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PHYTOCHEMICAL PROFILING AND EVALUATION OF IN VITRO ANTIOXIDANT AND IN VITRO/IN SILICO ANTIBACTERIAL ACTIVITIES OF *CYMBOPOGON CITRATUS* (DC.) STAPF LEAF EXTRACT AND ITS BIOACTIVE PHYTOCHEMICALS

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ABSTRACT

Cymbopogon citratus (DC.) Stapf is widely recognized for ethnomedicinal value, yet integrated evidence combining phytochemistry, antimicrobial testing, and computational validation remains limited. Leaf powders were extracted with methanol, ethanol, hexane, and water (1:10, 72 h). Qualitative metabolite screening, total phenolic (TPC), flavonoid (TFC), tannin (TTC), and protein estimation were performed. Antibacterial potential was evaluated by agar well diffusion. LC–MS characterized major constituents. Identified ligands were docked against bacterial proteins (1BVR, 1ENY, 1HZP, 6Y80) using AutoDock. The best complex underwent 100 ns molecular dynamics (MD) simulation and MM-GBSA free-energy estimation. Methanol extracted the widest spectrum of phytochemicals. TPC ranged from 42.0 ± 0.001 to 191.3 ± 0.002 µg/ml, TFC from 249.9 ± 0.003 to 994.0 ± 0.010 µg/ml, TTC from 227.5 ± 0.008 to 984.1 ± 0.063 µg/ml, and total protein from 248.2 ± 0.011 to 1070.9 ± 0.051 µg/ml. Zones of inhibition reached 12.5 mm for *S. aureus*, 11 mm for *Klebsiella*, 11 mm for *P. aeruginosa*, and 13 mm for *Bacillus*. LC–MS revealed monoterpenes, sesquiterpenes, and flavonoids, with farnesol prominent. Docking showed farnesol had the strongest affinity, especially with 1HZP (−6.7 kcal/mol). MD confirmed stability (protein RMSD ≈3.0 Å; ligand RMSD ≈2.8 Å), reduced Rg (19.7→19.4 Å), and persistent H-bonding. MM-GBSA yielded ΔG_bind −66.04 ± 2.63 kcal/mol, dominated by van der Waals and lipophilic terms. Polar extracts of lemon grass are rich in bioactives with measurable antibacterial activity, while in silico analyses identify farnesol as a stable, high-affinity inhibitor warranting further biological validation.

KEYWORDS: *Cymbopogon citratus*, Phytochemical analysis, LC-MS analysis, Molecular docking, Antibacterial activity.

1. INTRODUCTION

Medicinal herbs have long been used to treat and control a wide range of infectious microbial diseases. African traditional medicine, Ayurveda, and Traditional Chinese Medicine (TCM) have all utilized medicinal herbs for ages to treat bacterial infectious diseases and their symptoms (1). These methods are based on plant extracts and formulations that have demonstrated effectiveness in the treatment of infectious bacterial diseases. There are many bioactive chemicals found in plants that may help create novel medications to treat bacterial infectious diseases (2, 3). Many plants have secondary metabolites that have antibacterial qualities that can be used to fight germs, especially those that have become resistant to traditional medications (4). Medicinal plants can be more accessible and cost-effective in areas where bacterial infectious illnesses are common. Because plant-based remedies may be grown nearby, less reliance on pricey medications is required, and therapy becomes more accessible to those in need (5). *Eucalyptus globulus* (Eucalyptus), *Azadirachta indica* (Neem), *Curcuma longa* (Turmeric), *Nigella sativa* (Black Seed), and *Allium sativum* (Garlic) are examples of medicinal plants used to cure bacterial infectious diseases (6). To confirm these plants' effectiveness and safety, more study is essential (7). Clinical trials, phytochemical analysis, *in vitro* and *in vivo* investigations, and other rigorous scientific research are required to comprehend the mechanisms of action, ideal doses, and possible adverse effects of plant-based remedies for bacterial infectious diseases (8). Medicinal plants are essential in the battle against bacterial infectious diseases because

they may help with medication resistance, adjunct therapy, and the development of new drugs (9, 10). Their cost-effectiveness, accessibility, and historical use make them invaluable tools for managing bacterial infectious diseases worldwide, particularly in environments with low resources.

The tropical plant known as lemon grass is distinguished by its unique citrus scent and flavour. It has several uses in both medicine and cooking. Among its culinary applications are as a flavouring element for teas, marinades, soups, and curries. Magnesium, zinc, copper, iron, potassium, phosphorus, calcium, manganese, folate, and vitamins A and C are all found in lemongrass. It contains antioxidants that can aid in the fight against oxidative damage. Indigestion and bloating are two digestive problems that it might help with. Because of its anti-inflammatory qualities, lemongrass can help lessen discomfort and swelling. It has antibacterial qualities that help combat illnesses. Its relaxing properties, which can lessen tension and encourage sleep, make it a popular choice in aromatherapy. Lemongrass is a useful addition to kitchens and natural medicine closets because of its many uses. Quercetin is one of the flavonoids in lemongrass that possesses antioxidant and anti-inflammatory qualities. By lowering inflammation, quercetin stops the development of cancer cells and guards against heart disease.

A chemical is said to have antibacterial activity if it can stop germs from growing or kill them. This characteristic is essential for both treating and avoiding bacterial infections. Natural or manufactured substances having antibacterial properties are employed in a variety of industries, such as food preservation, agriculture, and medicine. Antibacterial qualities may be found in several plants, herbs, and their preparations. Garlic, honey, and essential oils like tea tree oil are a few examples. One of the fundamental and trustworthy techniques for separating pharmaceutically active biomolecules from their natural sources is spectroscopy and chromatography.

2. MATERIALS AND METHODS

2.1. Preparation of Plant Extract

Sterile distilled water was used to carefully wash the leaf in order to get rid of any debris and dirt particles. A blender was used to crush the leaves that were utilized for extraction after they had been shade-dried. Through Sieving, a fine powder was produced. The powder was gathered in a sterile, airtight container. For 72 hours, the fine powder was placed in an orbital shaker after being soaked in a 1:10 ratio of several solvents, including ethanol, methanol, hexane, and water. The crude extract was obtained by filtering the extract after three days and letting it evaporate. The resulting crude extract is kept for future research in an Eppendorf tube (Figure 1).

Dry leaves of lemon grass Powder of lemon grass

Figure 1: Dried form of *Cymbopogon citratus* (DC.) Stapf.

2.2. Qualitative phytochemical analysis

The qualitative screening was carried out to learn more about the primary and secondary metabolites found in the *Cymbopogan citratus* (DC.) Stapf. Antioxidant tests were conducted to determine the effect of the leaf. Alkaloids, flavonoids, tannins, proteins, amino acids, quinones and phenols in the extracts of hexane, methanol, ethanol, and aqueous extracts were analysed.

2.3. Liquid chromatography-mass spectrometry (LC-MS) assay

Lemon grass ethanolic extract phytochemical analysis was studied by using the LC-MS. The chemical constituents of the ethanolic extracts were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump. The HPLC was connected to a Q-TOF mass spectrometer with an ESI source. Utilising a 140°C source temperature, full-scan mode was run from m/z 100 to 1200. HPLC column Phenomenex 5µ C8 (150 × 2 mm i.d.) was used for the analysis. Solvent was acetonitrile with 0.1% formic acid in H_2O. Column: X-Bridge C18 (150*4.6) 3.5µm. Solvents were delivered at a total flow rate of 0.1 mL/min. The solvent was run by isocratic elution. The positive ion mode was used to obtain the MS spectra.

2.4. Antibacterial assay of crude extract of Lemon grass

To determine the microorganism's susceptibility in the plant extract, the well diffusion technique was employed [5]. The entire agar surface is covered with a volume of microbial inoculum to inoculate the agar plate. A plant extract is added to the well after a 6–8 mm diameter hole is aseptically punched with a sterile cork borer. Agar plates were incubated for twenty-four hours at 37°C. The well's edge was used to quantify the zone of inhibition, which was then measured in millimetres.

2.5. Molecular docking

Using PDB IDs of 1BVR, 1ENY, 1HZP, and 6Y80 for the four primary bacterial target proteins, the phytochemical compounds' binding characteristics were examined. The proteins were first curated using the What If interface to check

for any missing side-chain residues before conducting molecular interaction experiments. The Autodock v. 4.2.6 [12] was used for molecular docking studies to evaluate the compound's binding to target proteins. Based on the predetermined co-crystallized X-ray structure from the RCSB PDB, the binding cavity for chemical docking in each protein was identified. From the co-crystallised ligand, the residue locations were estimated within a 3 Å space. Following the selection of the cavity in each instance, the Chimaera tool was used to eliminate the co-crystallized ligands, and the conjugate gradient and steepest descent algorithms were used to reduce the energy of the protein structures. Next, the nonpolar hydrogens were combined to create the receptor and target molecules in pdbqt format. Using the Lamarckian Genetic Algorithm (LGA), docking tests of the protein-ligand complex were carried out to find the lowest free energy of binding (ΔG) between each protein and the chemical.

2.6. Molecular dynamics simulation

The 1HZP+FARNESOL combination was subjected to MD simulations using the Desmond 2020.1 [1]. The OPLS-2005 force field [2-4] and explicit solvent model with SPC water molecules [5] were utilized in this system in a 1.0 Å x 1.0 Å x 1.0 Å period boundary salvation box. The introduction of sodium ions (Na+) neutralized the electrical charge. By adding a solution containing 0.15 M NaCl to the instrument, the metabolic environment was reproduced [4]. To retrain the system, the protein-ligand complexes were first exposed to an NVT ensemble for ten nanoseconds. After the previous step, a short 12-ns run of equilibration and reduction was performed using an NPT ensemble [20]. Using the Nose-Hoover chain coupling approach, the NPT ensemble was created [6]. While keeping the pressure at 1 bar and the relaxation time at 1.0 picoseconds constant, the temperature was changed in each model. The simulation used a temporal increment of two femtoseconds. To control the pressure, the barostat approach [7] using the Martyna-Tuckerman-Klein chain coupling system was used. A relaxation time of two picoseconds was selected. We used the Ewald method with particle meshes to calculate long-range electrostatic interactions [8]. A constant value of 9Å was used for the coulomb interaction radius. The RESPA integrator was used to determine the bonded forces for each trajectory at a time step of two fs. A temporal period of 100 nanoseconds per unit was used in the most recent production cycle. The number of hydrogen bonds (H-bonds), radius of gyration (Rg), root mean square fluctuation (RMSF), and root mean square deviation (RMSD) were among the important metrics that were measured to evaluate the stability of the molecular dynamics (MD) simulations. To evaluate the stability of the molecular dynamics (MD) simulations, the previously indicated values were utilized [19].

2.7. Binding free energy analysis

The molecular mechanics combined with generalized Born surface area (MM-GBSA) approach was used to compute the binding free energies of the ligand-protein complexes. The MM-GBSA binding free energy was calculated using the Python script thermal mmgbsa.py in the simulation trajectory with the VSGB solvation model and the OPLS5 force field over the last 50 frames with a 1-step sampling size. The binding free energy of MM-GBSA (kcal/mol) was estimated using the principle of additivity, in which individual energy modules such as columbic, covalent, hydrogen bond, van der Waals, self-contact, lipophilic and solvation of ligand and protein were collectively added. The equation used to calculate ΔG_{bind} is the following:

$$\Delta G_{bind} = \Delta G_{MM} + \Delta G_{Solv} - \Delta G_{SA}$$

Were

- ΔG_{bind} designates the binding free energy,
- ΔG_{MM} designates the difference between the free energies of ligand-protein complexes and the total energies of protein and ligand in isolated form,
- ΔG_{Solv} designates the difference in the GSA solvation energies of the ligand-receptor complex and the sum of the solvation energies of the receptor and the ligand in the unbound state,
- ΔG_{SA} designates the difference in the surface area energies for the protein and the ligand.
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2.8. Statistical analysis

The experimental results were expressed as the total content ± SD of triplicates. The Biochemical parameters were analysed statistically using AGRES software, one-way analysis of variance (ANOVA). A p-value of < 0.05 was considered statistically significant.

3. RESULT AND DISCUSSION

3.1. Phytochemical analysis

The phytochemical profile of *C. citratus* (DC.) Stapf demonstrates a marked influence of solvent polarity on the recovery of bioactive constituents. Methanol proved to be the most efficient extractant, yielding strong reactions for flavonoids, phenols, tannins, certain amino acids, proteins, glycosides, and quinones, suggesting that many of the plant's secondary metabolites are relatively polar. Ethanol showed moderate extraction ability, particularly for alkaloids and flavonoids, but lacked several compounds detected in methanol. Ethyl acetate, a less polar solvent, selectively concentrated sterols and triterpenoids and also showed the presence of steroids and some tannins, indicating affinity of these lipophilic molecules for intermediate polarity media. In contrast, the aqueous fraction displayed limited diversity, with weak alkaloids and tannins but a distinctive reaction for terpenoids. The consistent absence of carbohydrates, saponins, coumarins, and carotenoids across most extracts may reflect low abundance or poor responsiveness to the applied assays. Overall, the findings highlight methanol as the most suitable solvent for broad-spectrum phytochemical recovery from this species.

3.2. Liquid chromatography-mass spectrometry (LC-MS) assay

The phytochemical analysis of lemon grass ethanolic extract using LC-MS identified a variety of compounds with distinct m/z values. The analysis detected Camphene and Limonene both with an m/z of 135.1, indicating the presence of monoterpenes. Farnesol, a sesquiterpene, was found with an m/z of 222.2. Methyl aloesin, a more complex molecule, had an m/z of 409.2. Neral, a monoterpene aldehyde, was detected with an m/z of 153.0. Isovitexin, a flavonoid glycoside, showed an m/z of 432.2. The sesquiterpenoid Loliolide was identified with an m/z of 197.1. Several monoterpene alcohols, including Nerol, Geraniol, and Linalool, were detected with similar m/z values around 154.1. Myrcene, another monoterpene, was found with an m/z of 136.0. Citronellal, a monoterpene aldehyde, was detected with an m/z of 154.9. The repeated identification of Linalool with the same m/z value suggests its abundance in the extract. These findings highlight the diverse range of phytochemicals present in lemon grass, each contributing to its potential medicinal and aromatic properties.

3.3. Antibacterial activity- well diffusion method

Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus sp*.) are more vulnerable to lemongrass compared to Gram-negative bacteria (*K. pneumoniae, E. coli*). Lemongrass inhibited gram-negative bacteria even at low doses, despite their susceptibility to numerous drugs. Lemongrass is effective against microorganisms that are resistant to medicine. Lemongrass may help treat infections caused by germs (Table 1).

Table 1: Antibacterial Activity of the extract (n=2).

S.no	Organisms	Zone of inhibition in diameter (mm)	
		Lemon grass extract	Ciprofloxacin
1.	*Staphylococcus aureus*	12.5 mm	30 mm
2.	*Klebsiella*	11 mm	34 mm
3.	*E. coli*	-	36 mm
4.	*Enterococcus*	-	-
5.	*Pseudomonas aeruginosa*	11 mm	34 mm
6.	*P. mirabilis*	-	-
7.	*Bacillus*	13 mm	-

According to (22), the antibacterial activity was found progressively increasing with the increase in concentration of essential oil. Gram-positive bacterial strains were more sensitive to the essential oil of *Cymbopogon citratus* (DC.) Stapf with respect to all concentrations of oil than the Gram-negative strain (*Escherichia coli*). The studies of showed that the gram-negative pathogens had demonstrated significant tolerance to many antibiotics; lemongrass oil was found to inhibit them even at lower concentrations. The earlier studies of (14) stated that the *C. citratus* (DC.) Stapf manifested a better MIC against *S. aureus* (0.78), similar to the combined ethanol extracts of the two plants against *S. aureus, K. pneumonia and P. mirabilis*

3.4. Molecular docking

The provided data lists the binding affinities of various ligands to four different proteins: 1BVR, 1ENY, 1HZP, and 6Y80 (Figure 2). Binding affinity, measured in negative values, indicates how strongly a ligand binds to a protein, with more negative values signifying stronger binding. For protein 1BVR, ligands such as Farnesol and Limonene show the strongest binding affinities at -6.4. For protein 1ENY, Farnesol also exhibits the highest affinity at -5.7, while other ligands generally show weaker binding. In protein 1HZP, Farnesol again shows the highest binding affinity at -6.7, with Neral and Limonene also displaying strong binding at -6. For protein 6Y80, Farnesol has a high affinity at -6.6, and Geraniol also shows strong binding at -6.1. These variations in binding affinities across different proteins and ligands suggest specific interactions that could be important for understanding their biochemical behaviours and potential applications in drug design or other biotechnological fields.

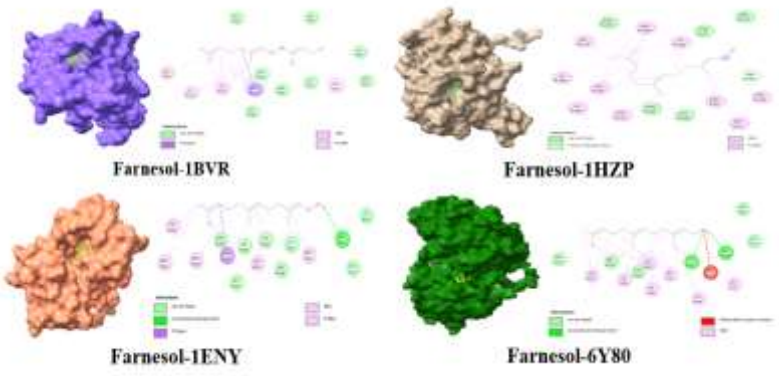

Figure 2: Comparative Binding Interactions of Farnesol with Proteins 1BVR, 1HZP, 1ENY, and 6Y80.

3.5. Molecular dynamics simulation (MDS)

Molecular dynamics and simulation (MD) studies were carried out to determine the stability and convergence of 1HZP+FARNESOL (Figure 3). Simulation of 100 ns displayed almost stable conformation, as compared to the root mean square deviation (RMSD). The RMSD of the Cα-backbone of 1HZP+FARNESOL stable curve and ended at 3.0 Å (Figure 2A), and ligand Farnesol also showed a stable RMSD of 2.8 Å (Figure 3A). A stable RMSD plot during simulation signifies good convergence and stable conformations. Therefore, it can be suggested that protein 1HZP was quite stable in the complex due to the higher affinity of the ligand FARNESOL. The plot for root mean square fluctuations (RMSF) displayed large spikes of fluctuation in the 1HZP protein, which are observed at 1-20, 170-180 residues, which may be due to higher flexibility (above 3 Å) of the residues (Figure 3B), but no significant fluctuations were observed later on. Most of the residues were less fluctuating during the entire 100 ns simulation (Figure 3B), indicating the rigid amino acid conformations during the simulation time. Therefore, from RMSF plots, it can be suggested that the protein structure is rigid during simulation in ligand-bound conformations. The radius of gyration (Rg) is the measure of compactness of the protein. Here in this study, 1HZP+FARNESOL Cα-backbone bound to displayed a lowering of radius of gyration (Rg) from 19.7 to 19.4 Å (Figure 3C). Lowering of gyration (Rg) indicates a highly compact orientation of the protein in the ligand-bound state. The number of hydrogen bonds between protein and ligand suggests a significant interaction and stability of the complex. The number of hydrogen bonds between 1HZP and FARNESOL upto one hydrogen bond observed on average through a 100 ns simulation (Figure 3D). The overall study of Rg signifies that the ligands bind to the respective proteins to become more compact.

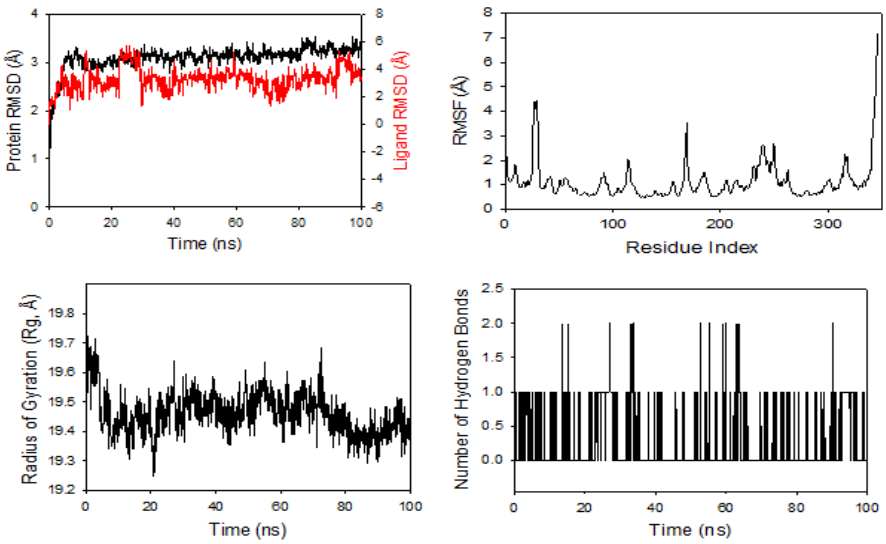

Figure 3: MD simulation analysis of 100 ns trajectories of (A) Cα backbone RMSD of 1HZP bound to ligand FARNESOL (B) RMSF of Cα backbone of 1HZP bound to ligand FARNESOL (C) Cα backbone radius of gyration (Rg) of 1HZP bound to ligand FARNESOL. (D) Formation of hydrogen bonds in 1HZP bound to ligand FARNESOL.

3.6. Molecular mechanics generalized born surface area (MM-GBSA) calculations

Utilizing the MD simulation trajectory, the binding free energy along with other contributing energies in the form of MM-GBSA, was determined for the complex of 1HZP bound to ligand FARNESOL. The results (Table 3) suggested that the maximum contribution to ΔG_{bind} in the stability of the simulated complexes was due to ΔG_{bind}Coulomb, ΔG_{bind}vdW and ΔG_{bind}Lipo, while, ΔG_{bind}Covalent and ΔG_{bind}SolvGB contributed to the instability of the corresponding complexes. 1HZP bound to ligand FARNESOL complex has significantly higher binding free energies (Table 2). Farnesol has a high affinity for binding to the protein, as well as efficiency in binding to the selected protein and the ability to form stable protein-ligand complexes.

Table 2. Binding free energy components for the 1HZP bound to ligand FARNESOL calculated from MM-GBSA.

Energies (kcal/mol)	1HZP+Farnesol
ΔG_{bind}	-66.04±2.63
ΔG_{bind}Lipo	-23.96±1.03
ΔG_{bind}vdW	-51.10±2.0
ΔG_{bind}Coulomb	-8.12±1.99
ΔG_{bind}SolvGB	-0.41±0.22
ΔG_{bind}Covalent	16.5±1.09

4. CONCLUSION

Cymbopogon citratus (DC.) Stapf leaves exhibited notable phytochemicals, attributable to the presence of phenolic compounds, and demonstrated strong antibacterial effects against *Bacillus* and *Staphylococcus aureus*. LC-MS profiling of the ethanolic extract confirmed diverse phytoconstituents. In silico antibacterial evaluation revealed Farnesol as a key bioactive compound, showing the highest binding affinity towards 1HZP in docking and molecular dynamics simulations. Its stability was supported by RMSD, RMSF, and Rg analyses, while MM-GBSA confirmed van der Waals, lipophilic, and Coulomb interactions as major contributors to complex stabilization. Collectively, the results emphasize *C. citratus* as a promising source of therapeutic agents, with Farnesol exhibiting strong potential in silico anti-bacterial activity. Further *in vivo* studies are required to confirm for human application for the treatment of bacterial infection.

CONFLICT OF INTEREST: Nil

ACKNOWLEDGEMENT: The authors sincerely thank Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore–641043, Tamil Nadu, India for providing the Minor Research Grant and the Department of Pharmaceutics, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram, Kerala, India, for providing the necessary facilities and support to carry out this work.

DATA AVAILABILITY: The datasets produced during this study can be obtained from the corresponding author upon reasonable request.

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