targets of highly successful β-lactam antibiotics for over 70 years. The increasing emergence of β-lactam-resistant microorganisms, coupled with advances in genomics, genetics, and immunofluorescence microscopy techniques, has spurred intensive studies of these PBPs from various bacterial species (Macheboeuf et al., 2006). Bacterial peptidoglycan is a three-dimensional reticular network lining the cell membrane that is synthesized rapidly during the cell

cycle and protects the bacterium from osmotic shock. Peptidoglycan determines the overall cell shape, functions as an attachment site for virulence and adhesion factors, helps bacteria undergo morphological changes in response to various stress-related factors, and ultimately, severe fragility or instability can lead to cell lysis and cell death (Höltje et al., 1998; Nanninga et al., 1998). Peptidoglycan consists of alternating N-acetylglucosamine and N-acetylmuramic acid glycan chains bridged by short-stem peptides linked to N-acetylglucosamine and N-acetylmuramic acid. Penicillin-binding proteins (PBPs) catalyze the polymerization of glycan chains (transglycosylation) and cross-linking between glycan chains (transpeptidation). Some PBPs hydrolyze the final D-alanine of the stem pentapeptide (DD-carboxypeptidation) or the peptide bond connecting the two glycan chains (endopeptidation) (Sauvage et al., 2008). Bacterial cell wall biosynthetic machinery is one of the most promising niches for antibiotic targets. Many of the proteins like PBPs are involved in their metabolisms that are essential for bacterial survival; similar macromolecular scaffolds are lacking in humans. These are two major prerequisites for the development of antibiotics.

Antibiotics are an important weapon in the fight against various bacterial infections and have significantly improved human health since their introduction. However, in recent years, the overuse of antibiotics has caused drug resistance, leading to dangerous harm to human health. Researchers try to develop new drugs without resistance (Mir et al., 2022). Due to the rise in antibiotic resistance, microorganisms have emerged as one of the most momentous and significant threats to the human population (Harris et al., 2022). In particular, gram-positive bacteria have commonly developed resistance to all currently available antibiotics and cause severe problems equally in the hospital and the community on the rampage. Gram-negative pathogens such as Pseudomonas aeruginosa and Escherichia coli escape the action of β-lactams by secreting β-lactamases into the cytoplasm. In another mechanism, the antibiotic cannot reach its macromolecule target because the bacteria are pushed out through the antibiotic ejector pump, such as the MexA, B-OprM pump in *Pseudomonas* strains (Contreras-Martel et al., 2017). Some Gram-positive bacteria, such as streptococci, do not secrete β-lactamases, produce highly mutant PBPs, and are insensitive to the drug. PBP is more or less sensitive to β-lactam antibiotics. Bacteria have several PBPs, some of which have begun to reveal their clear roles in the cell division cycle (Straume et al., 2020). The suppression of transpeptidation or carboxypeptidation reactions with β-lactam antibiotics depletes peptidoglycan and may induce cell death (Chan et al., 2016). This powerful mechanism, which has made penicillin and its analogs the most widely used antibiotics for all infectious diseases in the world over the past 70 years, has been challenged by the spread of resistance, highlighting the need for new natural antibiotic therapies. The natural product moenomycin has also been reported to inhibit glycosyltransferase reactions that degrade peptidoglycan and kill bacterial cells (Masters et al., 2020). Therefore, with their dominant role, PBPs are considered suitable targets during the development of bacterial inhibitors. Inhibiting the activity of the PBP protein will prevent bacterial replication. Since no PBP has been found in humans with comparable cleavage specificity, the inhibitors are unlikely to be considered toxic.

Therefore, traditional medicine systems are gaining popularity because they are more natural, eco-friendly, and free of side effects (Mir et al., 2022). Despite the many benefits of today's synthetic drugs, people still choose natural herbal remedies over synthetic drugs. The majority of medicinal plants are unique in that they contain multiple essential botanical components in different parts of the plant and thus have the potential to treat and cure various human health problems (Yuan et al., 2016; Sheikh et al., 2021). Natural plant products like essential oils, which are a rich source of bioactive compounds with molecular and biological diversity, play an important role in the drug discovery and development process (Hu et al., 2008). Numerous bioactive chemicals found in essential oil from medicinal plants have pharmacological activities like antimicrobial, anticancer, antioxidant, and anti-inflammation properties (Mir et al., 2022).

PALMAROSA (*Cymbopogon martinii*) is a tall perennial grass with a candy-like rose-like odor. It is a tropical plant that grows in hot, humid climates and is used in the perfumery, food flavoring, and pharmaceutical industry. Also used in Ayurvedic medicine to relieve skin problems and neuralgia (Murbach et al., 2014). In addition, it is a hardy plant that can be grown in a wide variety of soil types and climate conditions (Madan et al., 2015). Literature studies show that *Cymbopogon Spp* are used in the treatment of pharyngitis has insecticidal, anti-protozoal, anti-cancer, anti-HIV, anti-inflammatory, anti-diabetic effects, used as an antihypertensive agent for rheumatism. *C. martinii* has recently been prominent to scientists for its natural properties, such as antimicrobial, antigenic, and cancer-preventing agents (Murbach et al., 2014). Computational approaches to drug discovery have evolved into advanced technologies that can be used to screen drugs derived from phytochemicals found in various medicinal plants. Computational predictive models are critical in guiding the methodological selection process for drug and technology research. They have also been used in the in-silico prediction of pharmacokinetic, pharmacological, and toxicological parameters (Loza-Mejía et al., 2018). Molecular docking is now an effective and cost-effective strategy for the development of drugs and their

testing. This approach provides information on drug-receptor interactions that can be used to predict the direction of a candidate drug upon binding to a target protein (Lee et al., 2019). In addition, this technique facilitates systematic investigation by placing a non-covalent molecule at the binding site of a target macromolecule, resulting in specific binding at specific sites, the active site of each ligand (Mir et al., 2022). However, due to the complexity of *Cymbopogon martinii* essential oil (Palmarosa essential oil, PRO), its mechanism of antibacterial action is still not fully understood. We have presented our view that the abundant bioactive components in *C. martinii* have the potential to alleviate problems with gram-positive and gram-negative multi-drug resistant bacteria. Therefore, further research is needed to determine the potential therapeutic effects and possible mechanisms of action of essential oil from *C. martinii*. In response to all of the above, the present study was designed to (1) different identify potential bioactive components of PRO using the GC-FID technique, (2) *in-silico* analysis of the most abundant compounds against target proteins involved in the bacterial life cycle, and (3) evaluate the in vitro antibacterial activity of pure Palmarosa oil against bacterial strains. It will also add new insights to potential predictions for identifying key antimicrobial drugs during dosing.

Materials and Methods

Extraction of Palmarosa essential oil

From CSIR-AROMA nursery fresh leaves of *Cymbopogon martinii* were collected from naturally growing plants. The Palmarosa essential oil was extracted through the steam distillation method as described by Sharma et al. (2021).

Gas Chromatography

The Gas Chromatography (GC-FID) study of PRO was performed by using a Chemtron 2045 gas chromatograph coupled with a flame ionization detector. A stainless steel column (2 m long) filled with 10% OV-17 on 80-100% mesh Chromosorb W (HP) was used. Nitrogen gas was used as carrier gas a with flow rate of 30 ml/min. The detector and injector temperature were kept at 200°C and 250°C. 0.2 µl of the sample was injected. Ramping conditions for the oven were: 110°C maintained initially then ramped to 200°C at a rate of 2 °C/min. Bioactive compounds were identified by comparing the relative retention time with known standards or with data published in the literature (Adam, 2012).

Ligands

Eight (Geraniol, Geranial, Linalool, Fenchyl alcohol, 6-methylhept-5-en-2-one, Borneol, Elemol, -cadinol) bioactive compounds present in *Cymbopogon martinii* were ligands for six bacterial Penicillin-binding Proteins (PBP1a, PBP2a, PBP3, PBP4 PBP5, PBP6). Ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nig.gov/).3-D structures of ligands were made by using UCSF-chimera after retrieving SMILES from the NCBI-PubChem database.

Protein preparation

Six different bacterial penicillin-binding proteins (PBPs) were selected as targets. All six proteins (PBP1a, PBP2a, PBP3, PBP4, PBP5, and PBP6) play important roles in the bacterial life cycle. Target macromolecules (PDB IDs: PBP1a:3UDF, PBP2a:5m19, PBP3:4WEK, PBP4:5TW8, PBP5:6c84, PBP6:3ita) were obtained from the RCSB-PDB database (https://www.rcsb.org/). All PBP structures were cleaned of native inhibitors/water molecules and energy was minimized before the docking study using dock-prep in chimera software. Dock preparation is an optimization part that corrects for atomic bond length, structure, and charge anomalies.

Molecular Docking

Molecular docking analysis of all selected phytocompounds was performed using the CB-Dock2 tool (https://cadd.labshare.cn/cb-dock2/php/index.php). The protein receptors (PBPs) and ligand molecules were uploaded to the CB-Dock2 server in .pdb file format, after that docking was executed. The best-generated docked structure was downloaded in a .pdb file and saved.

Drug-likeness and toxicity

Drug likeness, pharmacokinetic studies, physiochemical properties, and ADMET (Absorption, Metabolism, Toxicity, and Excretion) were conducted by using SWISSADME (http://www.swissadme.ch/index.php). ProTox-II web server was used to study the toxicity profile (https://tox-new.charite.de/protox_II/). It calculates toxicity based on prediction from different levels such as oral toxicity, organ toxicity (hepatotoxicity), and toxicity endpoints (such as cytotoxicity, carcinogenicity, immunotoxicity, and mutagenicity). The Molinspiration tool was used to evaluate the bioactivity potential of all the ligands (https://www.molinspiration.com/cgi-bin/properties).

Active sites prediction in 3D modeled receptor

The active sites in the PBPs were predicted by using a web-based tool CASTp (The Computed Atlas of Surface Topography of proteins). The default value of 1.4 Angstroms as the probe radius was used to calculate the dimensions and identify cavities on 3D protein structures.

In-vitro Anti-bacterial Activity

Agar Disc Diffusion Method was used to determine the anti-microbial activity of PRO against four test organisms, Gram-negative *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424) and Gram-positive *Staphylococcus*

aureus (MTCC 3160), *Bacillus subtilis* (MTCC 121). Microorganisms were bought from IMTECH (Institute of Microbial Technology, Chandigarh). Sterilized paper discs (5mm in diameter) were impregnated with various amounts (1-50µl) of PRO. From 12-hour-old cultures fresh inoculums were prepared for all microbial strains. A swab of bacterial suspension was spread onto the LB-Agar plates and allowed to dry for 30 minutes. The paper discs impregnated with PRO were then placed in the centre of the petri plates. The plates were left at room temperature for 20 min for diffusion of oil from disc to media followed by incubation for 24 hrs at 37°C. Streptomycin (10 mg) was taken as Positive control. After incubation visualizes the plates under transilluminator and notes down the radius of the zone of inhibition formed around the disc, which indicates the antibacterial activity of PRO.

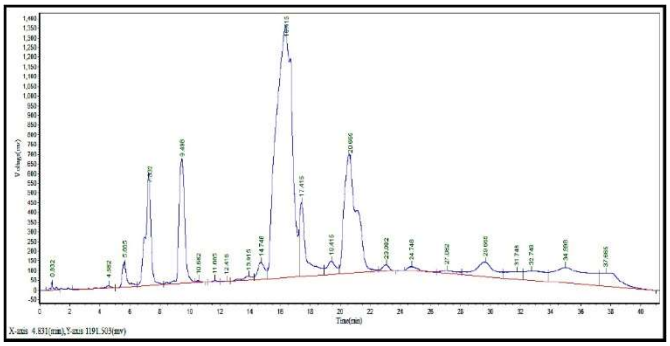
Results and Discussion

Aromatic Profiling

The aromatic profile of PRO obtained by GC-FID is depicted in Figure 1. The GC-FID analysis of PRO extracted from *Cymbopogon martinii* exposed 13 bioactive compounds. All identified compounds were: Terpinolene, 6-Methylhept-5-en-2-one, 2-Norbornaneacetic acid, Citronellyl Acetate, Geraniol, Borneol, Nerol, Epi-*α*-cadinol, *δ*-cadinol, Linalool, Fenchyl Alcohol. GC-FID chromatogram contained three major peaks along with many small peaks. The major compounds were Geraniol (45.8%), Geranial (16.9%), 6-methylhept-5-en-2-one (7.1%), and Linalool (6.3%) whereas Borneol (5.5%), Elemol (3.1%), Fenchyl alcohol (2.9%), and δ-cadinol (2.2%) were minor components. The GC analysis of *Cymbopogon martinii* revealed 3 major bioactive components and 10 minor bioactive components which are known for their biological properties. Literature studies also revealed the presence of geraniol as a major bioactive compound in palmarosa oil extracted from *Cymbopogon spp.* (Rajeswara et al., 2009). Geraniol likewise assumes a significant part in the clinical world as a compound that contains drugs antagonistic to cancer, leukemia, and hepatoma (Rihayat et al., 2020). During the course of time, the use of PRO has become a major area of health- and medical-related research due to the richness of bioactives. PRO also has been used as a therapeutic agent in pharmaceutical preparations as an anti-oxidative, antibacterial, antiviral, anti-diabetic, anti-tumor, antifungal, anti-obesity, anti-hypertensive**,** anti-histaminic, anti-cancer, anti-HIV and hepatoprotective agent (Murbach et al., 2014). In this study, for further docking studies, major components Geraniol, Geranial, 6-methylhept-5-en-2-one, and Linalool and some minor components like Elemol, *δ*-cadinol, Borneol, Fenchyl alcohol were selected as ligands against bacterial PBPs.

Molecular Docking

The docking score for each bioactive compound against the 6-PBPs, interaction of hydrogen bonds, hydrophobic interactions, and essential details are illustrated in Table 1. Molecular interactions and docking poses for *δ*-cadinol with six PBPs are shown in Figure 2, and for the rest of the ligand molecules, the docking poses are shown in Supplemental data, Figures S1-S7. The docking results revealed that for the PBP1a among all bioactive components delta-cadinol and Elemol showed strong docking with vina score of -6.3, followed by Fenchyl alcohol with vina score -5.8, Geraniol and Borneol with -5.5, Linalool, with -5.3, Geranial with -4.9, and 6-methyl-hept-5-em-2-one with least vina score -4.7. For PBP2a in-silico studies revealed robust docking with Delta-cadinol with -6.3 vina score, followed by Elemol (-6.2), Borneol ad Fenchyl Alcohol (-5.5), Linalool (-5.2), Geranial (-5.1), Geraniol (-5.0), and 6-methyl-hept-5-en-2-one (-4.9) vina score. For PBP3 *in-silico* studies depicted strong docking with Elemol with vina score of -6.2, followed by Delta-cadinol (-6.1), Geraniol and Geranial (-5.3), Linalool and Fenchyl alcohol with vina score (-5.2), Borneol (-5.0), and 6-methyl-hept-5-en-2-one with least docking score (-4.8). For PBP4 molecular docking studies revealed in the midst of all bioactive enzymes, Delta-cadinol possesses strong docking with vina score -7.1 followed by Elemol (-6.1), Fenchyl alcohol (-5.6), Borneol (-5.1), Linalool (-4.9), Geranial and 6-methyl-hept-5-en-2-one (-4.8), and geraniol (-4.6). For PBP 5 docking study revealed that Delta-cadinol



Peak No.	Retention time (min)	Bioactive compound	Conc.
1	5.6	Terpinolene	1.2
2	7.3	6-Methylhept-5-en-2-one	7.1
3	9.4	2-Norbornaneacetic acid	1.7
4	14.7	Citronellyl acetate	1.4
5	16.4	Geraniol	45.8
6	17.4	Borneol	5.5
7	19.4	Nerol	1.3
8	20.6	Geranial	16.9
9	29.6	Elemol	3.1
10	31.7	Epi-α-cadinol	1.3
11	32.7	δ-Cadinol	2.2
12	34.9	Linalool	6.3
13	37.6	Fenchyl alcohol	2.9

Figure 1. *GC-FID analysis of PRO.*

Table 1. *Molecular docking of bioactive components of Cymbopogon martinii essential oil against bacterial cell wall receptors.*

PBP	Ligand	Docking Score				Interacting Residues within 4A° radius	
		Vina Score	Cavity Volume (Å^3)	Center (x,y,z)	Docking Size (x,y,z)	Hydrophobic interactions	Hydrogen Bonds
	Geraniol	-5.5	1739	73, 98, -5	19, 19, 34	ASN134, LEU135, GLN551, HIS553, PRO571	TYR567
	Geranial	-4.9	1380	88, 31, 17	27, 33, 27	ASN134, GLN551, HIS553, PRO571	PHE72
	Linalool	-5.3	1739	73, 98, -5	18, 24, 34	HIS553, PRO571	GLU72, ARG628
PBP1a-	Elemol	-6.3	2357	93, 48, -1	29, 18, 32	LEU213, GLN514, 515	LEU513
3udf	Delta-cadinol	-6.3	2357	93, 48, -1	29, 18, 32	GLN212, LEU213, MET509, GLU515	GLY512
	Borneol	-5.5	1739	73, 98, -5	16, 24, 34	PHE71, ILE534	ARG549
	Fenchyl alcohol	-5.8	1739	73, 98, -5	16, 24, 34	PHE71, LEU532, ILE534	GLN535
	6-methyl-hept-5-en-2-one	-4.7	1739	73, 98, -5	18, 24, 34	ASN 134, LEU 135, GLN 551, PRO 571	LYS570
	Geraniol	-5.0	9752	-9, -13, 50	35, 32, 35	TYR446, THR582, 600	GLY640, ALA642, SER643
	Geranial	-5.1	1118	-28, -24, 5	19, 27, 19	TYR446, THR582, 600, MET641	SER403, 462; THR600
	Linalool	-5.2	9752	-9, -13, 50	35, 32, 35	THR165, VAL256,277	ARG151, 241
PBP2a-	Elemol	-6.2	9752	-9, -13, 50	35, 32, 35	LYS148, ARG241, VAL256, 277	SER149, ARG151
5m19	Delta-cadinol	-6.3	9752	-9, -13, 50	35, 32, 35	ARG151, GLU239, VAL256, 277	ARG151, HIS293
	Borneol	-5.5	9752	-9, -13, 50	35, 32, 35	ARG241, VAL256, 277	ARG151
	Fenchyl alcohol	-5.5	9752	-9, -13, 50	35, 32, 35	ARG241, VAL256, 277	---
	6-methyl-hept-5-en-2-one	-4.9	1118	-28, -24, 5	18, 27, 18	TYR446, THR600	----
	Geraniol	-5.3	2419	6, 13, -22	19, 30, 19	VAL323, ARG396, VAL414	TYR399, THR412, ARG517
	Geranial	-5.3	1439	3, 38, 14	28, 19, 19	ARG371,396, VAL414	TYR399, THR412, ARG517
	Linalool	-5.2	2419	6, 13, -22	18, 30, 18	VAL323, ARG371,396,397, TYR399, VAL414	THR412, ASP413, ARG517
PBP3-	Elemol	-6.2	1439	3, 38, 14	28, 18, 18	TYR 651,653	ARG575
4WEK	Delta-cadinol	-6.1	1439	3, 38, 14	28, 18, 18	VAL577, TYR651, 653	THR731
	Borneol	-5.0	1439	3, 38, 14	28, 16, 16	VAL577, 517, PHE777	LYS728
	Fenchyl alcohol	-5.2	1439	3, 38, 14	28, 16, 16	VAL715, PHE777	LYS728, SER729
	6-methyl-hept-5-en-2-one	-4.8	1439	3, 38, 14	28, 18, 18	VAL577, PHE777	GLY778, 779
	Geraniol	-4.6	1541	33, -60, 5	19, 19, 33	ALA74, ALA182	LYS78, ASN141, THR180, ALA182
	Geranial	-4.8	594	24, -63, 39	19, 19, 19	ALA74, GLU183	GLU114
	Linalool	-4.9	1541	33, -60, 5	18, 18, 33	TRP71, LEU96, ARG200, VAL202	------
PBP4-	Elemol	-6.1	594	24, -63, 39	18, 18, 18	PHE241	SER75, 262
5tw8	Delta-cadinol	-7.1	1541	33, -60, 5	18, 18, 33	LYS70, TRP 70,71, ASN72, VAL189, ARG200, VAL202	LYS70
	Borneol	-5.1	1541	33, -60, 5	22, 16, 33	TRP71, VAL89, LEU96	---
	Fenchyl alcohol	-5.6	1541	33, -60, 5	22, 16, 33	LYS70, TRP71, LEU96, VAL202	ASN72
	6-methyl-hept-5-en-2-one	-4.8	594	24, -63, 39	18, 18, 18	ALA74, LEU115, GLU183	LYS78, ASN141
	Geraniol	-5.6	5183	173, -28, 0	35, 19, 28	PHE155, PRO156, LEU319, ILE321, LEU332	LYS329, LYS330
	Geranial	-5.9	5183	173, -28, 0	35, 19, 28	PHE155, GLU157, ILE321, VAL 331, LEU 332	LYS329
	Linalool	-5.9	5183	173, -28, 0	35, 24, 28	PHE155, LEU319, ILE321, LYS330, VAL331, LEU332	GLU157
PBP5-	Elemol	-6.2	1666	171, 33, 46	25, 24, 18	LYS297	LYS 297, ARG213
6c84	Delta-cadinol	-7.0	2012	179, -12, 69	35, 18, 18	GLU170, ALA171, ARG173, LYS297	LYS 297, ARG312
	Borneol	-5.0	3087	144, -18, 0	28, 23, 27	ALA171, ARG321	SER 293, ASN 295, ARG312
	Fenchyl alcohol	-5.2	5183	173, -28, 0	35, 24, 28	PHE155, PRO156, ILE321, VAL331, LEU332	GLU157
	6-methyl-hept-5-en-2-one	-5.4	5183	173, -28, 0	35, 24, 28	LEU129, PHE155, LEU319, LYS330, LEU332	GLU157
	Geraniol	-5.6	3569	21, -47, 35	33, 19, 25	ALA39, ILE189, ARG190	ARG190, ARG194
	Geranial	-5.6	11049	6, -26, -1	35, 35, 35	PRO192, ARG196, TRP199, ARG242	---
	Linalool	-5.2	3569	21, -47, 35	33, 24, 25	ARG196, TRP199, ARG242, PHE245	ARG196
PBP6-	Elemol	-6.7	11049	6, -26, -1	35, 35, 35	GLU7, ASP91, GLN92, PHE186, 258	LYS88
3ita	Delta-cadinol	-7.4	3438	20, -39, 17	18, 35, 25	GLU7, PRO9, GLN92, LYS250, PHE186, 258	GLU7
	Borneol	-5.8	11049	6, -26, -1	35, 35, 35	LEU149, ARG190	SER40, LYS43, ASN108
	Fenchyl alcohol	-5.8	3569	21, -47, 35	33, 24, 25	ARG196,242, TRP199	ARG196
	6-methyl-hept-5-en-2-one	-4.8	3569	21, -47, 35	33, 24, 25	PRO192, ARG194, TRP199	ARG190

depicted strong docking with -7.0 vina score later Elemol with -6.2 vina score, Geranial and Linalool (-5.9), Geraniol with vina score -5.6, 6-methyl-hept-5-en-2-one (-5.4), Fenchyl alcohol (-5.2), and Borneol (-5.0). Among all bioactive components, Delta-cadinol depicted strong docking with PBP 6 with -7.4 vina score after that after that Elemol with -6.7, Borneol and Fenchyl alcohol (-5.8), Geraniol and Geranial (-5.6), Linalool (-5.2), and 6-methyl-hept-5-en-2-one (-4.8) respectively in that order. It was observed that all the ligands (Geraniol, Geranial, 6-methylhept-5-en-2-one, Linalool, Elemol, *δ*-cadinol, Borneol, Fenchyl alcohol) successfully docked with Penicillin binding domain of six Penicillin-binding Proteins (PBP1a, PBP2a, PBP3, PBP4, PBP5, and PBP 6). There are numerous targets to which antimicrobial compounds can inhibit cell wall synthesis. Such mechanisms have been considered important antimicrobial targets for many years (Bruning et al., 2011). The penicillin-binding (PB) domain at the C-terminus of all PBP classes has transpeptidase activity, which catalyzes peptide cross-linking between two adjacent glycan chains during cell wall peptidoglycan synthesis (Straume et al., 2020). In bacterial cells, penicillin-binding proteins (PBPs) polymerize and modify peptidoglycan, a stress-resistant component of the bacterial cell wall. As part of this process, PBPs help shape the exoskeleton morphology of peptidoglycan along with cytoskeleton proteins that regulate septum formation and cell shape. Many natural compounds have been reported to inhibit his PBP synthesis (Mir et al., 2022). The C-terminal module is responsible for the transpeptidase activity of PBP. The C-terminal domain shares its global fold with the transpeptidase

domain found in all PBPs (Contreras-Martel et al., 2017). It has been reported that blocking the transpeptidation or carboxypeptidation reactions by β-lactam antibiotics or therapeutic inhibitors, structurally similar to the D-peptide moiety, D-body, depletes peptidoglycan and possibly induces cell death (Costa et al., 2018). Once PBP is acylated by therapeutic inhibitors, it cannot catalyze the hydrolysis of the covalent acyl-enzyme intermediate and is inactivated; peptidoglycan transfer cannot occur and the cell wall is impaired (Moon et al., 2018). It has been speculated that the N-terminal domain interacts with other proteins or serves as a basis for PBPs to reach their target (Masters et al., 2020). These findings are in corroboration with those of (Yang et al., 2013) as they stated that molecular docking analyses were executed to clarify the anti-bacterial efficacy of the most active compounds of essential oil from *Pogostemon cablin* against PBPs. Similar docking studies using bioactive compounds against PBP have been cited. For example, in eucalyptus oil, eucalyptol, a major bioactive ingredient, is anti-PBP, and potent antibacterial activity has been reported (Sharma et al., 2022). Sripathi and Ravi (2017) also reported molecular docking studies of essential oil components *Plectranthus hadiensis* against PBP. Taken together; this study cited that PRO may be considered as the most important source of anti-bacterial compounds.

During docking, the ligand molecule exhibits hydrophobic interactions or forms hydrogen bonds with the active site residues of the receptor protein, determining the ligand's affinity for the receptor (Sharma et al., 2022). Therefore, the molecular interactions between all eight bioactive components and six PPBs were further investigated, as shown in Table 1. The average number of hydrophobic atoms in commercially available drugs is 16, with one to two donors and three to four acceptors (Davis & Teague, 1999). This determines the importance of hydrophobic interactions in drug design. They can increase the binding affinity between target drug interfaces. It has been previously reported that the binding affinity and efficacy of drugs concerning hydrophobic interactions can be optimized by combining them at the hydrogen binding site (Qian et al., 2009). Docking analysis also revealed that the interactions of all eight bioactive components with six protein receptors were mediated through both hydrophobic and hydrogen bonding interactions. As shown in Figure 3, delta-cadinol

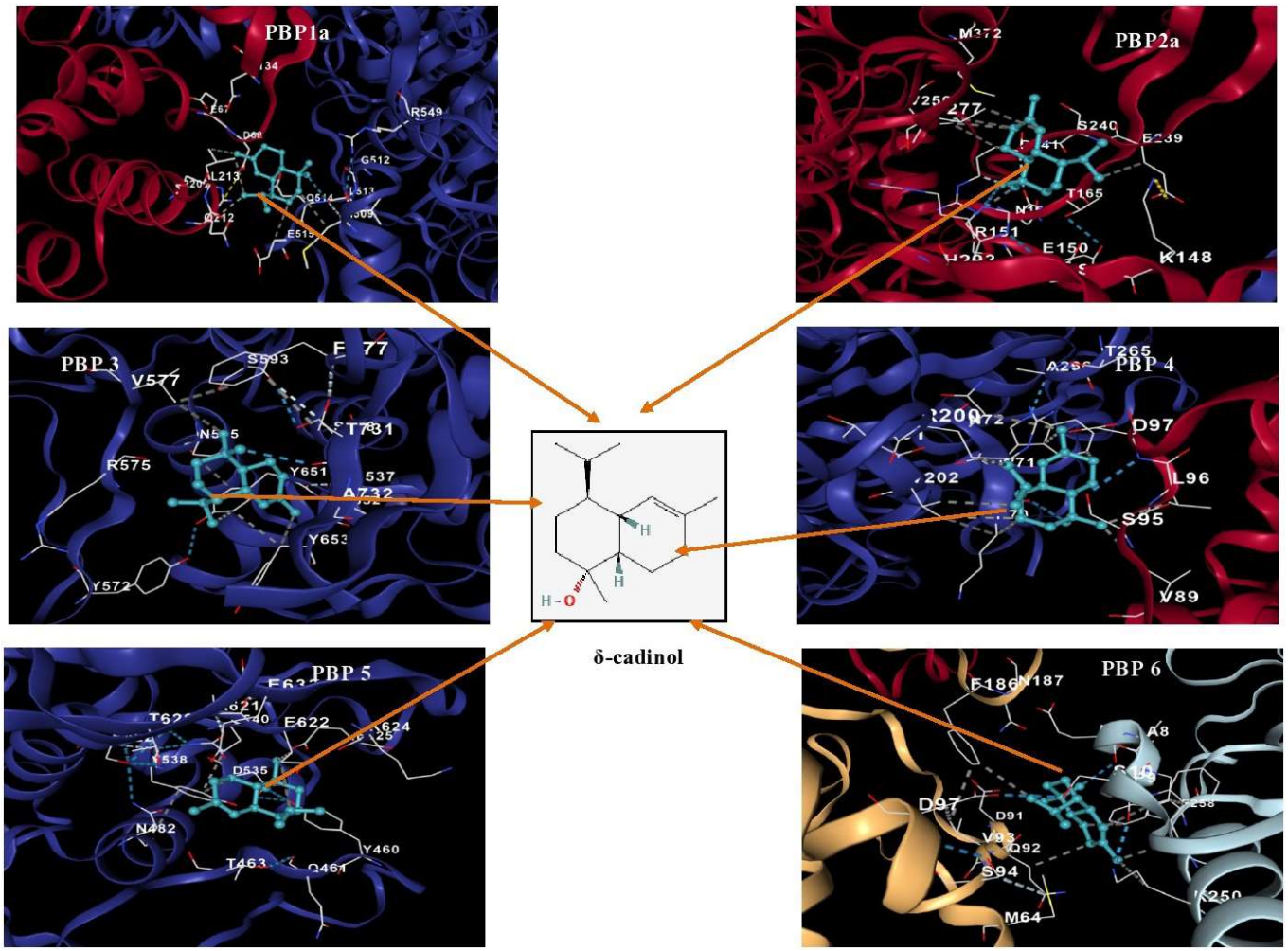


Figure 2. *Molecular docking of δ-cadinol with six different PBPs.*

showed hydrophobic interactions via GLN 212, LEU 213, MET 509, GLU 515 and Hydrogen bond interaction was seen through GLY 512 with PBP1a, hydrophobic interactions via ARG 151, GLU 239, VAL 256, 277 and Hydrogen bond interaction via ARG 151, HIS 293 with PBP2a, hydrophobic interactions via VAL 577, TYR 651, 653 Hydrogen bond interaction was seen through THR 731 with PBP 3, hydrophobic interactions via LYS70, TRP 70,71, ASN 72, VAL 189, ARG 200, VAL 202 and Hydrogen bond interaction was seen through LYS 70 with PBP4. With PBP5 delta-cadinol showed hydrophobic interactions via GLU 170, ALA 171, ARG 173, LYS 297 and Hydrogen bond interaction was seen through LYS 297, ARG 312. With PBP6 delta-cadinol showed hydrophobic interactions via GLU7, PRO9, GLN92, LYS250, PHE186, 258 and Hydrogen bond interaction was seen through GLU 7.

Hydrophobic and hydrogen bond interaction of Elemol against six PBP is shown in Supplemental data, Figure S8. Elemol showed hydrophobic interaction via LEU 213, GLN 514, 515 and Hydrogen bond interaction was seen through LEU 513 with PBP1a, hydrophobic interaction via LYS 148, ARG 241, VAL 256, 277 and Hydrogen bond interaction via SER 149, ARG 151 with PBP2a, hydrophobic interaction was observed via TYR 651,653 and hydrogen bond interaction via ARG 575 for PBP3. Hydrophobic interaction was seen through PHE 241 and Hydrogen bond interaction via SER 75, 262 for PBP4. Hydrophobic interaction was seen through LYS 297 and Hydrogen bond interaction via LYS

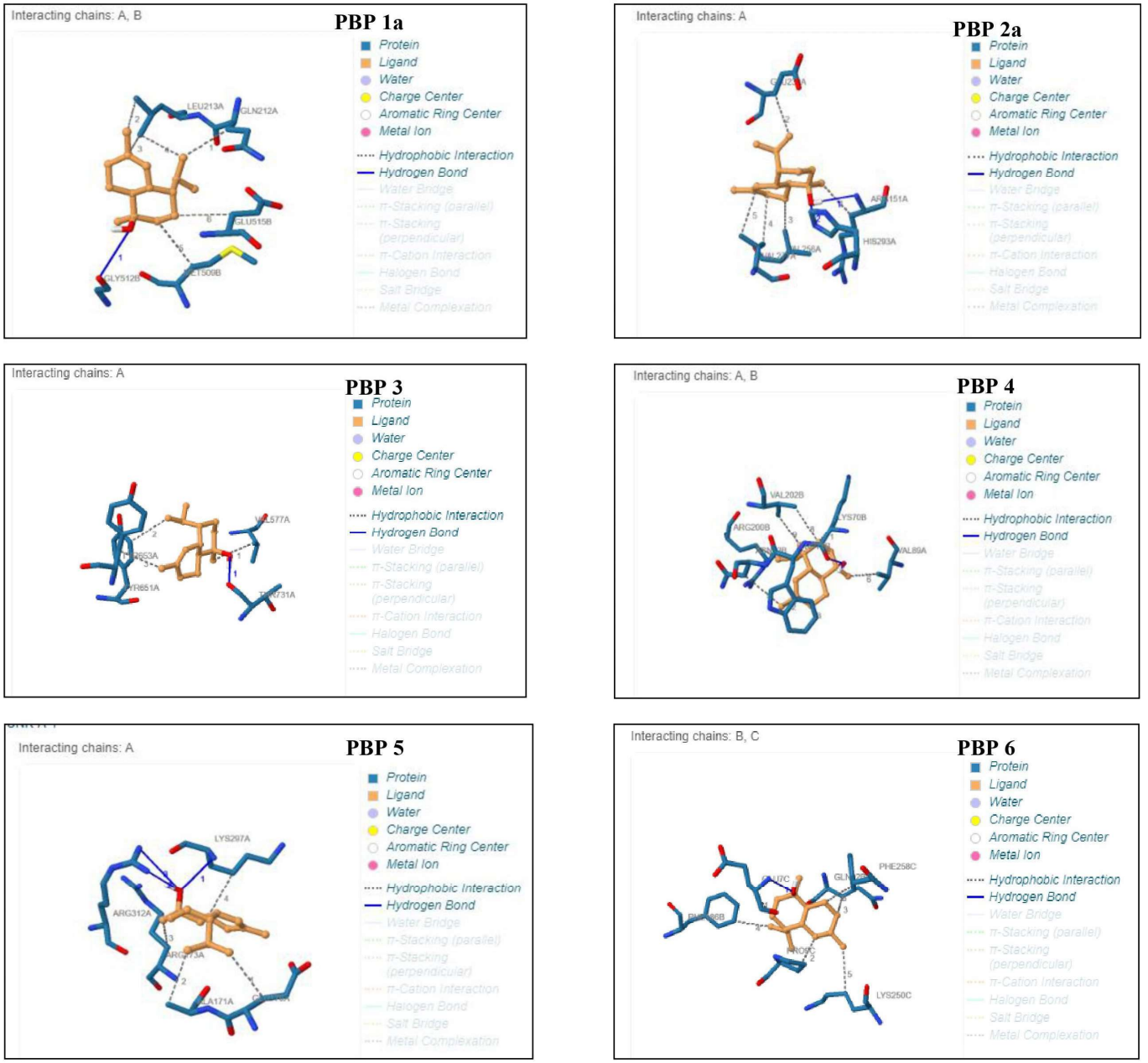


Figure 3. *3-D interactions of delta-cadinol with protein receptors.*

297 for PBP5. Hydrophobic interaction was seen through GLU7, ASP91, GLN92, PHE186, 258 and Hydrogen bond interaction via LYS 88 for PBP6.

Hydrophobic and hydrogen bond interaction of Fenchyl Alcohol against six PBP is shown in Supplemental data, Figure S9. Hydrophobic interaction was seen through PHE 71, LEU 532, ILE 534 and Hydrogen bond interaction via GLN 535 for PBP1a. Hydrophobic interaction was seen through ARG 241, VAL 256, 277 and no Hydrogen bond interaction was for PBP2a. Hydrophobic interaction was seen through VAL 715, PHE 777 and Hydrogen bond interaction via LYS 728, SER 729 for PBP3. Hydrophobic interaction was seen through LYS70, TRP71, LEU96, VAL 202 and Hydrogen bond interaction via ASN 72 for PBP4. Hydrophobic interaction was seen through PHE 155, PRO 156, ILE 321, VAL 331, LEU 332 and Hydrogen bond interaction via GLU 157 for PBP5.Hydrophobic interaction was seen through PHE 155 ARG 196,242, TRP 199 and Hydrogen bond interaction via ARG196 for PBP6.

Hydrophobic and hydrogen bond interaction of Geraniol against six PBP is shown in Supplemental data, Figure S10. Hydrophobic interaction was seen through ASN 134, LEU 135, GLN 551, HIS 553, PRO571 and Hydrogen bond interaction via TYR 567for PBP1a. Hydrophobic interaction was seen through TYR 446, THR 582, 600 and Hydrogen bond interaction via GLY640, ALA642, SER643 for PBP2a. Hydrophobic interaction was seen through VAL 323, ARG 396, VAL 414 and Hydrogen bond interaction via TYR 399, THR 412, ARG 517 for PBP3. Hydrophobic interaction was seen through ALA 74, ALA 182 and Hydrogen bond interaction via LYS 78, ASN 141, THR 180, ALA 182 for PBP4. Hydrophobic interaction was seen through PHE 155, PRO156, LEU319, ILE 321, LEU 332 and Hydrogen bond interaction via LYS 329, LYS 330 for PBP5.Hydrophobic interaction was seen through ALA39, ILE189, ARG190and Hydrogen bond interaction via ARG 190, ARG 194 for PBP6.

Hydrophobic and hydrogen bond interaction of Geranial against six PBP is shown in Supplemental data, Figure S11. Hydrophobic interaction was seen through ASN 134, GLN 551, HIS 553, PRO 571and Hydrogen bond interaction via PHE 72 for PBP1a. Hydrophobic interaction was seen through TYR 446, THR 582, 600, MET 641 and Hydrogen bond interaction via SER403,462; THR 600 for PBP2a. Hydrophobic interaction was seen through ARG371, 396, VAL414 and Hydrogen bond interaction via TYR 399, THR 412, and ARG 517 for PBP3. Hydrophobic interaction was seen through ALA 74, GLU183 and Hydrogen bond interaction via GLU 114 for PBP4. Hydrophobic interaction was seen through PHE155, GLU157, ILE321, VAL 331, LEU 332 and Hydrogen bond interaction via LYS 329 for PBP5.Hydrophobic interaction was seen through PRO 192, ARG196, TRP199, ARG242 and no Hydrogen bond interaction for PBP6.

Hydrophobic and hydrogen bond interaction of Linalool against six PBP is shown in Supplemental data, Figure S12. Hydrophobic interaction was seen through HIS 553, PRO 571 and Hydrogen bond interaction via GLU 72, ARG 628 for PBP1a. Hydrophobic interaction was seen through THR 165, VAL256, 277 and Hydrogen bond interaction via ARG 151, 241 for PBP2a. Hydrophobic interaction was seen through VAL323, ARG371, 396, 397, TYR 399, VAL 414 and Hydrogen bond interaction via ARG 517 for PBP3. Hydrophobic interaction was seen through TRP 71, LEU 96, ARG 200, VAL 202 and no Hydrogen bond interaction for PBP4. Hydrophobic interaction was seen through PHE155, LEU 319, ILE 321, LYS 330, VAL 331, LEU 332 and Hydrogen bond interaction via GLU 157 for PBP5. Hydrophobic interaction was seen through ARG196, TRP199, ARG242, PHE245 and Hydrogen bond interaction via ARG196 for PBP6.

Hydrophobic and hydrogen bond interaction of Borneol against six PBP is shown in Supplemental data, Figure S13. Hydrophobic interaction was seen through PHE 71, ILE 534 and Hydrogen bond interaction via ARG 549 for PBP1a. Hydrophobic interaction was seen through ARG 241, VAL 256, 277 and Hydrogen bond interaction via ARG 151 for PBP2a. Hydrophobic interaction was seen through VAL 577, 517, PHE 777 and Hydrogen bond interaction via LYS 728 for PBP3. Hydrophobic interaction was seen through TRP71, VAL 89, LEU 96 and no Hydrogen bond interaction for PBP4. Hydrophobic interaction was seen through ALA 171, ARG 321and Hydrogen bond interaction via ARG 312 for PBP5. Hydrophobic interaction was seen through LEU149, ARG190 and Hydrogen bond interaction via ASN108 for PBP6.

Hydrophobic and hydrogen bond interaction of 6-methyl-hept-5-en-2-one against six PBPs is shown in Supplemental data, Figure S14. Hydrophobic interaction was seen through ASN 134, LEU 135, GLN 551, PRO 571 and Hydrogen bond interaction via LYS 570 for PBP1a. Hydrophobic interaction was seen through TYR 446, THR 600 and no Hydrogen bond interaction for PBP2a. Hydrophobic interaction was seen through GLY 778, 779 and Hydrogen bond interaction via GLY 778, 779 for PBP3. Hydrophobic interaction was seen through ALA 74, LEU115, GLU 183 and Hydrogen bond interaction via LYS 78, ASN 141 for PBP4. Hydrophobic interaction was seen LEU129, PHE155, LEU319, LYS 330, LEU332 and Hydrogen bond interaction via GLU157 for PBP5. Hydrophobic interaction was seen through PRO192, ARG194, TRP199 and Hydrogen bond interaction via ARG190 for PBP6. Higher hydrogen bonding between the ligand and the protein determines the strength of the bond (Kortemme et al., 2003). Active site predictions revealed

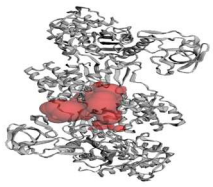
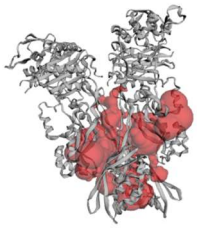
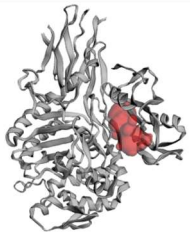
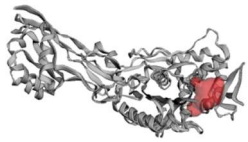
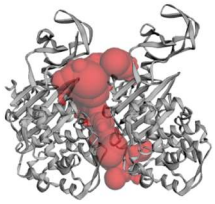
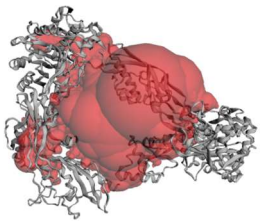
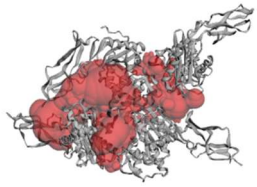
residues that interact with the major cavities of all six PBP as in Table 2. With CASTp, a major pocket was recognized with a Volume (SA) of 51218.649 and an Area (SA) of 11563.524 in PBP 5. For PBP 6 major pocket was recognized with a Volume of 22072.980 and an Area (SA) of 11436.474, in PBP 2a major pocket was documented with the Volume (SA) of 7926.773 and an Area (SA) of 5857.642; within PBP 4 major pocket was recognized with the Volume (SA) of 4186.682 and an Area (SA) of 2782.560; inside PBP 1a major pocket was documented with a Volume (SA) of 3464.850 and an Area (SA) of 1995.510, and for PBP 3 major pocket was observed at a Volume (SA) of 254.687 and an Area (SA) of 313.875 in that order. The interactive residues at the active site of all PBP are shown in Table 2. Hence due to owing hydrogen bond interaction; all the eight ligands possessed healthy binding with all the PBPs. It has been speculated that upon binding to the ligands, PBP ceases to function, thereby leading to a conformational change of the bacterial enzymes. All of these events suppress the viability of the bacteria, thereby reducing the infectivity of the bacteria in the host cell. Previous studies have also demonstrated through in- silico results citing the antibacterial ability of multi-pharmacological agents to control the growth of microbial strains (Lima et al., 2019).

In-silico ADMET properties

ADMET (Absorption, Distribution, Metabolism Excretion, and Toxicity) and Prediction of Activity Spectra for Substances (PASS) of the drug are prerequisites for its in vivo therapeutic use (Wu et al., 2020). Lipinski rule of 5 (RO5) is used to find out the drug-likeness of all the ligands (Geraniol, Geranial, Linalool, Elemol, δ-cadinol, Borneol, Fenchyl alcohol, and 6-methyl-hept-5-en-2-one). According to this rule to own drug-like properties ligand must have log P ≤ 5, number of H-bond acceptors ≤ 10, H-bond donor's ≤ 5, and no violation of more than 1. All the ligands followed Lipinski, Veber, and Egan's rule. Hence, it was claimed that bioactive compounds can be said to possess good oral bioavailability (Biswal et al., 2019). The oral activity of all the ligand molecules is predicted by calculating other molecular parameters like mlog P (Partition coefficient) and TPSA (Polar Surface Area) as illustrated in Table 3, all the ligands have revealed good agreement with RO5. PASS and ADMET analysis revealed that all eight ligands were low molecular weight ligands. The Log Po/w value of all the ligands was also in the satisfactory range. Molecular Lipophilicity potential (MLP) depicting a surface view of all the ligands is shown in Figure 4. TPSA of Geraniol, Fenchyl Alcohol, Borneol, Linalool, Elemol, and δ-cadinol was 20.23 Å2; and TPSA of Geranial and 6-methyl-hept-5-en-2-one was 17.07Å2. As obvious from Table-3, GI (Gastrointestinal tract absorption) of all the ligands was high. It was reported that low molecular weight ligands have a better tendency to diffuse quickly and be transported across biological membranes in contrast to high molecular weight ligands (Srimai et al., 2013). Wu et al., (2020) reported that TPSA is a noteworthy predictor of drug transport properties such as intestinal absorption, nice bioavailability, and permeability. In the human body, to exercise a toxic effect, drug molecules have to be absorbed potently. TPSA was found to be an exceptionally excellent descriptor of drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and BBB penetration (Veber et al., 2002). The Log Po/w value of all the ligands was also in the satisfactory range. Abraham 2003 cited that in pharmacokinetics analysis, Log Po/w is a crucial factor to assess the Lipophilicity of any drug and its distribution in the body after assimilation. Furthermore, all the ligands (Geraniol, Geranial, Linalool, Elemol, δ-cadinol, Borneol, Fenchyl alcohol, and 6-methyl-hept-5-en-2-one) were non-substrate to P-glycoprotein (P-gp) efflux transporters. Konig and Muller (2013) cited that in the gut, P-gp pumps drug back into the lumen, diminishing their absorption. Bioactive compounds Geraniol, Geranial, Linalool, Borneol, Fenchyl alcohol, and 6-methyl-hept-5-en-2-one expounded non-inhibitory potential against CYP450 series of enzymes, involved in liver detoxification in the body (Abraham, 2003; Srimai et al., 2013). Elemol showed an inhibitory effect against the CYP2C9 enzyme and delta-cadinol showed an inhibitory effect against the CYP2C19 enzyme and these two hold non-inhibitory potential against the rest of the CYP450 enzyme series. From this data, it is evident that all the eight ligands meet all the rules of Lipinski, Veber, and Egan's rule. All these data showed that all the ligands can easily interact with the target receptors and can be further taken in the assessment of biological activity score.

The biological activity is an important consideration that describes consequence of drug in living beings. In living systems, the drug is supposed to bind to biological targets which are as well-known as drug targets (Khan et al., 2017). The bioactivity was calculated using the online software Molinspiration based on the following parameters: GPCR ligand, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor, and Enzyme inhibitor and summarized in Table 4. The prescriptive bioactivity score is calculated in three different ranges: score > 0, the drug is active, if it is between -5.0 and 0, the drug is moderately active, and if the score is < -5.0, silent drug (Verma et al., 2012). Bioactivity score of Geraniol, Fenchyl alcohol,6-methyl-hept-en-2-one, and Borneol for Ion channel modulator was found to be in the range of -0.51 to -1.60, but the highest score of 0.47 was observed for Elemol followed by Geraniol and Linalool with bioactivity score 0.07, and δ-cadinol with score 0.02. Bioactivity score of all the eight ligands for Kinase Inhibitor was found to be in the range of -

Table 2. *Active site analysis of Penicillin-Binding Protein.*

Pdb id	Macromolecule	Interacting Active Site Residues	Cavity	
			Area	Volume
PBP1a-3udf		ARG 628, GLU 572, PRO 571, 552; LYS 570, ALA 569, ASN 134, LEU 135, SER 136,570; TYR 567; PHE 71, 72; ILE 555; HIS 553; GLN 551.	1995.510	3464.850
PBP2a-5m19		SER 643, 462, 598, 461; THR 582, 600; HIS 583; LYS 406; GLY 640, 599; MET 641; GLU 447; TYR 446; ALA 642	5857.642	7926.773
PBP2X-1qme		ILE 498; VAL 499, 662, 423; ARG 654, 426, 463; LYS 420; TRP 702; ASP 698; ILE 661, 498; PRO 654; TYR 700; THR 425; GLU 497	602.737	387.311
PBP3-4wek		ARG 371,514, 517, 396, 397; VAL 414, 323; ASP 413; PHE 411; TYR 399; GLU 394; THR 325, 412; PRO 514; GLY 372;	313.875	254.687
PBP4-5Tw8		TYR 291; SER 263, 116, 262, 75; GLU 114, 183; LEU 115; LYS 78; ALA 74; ASN 141; THR 180	2782.560	4186.682
PBP5-6c84		LEU 129, 319, 332; PHE 155; PRO 156; GLU 157; LYS 329, 330; ILE 321; SER 320; VAL 331;	11563.524	51218.649
PBP6-3ita		THR 212, 213; ALA 214; ASP 37, 150; GLY 211, 148; SER 40, 83; LEU 149; ARG 190, 194; LYS 188; ASN 108; ILE 189	11436.474	22072.980

3.31 to -0.84. Bioactivity score of Geraniol, Geranial, Linalool, Fenchyl alcohol, Borneol, and 6-methyl-hept-5-en-2-one for nuclear receptor Ligand was found to be in the range between -1.99 to -0.06, but the highest score of 0.80 was observed with Elemol followed by δ-cadinol with bioactivity score of 0.35. Bioactivity score of all the eight ligands for Protease Inhibitor was found to be in the range of -2.48 to -0.01. Bioactivity score of δ-cadinol, 6-methyl-hept-5-en-2-one, Borneol, and Fenchyl Alcohol was found to be in the range of -1.30 to -0.23, but the highest score of 0.52 was observed with Elemol followed by Geraniol with 0.28 bioactivity score, Linalool and Geranial with bioactivity score of 0.07 and 0.02 respectively. As illustrated in Table-4, the results of this study indicated that all eight ligands were biologically active and induced physiological effects by interacting with GPCR ligands, nuclear receptor ligands, inhibition of proteases, and other enzymes, indicating that they can as a potential drug. Bioactivity score of all the eight ligands for GPCR ligand was found to be in the range of -2.45 to -0.10, indicating them as a potential drug. In biological systems, drug targets mostly include general proteins such as enzymes, receptors and ion channels. Furthermore, it's been well established that bioactive compounds significantly influence the physiological process in the human body (increased or decreased). Therefore, a clear understanding of the physiological, biochemical, and metabolic role of these compounds in the human and animal systems is warranted (Kandeepan et al., 2021). For GPCR ligand all the bioactive components were moderately active. Elemol, Geraniol, Linalool, and δ-cadinol were highly active against Ion Channel Modulator. All ligands were moderately active against Kinase Inhibitor. Elemol and δ-cadinol were highly active against Nuclear Receptor Ligand. For Protease Inhibitor all the ligands were moderately active. Elemol, Linalool, Geraniol, and Geranial were found to be highly active against Enzyme Inhibitor rest of the ligands were moderately active. These observations indicated that studied bioactive compounds own such properties that are mandatory for the bioactive compounds to act as key drugs. Likely explanations have been given by researchers working on

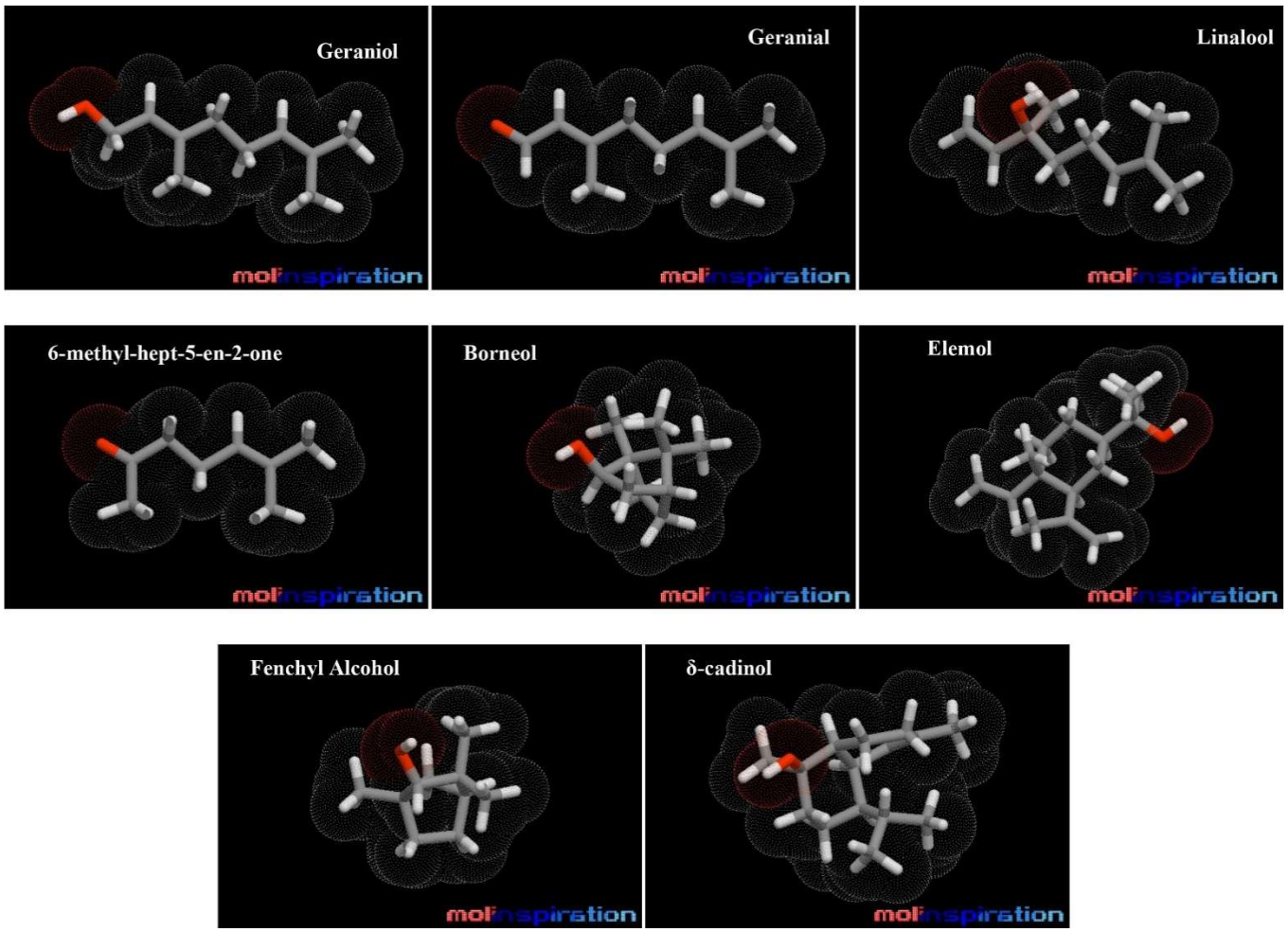


Figure 4. *Molecular Lipophilicity potential (MLP)/ Polar surface Area (PSA) views of ligands.*
Note: Hydrophobic area is encoded by Blue and Hydrophilic area is encoded by red.

RESEARCH ARTICLE

Table 3. *Physiochemical and pharmacokinetic properties of ligands.*

Physiochemical properties	Fenchyl Alcohol	Geraniol	Geranial	Linalool	Borneol	Elemol	Delta-cadinol	6-methyl-hept-5-en-2-one
Formula	$C_{10}H_{18}O$	$C_{10}H_{18}O$	$C_{10}H_{16}O$	$C_{10}H_{18}O$	$C_{10}H_{18}O$	$C_{15}H_{26}O$	$C_{15}H_{26}O$	$C_8H_{14}O$
Molecular weight g/mol	154.25	154.25	152.23	154.25	154.25	222.37	222.37	126.20
Num. heavy atoms	11	11	11	11	11	16	16	9
Num. arom.heavy atoms	0	0	0	0	0	0	0	0
Fraction Csp3	1.00	0.60	0.50	0.60	1.00	0.73	0.87	0.62
Num. rotatable bonds	0	4	4	4	0	3	1	3
Num. H-bond acceptors	1	1	1	1	1	1	1	1
Num. H-bond donors	1	1	0	1	1	1	1	0
Molecular Refractivity	46.60	50.40	49.44	50.44	46.60	72.10	70.72	40.30
TPSA	20.23 Å²	20.23 Å²	17.07 Å²	20.23 Å²	20.23 Å²	20.23 Å²	20.23 Å²	17.07 Å²
Lipophilicity								
Log $P_{o/w}$ (iLOGP)	2.42	2.75	2.47	2.70	2.29	3.20	3.15	2.23
Log $P_{o/w}$ (XLOGP3)	3.17	3.56	3.03	2.97	2.72	4.41	3.34	1.88
Log $P_{o/w}$ (WLOGP)	2.19	2.67	2.88	2.67	2.19	3.94	3.78	2.32
Log $P_{o/w}$ (MLOGP)	2.45	2.59	2.49	2.59	2.45	3.56	3.67	1.97
Log $P_{o/w}$ (SILICOS-IT)	2.27	2.35	2.65	2.35	2.27	3.74	3.22	1.96
Consensus Log $P_{o/w}$	2.50	2.78	2.71	2.66	2.38	3.77	3.43	2.07
Water solubility								
Log *S* (ESOL)	-2.79	-2.78	-2.43	-2.40	-2.51	-3.80	-3.26	-1.61
Solubility	2.48e-01mg/ml 1.61e-03mol/l	2.59e-01mg/ml 1.68e-03mol/l	5.67e-01mg/ml 3.73e-03mol/l	6.09e-0mg/ml 3.95e-03mol/l	4.77e-01mg/ml 3.09e-03mol/l	3.53e-02mg/ml 1.59e-04mol/l	1.23e-01mg/ml 5.54e-04mol/l	3.11e+00mg/ml 2.46e-02mol/l
Class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Very soluble
Log *S* (Ali)	-3.27	-3.67	-3.05	-3.06	-2.80	-4.55	-3.44	-1.86
Solubility	8.37e-02 mg/ml 5.43e-04 mol/l	3.30e-02mg/ml 2.14e-04mol/l	1.34e-01mg/ml 8.83e-04mol/l	1.35e-01mg/ml 8.75e-04mol/l	2.45e-01mg/ml 1.59e-03mol/l	6.23e-03mg/ml 2.80e-05mol/l	8.04e-02mg/ml 3.61e-04mol/l	1.74e+00mg/ml 1.38e-02mol/l
Class	Soluble	Soluble	Soluble	Soluble	Soluble	Moderately soluble	Soluble	Very soluble
Log *S* (SILICOS IT)	-1.91	-1.84	-1.96	-1.84	-1.91	-3.00	-2.73	-1.85
Solubility	1.92e+00g/ml; 1.24e-02mol/l	2.20e+00mg/ml 1.43e-02mol/l	1.66e+00mg/ml1 .09e-02 mol/l	2.20e+00mg/ml 1.43e-02 mol/l	1.92e+00g/ml 1.24e-02 mol/l	2.24e-01mg/ml 1.01e-03mol/l	4.10e-01mg/ml 1.85e-03mol/l	1.79e+00mg/ml 1.42e-02mol/l
Class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
Pharmacokinetics								
GI absorption	High	High	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P-gp substrate	No	No	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	Yes	No
CYP2C9 inhibitor	No	No	No	No	No	Yes	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No
LogK_p(skin permeation)	-4.99 cm/s	-4.71 cm/s	-5.08 cm/s	-5.13 cm/s	-5.31 cm/s	-4.53 cm/s	-5.29 cm/s	-5.73 cm/s
Druglikeness								
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	No; 1 violation: MW<160	No; 1 violation: MW<160	No; 1 violation: MW<160	No; 1 violation: MW<160	No; 1 violation: MW<160	Yes	Yes	No; 1 violation: MW<160
Veber	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Egan	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Muegge	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 1 violation: Heteroatoms<2	No; 1 violation: Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Medicinal chemistry								
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	1 alert: isolated_alkene	3 alerts: aldehyde, isolated_alkene, michael_acceptor_1	1 alert: isolated_alkene	0 alert	1 alert: isolated_alkene	1 alert: isolated_alkene	1 alert: isolated_alkene
Leadlikeness	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5	No; 1 violation: MW<250	No; 1 violation: MW<250
Synthetic accessibility	3.43	2.58	2.49	2.74	3.43	3.54	4.29	2.22

Table 4. *Bioactivity score of Ligands.*

Ligand name	Bioactivity	Score
Geraniol	GPCR ligand	-0.6
	Ion channel modulator	0.07
	Kinase inhibitor	-1.32
	Nuclear receptor ligand	-0.20
	Protease inhibitor	-1.03
	Enzyme inhibitor	0.28
Geranial	GPCR ligand	-0.86
	Ion channel modulator	-0.25
	Kinase inhibitor	-1.29
	Nuclear receptor ligand	-0.42
	Protease inhibitor	-0.57
	Enzyme inhibitor	0.02
Linalool	GPCR ligand	-0.73
	Ion channel modulator	0.07
	Kinase inhibitor	-1.26
	Nuclear receptor ligand	-0.06
	Protease inhibitor	-0.94
	Enzyme inhibitor	0.07
Fenchyl alcohol	GPCR ligand	-0.56
	Ion channel modulator	-0.46
	Kinase inhibitor	-1.71
	Nuclear receptor ligand	-0.80
	Protease inhibitor	-0.67
	Enzyme inhibitor	-0.59
Elemol	GPCR ligand	-0.10
	Ion channel modulator	0.47
	Kinase inhibitor	-0.84
	Nuclear receptor ligand	0.80
	Protease inhibitor	-0.01
	Enzyme inhibitor	0.52
6-methyl-hept-5-en-2-one	GPCR ligand	-2.45
	Ion channel modulator	-1.60
	Kinase inhibitor	-3.31
	Nuclear receptor ligand	-1.99
	Protease inhibitor	-2.48
	Enzyme inhibitor	-1.30
Borneol	GPCR ligand	-0.47
	Ion channel modulator	-0.51
	Kinase inhibitor	-1.57
	Nuclear receptor ligand	-0.84
	Protease inhibitor	-0.80
	Enzyme inhibitor	-0.23
δ-cadinol	GPCR ligand	-0.12
	Ion channel modulator	0.02
	Kinase inhibitor	-1.07
	Nuclear receptor ligand	0.35
	Protease inhibitor	-0.67
	Enzyme inhibitor	-0.67

various drug formulations (Khan et al., 2017). The bioactivity score delivered the indication about the binding cascade of the bioactive compounds that is used for the development of a new functional drug with more binding selectivity profile and less undesirable effects (Khan et al., 2017).

Accordingly, toxicity profile of all the ligands (Geraniol, Geranial, 6-methylhept-5-en-2-one, Linalool, Elemol, δ-cadinol, Borneol, Fenchyl alcohol) was assessed and toxicity profile revealed that all the ligands were non-toxic to organs such as inactive prediction was observed with hepatotoxicity as shown in Table 5. Furthermore, all the eight bioactive compounds were non-carcinogenic and non-mutagenic in nature. In addition, bioactive compounds showed inactiveness towards the target-based biological pathways for instance: Stress response pathways and nuclear receptor signalling pathways. Toxicity is the extent to which a substance can harm the body or its organs, such as cells and tissues, and is one of the most important reasons for failure in drug development last stage. Therefore, early identification of toxicity would be very valuable (Lagorce et al., 2017). To evaluate the safety profile of a therapeutic drug in the pharmaceutical industry, a prerequisite is to obtain appropriate informed consent for the risks of chemical drugs (Banerjee et al., 2018). Taking into account, toxicity in silico is an important manifesto for predicting the toxicity of drugs that can harm humans, animals, and the environment (Raies et al., 2016). Drug-induced hepatotoxicity is a major cause of liver damage and a major cause of failure of major marketed drugs (Siramshetty et al., 2016). Lea et al. (2017) reported that the mutagenic nature of the biologically active substance is harmful to cells and is the main driver behind several diseases, such as cancer. Huang et al. (2016) reported that all these targets such as Androgen receptor-ligand-binding domain (AR-LBD), Aryl hydrocarbon receptor (AhR), Androgen receptor, weak response element Heat shock factor (HSE), Nuclear Factor (derived from Erythroid2)-antioxidant/antioxidant response factor (nrf2/ARE) and mitochondrial membrane potential (MMP) are principal components of biological systems inside the human body.

Antibacterial activity

To validate the *in-silico* findings, a wet-lab experiment was designed to evaluate anti-bacterial potential of Palmarosa essential oil against four bacterial strains. The present study reveals that Palmarosa essential oil has shown strong antibacterial activity than antibacterial drug. The results are shown in Figure 5 (Supplemental data, Figures S15-S18), and Table 6. PRO showed substantial antibacterial activity against *E. coli* (MTCC 40 Gram –ve) while strong inhibition against *S. aureus* (MTCC 3160 Gram +ve), and moderate activity against *B. subtilis* (MTCC 121 Gram +ve) *and P. aeruginosa* (MTCC 424 Gram –ve). The present study shows

that the PRO showed nil inhibition at lower concentration (1µl), but antibacterial activity increased substantially with the increase in the volume of PRO (3, 5, 10, 15, 20, 50µL) in case of MTCC 40, MTCC 424, and MTCC 121 (data not shown). The strong antibacterial activity of Palmarosa essential oil may be due to the presence of major and minor bioactive components *in toto* that affected hydrolytic enzyme inhibition (proteases) or inhibited partners like cell wall envelop proteins, microbial adhesions, and non-specific interactions with carbohydrates (Siramon & Ohtani, 2007). Earlier studies also have cited that anti-microbial activity was not always related to the high content of one chemical compound, rather than to synergic effects between major and minor components (Elaissi et al., 2012). It is possible that PRO easily enters bacterial cell walls thus causing membrane leakage of electrolytes or lipid peroxidation of the membrane which eventually lysed or hindered growth of bacterial strains. Siramon & Ohtani (2007) also cited that bioactive molecules have the potential to cross across the cell membranes and to induce biological reactions such as upsetting electron flow, the proton motive force, active transport and coagulation of the cellular contents. Anti-microbial effect of PRO has also been pronounced for other pathogenic organisms like: *Saccharomyces cerevisiae, Botrytis cinerea, Penicillium digitatum and P. italicum* (Prashar et al., 2003; Zhang et al., 2019). The difference in antibacterial activity of PRO against bacterial strains could be due to incidence of multiple targets or single target for their activity. Geraniol, major component of Palmarosa essential oil is a lipophilic compound which inhibits microbial growth by adhering and interacting with the lipids present in the cell membrane of the bacteria, thus making it more permeable and binds with the intracellular sites to destroy the structure (Lira et al., 2020). PRO exhibited very high antibacterial activity against *Staphylococcus aureus. S. aureus* causes different suppurative diseases in people. It causes shallow skin sores; more serious diseases like pneumonia, meningitis, also, urinary plot diseases; food contamination by delivering enterotoxins into food and poisonous shock disorder by arrival of superantigens into the blood stream (Lodhia et al., 2009). Literature studies also revealed the antibacterial activity of essential oils against methicilin-resistant *S. aureus*

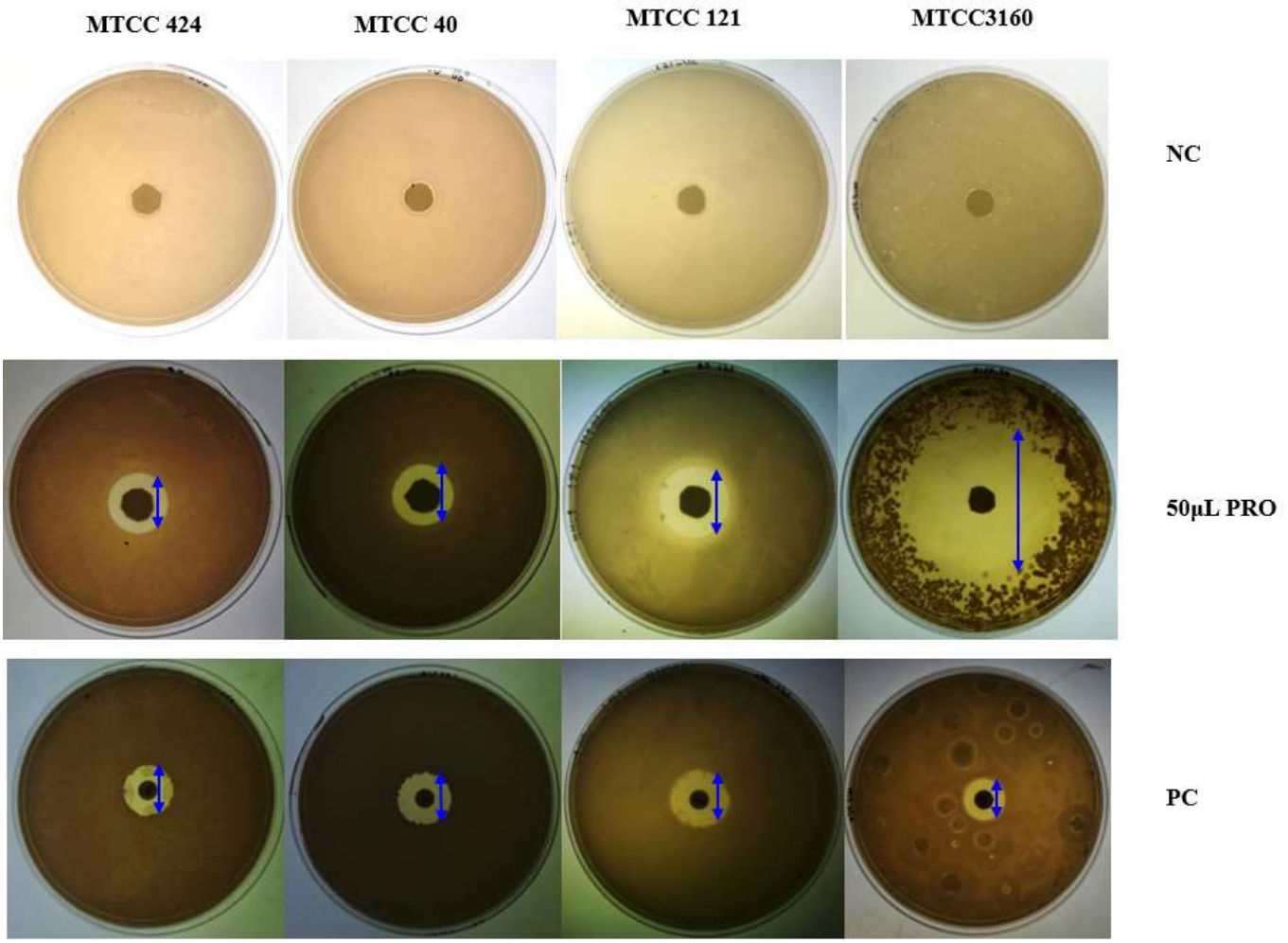


Figure 5. *Antibacterial activity of PRO against different bacterial strains. NC: Negative Control; PC: Positive control (Streptomycin 10 mg/disc). Arrow indicates zone of inhibition (values are given in Table 6).*

(MRSA) derived from medicinal plants (Horvath et al., 2017; Lin et al., 2021). In the present study, high antimicrobial toxicity of PRO toward gram negative bacteria (like *E. coli*) was observed which is a noteworthy observation as most studies suggest that the gram negative bacteria are more resistant than gram positive bacteria due to the thick peptidoglycan layer, lipopolysaccharides, phospholipids, of the cell wall that permit gram negative bacteria to be added resistant to most of the hydrophobic antibiotics and toxic drugs (Su et al, 2006). Escherichia coli is ordinarily present in the human digestive system yet at last reason for food poisoning (Lodhia et al., 2009).

Conclusions

The chemical composition and biological activity of the essential oil obtained from the tropical species *Cymbopogon martinii* was investigated.

GC-FID detected major components Geraniol, Geranial, 6-methylhept-5-en-2-one, and Linalool and some minor components like Elemol, δ-cadinol, Borneol, Fenchyl alcohol as the major bioactive compound in Palmarosa essential oil. Docking and *in-vitro* studies have shown effective docking of all the ligands with PBPs and antibacterial activity. We, therefore, propose that Palmarosa essential oil may represent potential therapeutic options and be found in herbal medicines that may act as inhibitors of bacterial PBP. However, further studies should be conducted to confirm these compounds using *in vitro* and *in vivo* models to pave the way for these compounds in drug discovery.

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Table 5. *Toxicity model report of Ligands.*

Ligand name	Classification	Target	Prediction	Probability
Geraniol	Organ Toxicity	Hepatotoxicity	Inactive	0.79
	Toxicity End Points	Carcinogenicity	Inactive	0.76
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.97
	Toxicity End Points	Cytotoxicity	Inactive	0.85
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	1.0
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	0.98
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	0.98
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.99
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	1.0
	Tox-21 -Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	1.0
Geranial	Organ Toxicity	Hepatotoxicity	Inactive	0.69
	Toxicity End Points	Carcinogenicity	Inactive	0.88
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.98
	Toxicity End Points	Cytotoxicity	Inactive	0.82

	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.95
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.94
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.96
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	1.0
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	0.99
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	0.99
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.98
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	1.0
	Tox-21 -Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	1.0
Borneol	Organ Toxicity	Hepatotoxicity	Inactive	0.77
	Toxicity End Points	Carcinogenicity	Inactive	0.78
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.98
	Toxicity End Points	Cytotoxicity	Inactive	0.88
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.97
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.97
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	1.0
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	1.0
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	1.0
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.95
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	1.0
	Tox-21 -Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	1.0
Linalool	Organ Toxicity	Hepatotoxicity	Inactive	0.76
	Toxicity End Points	Carcinogenicity	Inactive	0.64
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.95
	Toxicity End Points	Cytotoxicity	Inactive	0.82
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	1.0

	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	1.0
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	0.99
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	0.99
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.86
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	1.0
	Tox-21- Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	1.0
Fenchyl Alcohol	Organ Toxicity	Hepatotoxicity	Inactive	0.78
	Toxicity End Points	Carcinogenicity	Inactive	0.71
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.94
	Toxicity End Points	Cytotoxicity	Inactive	0.84
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.97
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.97
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	0.98
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	0.99
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	0.99
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.90
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	0.99
	Tox-21- Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	0.99
6-methyl-hept-5-en-2-one	Organ Toxicity	Hepatotoxicity	Inactive	0.70
	Toxicity End Points	Carcinogenicity	Inactive	0.82
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.98
	Toxicity End Points	Cytotoxicity	Inactive	0.82
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	1.0

	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	1.0
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.98
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	1.0
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	1.0
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	1.0
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.99
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	1.0
	Tox-21- Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	1.0
Elemol	Organ Toxicity	Hepatotoxicity	Inactive	0.74
	Toxicity End Points	Carcinogenicity	Inactive	0.77
	Toxicity End Points	Immunotoxicity	Inactive	0.99
	Toxicity End Points	Mutagenicity	Inactive	0.85
	Toxicity End Points	Cytotoxicity	Inactive	0.73
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.99
	Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.92
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.95
	Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.93
	Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	0.94
	Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	0.87
	Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	0.87
	Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.80
	Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	0.99
	Tox-21- Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	0.99
δ-cadinol	Organ Toxicity	Hepatotoxicity	Inactive	0.80
	Toxicity End Points	Carcinogenicity	Inactive	0.65
	Toxicity End Points	Immunotoxicity	Active	0.77
	Toxicity End Points	Mutagenicity	Inactive	0.93
	Toxicity End Points	Cytotoxicity	Inactive	0.86
	Tox21-Nuclear receptor signalling pathways	Aryl Hydrocarbon Receptor (AhR)	Inactive	0.98
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor	Inactive	0.70
	Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.80

Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.86
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive	0.53
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Biding Domain(ER-LBD)	Inactive	0.59
Tox21-Nuclear receptor signalling pathways	Peroxisome proliferator Activated receptor Gamma (PPAR-Gamma)	Inactive	0.99
Tox-21- Stress response pathways	Nuclear Factor (Erythroid-derived 2)-like/antioxidant responsive element (nrf2/ARE)	Inactive	0.80
Tox-21 -Stress response pathways	Heat Shock factor response element (HSE)	Inactive	0.80
Tox-21- Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.63
Tox-21- Stress response pathways	Phosphoprotein (Tumour Supressor) p53	Inactive	0.97
Tox-21- Stress response pathways	ATPase family AAA domain-containing protein 5 (ATADS)	Inactive	0.99

Table 6. *Anti-bacterial activity of Palmarosa essential oil against bacterial strains.*

S. №.	Microbial culture	Sample name	Pro/control volume (µl)	Zone of inhibition (cm)
1	MTCC 40	NC	0	NI
2	MTCC 40	PC	Streptomycin(10mg)	0.3
3	MTCC 40	PRO	50	0.7
4	MTCC 121	NC	0	NI
5	MTCC 121	PC	Streptomycin(10mg)	0.8
6	MTCC 121	PRO	50	1.1
7	MTCC 424	NC	0	NI
8	MTCC 424	PC	Streptomycin(10mg)	0.3
9	MTCC 424	PRO	50	0.7
10	MTCC 3160	NC	0	NI
11	MTCC 3160	PC	Streptomycin(10mg)	1.1
12	MTCC 3160	PRO	50	3

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