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ANTIMICROBIAL POTENTIAL, GC-MS ANALYSIS, AND MOLECULAR DOCKING STUDIES OF POGOSTEMON BENGHALENSIS LEAF EXTRACT

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ABSTRACT

Background: *Pogostemon benghalensis* has several medicinal uses in Northeast India, including wound healing activity. Currently, no molecular modeling research has examined the antimicrobial potential of its phytoconstituents. This molecular docking research identifies bioactive chemicals and evaluates their antibacterial properties. **Methodology:** Phytochemical screening and *in vitro* antibacterial tests were performed on a crude ethyl acetate extract of Pogostemon benghalensis leaves. After GC-MS analysis revealed the phytoconstituents, in-silico molecular docking was performed against the dihydrofolate reductase (DHFR) enzymes of *Escherichia coli* and *Staphylococcus aureus*. **Results and discussion:** The crude ethyl acetate extract of Pogostemon benghalensis leaves included alkaloids, carbohydrates, flavonoids, glycosides, tannins, and phenolic compounds. The extract also demonstrated potent *in vitro* antibacterial activity against *E. coli* and *S. aureus*. GC-MS data demonstrated that Phytol was the most abundant compound (53.72%) followed by Oxirane, dodecyl (13.51%.). Molecular docking studies demonstrated identified compounds have high binding affinity (BA) to the bacterial DHFR enzyme. Notable compounds are Androst-5-ene-3,19-diol, 3-acetate (3 β) with -7.4 kcal/mol BA against *E. coli* DHFR and -10.1kcal/mol against *S. aureus* DHFR; Retinol acetate with -8.7 kcal/mol BA against *E. coli* DHFR and Phytol with -6.5 kcal/mol BA against *E. coli* DHFR and -6.7 kcal/mol BA for *S. aureus* DHFR respectively. **Conclusion:** The results show that *Pogostemon benghalensis* contains valuable bioactive compounds with high antibacterial activity which further validates the use of this plant as a wound healing medication. However, further *in vivo* experimental validation of these results and their toxicological implications are required.

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INTRODUCTION

Assam, which is in the northeast of India, is known for its incredible biodiversity and rich cultural heritage [1]. For centuries, indigenous plant-based traditional medicinal practices have been a part of the healthcare system in the region [2]. The indigenous people have been aware of the medicinal value of many plant species found in Assam's plains and forests for centuries and employed them to treat a myriad of diseases [3]. Despite developments in new medicine, the local knowledge about the medicinal properties of these plants continues to exist, an indication of a symbiotic relationship between traditional wisdom and new healthcare practice [4]. Of these medicinal plants, *Pogostemon benghalensis* (figure 1) is extensively used in tribal as well as rural communities for its medicinal, agrochemical, and pharmaceutical properties [5]. This essential oil plant is renowned for its distinctive, powerful scent and high essential oil content in both the leaves and flowers. It exhibits several biological activities, including anti-inflammatory, antipyretic, antinociceptive, and insecticidal effects. It displays various biological effects, such as anti-inflammatory, antipyretic, and antinociceptive activities, and insect-killing properties [6].

Phytochemical analysis previously established the occurrence of numerous secondary metabolites like steroids, terpenoids, saponins, alkaloids, flavonoids, and phenolic compounds, attributing to its wide range of biological effects [5]. Recent studies have demonstrated the plant's high antimicrobial activity against a variety of pathogenic microbes, including *Staphylococcus aureus, Escherichia coli, Aspergillus parasiticus, Proteus vulgaris, Candida albicans*, and certain viruses. Due to the increasing spread of antimicrobial resistance all over the world, bioactive compounds found in plants are being considered as sources of alternative drugs to conventional drugs [7]. Medicinal plant-derived secondary metabolites are a primary source of new drug leads. These consist of classes of compounds like alkaloids, flavonoids, tannins, coumarins, quinones, carotenoids, and steroids [8]. Advanced analytical methods, such as Gas Chromatography-Mass Spectrometry (GC-MS), have become extremely useful, quick, and precise techniques for identifying bioactive compounds. Realizing the significance of phytochemical investigation in the discovery of antimicrobial agents, this work aimed at GC-MS analysis of Pogostemon benghalensis leaves to identify its chemical constituents & determine their antimicrobial activity [9].

Figure 1: *Pogostemon benghalensis* leaves

MATERIALS & METHODS

Chemicals

All the compounds and reagents used in this study were of analytical quality.

Collection and authentication of plant material

Fresh leaves of Pogostemon benghalensis were freshly collected from the Lakhimpur district of Assam, India (27.2340° N, 94.1022° E) during August and September. The plant material was taxonomically identified and authenticated at the Botanical Survey of India, Eastern Regional Centre, Shillong, with identification number BSI/ERC/Tech/2024-25/213. A voucher specimen was deposited at the herbarium repository *of* NETES Institute of Pharmaceutical Science under the voucher specimen number NIPS/HS/080.

Processing and extraction of plant material

The collected leaves were thoroughly washed with tap water to remove dust and contaminants, then cut into small fragments and dried under partial sunlight until a constant weight of the crude drug was achieved. The dried crude drug was ground into a fine powder using a mechanical grinder and stored in tightly sealed containers for later use. 50g of the powdered crude drug was subjected to successive solvent extraction in the order of n-hexane, ethyl acetate, and ethanol in a Soxhlet apparatus. Fifty grams of powdered material was packed in a Soxhlet extractor and extracted with 500 mL of n-hexane at a temperature of 65°C, followed by 500 mL of ethyl acetate (70°C) for 24 hours, and finally extracted in ethanol at 80°C for 24 hours. Between each successive solvent change, the marc was air-dried in an oven at temperatures not exceeding 50°C for 24 hours. Each solvent extract was concentrated individually in a rotary evaporator under vacuum to eliminate the solvents and transferred to a desiccator (containing activated silica) to remove any solvent

residue. Initial antimicrobial screening revealed that the ethyl acetate extract had the most potent activity. This extract was set aside for further phytochemical analysis and determination of antimicrobial activity.

Bacterial strains and growth media

The bacterial strains *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were obtained from the Dept of Microbiology, NEMCARE Hospital, Bhangagarh, Guwahati, Assam, India, to assess the antibacterial activity of the plant extracts. The bacterial cultures were first incubated overnight at 37°C in Mueller-Hinton Broth (MHB) under constant agitation through a rotary shaker. After incubation, the cultures were diluted to a final concentration of 1×10^5 CFU/mL using sterile MHB by dilution. The density of the bacterial cells was also verified spectrophotometrically based on measurement of the optical density at 620 nm (OD_{620}) to ensure conformity with the standardized inoculum concentration used in antimicrobial susceptibility testing. For the disc diffusion assay, sterilized discs impregnated with the plant extract were applied to Mueller-Hinton Agar (MHA) plates that had been inoculated with a standardized bacterial suspension. Experimental replicates per test were n = 3, and results were presented as mean zone of inhibition (in millimeters) ± standard deviation (SD). Dimethyl sulfoxide (DMSO) was used as the negative control to compensate for solvent effects, while standard antibiotics served as the positive control. All experiments were performed in triplicate to ensure reproducibility and statistical significance of the results.

Disc-diffusion test

Antibacterial activity of the plant extracts was determined by the standard agar disc diffusion method on Mueller-Hinton Agar (MHA) plates. Bacterial suspensions of Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* were made in sterile physiological saline and diluted to a turbidity corresponding to 0.5 McFarland standard (about 1×10^8 CFU/mL). Next, 100 µL of the suspension of each bacterium was spread evenly over the surface of MHA plates with a sterile swab, and the plates were left at room temperature for 5 minutes to dry. Sterile filter paper discs (diameter 6 mm) were impregnated with 30 µg/disc of plant extract dissolved in dimethyl sulfoxide (DMSO). Amoxicillin (30 µg/disc) served as the positive control, and discs containing only DMSO were used as the negative control to eliminate solvent interference. The discs that were impregnated were aseptically applied to the inoculated MHA plates and incubated at 37°C, with the plates inverted, for 24 hours. After incubation, antibacterial activity was quantified by measuring the diameter of the zone of inhibition (mm) surrounding each disc, including the diameter of the disc. All experiments were performed in triplicate (n = 3) and repeated three times, and the results are presented as the mean ± standard deviation (SD). Statistical analysis was carried out to compare the significance of differences in antibacterial activity between the extracts and control groups [10].

Phytochemical screening and GC-MS examination of the ethyl acetate extract

Phytochemical analysis of the extract was done using previously established protocols [11]. Further identification and characterization of the chemical constituents were carried out by performing GC-MS analysis on the extract using a Trace 1300 GC system in combination with a Thermo TSQ8000 Triple Quadrupole MS detector. The chromatographic analysis utilized an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was initially set at 60°C for 2 minutes, followed by a rise to 280°C at a rate of 10°C/min and a hold for 10 minutes. Helium (99.99% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 0.5 µL, carried out in splitless mode with an injector temperature of 250°C. Mass spectra were obtained under electron ionization (EI) conditions at 70 eV, with a mass range of 40 to 550 m/z and a scan interval of 0.5 seconds. Phytochemical component identification was performed by comparing the mass spectra of the identified compounds with those in the National Institute of Standards and Technology (NIST) Library database (version containing more than 62,000 reference spectra). Each compound identified was noted for retention time, molecular weight, and molecular formula. All GC-MS measurements were conducted in triplicate (n = 3) for reproducibility, and values were reported as mean relative percentage composition after total peak area normalization [12].

***In-silico* molecular docking for antimicrobial activity**

Molecular Docking was performed for all biologically active GC-MS constituents with high % peak areas to analyse their anti-microbial activity. For docking Dihydro Folate Reductase (DHFR) protein, docking was conducted for all constituents against the DHFR protein of both Gram-positive bacteria *Staphylococcus aureus* (PDB code: 3FRD) and Gram-negative

bacteria *Escherichia coli* (PDB code: 1DDS). Docking was conducted via PyRx software to check the binding affinity of all constituents, in which protein was added, sourced from RCSB Protein Data Bank https://www.rcsb.org/ and prepared with Discovery Studio tool by removing hetero atoms and water molecule and adding a polar hydrogen the added protein was converted to Pdbqt format with autodock tool and ligands (all constituents), sourced from PubChem database format (https://pubchem.ncbi.nlm.nih.gov/) and prepared with ChemDraw and added in PyRx and converted with open babel software (https://openbabel.org/wiki/Main_Page) & Discovery Studio was used to check the 2D & 3D interactions [13].

RESULTS

Preliminary phytochemical screening

The ethyl acetate extract of *Pogostemon benghalensis* leaves resulted in a yield of 9.53% of dried crude plant material. The extract of leaves revealed the presence of a variety of phytochemicals, including flavonoids, tannins, glycosides, carbohydrates, phenolic compounds, and alkaloids.

In vitro antimicrobial activity

The agar disc diffusion technique was employed to ascertain the antibacterial activity of the ethyl acetate extract of *Pogostemon benghalensis*. To determine its efficacy, the zones of inhibition were quantified. The results indicated that the extract exhibited greater antibacterial activity against the Gram-negative bacterium *Escherichia coli* than the Gram-positive bacterium *Staphylococcus aureus*. Table 1 summarizes the findings of the *in vitro* antimicrobial assay.

GC-MS Analysis

The GC-MS profiling of the ethyl acetate extract from *Pogostemon benghalensis* identified 11 bioactive constituents with prominent peak intensities, as shown in Figure 2. These compounds were subsequently chosen for *in silico* molecular docking studies, with specific details presented in Table 2. The constituents that have been identified are hexadecanoic acid; hexadecen-1-ol acetate; Pregnan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-; 11,14,17-Eicosatrienoic acid, methyl ester; 9,12-Octadecadienoic acid, methyl ester; Retinol acetate; phytol; oxirane, dodecyl-; Strophanthidol, 3,19-diacetate; Z-4-Nonadecen-1-ol acetate and Androst-5-ene-3,19-diol, 3-acetate (3β). Among these, Phytol exhibited the highest abundance with a % peak area of 53.72, followed by Oxirane, dodecyl- at 13.51%. Other constituents showed substantial % peak areas, ranging from 2.04 to 6.80%.

Table 1: Zone of inhibition of ethyl acetate extract of *Pogostemon benghalensis* leaves.

Micro-organism	Zone diameter (mm) Expressed as Mean ± S.D., (n = 3)		
	Ethyl acetate *Pogostemon benghalensis* leaves (30 µg/ml)	Amoxycillin (30 µg/ml)	Negative Control (DMSO)
E. coli	19.43 ± 0.55	21.12 ± 0.21	1.30 ± 0.10
S. aureus	18.03 ± 0.51	22.11 ± 0.59	1.37 ± 0.35

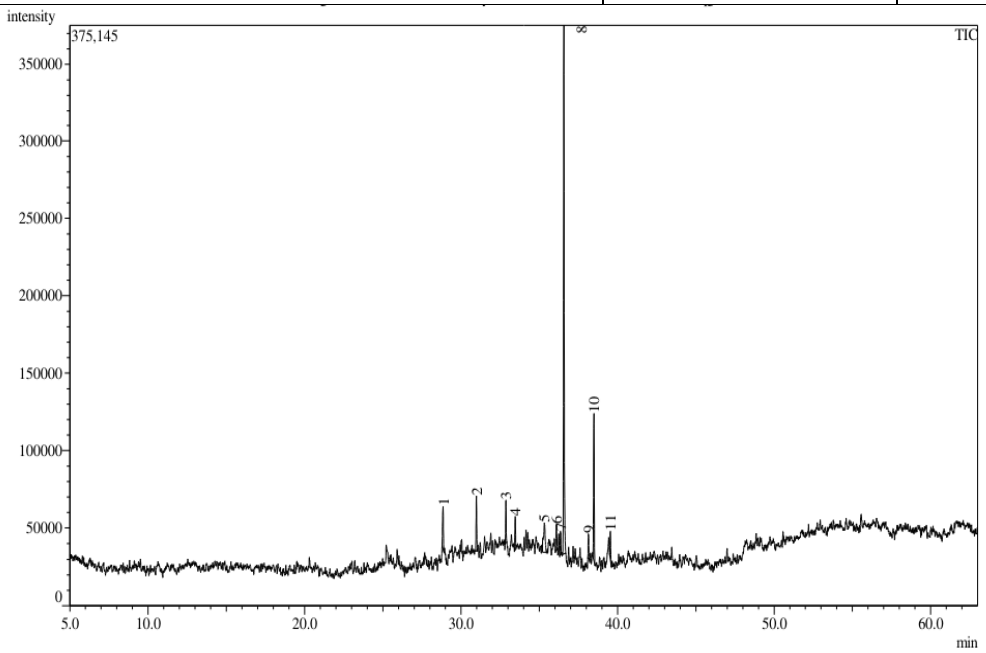


Figure 2: GC-MS Spectrum of ethyl acetate extract of *Pogostemon benghalensis* leaves.

Table 2: Highly abundant phytoconstituents of *Pogostemon benghalensis* leaves identified using GC-MS analysis.

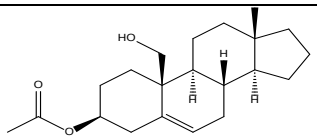
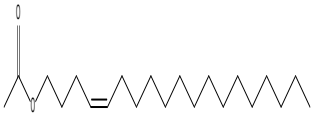
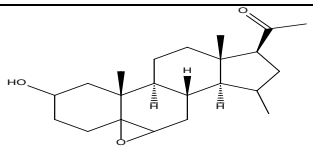
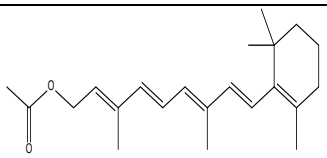
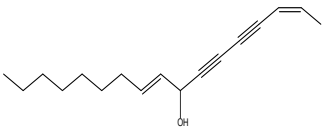
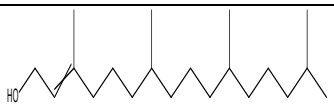
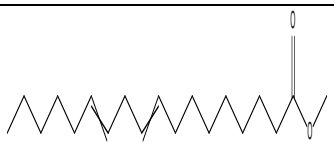
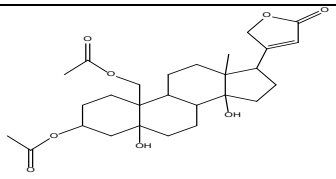
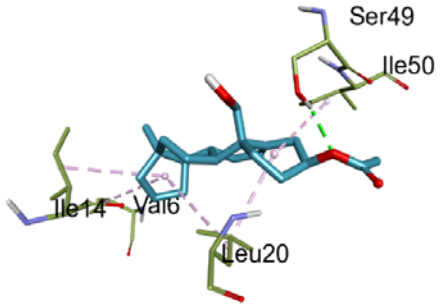
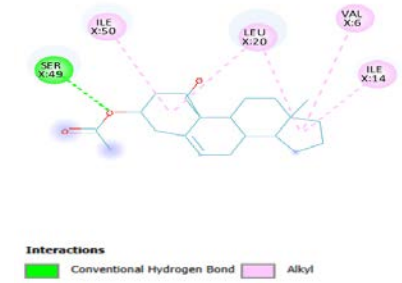
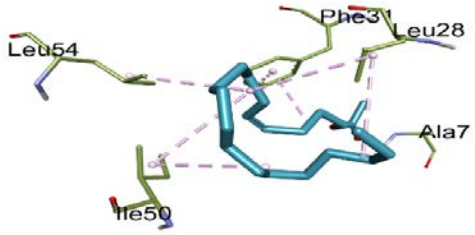
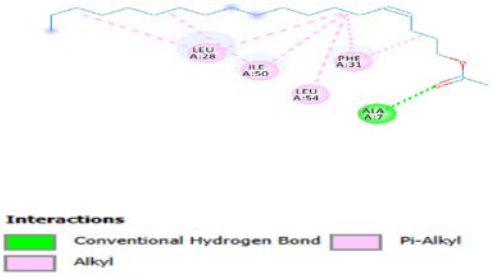
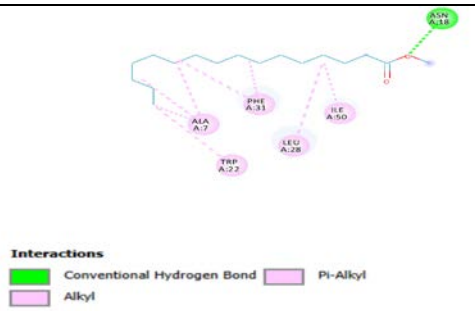
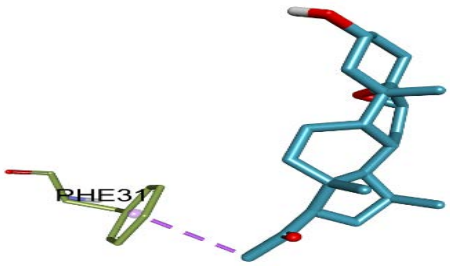
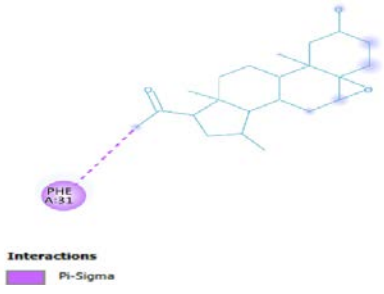
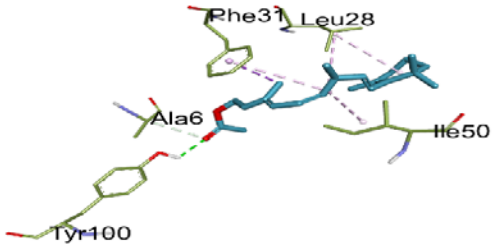
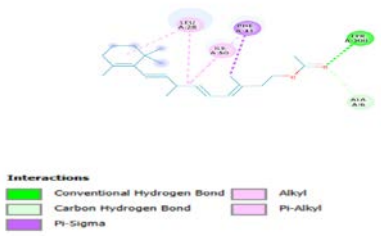
Sl. No	Compounds	Structure	Binding Affinity (Receptor- DHFR) Kcal/mol		RT (min)	%Peak Area
			E. coli (Gram -ve)	*S. aureus* (Gram +ve)		
1	Androst-5-ene-3,19-diol, 3-acetate,(3. beta.)-		-7.4	-10.1	28.844	4.62
2	Z-4-Nonadecen-1-ol acetate		-6.2	-6.3	30.977	5.24
3	Hexadecanoic acid, methyl ester		-6.1	-6.2	32.864	3.58
4	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-		-8.1	-7.3	33.456	3.34
5	Retinol, acetate		-8.1	-8.7	35.318	6.80
6	2,9-Heptadecadiene-4,6-diyn-8-ol, (Z,E)-		-6.2	-6.4	36.088	2.47
7	11,14,17-Eicosatrienoic acid, methyl ester		-6.6	-6.7	36.349	2.27
8	Phytol		-6.5	-6.7	36.566	53.72
9	9,12-Octadecadienoic acid, methyl ester		-6.5	-6.4	38.136	2.04
10	Oxirane, dodecyl-		-5.9	-5.3	38.484	13.51
11	Strophanthidol, 3,19-diacetate		-6.6	-7.4	39.532	2.41

Table 3: 3D & 2D Interaction between highly abundant phytoconstituents (Ligands) present in ethyl acetate extract *Pogostemon benghalensis* leaves with high binding affinity against Dihydro folate reductase (Receptor) of Gram-negative Bacteria (*Escherichia coli)*

Constituents	3D Interactions	2D interactions
Androst-5-ene-3,19-diol, 3-acetate, (3. beta.)-		
Z-4-Nonadecen-1-ol acetate		
Hexadecanoic acid, methyl ester		
Pregan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-		
Retinol, acetate		

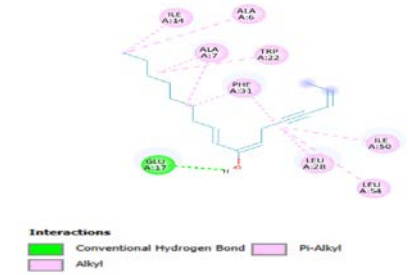
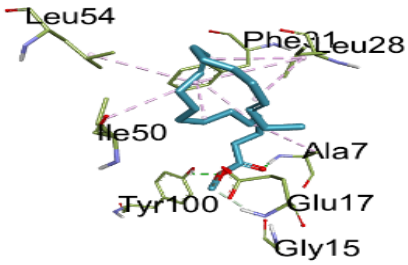
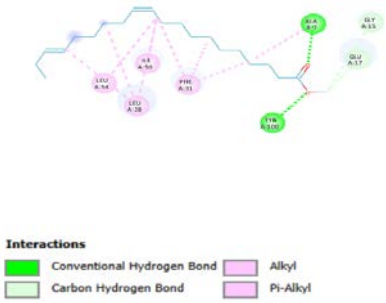
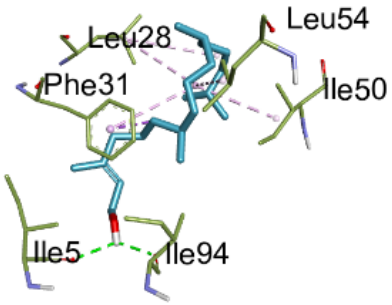
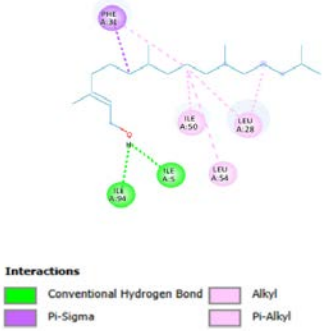
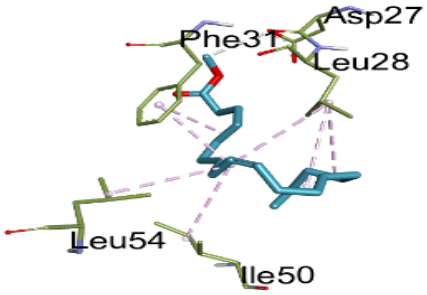
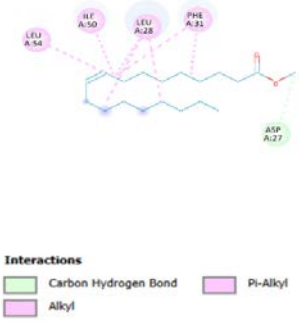
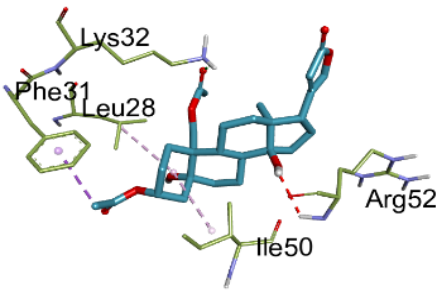
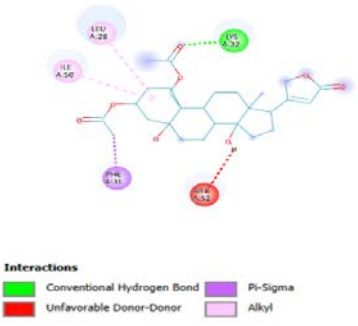
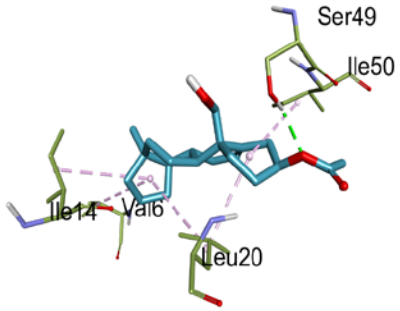
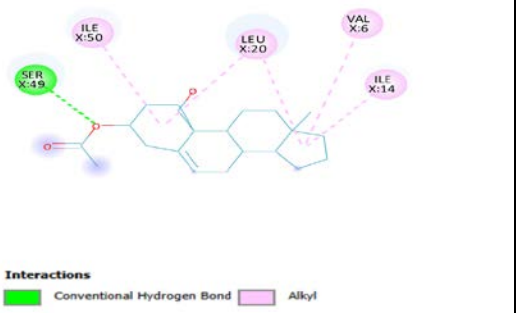
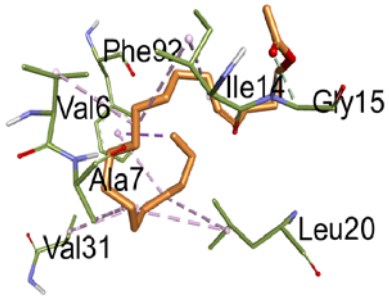
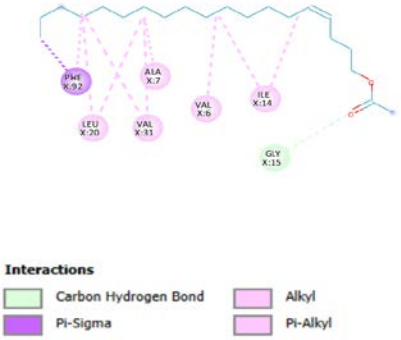
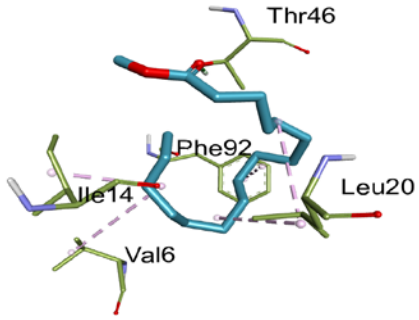
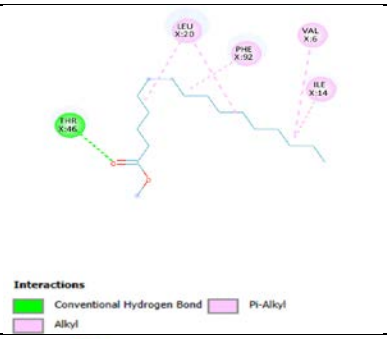
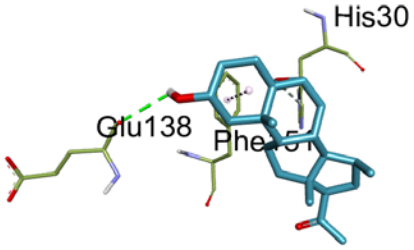
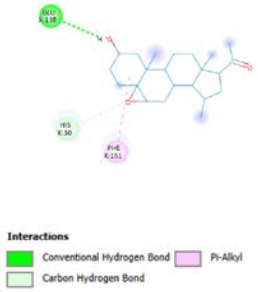
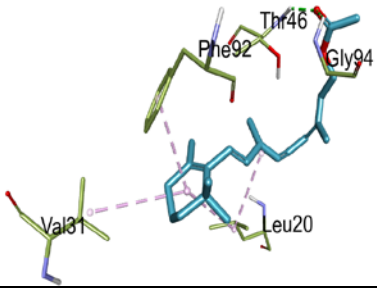
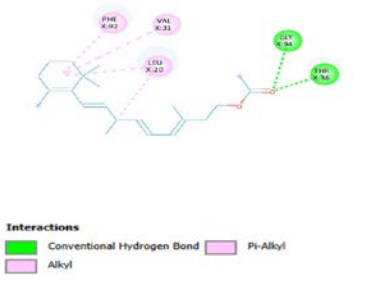
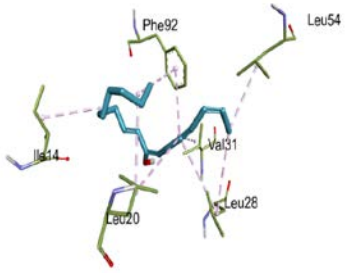
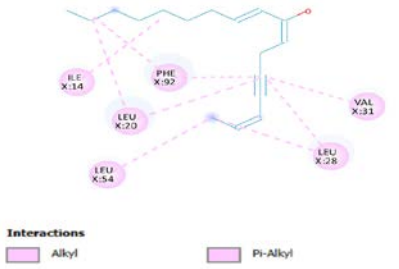
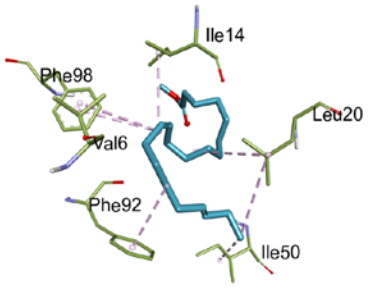
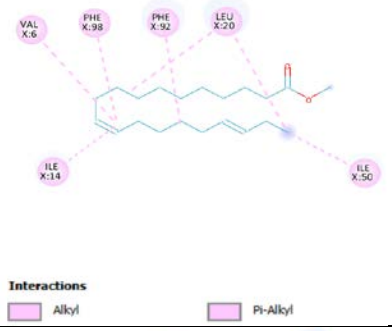
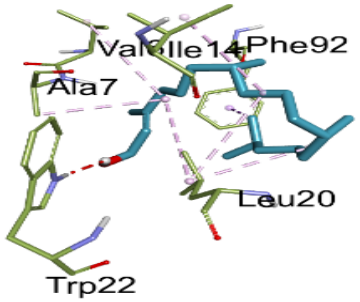
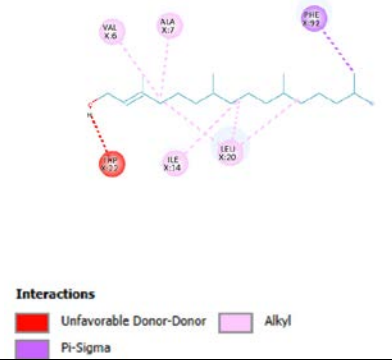
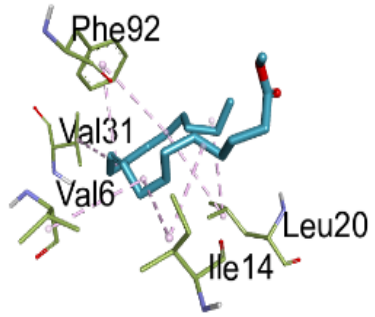
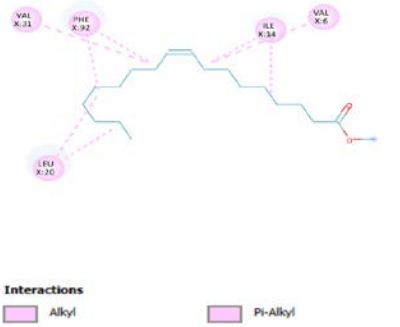
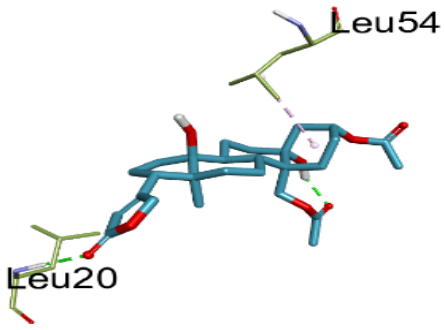
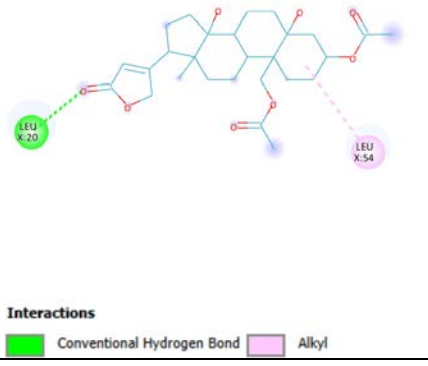
Constituents	3D Interactions	2D interactions
2,9-Heptadecadiene-4,6-diyn-8-ol, (Z, E)-		
11,14,17-Eicosatrienoic acid, methyl ester		
Phytol		
9,12-Octadecadienoic acid, methyl ester		
Strophanthidol, 3,19-diacetate		

Table 4: 3D & 2D Interaction between highly abundant phytoconstituents (Ligands) present in ethyl acetate extract *Pogostemon benghalensis* leaves with high binding affinity against Dihydro folate reductase (Receptor) of Gram-positive Bacteria (*Staphylococcus aureus*)

Constituents	3D Interactions	2D interactions
Androst-5-ene-3,19-diol, 3-acetate, (3. beta.)-		
Z-4-Nonadecen-1-ol acetate		
Hexadecanoic acid, methyl ester		
Pregan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-		
Retinol, acetate		

Constituents	3D Interactions	2D interactions
2,9-Heptadecadiene-4,6-diyn-8-ol, (Z,E)-		
11,14,17-Eicosatrienoic acid, methyl ester		
Phytol		
9,12-Octadecadienoic acid, methyl ester		
Strophanthidol, 3,19-diacetate		

In-Silico Molecular Docking

A molecular docking study evaluating the antimicrobial potential of 11 bioactive constituents against Dihydrofolate reductase (DHFR) enzymes from E. coli and S. aureus revealed that 10 constituents exhibited significant binding affinities exceeding -6 kcal/mol. The compound Androst-5-ene-3,19-diol, 3-acetate, (3β) was identified as the lead candidate, demonstrating the highest binding affinities of -10.1 kcal/mol for S. aureus DHFR and -7.4 kcal/mol for E. coli DHFR, suggesting considerable potential against Gram-negative bacteria. Retinol acetate exhibited a binding affinity of -8.7 kcal/mol, demonstrating notable effectiveness against *E. coli* DHFR. Phytol, recognized as the predominant component, exhibited binding affinities of -6.5 and -6.7 kcal/mol for E. coli and S. aureus DHFR, respectively. The binding affinities for all tested constituents varied from -5.3 to -10.1 kcal/mol, highlighting their significant interactions with DHFR enzymes in both bacterial species [13].

DISCUSSIONS

In India, the use of plant extracts to treat microbial, fungal, and deficiency diseases has a long history, and this approach has now gained global recognition [14]. The search for compounds with potent antimicrobial activity has emerged as a primary area of investigation to mitigate the threat of infectious diseases caused by harmful bacteria, fungi, viruses, and parasites. Plant extracts continue to serve as a primary source of various therapeutic agents, including those used to combat infectious diseases [15].

The ethyl acetate extract of *Pogostemon benghalensis* leaves yielded an adequate extraction of 9.53%, indicating that ethyl acetate was an effective solvent for extracting bioactive compounds. Phenolic compounds, glycosides, carbohydrates, flavonoids, tannins, and alkaloids were identified through phytochemical analysis, each exhibiting distinct pharmacological properties. Such outcomes were in line with previous studies reported by Sandhya et al. (2023), which identified the main classes of compounds as steroids, alkaloids, phenolic compounds, saponins, tannins, and various sugars [16].

Antibacterial evaluation of the ethyl acetate extract of *Pogostemon benghalensis* leaves using the disc diffusion agar method revealed that this particular extract displayed stronger activity against the Gram-negative bacterium *Escherichia coli* as compared to the Gram-positive bacterium *Staphylococcus aureus*. Such a difference may be explained by structural features of cell membranes in bacteria, which suggests that the phytoconstituents present in *Pogostemon benghalensis* leaves may penetrate or disrupt the outer membrane of *E. coli* more effectively than the thicker peptidoglycan layer of *S. aureus* [17,18]. From GC-MS analysis, 11 bioactive constituents were identified. Phytol is the major constituent, constituting 53.72% of the entire composition. Oxirane, dodecyl- 13.51%, and Retinol acetate were other significant compounds detected. The diversity of compounds identified suggests that the mixture is complex and may contain synergistically active agents that contribute to the observed antimicrobial activity.

The *in silico* molecular docking analysis revealed that phytoconstituents present in *Pogostemon benghalensis* leaves significantly bind with DHFR enzyme, which ascertains their potency in inhibiting the enzyme. Androst-5-ene-3,19-diol, 3-acetate, (3β) emerged as the most potent compound, demonstrating the highest binding affinities of -10.1 and -7.4 kcal/mol against the DHFR enzymes of *Escherichia coli* and *Staphylococcus aureus*, respectively. This demonstrates its strong antibacterial effectiveness against both Gram-negative and Gram-positive bacteria. Retinol acetate exhibited a notable binding affinity of -8.7 kcal/mol for *Staphylococcus aureus*. The prevalent compound, phytol, demonstrated moderate affinities of -6.5 kcal/mol for *Staphylococcus aureus* and -6.7 kcal/mol for *Escherichia coli*, suggesting its potential to augment the antimicrobial efficacy of the extract. DHFR is a key enzyme targeted in antibacterial activity testing assays because it is essential for bacterial DNA synthesis and cell growth. Inhibiting DHFR disrupts folate metabolism, thereby inhibiting bacterial growth and making it a validated target for antibacterial drug discovery. DHFR catalyzes the reduction of dihydrofolate to tetrahydrofolate, a critical step in the synthesis of nucleotides required for DNA replication. Inhibiting this enzyme blocks bacterial proliferation [19,20]. Therefore, the experiment proves the possibility of the ethyl acetate extract of *Pogostemon benghalensis* as an antimicrobial resource. The identified bioactive constituents, which exhibit substantial binding affinities to DHFR enzymes, provide the molecular basis for the observed antimicrobial activity and justify further studies, including in vivo assessments and formulation development, to extract the therapeutic potential of these compounds in combating bacterial infections.

However, this study has certain limitations regarding its practical application in clinical settings. *In vitro* and *in silico* studies, while valuable for preliminary screening, often lack the biological complexity of whole organisms, limiting the translatability of their results. *In vitro* assays typically involve isolated cells or microorganisms and therefore miss critical physiological interactions such as immune responses, metabolic processes, and tissue barriers. Similarly, *in silico* models rely on computational predictions and molecular simulations based on simplified assumptions and existing data, which cannot fully capture the dynamic and multifactorial nature of living systems. As a result, compounds that exhibit strong antimicrobial activity *in vitro* or show promising molecular docking results *in silico* may fail *in vivo* due to factors like poor absorption, rapid metabolism, unexpected toxicity, or an inability to reach the infection site effectively. Since this current study does not assess pharmacokinetics, toxicity, or side effects within the context of an intact biological system, the need for subsequent *in vivo* validation is of prime importance.

CONCLUSION

The present study indicates that the bioactive constituents derived from *Pogostemon benghalensis* leaves possess strong interactions with DHFR enzymes, suggesting a multifaceted approach to combating microbial diseases. These compounds include retinol, phytol, and Androst-5-ene-3,19-diol, 3-acetate, (3β). The varying degrees of binding affinities to DHFR of *E.coli* and *S. aureus* also indicate that these compounds could be explored further for their synergistic effects, potentially leading to the development of new antimicrobial agents. However, to verify these results and investigate the potential therapeutic use of these phytoconstituents in clinical settings, further experimental validation is necessary.

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FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHORS CONTRIBUTIONS

Bhaswati Kashyap and Koushik Nandan Dutta conceived the idea. They developed the theoretical framework. Siddhartha Sankar Das and Dipjyoti Sharma performed the in-silico molecular docking studies and analysis of GC-MS results, Bhaswati Kashyap, Nilutpal Sharma Bora, and Sameeran Gam were involved in data curation and drafted the original manuscript. All authors have read and approved the final manuscript.

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