Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 02 (Mar–Apr 2024); pp 563–570

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Identification of Antibacterial Leads from *Andrographis paniculata* Leaves via *in vitro* and *in silico* studies

T. K. Bindu[1], Vinod P. Raphael*[2], K. S. Shaju[3] and Sherry Sebastian[4]

[1-2, 4] Department of Chemistry, Government Engineering College, Thrissur - 680 009, Kerala, India
[3] Department of Chemistry, Panampilly Memorial Government College, Thrissur - 680 722, Kerala, India

Received: 23 Jan 2024; Revised accepted: 31 Mar 2024; Published online: 19 Apr 2024

Abstract

Pharmaceutical researchers are continually exploring potent compounds that could lead to the discovery of effective treatments against pathogens. Scientists maintain an unwavering interest in identifying effective molecules from the vast array of phytochemicals. In this study, we prepared hexane and acetone extracts from the medicinal herb *Andrographis paniculata*. These extracts were screened against the pathogens *E. coli*, *S. aureus*, and *P. aeruginosa*. Interestingly, the hexane extract exhibited no activity against the pathogens, while the acetone extract demonstrated significant inhibitory effects on the growth of all three pathogens. To identify the specific phytochemicals, present in the extract, we conducted GC-MS analysis. Among the compounds identified, three stood out due to their favorable ADME properties and bioavailability: alpha-bisabolene epoxide, alpha-bulnesene, and andrographolide. Further investigations involved *in silico* studies on selected structural proteins of microbes with these drug-like molecules. These studies revealed that andrographolide, a diterpene lactone, exhibited high binding affinity on the structural receptors of pathogens. Additionally, computational methods were employed to identify the most likely receptor involved in inhibiting the growth of microorganisms.

***Key words*:** Andrographolide, GC-MS, Molecular docking, *P. aeruginosa*, ADME

Bacterial infections serve as the underlying cause of numerous diseases. Antibacterial agents, also known as antibiotics, possess the capability to inhibit or eradicate the growth of microorganisms. However, due to repeated or unwise usage, bacterial resistance has emerged [1-5]. Contemporary antibiotics struggle to completely suppress mutated bacterial growth. Their effectiveness against bacterial strains may be compromised due to the resistant nature of pathogens. As we move forward, the era may witness a surge in antibiotics with diminished efficacy. To tackle this challenge, pharmaceutical scientists and researchers are actively exploring novel and potent compounds.

Plants serve as abundant sources of medicinal compounds [6-8]. Numerous medicinal phytochemicals have been isolated and characterized by various researchers, with a focus on their antimicrobial properties. Notable medicinal herbs, such as echinacea [9-10], garlic [11-12], and oregano [13-14], contain highly efficient compounds with presumed antimicrobial potency. *Andrographis paniculata* (AP) [15-17], an herb found in various regions across Southeast Asia, including India, Sri Lanka, and parts of China, plays a significant role. It is also native to other countries in the Indian subcontinent and has been introduced to several tropical and subtropical areas around the world [18-19].

Some research in the literature has explored the efficacy of extracts from this plant in relation to cancer, diabetes, and microbial activity. However, an extensive literature survey reveals that the potency of the leaf extract from *Andrographis paniculata* and the identification of its active components have not been systematically investigated. In the present study, we aim to assess the efficacy of the acetone extract from *Andrographis paniculata* leaves against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Details of the pathogens, their characteristics and antibacterial agents used to control are given in the subsequent paragraphs.

Staphylococcus aureus is a spherically shaped Gram-positive bacterium that is a major cause of blood infections known as bacteremia [20-21]. It also contributes to skin and soft-tissue infections in humans, which can be managed using antibiotics such as beta-lactams, Vancomycin, and Clindamycin. Various strains of *E. coli* can lead to conditions like diarrhea, stomach upset, urinary tract infections, prostate gland infections, and pelvic inflammatory diseases [22-23]. Antibiotics such as Ciprofloxacin, Levofloxacin, and Amoxicillin are commonly used for treatment. *Pseudomonas aeruginosa* is a Gram-negative, aerobic, rod-shaped bacterium capable of causing diseases in plants, animals, and humans [24-25]. It has adapted well to anaerobic conditions. Being a multidrug-resistant pathogen, it plays a central role in serious hospital-acquired infections, including ventilator-associated pneumonia and sepsis syndromes. *P. aeruginosa* selectively inhibits various antibiotics (such as penicillin or cephalosporins) from penetrating its outer membrane, leading to high resistance. According to the World Health Organization,

***Correspondence to:** Vinod P. Raphael, E-mail: vinodpraphael@gectcr.ac.in; Tel: +91 9287560416

Citation: Bindu TK, Raphael VP, Shaju KS, Sebastian S. 2024. Identification of antibacterial leads from *Andrographis paniculata* leaves via *in vitro* and *in silico* studies. *Res. Jr. Agril. Sci.* 15(2): 563-570.

P. aeruginosa poses one of the greatest threats to humans in terms of antibiotic resistance.

Fig 1 *Andrographis paniculata* plant

MATERIALS AND METHODS

Preparation of plant extract

The plant *Andrographis paniculata* was sourced from Mannuthy, Thrissur, Kerala, India. Following a thorough wash with water, the leaves were air-dried in a shaded environment for 7 days. Subsequently, the dried leaves underwent fine powdering, and extraction was carried out using a Soxhlet apparatus with 200 g of the powder. Initially, the powdered specimen was subjected to hexane extraction to collect chlorophyll and other hydrophobic components. After hexane extraction, the sample was dried in a hot air oven at 50°C. Next, acetone was used for extraction using the Soxhlet extractor. The surplus solvent present in the extract was eliminated via a rotary evaporator under reduced pressure conditions. The resultant dried solid mixture was dissolved in 2% DMSO, yielding a stock solution with a concentration of 1 mg/L. This stock solution, ranging from 50 to 80 microliters, was then applied for antibacterial screening.

Antibacterial studies

A pure culture of pathogens, including *P. aeruginosa*, *E. coli*, and *S. aureus*, was sourced from the Microbial Type Culture Collection and Gene Bank in Chandigarh, India. To maintain viable cultures for antibacterial testing, a nutrient broth was formulated with specific nutrients and adjusted to a neutral pH. The well-diffusion method, adapted from Collins and Lyner's 1987 protocol, served as the platform for evaluating the antibacterial potential of various plant extracts. The bacterial cultures were sustained in nutrient broth as the medium. Using sterile swabs, the bacterial culture was evenly distributed on nutrient agar plates. Wells, each with a 3 mm diameter, were created in the solidified agar surface using a well borer, maintaining a 2 cm separation between each well. To form a stock solution, the solid extract was dissolved in a 2% DMSO solution at a concentration of 1 mg/L. Plant extracts of different concentrations (50, 60, 70, and 80 µL) were then administered to the wells on the nutrient agar plates through a micropipette. Following incubation at 37°C for 24 hours, the size of any clear zones surrounding the wells, marking areas of suppressed bacterial growth, was meticulously measured. This revealed the effectiveness of each extract concentration against this resilient pathogen.

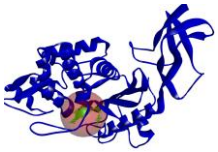
Gas chromatography-Mass spectroscopic studies

To analyze the phytochemicals in the acetone extract of the *Andrographis paniculata* (AP) plant, GC-MS analysis was conducted using a fused silica packed capillary column (30 m x 0.25 mm x 0.25 micrometers). The analysis temperature followed this program: initially held at 110°C for 2 minutes, then increased to 150°C at a constant rate of 15°C/min, and subsequently continued to rise until reaching 250°C at a rate of 10°C per minute. Helium served as the carrier gas with a constant flow rate of 1 mL/min. The chromatographic separation was completed in 42 minutes. Phytochemical identification in the extract relied on retention time indices obtained from the NIST library.

Computational studies

Driven by the power of computational tools, this study delves into the intricacies of *Andrographis paniculata* (AP) leaves, seeking potent warriors against bacterial species. Gas chromatography-mass spectrometry (GC-MS) meticulously reveals the hidden compounds within the leaf's acetone extract. The optimized molecular structures of these identified compounds were downloaded from the vast library of PubMed, in the SDF format. Subsequently, we evaluated the drug-like properties of these compounds using the Swiss ADME webserver [26]. Only those molecules demonstrating pharmaceutical potential underwent further computational evaluation. To explore the interaction mechanisms, we obtained crystal structures of the structural receptors from various pathogens via the Protein Data Bank (PDB) [27]. Data presented in (Table 1-3) provides details on the structures, including the major binding pocket, characterization methods, functions, and sequence lengths of target proteins for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These crystal structures were meticulously prepared for docking studies using Biovia Discovery Studio 2024 [28]. The drug-like molecules were docked [29-30] with the receptors of pathogens using the CB-dock2 webserver, which is assisted by the docking software AutoDock. Each docking simulation unfolded a breathtaking display of potential interactions and was subsequently analyzed using Discovery Studio 2024.

Table 1 Structure and function of target proteins of *Staphylococcus aureus*

Target receptor	Binding pocket
The protein with PDB ID 5TW8 in *S. aureus* is Penicillin-Binding Protein 4 (PBP4), also known as MecA. Its function is crucial for bacterial cell wall synthesis and plays a significant role in the notorious methicillin-resistant *Staphylococcus aureus* (MRSA) strains. (X-ray diffraction; Resolution: 1.72 Å; Sequence length 359)	

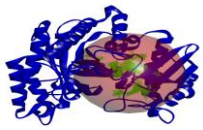
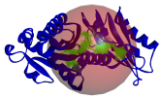
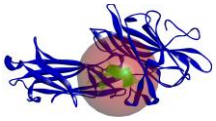
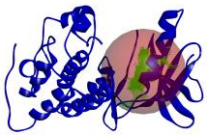
The protein with PDB ID 5BOE in *S. aureus* is Staphylococcal Protein G (SpG). SpG doesn't directly participate in vital bacterial functions like cell wall synthesis. Instead, its function is related to immune evasion, making it a crucial virulence factor for *S. aureus* infections. (X-ray diffraction; Resolution: 1.60 Å; Sequence length 442)	
3VOB in *S. aureus* is FtsZ, a crucial player in bacterial cell division. Its function is essential for the bacteria to replicate and spread, making it a potential target for antibiotic development. (X-ray diffraction; Resolution: 2.70 Å; Sequence length 308)	
The protein with PDB ID 1N67 in *S. aureus* is ClfA, short for clot formation-associated fibrinogen-binding protein. Its function is related to adhesion and virulence, playing a crucial role in *S. aureus*' ability to form blood clots and evade the immune system. (X-ray diffraction; Resolution: 1.90 Å; Sequence length 359)	
4EQM belongs to the protein kinase family and shares a similar structure with other kinases. It has a conserved catalytic domain responsible for phosphorylating target proteins and regulating their activity. (X-ray diffraction; Resolution: 3.0 Å; Sequence length 294)	

Table 2 Structure and function of target proteins of *E. coli*

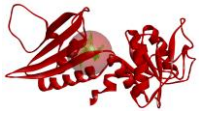
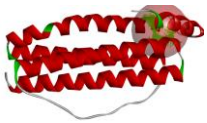
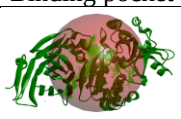
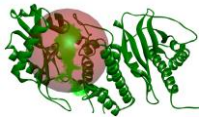
Target receptor	Binding pocket
The protein identified by the PDB ID 4YCP in *E. coli* is phosphoenolpyruvate carboxylase (PEPC), also referred to as PycA. This enzyme holds significant importance in the gluconeogenesis pathway, playing a crucial role in bacterial metabolism. (X-ray diffraction; Resolution: 2.55 Å; Sequence length 321)	
The protein designated by the PDB ID 4QR8 in *E. coli* is Xaa-Pro dipeptidase (PepQ). It assumes a pivotal role in the bacterium's processes of protein degradation and recycling. Delving into its functionality and examining its potential as a target for drug development reveals intriguing aspects. (X-ray diffraction; Resolution: 2.0 Å; Sequence length 443)	
1DIH protein in *E. coli* is dihydrodipicolinate reductase (DHDPR). It holds a pivotal role in the biosynthesis of diaminopimelic acid (DAP), which is an indispensable amino acid required for the synthesis of the bacterial cell wall. (X-ray diffraction; Resolution: 2.20 Å; Sequence length 273)	
The protein with PDB ID 1EUM is FtsZ, a key player in bacterial cell division. Its function and potential as a drug target are well-studied and crucial for bacterial survival. (X-ray diffraction; Resolution: 2.05 Å; Sequence length 165)	
1Q8I is the PDB code of the protein of *E. coli* is DNA polymerase II in *E. coli*. It plays a crucial role in DNA repair processes within the bacterium. (X-ray diffraction; Resolution: 2.0 Å; Sequence length 783)	

Table 3 Structure and function of target proteins of *P. aeruginosa*

Target receptor	Binding pocket
Pseudomonas aeruginosa's Exotoxin A (1IKQ) causes cellular harm by ADP-ribosylating translation elongation factor 2. This process relies on binding to specific cell surface receptors and follows a complex pathway of intracellular trafficking. (X-ray diffraction; Resolution: 1.62 Å; Sequence length 613)	
The 3BJZ protein in *P. aeruginosa* is sedoheptulose-7-phosphate isomerase, an essential enzyme involved in the biosynthesis of lipopolysaccharide (LPS), a major component of the outer membrane of the bacterium. (X-ray diffraction; Resolution: 2.4 Å; Sequence length 199)	
The protein with PDB ID 3SY7 is an outer membrane protein D (OprD), a porin, and its function is to facilitate the diffusion of small molecules across the outer membrane. This includes nutrients for the bacteria, as well as some antibiotics (X-ray diffraction; Resolution: 2.15 Å; Sequence length 428)	

The protein designated with PDB ID 5WZE in *P. aeruginosa* is PepP, also known as aminopeptidase P that specifically cleaves peptide bonds involving an amino acid residue. (X-ray diffraction; Resolution: 1.78 Å; Sequence length 452)

6P8U protein present in *P. aeruginosa* is a complex called the CdnD:HORMA2:Peptide 1 complex. Its function is related to a bacterial defense pathway against bacteriophages, viruses that infect bacteria. (X-ray diffraction; Resolution: 1.89 Å; Sequence length 300)



The protein with PDB ID 7JWL in *P. aeruginosa* is Penicillin-Binding Protein 3 (PBP3). Its function is crucial for bacterial cell wall synthesis and, as the name suggests, plays a role in resistance to some penicillin antibiotics. (X-ray diffraction; Resolution: 2.20 Å; Sequence length 538)

RESULTS AND DISCUSSION

Antibacterial screening

Antibacterial studies of the hexane extract and acetone extract were conducted using the well diffusion method on *E. coli*, *P. aeruginosa*, and *S. aureus*. The hexane extract did not exhibit activity against the growth of these pathogens. However, the acetone extract of AP leaves demonstrated appreciable antibacterial activity against these microorganisms. (Table 4) represents the antibacterial screening results of the acetone extract of AP leaves. The extract was highly active against *E. coli* according to the well diffusion method, where a volume of 80 µl showed 18 mm zone of inhibition for *E. coli*. The extract also exhibited good antibacterial efficiency towards other pathogens, namely *P. aeruginosa* and *S. aureus*. The activity of the acetone extract on the bacterial strains follows the order *E. coli* > *P. aeruginosa* > *S. aureus*.

Table 4 Antibacterial activity of acetone extract of AP leaves

Species	zone of inhibition (mm) of extract			
	50µl	60µl	70µl	80µl
S. aureus	11	12	14	14
E. Coli	15	17	17	18
P. aeruginosa	12	14	15	15

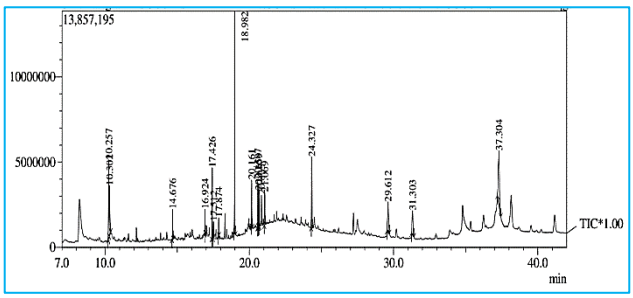


Fig 2 Gas chromatogram of the acetone extract of AP leaves

GC-MS analysis

The gas chromatogram presented in (Fig 2) illustrates the acetone extract of AP leaves. Mass spectral analysis identified 18 compounds within the extract, including 1,2,3-propanetriol-1-acetate (10.257)*, d-mannitol,1,4-anhydro (10.302), 1-hexadecene (14.676), E-15-heptadecenal (16.924), neophytadiene (17.426), hexahydrofarnesylacetone (17.512), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (17.874), ethyl palmitate (18.982), phytol (20.161), ethyl (9Z,12Z)-9,12-octadecadienoate (20.587), 9-octadecenoic acid (20.626), 9,12,15-octadecatrienoic acid (20.659), ethyl stearate (20.835), phytol acetate (21.069), 1,2-benzenedicarboxylic acid (24.327), cis-Z-.alpha.-bisabolene epoxide (29.612), alpha.-bulnesene (31.303) and andrographolide (37.304). (*Retention time is given in the bracket)

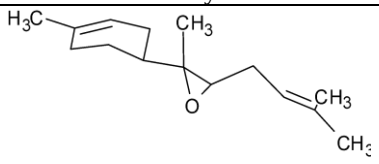
ADME predictions

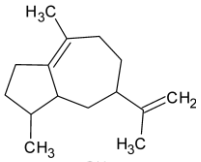
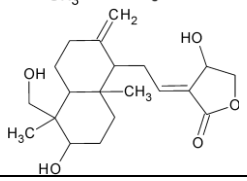
Based on drug-likeness screening and ADME predictions, we selected three promising molecules from the acetone extract: α-bisabolene epoxide, α-bulnesene, and andrographolide. Table 5 lists the identified phytochemicals with their names, retention times in the chromatogram, and structures. The ADME predictions for these compounds indicated favorable drug-likeness properties, with molecular descriptors and parameters falling within the webserver's predicted limits. This includes factors like lipophilicity, unsaturation, molecular size, flexibility, and solubility. (Fig 3) presents a bioavailability radar given by the SwissADME webserver for these compounds, visually summarizing their predicted absorption and distribution characteristics.

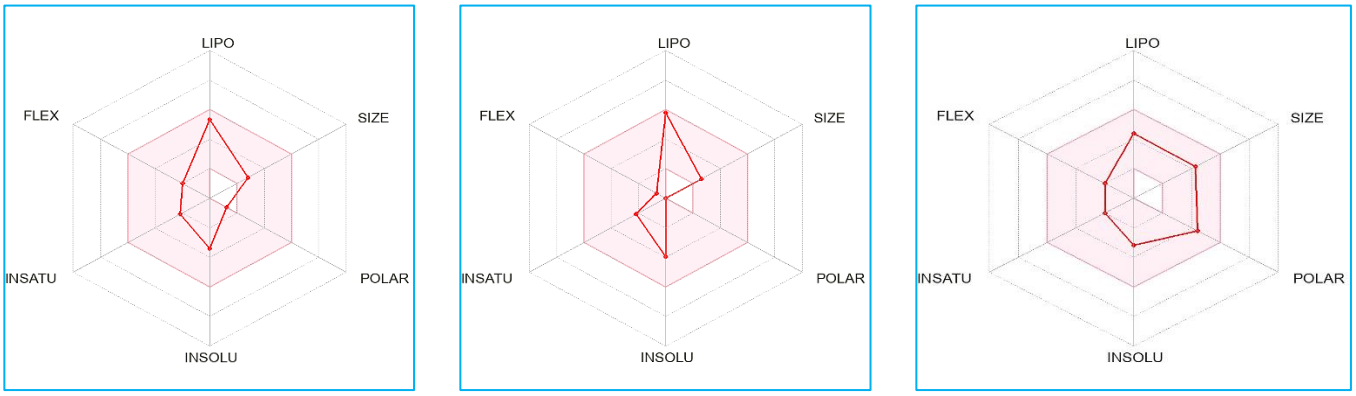
In silico studies

Antibacterial studies revealed the potency of the acetone extract from AP leaves in inhibiting the growth of pathogens *E. coli*, *P. aeruginosa*, and *S. aureus*. This piques the interest of medicinal chemists and pharmaceutical scientists to identify the active component(s) responsible for this growth inhibition. The main objective of the computational study is to gain insight into the active components present in the acetone extract. The in-silico study also aims to provide an idea about the most probable structural target proteins of the pathogens vulnerable to the active compounds in the extract. The subsequent sections will depict the molecular docking studies of three drug-like molecules, bisabolene epoxide, bulnesene, and andrographolide, present in the acetone extract of AP leaves, on the selected structural protein receptors of the pathogens.

Table 5 Structures of drug like compounds present in the acetone extract of AP leaves

S. No.	Name	Structure of Phytochemical	Retention time (min) and area %
1.	cis-Z-.alpha.-bisabolene epoxide		29.612; 6.49%

2.	alpha.-bulnesene		31.303; 5.56%
3.	andrographolide		37.304; 14.08%



Bisabolene epoxide Bulnesene Andrographolide

Fig 3 Bioavailability radars of drug like compounds present in the acetone extract of AP leaves

Table 6 Binding energies (-kcal/mol) of three molecules on the structural receptors of *S. aureus*

PDB	Bisabolene epoxide	Bulnesene	Andrographolide
5TW8	6.6	6.9	7.7
5BOE	6.6	5.6	6.6
3VOB	7.2	6.9	8.1
1N67	7.2	6.9	8.5
4EQM	7.1	7.9	7.4

Molecular docking studies on the receptors of S. aureus

In silico studies on the receptors of *S. aureus* revealed that the phytochemicals bisabolene epoxide and bulnesene showed moderate inhibition potential. Bulnesene demonstrated a maximum binding score of -7.9 kcal/mol (a measure of binding affinity) on protein kinase receptor 4EQM. Andrographolide present in the acetone extract exhibited very good binding energies, of -8.1 and -8.5 kcal/mol on the 3VOB and 1N67 receptors, respectively (Table 6). The 3VOB receptor in *S. aureus*, crucial for bacterial cell division and essential for replication and spread, and the 1N67 receptor, a fibrinogen-binding protein that enables *S. aureus* to evade the immune system, were targeted in this study. Table 6 represents the binding scores of the three molecules, bisabolene epoxide, bulnesene, and andrographolide, on these selected receptors of *S. aureus*. (Fig 4) displays the interaction plot of andrographolide with the receptors 1N67 and 3VOB. The high binding score of the 1N67-andrographolide complex (-8.5 kcal/mol) is due to the two conventional hydrogen bonds originating from the two hydroxyl groups of andrographolide to the amino acid residue aspartic acid (Asp 385), established at distances of 2.24 and 3.02 Å. In addition to these interactions, a non-conventional hydrogen bond formed between Val288 and the five-sided membered ring of the molecule (3.32 Å). Furthermore, an alkyl-π interaction (a weak attraction between electron-rich and electron-poor regions of the molecules) was noted between one of the cyclohexyl rings of andrographolide and the five-sided pyrrole ring of the amino acid Pro341. The 3VOB receptor is also strongly bound with andrographolide, forming two conventional hydrogen bonds with threonine residue at position 102 (Thr102: 2.14 Å) and aspartic acid residue at position 187 (Asp187: 2.85 Å). In these interactions, threonine and aspartic acid residues acted as hydrogen bond donor and acceptor, respectively. Gly23 and Phe183 residues also interacted well with the andrographolide molecule using carbon-hydrogen interaction (2.1 Å) and pi-alkyl interaction (4.92 Å), respectively.

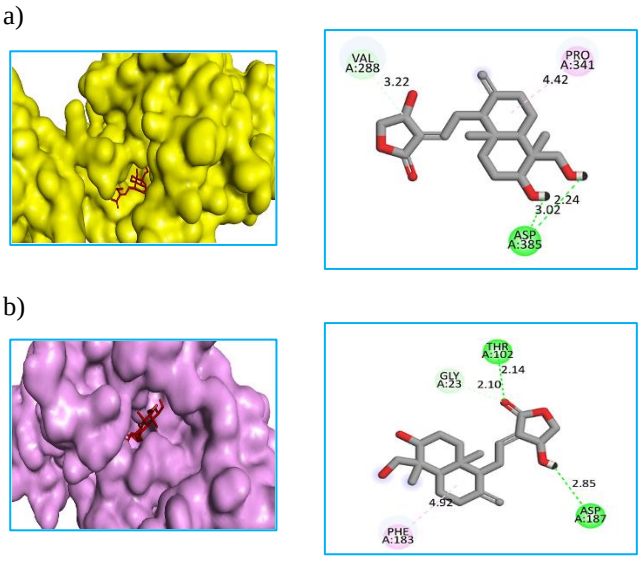


Fig 4 3D and 2D images of various interactions present in a) 1N67-andrographolide and b) 3VOB-andro complexes

Molecular docking studies on the receptors of E. coli

Bisabolene epoxide and bulnesene molecules exhibited low binding scores (-5 to -7 kcal/mol) with the five targeted structural proteins of *E. coli*, likely enzymes. The compound andrographolide also showed low binding scores with some structural proteins such as 4YCP (-7.3 kcal/mol), 1DIH (-7.1 kcal/mol), and 1EUM (-6.6 kcal/mol). However, this molecule displayed significantly higher binding scores (>8 kcal/mol)

with 4QR8 and 1Q8I receptors. The binding scores of these important molecules present in the AP extract with the structural receptors of *E. coli* are tabulated in Table 7. The protein with PDB ID 4QR8 plays a major role in E. coli's degradation and recycling processes. Inhibiting this protein's activity could be significant for controlling *E. coli* growth. Similarly, 1Q8I, a DNA polymerase, is crucial for DNA repair in *E. coli*. Andrographolide binding to these receptors could potentially lead to increased inhibition of *E. coli* growth.

Upon analyzing the complex 4QR8-andrographolide (Fig 5), we can understand that three conventional hydrogen bonds (1.89, 2.13, 2.89 Å) formed between the amino acid residues Ser215, Arg370, and Asp246 with the ligand molecule make the complex strong, even in the presence of a less favorable interaction with Glu384 involving the oxygen of the hydroxyl group present in the compound.

The 1Q81-andrographolide complex was stabilized by two strong hydrogen bond interactions with the aspartic acid residues 156 and 335 of the receptor (2.31, 2.25 Å). Both hydrogen bonds originated from the hydroxyl groups of the andrographolide molecule. In addition to this, a pi-sigma electron donation interaction was identified between the Phe228 residue of the receptor and the cyclohexane ring of the ligand molecule. These interactions between andrographolide and the 1Q81 receptor significantly inhibited the growth of *E. coli*. The 2D and 2D interaction plots for both 4QR8-andrographolide and 1Q81-andrographolide complexes are given in (Fig 5).

Table 7 Binding energies (-kcal/mol) of three molecules on the structural receptors of *E. coli*

PDB	Bisabolene epoxide	Bulnesene	Andrographolide
4YCP	7	5.7	7.3
4QR8	6.6	6.2	8.3
1DIH	6	6.1	7.1
1EUM	6.8	6.6	6.6
1Q81	6.1	6.3	8

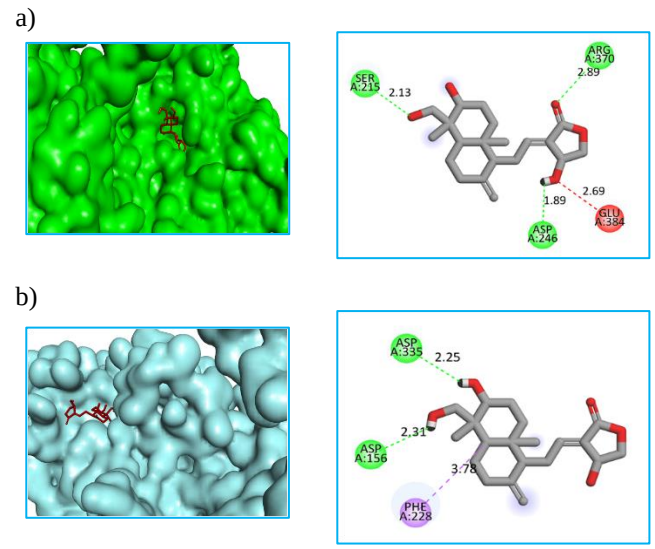


Fig 5 3D and 2D images of various interactions present in a) 4QR8-andrographolide and b) 1Q81-andrographolide complexes

Molecular docking studies on the receptors of P. aeruginosa

Similar to its effect on *E. coli* and *S. aureus*, andrographolide exhibited significant inhibition of *P. aeruginosa* growth. Bisabolene epoxide and bulnesene molecules showed no remarkable binding scores compared to andrographolide. Potentially, andrographolide significantly hinders exotoxin A - a protein in *P. aeruginosa* responsible for cellular harm. This binding is supported by a high binding score (-9.9 kcal/mol). The enhanced binding efficacy of this molecule can be attributed to the formation of four strong hydrogen bonds between the C=O and -OH groups of andrographolide with Arg458 (2.81 Å), Thr449 (2.75 Å), His440 (2.88 Å) and Gly441 (2.04 Å). The molecule orients itself within the receptor cavity, allowing adjacent amino acid residues 440, 441, and 442 of the protein chain to form strong hydrogen bonds with the hydroxyl and carbonyl groups of andrographolide. Additionally, a pi-alkyl hydrophobic interaction further strengthens the 1Q81-Andrographolide complex.

Andrographolide also bound strongly to the antibacterial defense protein (pdb Id: 6P8U) and penicillin binding protein (pdb Id: 7JWL) with binding scores of -8.9 and -8.6 kcal/mol, respectively. Due to its very good binding score, only the structural details of the 6P8U-andrographolide complex are illustrated here. The complex is stabilized by a strong hydrogen bond between Ser199 and one of the hydroxyl groups of andrographolide at 2.6 Å. Additional hydrogen interactions were also observed between the amino acid residues Asp64 and Ile247 and the molecule at distances of 2.97 and 2.83 Å, respectively. The presence of a hydrophobic pi-alkyl interaction between the amino acid residue Phe200 and the cyclohexane part of the andrographolide further stabilizes the complex. The binding energies of three molecular species on the selected structural receptors of *P. aeruginosa* are exhibited in (Table 8). The interaction plots of 1IKQ and 6P8U with andrographolide are depicted in (Fig 6).

Table 8 Binding energies (-kcal/mol) of three molecules on the structural receptors of *P. aeruginosa*

PDBID	Bisabolene epoxide	Bulnesene	Andrographolide
1IKQ	7.2	7.8	9.9
3BJZ	5.8	6	6.6
3SY7	6.6	6.3	7.7
5WZE	6.7	6.6	7
6P8U	7	7	8.9
7JWL	6.4	6.8	8.6

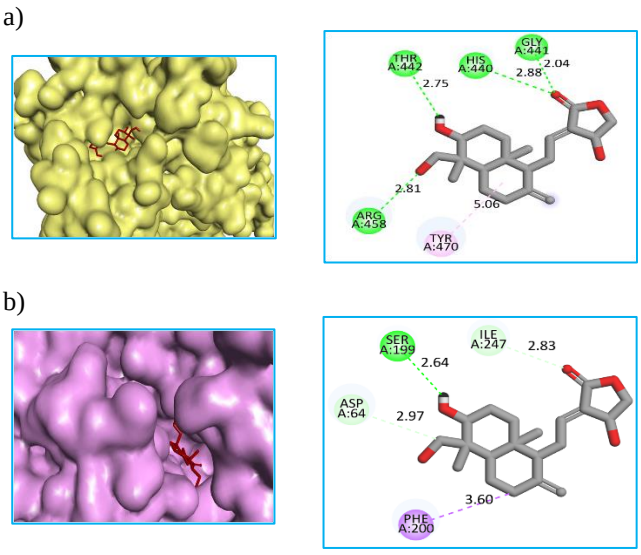


Fig 6 3D and 2D images of various interactions present in a) 1IKQ-andrographolide and b) 6P8U-andrographolide complexes

CONCLUSION

In this study, we prepared hexane and acetone extracts of *Andrographis paniculata* leaves. The solid residue after

extraction was collected and prepared as a 2% DMSO solution for screening against bacterial growth. While the hexane extract showed no activity, the acetone extract exhibited significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. To identify potential phytochemicals, GC-MS analysis of the acetone extract was performed. Out of the 18 identified compounds, only three (Bisabolene Epoxide, bulnesene, and andrographolide) exhibited drug-like properties as predicted by computational ADME studies. To validate and identify the active antibacterial agent(s), these molecules were subjected to molecular docking studies against relevant receptors of these pathogens. Based on the *in-silico* investigations, the andrographolide molecule present in the acetone extract appears to have the highest binding scores on the structural receptors of the microorganisms. In *E. coli*, the prodipeptidase receptor (4QRS) showed the strongest affinity towards andrographolide (-8.3 kcal/mol). *P. aeruginosa's* exotoxin A (PDB ID: 1IKQ) could be potentially inhibited by the andrographolide molecule (-9.9 kcal/mol). Similarly, the complex 1N67-andrographolide (*S. aureus*) showed a binding score of -8.5 kcal/mol, potentially preventing the biological action of the 1N67 protein, which plays a crucial role in destroying the immune system.

LITERATURE CITED

1. Prestinaci F, Pezzotti P, Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health* 109(7): 309-318. https://doi.org/10.1179/2047773215Y.0000000030
2. Levy S, Marshall B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med*. 10(Suppl 12): S122-S129. https://doi.org/10.1038/nm1145
3. Ventola CL. 2015. The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics* 40(4): 277-283.
4. Antimicrobial Resistance Collaborators. 202. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* 399(10325): 629-655. https://doi.org/10.1016/S0140-6736(21)02724-0
5. Llor C, Bjerrum L. 2014. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *Ther. Adv. Drug Saf.* 5(6): 229-241. https://doi.org/10.1177/2042098614554919
6. Ahmada W. 2020. Preliminary phytochemical, antimicrobial and photochemical study of Calotropis gigantea leaf extract. *Curr. Chem. Letters* 9: 105-112.
7. Dubeya S, Shuklaa A, Shukla RK. 2023. Green synthesis, characterization, and biological activities of Zn, Cu monometallic and bimetallic nanoparticles using Borassus flabellifer leaves extract. *Curr. Chem. Letters* 12: 799-811.
8. Ahmad MA, Ahmad N, Bello OS. 2015. Modified durian seed as adsorbent for the removal of methyl red dye from aqueous solutions. *Appl. Water Science* 5: 407-423.
9. Sharifi-Rad M, Mnayer D, Morais-Braga MFB. 2018. Echinacea plants as antioxidant and antibacterial agents: From traditional medicine to biotechnological applications. *Phytother Research* 32(9): 1653-1663. https://doi.org/10.1002/ptr.6101
10. Moghtaderi M, Mirzaie A, Zabet N. 2021. Enhanced antibacterial activity of *Echinacea angustifolia* extract against multidrug-resistant *Klebsiella pneumoniae* through Niosome Encapsulation. *Nanomaterials* (Basel) 11(6): 1573. https://doi.org/10.3390/nano11061573
11. Bhatwalkar SB, Mondal R, Krishna SBN. 2021. Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). *Front. Microbiology* 12: 613077. https://doi.org/10.3389/fmicb.2021.613077
12. Kshirsagar MM, Dodamani AS, Karibasappa GN. 2018. Antibacterial activity of garlic extracts on cariogenic bacteria: An in vitro study. *Ayu*. 39(3): 165-168. https://doi.org/10.4103/ayu.AYU_193_16
13. Yuan Y, Sun J, Song Y. 2023. Antibacterial activity of oregano essential oils against Streptococcus mutans in vitro and analysis of active components. *BMC Complement Med Ther*. 23: 61. https://doi.org/10.1186/s12906-023-03890-4
14. Lu M, Dai T, Murray CK, Wu MX. 2018. Bactericidal property of oregano oil against multidrug-resistant clinical isolates. *Front Microbiology* 9: 2329. https://doi.org/10.3389/fmicb.2018.02329
15. Dai Y, Chen SR, Chai L, Zhao J, Wang Y, Wang Y. 2019. Overview of pharmacological activities of Andrographis paniculata and its major compound andrographolide. *Critical Review of Food Science and Nutrition* 59(Sup1): S17-S29. https://doi.org/10.1080/10408398.2018.1501657
16. Bokelmann JM. 2022. Andrographis (Andrographis paniculata): Whole Plant. *In*: (Eds) J. M. Bokelmann, Medicinal Herbs in Primary Care. pp 189-194. Elsevier. https://doi.org/10.1016/B978-0-323-84676-9.00025-8
17. Hossain MS, Urbi Z, Sule A, Rahman KMH. 2014. Andrographis paniculata (Burm. f.) Wall. ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. *The Scientific World Journal* 2014: Article ID 274905. https://doi.org/10.1155/2014/274905
18. Bhaisare S, Pathak S, Ajankar VV. 2023. Physiological activities of the king of bitters (*Andrographis paniculata*): A review. *Cureus* 15(8): e43515. https://doi.org/10.7759/cureus.43515
19. Tandon C, Mathur P, Sen M. 2015. Andrographis paniculata Nees (Kalmegh): A review on its antibacterial activities and phytocompounds. *European Journal of Medicinal Plants* 8(1): 1-10. https://doi.org/10.9734/EJMP/2015/16280
20. Taylor TA, Unakal CG. 2024. *Staphylococcus aureus* infection. *In*: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441868/
21. Foster T. 1996. Staphylococcus. *In*: (Eds) Baron S. Medical Microbiology. 4th Edition. Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 12. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8448/
22. Kaper J, Nataro J, Mobley H. 2004. Pathogenic Escherichia coli. *Nat. Rev. Microbiology* 2: 123-140. https://doi.org/10.1038/nrmicro818
23. Thankia PH, Hingrajiya DV, Modha JJ, Vadgama JH. 2023. Synthesis, characterization and antibiotic evaluation of various biologically active derivatives of 4-Alkylpyrimidine-5-Carbonitrile. *Curr. Chem. Letters* 12: 537-544.
24. Lister PD, Wolter DJ, Hanson ND. 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin. Microbiol. Rev.* 22(4): 582-610. https://doi.org/10.1128/CMR.00040-09

25. Wilson MG, Pandey S. 2023. *Pseudomonas aeruginosa*. [Updated 2023 Aug 8]. *In*: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557831/
26. Daina A, Michielin O, Zoete V. 2017. Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Science Reporter* 7: 42717. https://doi.org/10.1038/srep42717
27. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. 2000. The protein data bank. *Nucleic Acids Research* 28(1): 235-242. https://doi.org/10.1093/nar/28.1.235
28. Dassault Systemes BIOVIA. 2024. Discovery Studio Modeling Environment, Release 2024.
29. Liu, Yang X, Gan J, Chen S, Xiao ZX, Cao Y. 2022. CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Research* 50(W1): W159-W164. https://doi.org/10.1093/nar/gkac394
30. Meghaa GV, Bodkea DY, Shanavaz H. 2023. Synthesis of novel 2,5-disubstituted tetrazole derivatives as potent biological agents. *Current Chem. Letters* 12: 397-412.
31. Ahmad MA, Ahmad N, Bello OS. 2015. Modified durian seed as adsorbent for the removal of methyl red dye from aqueous solutions. *Applied Water Science* 5: 407-423.