BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 12, Number 1, April 2023 | Pages: 225-232 | DOI: 10.14421/biomedich.2023.121.225-232 ISSN 2540-9328 (online)

Chemical Profile and *in-silico* Docking Studies on Bioactives from Essential Oil of *Cymbopogan pendulus* Targeting Penicillin Binding Proteins (PBPs) in Bacteria

Arun Dev Sharma*, Inderjeet Kaur
PG Department of Biotechnology, Lyallpur Khalsa College Jalandhar, India.

Corresponding author*
arundevsharma47@gmail.com

Manuscript received: 25 January, 2023. Revision accepted: 15 February, 2023. Published: 17 February, 2023.

Abstract

Antibiotic resistance in bacteria is the major concern worldwide. PBP (Penicillin binding proteins) have been cited as an appropriate target for therapeutic drug design. In the present study molecular docking followed by wet lab validation was designed to estimate the effect of potent bioactive molecules from *Cymbopogan pendulus* essential oil against PBP5 protein. GC-FID (*gas chromatography* with flame-ionization detection) based composition profile, and *in-silico* docking study was conducted by using CB-dock 2 analysis followed by 2D and 3D interactions. GC-FID revealed Limonene, Neral, Geranial, Linalool, Myrcene as major and minor compounds in *Cymbopogan pendulus* essential oil. The docking score indicated effective binding of ligands to PBP5. Interactions results indicated that, PBP5/ligand complexes form hydrogen and hydrophobic interactions. Wet lab study validated the anti-bacterial potential of oil against gram-positive and gram-negative bacteria. Therefore, essential oil from *Cymbopogan pendulus* essential oil may represent potential herbal treatment to mitigate bacterial infections.

Keywords: Bacteria; Docking; lemon grass oil; geranial; Herbal Drug.

INTRODUCTION

Bacterial peptidoglycan, which is repeating disaccharide unit of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with NAM bearing a peptide stem, provide resistance to bacteria not only by providing capability to resist against internal intracellular pressure but also helps to maintain well-defined cell-shape (Brockhurst et al., 2019). The final steps involved in cell wall bio synthesis and remodeling are mediated by penicillin-binding proteins (PBPs), which comprise high-molecular-mass (HMM) and low-molecular-mass (LMM) PBP subgroups. The term 'PBP' has been cited in manuscripts to refer to any enzyme that identifies and/or metabolizes β-lactams, independently of its function in the cell (Fair and Tor, 2014). PBPs are implicated in catalysis such as: in trans-glycosylation (polymerization of the glycan strands) and trans-peptidation (the cross-linking between glycan chains), DD-carboxy-peptidation (hydrolyze the last D-alanine of stem pentapeptides) and endo-peptidation (hydrolyze the peptide bond connecting two glycan strands) (Simoes et al., 2017). PBPs represents excellent targets for β-lactam antibiotics such as penicillin's, thus inhibiting penultimate physiological role of enzymes involved in bacterial cell wall synthesis and leading to cell death. However, worldwide uncontrolled use of β-lactam antibiotics has led to bacterial resistance to antibiotics which is a swiftly growing apprehension. This problem emerged due to the emergence spread, and persistence of multidrug-resistant (MDR) bacteria, which are frequently isolated in hospitals collectively known as "ESKAPE", which constitutes Gram-positive and Gram-negative species (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*.), also known as "superbugs". "ESKAPE" are also resistant to traditional and conventional treatments involved in majority of nosocomial infections (Brockhurst et al., 2019).

Bacteria possess a variable number of PBPs and among all PBP5, an LMM trans-peptidase, is the most abundant and a vital enzyme involved in peptidoglycan synthesis in cell wall of bacteria (Welsh et al., 2017). In the cell wall, the major function of PBP5 is a DD-carboxypeptidase reaction, that regulates the degree of cross-linking by hydrolytically shortening the peptide stem of the nascent peptidoglycan. It was reported that blocking of either the carboxypeptidation, transpeptidation or reactions by β-lactams antibiotics, worsens the peptidoglycan and may cause cell death (Chan et al., 2016). This process has widened the use of antibiotics and its analogs maximally but has been challenged by the transmission of drug-resistant strains,

highlighting the inevitability for novel natural antibiotic therapies. In this regard, inhibition of the glycosyltransferase reaction by the natural product moenomycin also has been reported that weakens the peptidoglycan and kills bacterial cells (Masters et al., 2020). PBP5, topology constitutes: a trans-membrane anchor, a cytoplasmic tail, and two domains joined by a beta-rich linker located on the outer surface of the cytoplasmic membrane where cell wall peptidoglycan synthesis takes place (Lu et al., 2020). The antibacterial activity of β-lactams is arbitrated by covalent binding to PBPs, thus inhibiting the transpeptidase (TPase) activity of PBP-mediated bacterial cell wall synthesis (da Costa et al., 2018). Since bacterial resistance to multiple drugs, including b-lactam antibiotics, is a main therapeutic problem thus development of new chemical entities as antibacterial agents are urgently needed (da Costa et al., 2018). It was argued that gram-positive and gram-negative bacteria have mostly established resistance to all the available antibiotics and pose a grave problem not only in hospitals but also for the general population (Contreras-Martel et al. 2017). Therefore, by virtue of its key role, PBPs are considered as an appropriate objective for developing bacterial inhibitor. Inhibition of PBPs protein activity would block replication of bacteria. Since in humans, not at all any PBPs with comparable cleavage specific are recognized, so inhibitors are improbable to be considered as toxic.

Lemon grass essential oil (LGO) from *Cymbopogon* species, also known as lemon grass, encompasses a number of bio- actives. Due to complex nature of essential oil, their anti-fungal mechanism of action is still not completely understood (Bhatnagar, 2008, Mancianti et al., 2020)]. LGO has long history to be used as complementary and traditional medicine in ancient times. In addition, various potent biological activities like anti-amoebic, anti-inflammatory, anti-filarial, anti-diarrheal, anti-malarial, anti-fungal and anti-bacterial agent, anti-HIV have been attributed to LGO, hence playing a major role as therapeutics in the scientific community (Oladeji et al., 2019). This study postulated that due to richness of geranial, essential oil from *Cymbopogon pendulus* plants have potential to inhibit bacterial infections. Hence as an objective this study was designed to study molecular docking of geranial and wet lab validation of anti-bacterial potential of lemon grass oil in relation with PBP5. The present study outcomes would offer scientists and doctors with prospects to identify the key anti-bacterial drugs to combat MDR.

EXPERIMENTAL

GC-FID Analysis

LGO was extracted from fresh leaves of *Cymbopogan pendulus* growing naturally at nearby areas of Lyallpur Khalsa College, Jalandhar. The *Cymbopogan pendulus* was authenticated by Dr Upma from Botany Dept and voucher with number BT103 was deposited in Dept of Biotechnology. Hydro-distillation method was used for extraction of essential oil by using clevenger-type apparatus (Borosil, India) (Sharma and Kaur 2021). To identify bioactive compounds in EO, GC-FID study was carried out (GC-FID, Chemtron 2045). The specifications of column was: 2 m long, stainless steel having 10% OV-17 on 80-100% mesh Chromosorb W (HP). Nitrogen was used as carrier gas at flow rate of 35 ml/min. 0.2 µl LEO sample was used. The temperatures for detector and injector were: 220 °C and 270 °C. Oven ramping conditions were: 100°C (firstly maintained) ramped to 2100 °C at 3 °C/min. Bioactive constituents in LEO were identified by comparing relative retention times (RT) of GC-FID spectra of LEO with authentic standards and literature data.

Ligand preparation

For bacterial receptors (PBP5, pdb id: 3MZF), various bioactive compounds such as: Limonene, Neral, Geraniaol, Linalool, Myrcene which are present in lemon grass essential oil as major and minor amounts were used as ligands for structures. To build 3D structure of ligand, SMILES of ligands was recovered from NCBI-Pubchem database. The structure was built by using UCSF-chimera.

Molecular Docking

Crystal structures of PBP5 bacterial penicillin binding protein recovered from PDB (https://www.rcsb.org/). Before docking analysis, all target enzymes were cleaned from selected H_2O molecules, cofactors, co-crystallized ligand, and energy minimized. Then all protein target structures were prepared by means of the dock prep set up in UCSF-chimera. It is the process under optimization that bond length, charges anomalies and corrects atomic structure. For docking, CB-DOCK 2 tool was used for docking of ligands over PBP5 (https://cadd.labshare.cn/cb-dock2/php/index.php). To execute docking, both receptors and ligand molecules as "pdb files" were uploaded to the CB-DOCK 2 and docking was performed. For 2D and 3D interactions in docked complexes, Biovia 2020, UCSF Chimera and Plip tools were used.

Active sites prediction

In fungal receptors, identification and dimension of cavities on 3D active sites were computed by using CASTp web tool. For this all structures in "pdb" format were uploaded to server and prediction was executed with probe radius value of 1.4 Angstroms.

In-Vitro Anti-Bacterial Activity

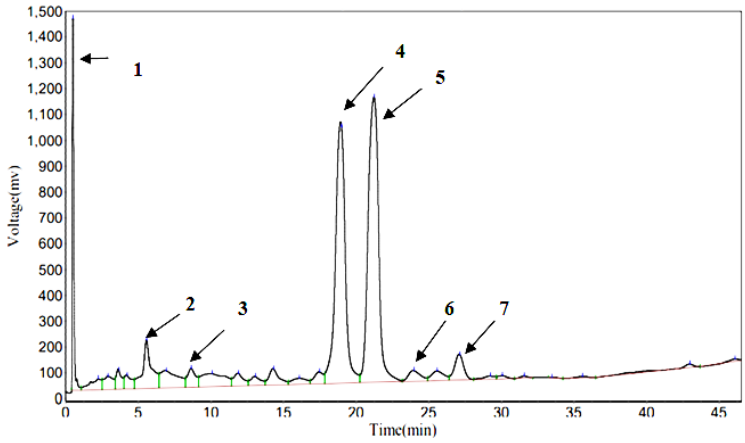
The in-vitro antimicrobial activity of LGO was determined through agar disc diffusion method against four test organisms, gram-negative *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), and

Gram-positive *Staphylococcus aureus* (MTCC 3160) and *Bacillus subtilis* (MTCC 121). Pathogens were purchased from Institute of Microbial Technology, Chandigarh. Sterile paper discs (10 mm in diameter) were impregnated with 100 µl LGO. 12-h cultures were used Inoculums and OD of suspensions was adjusted to 0.6. A swab of bacteria suspension was spread on to LB-Agar plates and allowed to dry for 30 min. The discs with essential oil were then applied and plates were left for 20 min at room temperature to allow to diffusion of oil followed by incubation at 37°C for 24 hours. Vancomycin antibiotic, (10mg) was taken as positive control. Zone of inhibition was measured.

RESULT AND DISCUSSION

GC-FID analysis of bioactive molecules in LGO

The GC- FID chromatogram obtained was depicted in Figure 3. The peaks observed and their respective retention time was also displayed. The GC- FID analysis of lemon grass oil obtained from *Cymbopogon pendulus* revealed 26 compounds for the total of 100%. In the present study, all identified compounds were micrene, limonene, linalool, geranial, neral, undececanone and geranial acetate. GC-FID chromatogram contained three major peaks along with many small peaks indicating the presence of minor compounds. The major constituents were Geranial (27%), Neral (31%), Myrcene (6.7%), Limonene (4.9%) and Linalool (3.6%). The small peaks may be ascribed to the disintegrated major compounds bioactive compounds or present in small quantities. The literature studies also showed the presence and identification of Mycrene, Geranial and Neral in lemongrass oil obtained from *C. flexuous*, (Oladeji et al., 2019). During the course of time, use of LGO has become a major area of health- and medical-related research due to richness of bioactives. LGO also has been used as therapeutic agent in pharmaceutical preparations as anti-oxidative, antibacterial, antiviral, anti-diabetic, anti-tumor, antifungal, anti-obesity, anti-hypertensive**,** anti-histaminic, anti-cancer, anti-HIV and hepatoprotective agent (Oladeji et al., 2019). In this study 2 major and 3 minor bioactive compounds as cited above were selected for 3D docking.



PEAK NO.	RETENTION TIME (min)	BIACTIVE COMPOUND	Conc.
1	0.498	MICRENE	6.7075
2	5.582	LIMONENE	4.9152
3	6.915	LINALOOL	3.6378
4	18.998	GERANIAL	27.0440
5	21.248	NERAL	31.5752
6	23.998	UNDECECANONE	1.5138
7	27.165	GERANIAL ACETATE	1.3755

Figure 1. GC-FID analysis of Lemongrass essential oil (LGO).

Molecular docking

Structure-based drug design (SBDD) is most widely used *In-silico* technique in making drugs which is based on 3-D structures (Srimai *et al*., 2013). *In-silico* docking has simplified investigators to screen conformations and affinities of an assembly of bioactive components against receptors (Barcellos *et al*., 2019). Present study aimed at docking of limonene, neral, geranial, linalool, and myrcene bioactive molecules from LGO as key anti-bacterial inhibitor candidates against PBP5. From docking analysis, it was apparent that ligands efficiently docked with PBP5 bacterial enzyme. 3D docking results illustrated that PBP5 depicted strong binding with ligand limonene (Table 1) as apparent from its docking score of -5.3. Based on docking score, the order to docking of ligand with PBP5 receptor was: limonene>, neral>geranial>linalool> myrcene. The best pose displaying 3D model of PBP5-ligand based on docking

score are displayed in Figure 2. 2D/3D interaction of ligands with PBP5 is displayed in Figure 3. With PBP5, it was revealed that ligands docked with Penicillin binding domain of PBP5. The C-terminal module is responsible for the transpeptidase activity of PBPs catalyzing peptide cross-linking between two adjacent glycan chains catalyzing peptide cross-linking between two adjacent glycan chains in peptidoglycan cell wall synthesis (Contreras-Martel et al., 2017). It was cited that blocking of either the transpeptidation or carboxypeptidation reactions by b-lactam antibiotics or therapeutic inhibitors, weakens the peptidoglycan and may engender cell death (Straume et al., 2020). Once a PBP is acylated by a by therapeutic inhibitors, it is unable to catalyze hydrolysis of the covalent acyl-enzyme intermediate and is inactivated; peptidoglycan transpeptidation cannot occur, and the cell wall is weakened (Masters et al., 2020, Moon et al., 2018). Based on analysis, it was highlighted that LGO can be used as effective source of anti-bacterial compounds.

Table 1. Docking score of Ligands with PBP5.

Ligand	Vina score (kcal/mol)	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)	Interacting residues (4 A˚)	
					H-bond interactions	Hydrophobic interactions
LIMONENE	-5.3	1721	39, 4, 27	26, 17, 17	-	214ATHR,222ATYR,222ATYR,224ALEU 245APHE, 248AARG, 248AARG
NERAL	-5.1	1721	39, 4, 27	26, 19, 19	216A HIS	198AARG,214ATHR,216AHIS,222ATYR, 222ATYR,224ALEU,248AARG, 248AARG,249AGLU
GERANIAOL	-4.9	1721	39, 4, 27	26, 19, 19	216A HIS	198ARG, 214ATHR 216AHIS, 222ATYR 222ATYR, 224ALEU 248AARG,249A,GLU
LINALOOL	-4.7	1721	39, 4, 27	26, 18, 18	216AHIS	198AARG,214ATHR,222ATYR,248AARG, 248AARG,249AGLU
MYRCENE	-4.7	1721	39, 4, 27	26, 18, 18	-	214ATHR,216AHIS,222A TYR,222ATYR,222ATYR, 248AARG,248AARG,249AGLU

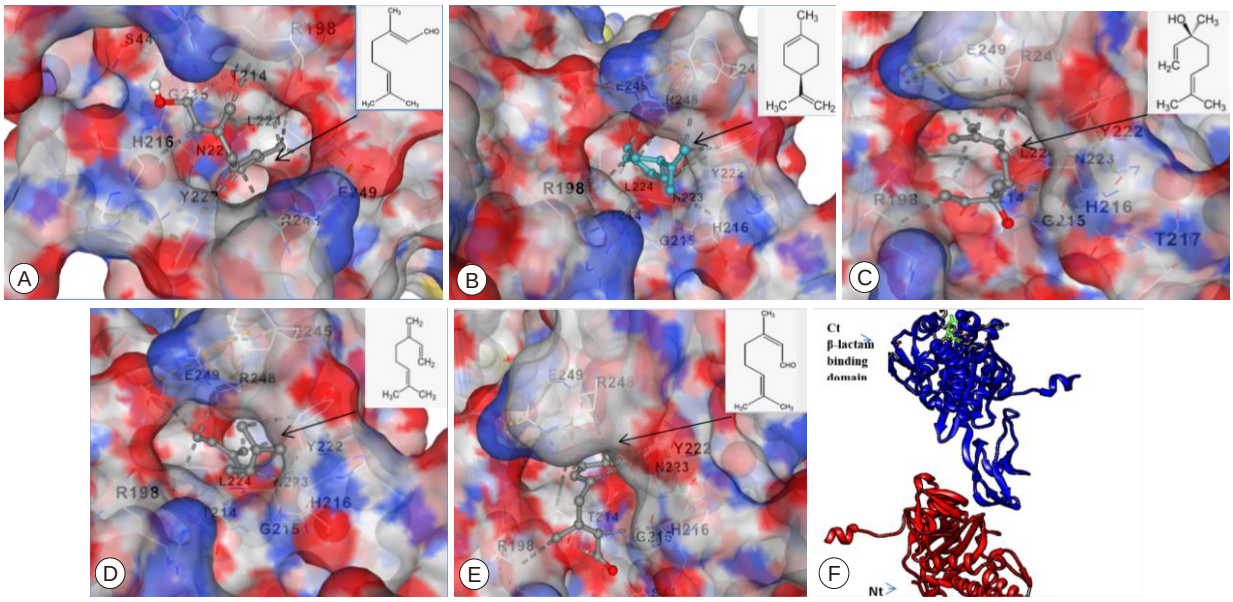


Figure 2. Pictorial view of 3D model of PBP-ligand interactions. A: Geranial; B: Limonene; C: Linalool; D: Myrcene; E: Neral; F: General pictorial view of PBP1 exhibiting domains.

Through 3D docking, with site residues of receptors, ligand could form H-bonds or hydrophobic bonds which designate affinity of ligand with receptor (Lima et al., 2019). Hence, docking interactions of limonene, neral, geranial, linalool, myrcene with PBP5 was further evaluated. It was observed that most effective ligand limonene forms hydrophobic interactions with PBP. With PBP5 receptors, hydrophobic interactions were detected

via 214THR, 222TYR, 224LEU, 245PHE, and 248ARG active site residues at 3.38, 3.84 3.69, 3.68 and 3.56 Å (Figure 2). It was noted in addition to hydrophobic interactions that ligands neral, geranial, linalool exhibited H-bond interactions also by 216HIS. CASTp active sites prediction quantified interacting residues in the active site cavities of PBP1 receptors (data not shown). In PBP5 enzymes, a main pocket was documented with Volume (SA) of 1721**(Å^3)** and Area (SA) of 376 **(Å^3)** Main pocket contained active site residues such as SER44 ARG198 THR214 GLY215 HIS216 TYR222 ASN223 LEU224 ARG248 GLU249. Meanwhile, as all ligands shown good affinity to PBP5 enzyme via active site residues so it was conjectured that upon binding with ligand PBP5 becomes closed thus in-turn persuades change in conformation of bacterial enzymes and inhibit biosynthetic pathway involved in cell wall synthesis. Earlier studies also documented that β-lactam antibiotics irreversibly acylate the active-site serine of PBPs, which deprives bacteria of their biosynthetic functions and results in bacterial death (O'Daniel et al., 2014). All these events halts bacterial viability thus mitigate infectivity of bacteria into the host cell. Similar *in-silico* results citing antibacterial potential of polypharmacological natural agents like flavonoids, phenolics, steroids, and terpenoids, which have the ability to inhibit and kill bacteria strains have been, stated (Soviati and Widyarman, 2020, Apriyanti and Kurnia, 2020, Kurnia and Apriyanti, 2019, Gartika and Pramesti, 2018).

Anti-bacterial Activity

In the present study the *in-vitro* anti-bacterial activity of LGO was quantitatively assessed against drug resistant microbial strains of *Escherichia coli* (MTCC-40), *Bacillus subtilis* (MTCC-121), *Pseudomonas aeruginosa* (MTCC-424) and *Staphylococcus aureus* (MTCC-3160), the results of which are depicted in Table-2 and Figure 4. The present study shows that LGO exhibits substantial antimicrobial activity against gram negative *Escherichia coli* (MTCC-40) while total inhibition was seen for gram positive *Bacillus subtilis* (MTCC-121), *Pseudomonas aeruginosa* (MTCC-424) and *Staphylococcus aureus* (MTCC-3160) as indicated in Figure 3. The variance action of LGO might be owing to the incidence of single target or multiple targets for their activity. The antimicrobial activity of LGO may arise due to the presence of major and minor bioactive component that affected hydrolytic enzyme inhibition (proteases) or inhibited partners like: cell wall envelop proteins, microbial adhesions, and non-specific interactions with carbohydrates (Silva et al., 2003, Siramon et al, 2007). Earlier studies also have cited that anti-microbial activity was not always related to the high content of one major chemical compound, rather than to synergic effects between major and minor components (Silva et al., 2003). Siramon et al, (2007) also cited incidence of potent bioactive molecules like flavonoids, and terpenoids behind the antimicrobial activity. Same authors cited that bioactive molecules have tendency to cross across the cell membranes and to induce biological reactions, thus upsetting electron flow, the proton motive force, active transport and coagulation of the cellular contents. LGO also exhibits high antifungal, insecticidal and bactericidal activity (Chen et al, 2016). In present study high antimicrobial toxicity of LGO toward gram negative bacteria was observed which is a noteworthy observation as most studies suggests that the gram negative bacteria are more resistant than gram positive bacteria due to thick peptidoglycan layer, lipopolysaccharides, phospholipids, of cell wall that permit gram negative bacteria to be added resistant to most of the hydrophobic antibiotics and toxic drugs (Su et al, 2006).

Table 2. Antimicrobial analysis of lemon grass essential oil.

Strain	Strain type	Zone of inhibition (cm)	
		C	**EO**
Pseudomonas aeruginosa	Gram negative	3.0	FI
Escherichia coli	Gram negative	2.5	6.5
Bacillus subtilis	Gram positive	2.6	FI
Staphylococcus aureus	Gram positive	2.5	FI

Here: C= Positive control (Vancomycin antibiotic, 10mg), EO= essential oil, FI: 100% inhibition, values are expressed as mean±SD (*n*=3),

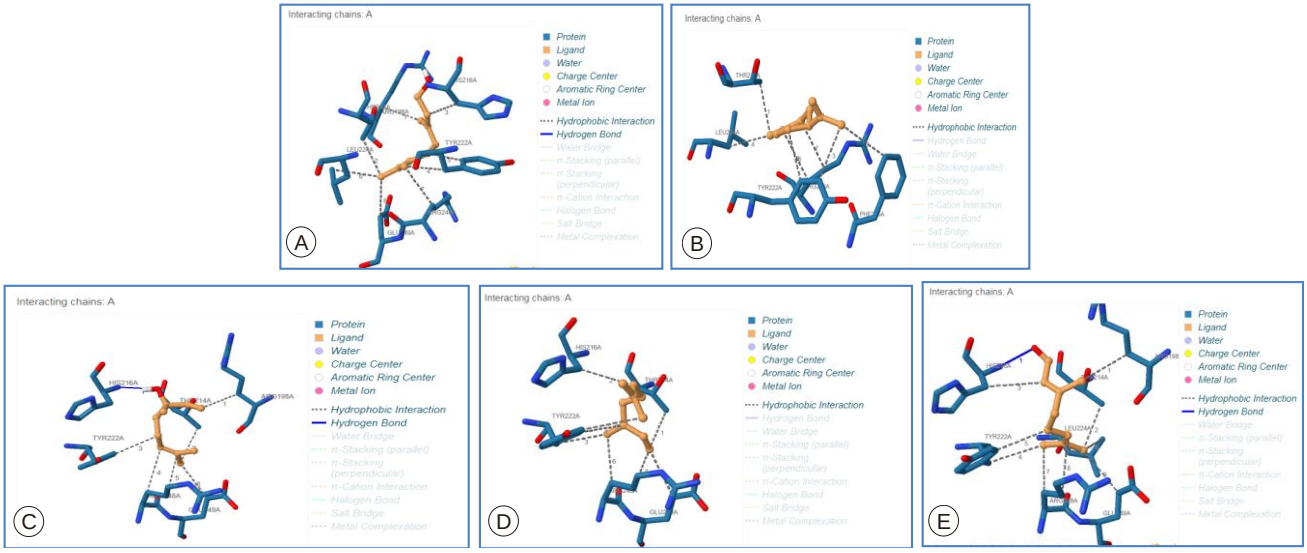


Figure 3. 3D interaction's of PBP5 with ligands. A: Geranial; B: Limonene; C: Linalool; D: Myrcene; E: Neral.

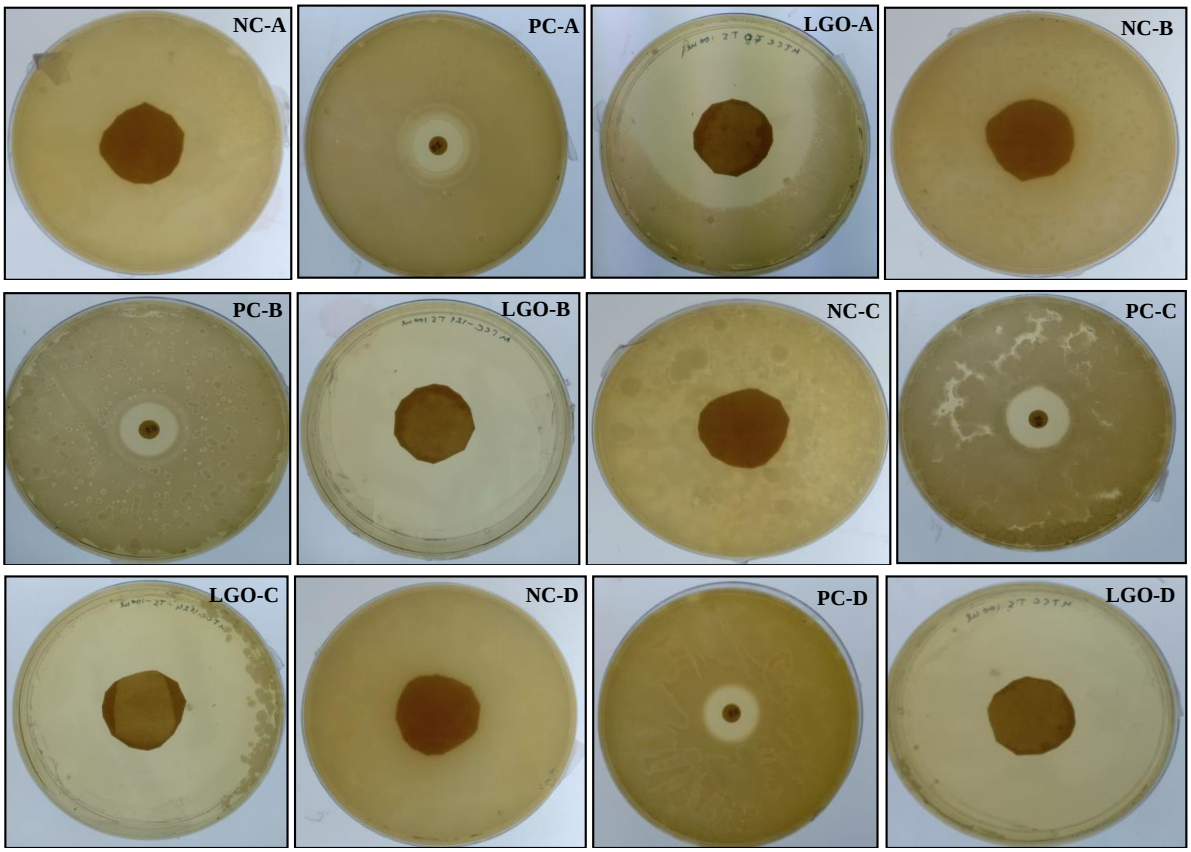


Figure 4. Anti-bacterial activity of LGO against MTCC-121, MTCC-40, MTCC-424 and MTCC-3160. Code- NC, PC and LGO are for negative control (blank), positive control and lemongrass oil and code- LGO-A, LGO-B, LGO-C and LGO-D represents MTCC-40, MTCC-121, MTCC-424, and MTCC-3160 respectively.

CONCLUSIONS

Currently, antibiotic resistance against gram-positive and gram-negative bacteria has emerged in the human population, and is a potential threat to global health, worldwide. Currently, the main target for bacterial infections is primarily PBPs. The aim of this study was to examine bioactive molecules from lemon grass essential oil that may be used to inhibit the bacterial infection pathway. Compositional analysis revealed the presence

of bioactive compounds in lemon grass oil. *In-silico* docking depicted effective docking of all bioactive compounds. Wet lab validation documented anti-bacterial role of LGO. Therefore, we suggested that bioactives compounds from lemon grass essential oil may represent potential treatment options, and found in medicinal plants that may act as potential inhibitors of bacterial PBPs. However, further studies should be conducted for the validation of these compounds using in vitro and in vivo models to pave a way for these compounds in drug discovery.

***Conflict of interest*:** Authors declares no conflict of interest.

Funding: DST Govt of India.

Author contributions: ADS: designed study, IJK: interpreted study.

REFERENCES

Apriyanti E, Kurnia D. (2020). Potential of MurA enzyme and GBAP in Fsr quorum sensing system as antibacterial drugs target: In vitro and *in silico* study of antibacterial compounds from *Myrmecodia pendans*. Comb. Chem. Highthroughput Screen. 23:1–10.

Barcellos MP, Santos CB, Federico LB, Almeida PF, da Silva CHTP, Taft CA. (2019). Pharmacophore and structure-based drug design, molecular dynamics and admet/tox studies to design novel potential pad4 inhibitors. J Biomol. Struc.Dyn. 37:966–981

Bhatnagar A. (2018). Composition variation of essential oil of Cymbopogon spp. growing in Garhwal region of Uttarakhand, India. J App Nat Sci 10: 363–366.

Brockhurst MA, Harrison F, Veening JW, Harrison E, Blackwell G, Iqbal Z, Maclean C. (2019). Assessing evolutionary risks of resistance for new antimicrobial therapies. Nat. Ecol. Evol. 3: 515–517

Chan LC, Gilbert A, Basuino L, da Costa TM, Hamilton SM, Dos Santos KR, Chambers HF, Chatterjee SS. (2016). PBP 4 Mediates High-Level Resistance to New-Generation Cephalosporins in Staphylococcus aureus. Antimicrob Agents Chemother. 60:3934-41

Chen J.B, Sun S.Q., Tang X.D, Zhang J.Z, Zhou Q. (2016). Direct and simultaneous detection of organic and inorganic ingredients in herbal powder preparations by Fourier transform infrared microspectroscopic imaging. *Spectrochimica Acta*, **165**, 176–182

Chen Y, Zhang W, Shi Q, Hesek D, Lee M, Mobashery S, Shoichet BK. (2009). Crystal structures of penicillin-binding protein 6 from Escherichia coli. J Am Chem Soc. 131:14345-54

Contreras-Martel C, Martins A, Ecobichon C, Trindade DM, Mattei PJ, Hicham S, Hardouin P, Ghachi ME, Boneca IG, Dessen A. (2017). Molecular architecture of the PBP2-MreC core bacterial cell wall synthesis complex. Nat Commun 8: 776-776

da Costa TM, de Oliveira CR, Chambers HF, Chatterjee SS. (2018). PBP4: A New Perspective on *Staphylococcus aureus* β-Lactam Resistance. Microorganisms. 22:57.

Fair RJ, Tor Y. (2014). Antibiotics and Bacterial Resistance in the 21st Century. Perspect. Medicin. Chem.6: S14459

Gartika M, Pramesti HT. (2018). A terpenoid isolated from sarang semut (*Myrmecodia pendans*) bulb and its potential for the inhibition and eradication of *Streptococcus mutans* biofilm. BMC Complement. Altern. Med.18:1–8

Kurnia D, Apriyanti E. (2019). Antibacterial flavonoids against oral bacteria of *Enterococcus faecalis* ATCC 29212 from Sarang Semut (*Myrmecodia pendans*) and its inhibitor activity against enzyme MurA. Curr. Drug Discov. Technol.16:290–296

Lima SL, Colombo AL and de Almeida Junior JN. (2019). Fungal Cell Wall: Emerging Antifungals and Drug Resistance. Front. Microbiol. 10:2573

Lu Z, Wang H, Zhang A, Liu X, Zhou W, Yang C, Guddat L, Yang H, Schofield CJ, Rao Z. (2020). Structures ofMycobacterium tuberculosisPenicillin-Binding Protein 3 in Complex with Fivebeta-Lactam Antibiotics Reveal Mechanism of Inactivation. Mol Pharmacol **97**: 287-294

Mancianti, F., Valentina, V. (2020). Biological Activity of Essential Oils" Molecules 25: no. 3: 678.

Masters EA, de Mesy Bentley KL, Gill AL, Hao SP, Galloway CA, Salminen AT (2020). Identification of Penicillin Binding Protein 4 (PBP4) as a critical factor for *Staphylococcus aureus* bone invasion during osteomyelitis in mice. PLoS Pathog 16(10): e1008988

Moon TM, D'Andréa ÉD, Lee CW, Soares A, Jakoncic J, Desbonnet C, Garcia-Solache M, Rice LB, Page R, Peti W. (2018). The structures of penicillin-binding protein 4 (PBP4) and PBP5 from *Enterococci* provide structural insights into β-lactam resistance. J Biol Chem. 293:18574-18584.

O'Daniel PI, Peng Z, Hualiang Pi, Sebastian A, Derong D, Edward S, Erika L, Marc A,, Takao Y, Valerie A, et al., (2014). Discovery of a New Class of Non-β-lactam Inhibitors of Penicillin-Binding Proteins with Gram-Positive Antibacterial Activity. *Journal of the American Chemical Society* 2014 *136* (9), 3664-3672

Oladeji, O.S., Adelowo, F.E., Ayodele, D.T., Odelade, K.A. (2019). Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. Scientific African, 6: e00137

Sauvage E, Derouaux A, Fraipont C, Joris M, Herman R, Rocaboy M, Schloesser M, Dumas J, Kerff F, Nguyen-Distèche M, Charlier P. (2014). Crystal structure of penicillin-binding protein 3 (PBP3) from Escherichia coli. PLoS One. 9(5): e98042.

Sharma AD, Inderjeet Kaur. (2021). By-product 'water' of Eucalyptus globulus essential oil distillation as source of botanical insecticides: wealth from waste. Notulae Scientia Biologicae, 13, Article ID 10854.

Silva J., Abebeb W., Sousa S.M., Duarte V.G (2003). Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *Journal of Ethnopharmacol*., **89,** 277–283

Simoes NG, Bettencourt AF, Monge N, Ribeiro IAC. (2017). Novel Antibacterial Agents: An Emergent Need to Win the Battle Against Infections. Mini Rev. Med. Chem. 17: 1364–1376

Siramon P., Ohtani Y. (2007). Antioxidative and antiradical activities of Eucalyptus camaldulensis leaf oils from Thailand. *The Journal of Wood Science*, **53**: 498–504

Soviati N., Widyarman A.S. T (2020) he Effect Ant-Nest Plant (*Myrmecodia pendans*) Extract on *Streptococcus sanguinis* and *Treponema denticola* Biofilms. J. Indones. Dent. Assoc. 2020;3:11

Srimai V, Ramesh M, Parameshwar KS, Parthasarathy T. (2013). Computer-aided design of selective Cytochrome P450 inhibitors and docking studies of alkyl resorcinol derivatives. Med. Chem. Res., 22: 5314-5323

Straume D, Piechowiak KW, Olsen S, Stamsås GA, Berg KH, Kjos M, Heggenhougen MV, Alcorlo M, Hermoso JA, Håvarstein LS. (2020). Class A PBPs have a distinct and unique role in the construction of the pneumococcal cell wall. Proc Natl Acad Sci U S A. 17:6129-6138

Su Y.C., Ho C.L., Wang E.I., Chang S.T. (2006). Antifungal activities and chemical compositions of essential oils from leaves of four *Eucalyptus*. *Taiwan J for Sci*. **21**, 49–61.

Welsh MA, Taguchi A, Schaefer K, Van Tyne D, Lebreton F, Gilmore MS, Kahne D, Walker S. (2017). Identification of a Functionally Unique Family of Penicillin-Binding Proteins. J Am Chem Soc. 13:17727-17730