Bulgarian Chemical Communications, Volume 55, Issue 1 (pp. 40-47) 2023 *DOI: 10.34049/bcc.55.1.5528*

Targeting penicillin binding proteins (PBPs) by using bioactive geranial from essential oil of *Cymbopogan pendulus* against gram-positive and gram-negative bacteria: molecular docking and experimental approach

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Received: September 13, 2022; Revised: February 22, 2023

Worldwide antibiotic resistance developed in bacteria in response to the uncontrolled use of currently available antibiotics is a major concern. Therefore, by using computational biochemistry, it is necessary to discover and design novel antibacterial compounds with new formulations to overcome this problem. PBP (Penicillin binding proteins) have been cited as an appropriate target for therapeutic drug design. In this study, molecular docking followed by wet lab authentication was designed to estimate the effect of the potent bioactive molecule of geranial from *Cymbopogan pendulus* **e**ssential oil (LGO) against PBP1 protein. GC-FID (gas chromatography with flame-ionization detection) based composition profile, *in-silico* docking study was conducted by using Patchdock analysis followed by 2D and 3D interactions. GC-FID revealed geranial as main compound in *Cymbopogan pendulus* essential oil. The docking score indicated effective binding of geranial to PBP1. Interaction results indicated that PBP1 / eucalyptol complexes participate in both H-bond and hydrophobic interactions. Wet lab study validated the anti-bacterial potential of oil against gram-positive and gram-negative bacteria. Therefore, essential oil from eucalyptus plant may provide potential herbal treatment to mitigate bacterial infections.

Keywords: Bacteria, docking, lemon grass oil, geranial, herbal drug

INTRODUCTION

Worldwide bacterial resistance to antibiotics is a swiftly growing apprehension. It happened due to the emergence spread, and persistence of multidrug-resistant (MDR) bacteria, collectively known as "ESKAPE", which includes gram-positive and gram-negative species (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*.), also known as "superbugs". These bacteria were frequently isolated in hospital environments and are resistant to traditional and conventional treatments [1]. They have been known to cause the majority of nosocomial infections. To mitigate bacterial infections, β-lactam antibiotics are excellent drugs for treatments. β-lactams were known to inhibit bacterial infections by binding to penicillin binding proteins (PBPs) which consist of high-molecular-mass (HMM) and low-molecular-mass (LMM) PBP subgroups and execute the penultimate steps of bacterial cell wall synthesis, so playing vital roles in cell survival [2]. Bacterial peptidoglycan provides resistance to bacteria not only by a capability to resist against internal intracellular pressure but also helps to maintain well-defined cell shape. The term 'PBP' has been cited in manuscripts to refer to any enzyme that recognizes and/or metabolizes β-lactams, independently of its function in the cell [3]. PBPs are involved in catalysis in transglycosylation (polymerization of the glycan strands) and transpeptidation (cross-linking between glycan chains), DD-carboxypeptidation (hydrolysis of the last D-alanine of stem pentapeptides) and endopeptidation (hydrolysis of the peptide bond connecting two glycan strands) and hence a major target for antibiotics [2].

Antibiotic resistance has emerged in response to the misuse of currently available antibiotics. Hence, by using computational biochemistry, it is indispensable to discover and design novel antibacterial compounds with new formulations to overcome serious infections [5, 6]. Bacteria possess a variable number of PBPs and among all PBP1 is a high-molecular-weight trans-peptidase, a vital enzyme involved in peptidoglycan synthesis in bacteria cell wall [4]. It was reported that blocking of either carboxypeptidation or transpeptidation reactions by β-lactam antibiotics, deteriorates the peptidoglycan and may cause cell death [5]. This influential process, which has made penicillin and its analogues the most extensively engaged antibiotics for any infectious worldwide over the past, has been confronted by the broadcast of drug-resistant strains, emphasizing the necessity for novel natural antibiotic therapies [5, 6]. In this regard, inhibition of the glycosyltransferase reaction by the natural product moenomycin against

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PBP4 has also been reported to weaken the peptidoglycan and kill bacterial cells [6]. Earlier docking studies on flavonoids like kaempferol, 3-rutinoside-7-sophoroside and rutin also demonstrated a considerable binding affinity to PBP2 and suggested that kaempferol, 3-rutinoside-7-sophoroside, amentoflavone and rutin may be considered as drug candidates for therapeutic aims in several human infections associated with *Staphylococcus aureus* [7]. Hence, by virtue of its crucial role, PBP1 is considered as an appropriate target for developing bacterial inhibitors. 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. PBP1 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 [3]. 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 [5, 6]. Since bacterial resistance to multiple drugs, including β-lactam antibiotics, is a main therapeutic problem, thus, development of new chemical entities as antibacterial agents is urgently needed [8]. 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 [2, 9].

Lemon grass essential oil (LGO) from *Cymbopogon* species also known as lemon grass, encompasses a number of bio-activities. Due to the complex nature of the essential oil, its anti-fungal mechanism of action is still not completely understood [10]. LGO has long history of being 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 anti-HIV and anti-bacterial agent, have been attributed to LGO, hence playing a major role as a therapeutic in the scientific community [11]. This study postulated that due to the richness of geranial, essential oil from *Cymbopogon pendulus* plants has a potential to inhibit bacterial infections. Hence, as an objective this study was designed to study molecular docking of geranial and carry out wet lab validation of the anti-bacterial potential of lemon grass oil in relation with PBP1. 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 on nearby areas of Lyallpur Khalsa College, Jalandhar. The *Cymbopogan pendulus* was authenticated by Dr. Upma from the Botany Department and voucher with number BT103 was deposited in the Department of Biotechnology. Hydro-distillation method was used for extraction of essential oil by using a clevenger-type apparatus (Borosil, India) [12]. To identify bioactive compounds in EO, GC-FID study was carried out (GC-FID, Chemtron 2045). The column specification was: 2 m long, stainless steel having 10% OV-17 on 80-100% mesh chromosorb W (HP). Nitrogen was used as carrier gas at a 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 210 °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 (PBP1), geranial was used as a ligand for structures. To build 3D structure of the ligand, SMILES of geranial was recovered from NCBI-Pubchem database. The structure was built by using UCSF-chimera.

Molecular docking

Crystal structures of PBP1 bacterial penicillin binding protein were recovered from PDB (https://www.rcsb.org/). Before docking analysis, all target enzymes were cleaned from 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 determines bond length, charges anomalies and corrects atomic structure. PatchDock tool was used for docking of ligands over PBP1 (https://bioinfo3d.cs.tau.ac.il/PatchDock/). To execute docking, both receptors and ligand molecules as “pdb files” were uploaded to the PatchDock and docking was performed. For 2D and 3D interactions in docked complexes, Biovia 2020, UCSF-chimera and Plip tools [13] 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 Å.

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 the Institute of Microbial Technology, Chandigarh. Sterile paper discs (10 mm in diameter) were impregnated with 100 µl of LGO. 12-h cultures were used. Inocula and OD of suspensions were 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 diffusion of oil followed by incubation at 37ºC for 24 hours. Zone of inhibition was measured. Vancomycin antibiotic (10 mg) was used as positive control.

RESULTS AND DISCUSSION

GC-FID analysis of bioactive molecules in LGO

The GC-FID chromatogram obtained is depicted in Figure 1. The peaks observed and their respective retention times are 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, geraniol, 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 and minor constituents were geraniol (45%, geranial), neral (20%), undecanone (20%), linalool (8%), myrcene (6.7%), cuparene (11%), limonene (2.3%) and micrene (2%). The small peaks may be ascribed to disintegrated major bioactive compounds. The literature studies also showed the presence and identification of mycrene, citral-a, and citral-b in lemon grass oil obtained from *C. flexuous* [11]. During the course of time, use of LGO has become a major area of health- and medical-related research due to richness of bioactivities. LGO has also 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 [11]. In this study two major and 3 minor bioactive compounds as cited above were selected for 3D docking.

Molecular docking

Structure-based drug design (SBDD) is most widely used as *in-silico* technique in making drugs, which is based on 3-D structures. *In-silico* docking has simplified investigators to screen conformations and affinities of an assembly of bioactive components against receptors [14]. Present study aimed at docking of geranial bioactive molecule from LGO as key anti-bacterial inhibitor candidate against PBP1. From docking analysis, it was apparent that geranial ligand efficiently docked with PBP bacterial enzyme. 3D docking results illustrated that PBP1 depicted strong binding with geranial ligands (Table 1) as apparent from its docking score. 3D model displaying docking poses and 2D/3D interaction of eucalyptol with PBP1 is shown in Figure 2. Hydrophobicity view depicted that ligands sre firmly bound within binding pocket of receptors. With PBP1, geranial ligand docked with penicillin binding domain of PBP1. The C-terminal module is responsible for the transpeptidase activity of PBPs catalyzing peptide cross-linking between two adjacent glycan chains in peptidoglycan cell wall synthesis [9, 15]. It was cited that blocking of either the transpeptidation or carboxypeptidation reactions by β-lactam antibiotics or therapeutic inhibitors weaken the peptidoglycan and may engender cell death [7]. Once a PBP is acylated 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 [16, 17]. Based on analysis, it was highlighted that LGO can be used as effective source of anti-bacterial compounds.

Through 3D docking, with site residues of receptors, the ligand could form H-bonds or hydrophobic bonds which designate the affinity of ligand toward receptor [18]. Hence, docking interactions of geranial with PBP1 were further evaluated. It was observed that geranial ligand participates in both H-bond and hydrophobic interactions with PBP1. With PBP1 receptors, hydrophobic interactions were detected *via* TRP411, ASN562 and PHE577 at 3.74, 2.68 and 3.69 Å (Figure 2).

Table 1. Molecular docking, 3D interactions of geranial with PBP1

Solution No	Score	Area	ACE	Transformation
1	3424	396.50	-90.00	0.73 -0.72 2.66 94.93 10.88 42.52
2	3274	346.10	-105.92	2.24 -0.02 3.01 98.42 39.00 54.25
3	3262	352.80	-81.80	-2.87 -0.44 -1.23 87.53 14.38 47.09
4	3122	336.50	-115.80	0.63 -1.14 0.89 96.06 38.17 55.23
5	3112	369.50	-80.45	-1.95 0.07 2.39 96.01 10.41 40.13
6	3106	414.80	-89.98	1.32 0.20 -0.05 95.51 11.70 41.86
7	2988	376.00	-88.97	-0.77 -0.66 -0.38 96.51 9.59 40.06
8	2916	340.80	-122.79	-2.03 0.95 2.88 95.65 37.49 55.58
9	2904	340.90	-144.34	-1.26 0.79 -0.38 98.08 9.86 46.52
10	2894	324.90	-95.87	0.36 0.74 2.92 98.37 7.83 39.82
11	2876	347.30	-91.53	-1.95 0.91 -2.64 75.44 28.61 24.83
12	2872	329.10	-69.69	1.89 -0.13 -1.39 101.39 34.97 51.74
13	2856	319.80	-81.93	-0.98 -0.58 0.19 99.45 37.45 52.81
14	2848	311.30	-3.62	1.42 -0.51 -2.24 102.98 14.24 39.92
15	2824	320.40	-61.45	2.08 0.71 2.31 86.56 14.71 47.20

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	411B	TRP	3.74	3170	1254
2	562B	ASN	2.68	3172	2420
3	577B	PHE	3.69	3170	2551

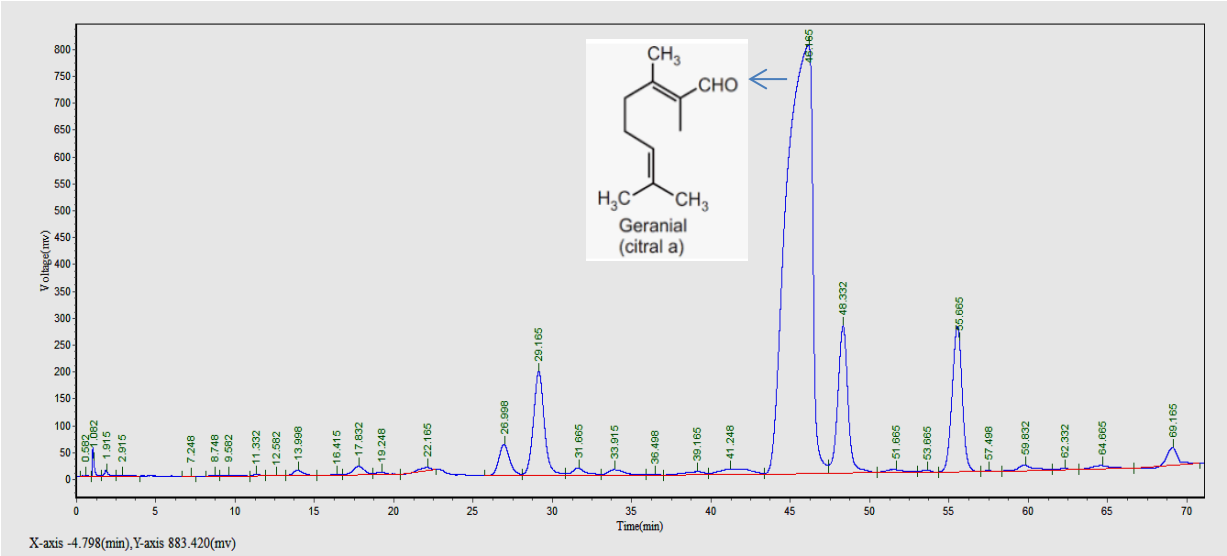
Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	370B	SER	3.11	3.63	115.24			933 [O3]	3178 [O3]
2	428B	SER	2.21	3.10	152.67			3178 [O3]	1400 [O2]

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, 10 mg), EO= essential oil, FI: 100% inhibition, values are expressed as mean±SD (*n*=3).



RT (min)	Compound	Concentration (%)
13	micrene	2.2
17	limonene	2.3
22	caryophyllene oxide	1.2
26	linalool	8.2
29	cuparene	11.2
31	epi-α-cadinol	2.0
33	δ-cadinol	2.1
39	aristolone	0.8
41	santalol acetate	1.9
46	geranial	45.2
48	neral	20.1
51	methyl octadecanoate	0.9
53	incensoleacetate	0.8
55	undececanone	20.0
59	heyderiol	1.3
64	hinokiol	0.6
69	geranial acetate	6.7

Figure 1. GC-FID analysis of lemon grass essential oil

It was noted that geranial exhibited H-bond by SER 378, and SER 428. CASTp active sites prediction quantified interacting residues in the active site cavities of PBP1 receptors (data not shown). In PBP1 enzymes, a main pocket was documented with volume (SA) of 214 and area (SA) of 376. Main pocket contained active site residue SER 378. Earlier study documented the role of active site residue SER 378 as a nucleophile that has been implicated in the catalytic mechanism in cell wall synthesis [4]. Meanwhile, geranial showed good affinity to PBP1 enzyme *via* SER residue, so it was conjectured that upon binding with ligand PBP1 becomes closed, thus, in-turn persuades change in conformation of bacterial enzymes and inhibits 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 [19]. All these events halt 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 [20-22].

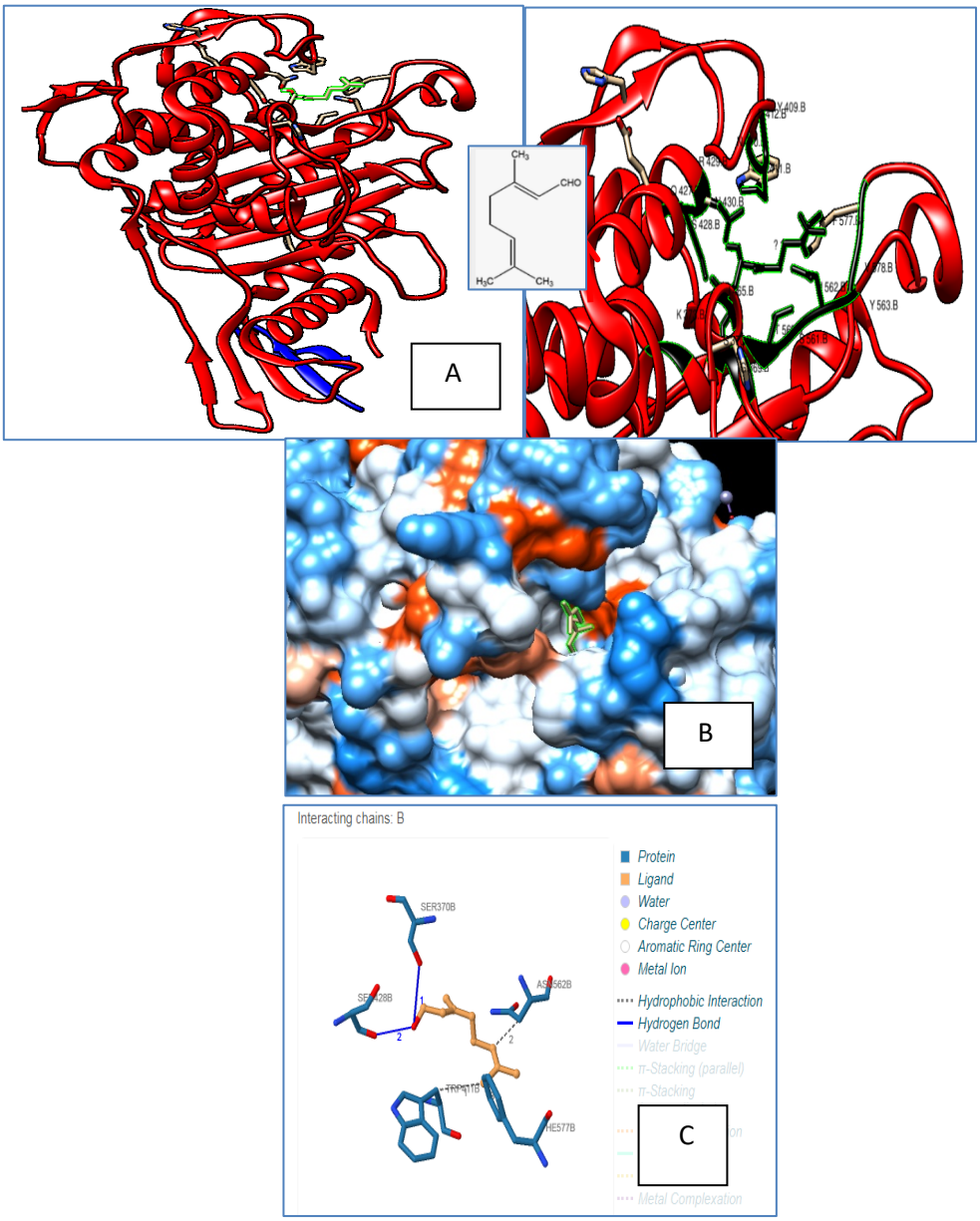


Figure 2. PBP1-citral model (A), Hydrophobic view (B) and 3D interactions of ligand-receptor. Red and green arrows indicate ligand geranial

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 3. The present study shows that LGO exhibits substantial antimicrobial activity against gram-negative *Escherichia coli* (MTCC-40) while total inhibition is 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 due to the incidence of a single target or multiple targets for their activity. The antimicrobial activity of LGO may arise due to the presence of major and minor bioactive components that affect hydrolytic enzyme inhibition (proteases) or inhibit partners like: cell wall enveloped proteins, microbial adhesions, and non-specific interactions with carbohydrates [23]. 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) [24]. Siramon *et al.*, [25] also cited incidence of potent bioactive

molecules like flavonoids, and terpenoids behind the antimicrobial activity.

Same authors cited that bioactive molecules have tendency to pass 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. geranial, the major constituent of LGO also exhibits high antifungal, insecticidal and bactericidal activity [25]. In the present study high antimicrobial toxicity of LGO toward gram-negative bacteria was established which is a noteworthy observation as most studies suggest that the gram-negative bacteria are more resistant than the gram-positive bacteria due to the thick peptidoglycan layer, lipopolysaccharides, phospholipids of the cell wall that permit gram-negative bacteria to be resistant to most of the hydrophobic antibiotics and toxic drugs [26].

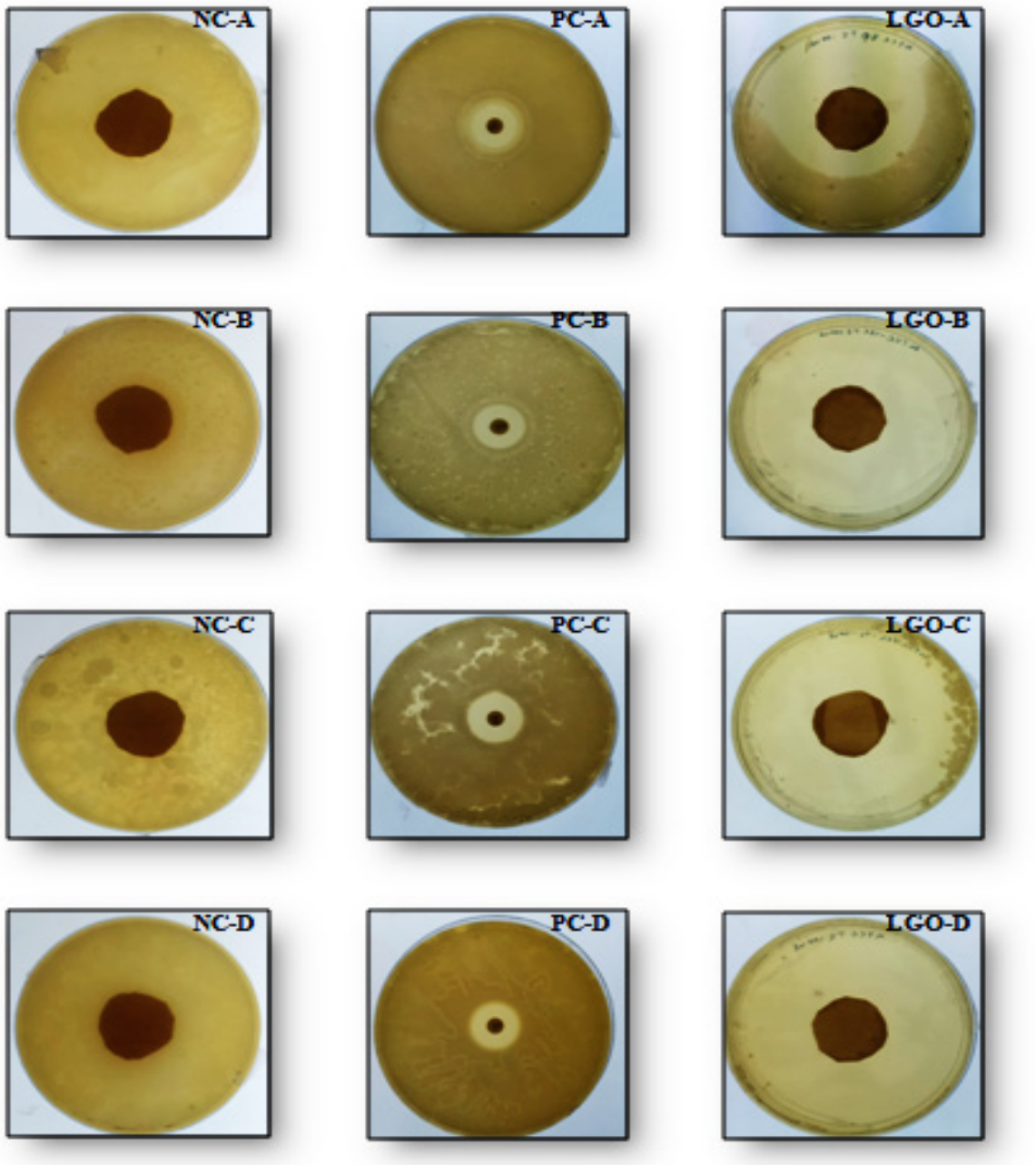


Figure 3. Anti-bacterial activity of LGO against MTCC-121, MTCC-40, MTCC-424 and MTCC-3160. Codes NC, PC and LGO are for negative control (blank), positive control and lemon grass oil and codes LGO-A, LGO-B, LGO-C and LGO-D represent MTCC-40, MTCC-121, MTCC-424, and MTCC-3160, respectively.

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. The main target for bacterial infections are 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. Docking study revealed effective binding of ligand geranial with PBP1. Wet-lab study indicated that *Cymbopogan pendulus* **e**ssential oil was effective to inhibit tested pathogens. Therefore, we suggested that geranial may offer potential treatment options. It is found in medicinal plants that may act as potential inhibitors of bacterial PBPs. This study paved the way for researchers and a specific direction in the field of computational biochemistry and computational drug design. The present study described a new perspective that gives other researchers the opportunity to evaluate the proposed structures with more detailed studies in the field of computational biochemistry. Hence, further studies may 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 declare no conflict of interest.*

Funding*: DST SEED Government of India.*

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

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