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ANTIBACTERIAL POTENTIAL OF *CASSIA FISTULA* LEAF EXTRACTS BY EXPERIMENTAL AND IN SILICO APPROACH

[1]Sahithya, [2]Aravind Ganessin
[1]Former PG Student
[1]Department of Microbiology,
[1]JSS Academy of Higher Education and Research, Mysuru, India
[2]Managing Director,
[2]Dextrose Technologies Pvt. Ltd., Bengaluru, Karnataka, India

Abstract: The emergence of antibiotic resistance especially by multi-drug resistant pathogens has created a crucial need for novel antibacterial agents from natural sources. This study explores the antibacterial efficacy of methanol leaf extract of *Cassia fistula* against clinical pathogens and evaluates its mechanism of action using bioinformatics tools. The leaves were subjected to methanol extraction and the extracts were tested by agar well diffusion method, phytochemical analysis and in silico analysis. The extract showed antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* with an inhibition zone ranging from 10 to 17 mm. Phytochemical screening identified the presence of tannins, glycosides, phenols, alkaloids, flavonoids and steroids which was further supported by thin layer chromatography. GC-MS analysis identified 90 compounds, of which 21 exhibited potential antibacterial activity. Six phytocompounds were selected for molecular docking based on ADME profiling. Myo-inositol 4-C-methyl exhibited the highest binding affinity especially with elongation factor P and the UDP-N-acetylglucosamine 1-carboxyvinyltransferase of *E. coli* and *E. faecalis*. These findings show that *C. fistula* could be a potential source of antibacterial agents. Additional *in vivo* and clinical testing is required to validate these results and explore synergistic effect with standard antibiotics.

Index Terms - *Cassia fistula*, antibacterial activity, phytocompounds, GC-MS, molecular docking, ADME profiling.

1. INTRODUCTION

Antibiotics have transformed the field of healthcare, having a critical impact on the effectiveness of bacterial infections and a substantial improvement in human life expectancy. The rising concern of antimicrobial resistance (AMR) is a serious global health challenge. It is estimated that by 2050, AMR will lead to 10 million deaths per year and incur costs to 100 trillion dollars [1, 2]. The emergence of the multidrug-resistant (MDR) strains at an alarming rate renders the issue, increasing the risk of returning to the pre-antibiotic period [3]. In this context, it has been established that over the last 5,000 years, plants have contributed as an excellent source of medicinal agents to human health [4]. Traditional medicine, mainly plant-based medicine still remains the major health care practice in 70% to 90% of the world in most developing regions [5]. Medicinal plants are also abundant in secondary metabolites including flavonoids, tannins, alkaloids, and glycosides, which have considerable antimicrobial activity [6]. This increasing worldwide demand has led to investigation of such natural products as alternative therapeutics [7]. *Cassia fistula*, more popularly recognized as golden shower tree, is one of them due to its extensive scope in pharmacological uses especially antibacterial properties. *C. fistula* has been utilized traditionally in folk medicine all over the world to treat many diseases, including skin pathologies, diabetes and intestinal diseases [8, 9]. Its traditional applications have been confirmed by scientific studies that have demonstrated potent antimicrobial activity, especially against the Gram-positive bacteria and Gram-negative bacteria including MDR strains such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococci* [10]. Aqueous extracts, methanol and ethanol have all proven to have good antibacterial activity and in some cases similar to the antibacterial activity of standard antibiotics [11]. Phytochemical analysis has discovered some of the major bioactive products of *C. fistula* like flavonoids (quercetin and kaempferol), anthraquinones (rhein and emodin), phenolic acids and tannins [12]. These compounds disrupt the bacterial membranes, inhibit nucleic acid and protein synthesis, and denature vital proteins, therefore validating their antimicrobial activity [13, 14]. Thin layer chromatography is among the techniques that have contributed towards the isolation and identification of phytochemicals [15]. Gas chromatography-mass spectrometry has further explained the composition of *C. fistula* extracts [16], confirming the presence of flavonoids and anthraquinones with known antimicrobial effects [17]. The composition of *C. fistula* extracts has also been studied using gas chromatography-mass spectrometry, which revealed several bioactive constituents with antimicrobial potential [16, 17]. Emodin and rhein especially have been demonstrated to inhibit the synthesis of the bacterial cell wall and protein synthesis [18], and flavonoids like quercetin, kaempferol disrupt membranes and prevent nucleic acid synthesis [19]. The study of molecular docking has revealed the mechanism of action through the modeling of interaction of these compounds with the bacterial targets. Both rhein and emodin have high affinity with DNA gyrase and prevent replication of bacterial DNA [20]. Quercetin and kaempferol inhibit β-lactamase, which leads to antibiotic resistance, and thus increases the effect of β-lactam antibiotics. There are also certain compounds which disturb the efflux pumps, thereby causing antibiotic concentration and potency to rise within the cells [21]. Studies highlight the antibacterial and antifungal efficacy of *C. fistula* extracts, supporting its traditional use as a natural antimicrobial agent [22]. Even though extensive research has been conducted on the antibacterial potential of *C. fistula*, there remains a significant gap in the combination of experimental antimicrobial testing with advanced computational methods to explain the exact modes of action at the molecular level. A majority of the significant literature published in the past has addressed antibacterial activity in vitro or phytochemical profiling separately without identifying the bioactive constituents and the corresponding bacterial protein targets with bioinformatics tools. The innovative aspect of the study is the multidisciplinary study design, which allows not only determining active components in the extract of the leaf but also the prediction of its pharmacokinetic distribution and the molecular interaction with vital bacterial proteins. Importantly, it also associates abundance of compounds (as observed in GC-MS) with the antibacterial potency and identifies compounds with high binding affinity to unexplored bacterial targets including myo-inositol 4-C-methyl with their high binding affinity to elongation factor P and UDP-N-acetylglucosamine 1-carboxyvinyltransferase. Such integrative strategy contributes to the improved insight into the possible modes of action and allows targeted development of plant-derived antibacterial agents.

2. MATERIALS AND METHODS

2.1 Leaf Sample Collection and Extract Preparation

Fresh and healthy *C. fistula* leaves were collected from Lalbagh Botanical Garden, Bengaluru, India. The tree was identified and labelled by the Department of Horticulture of the Government of Karnataka. The leaves (20 g) were sun-dried for 5 to 10 days which yielded 12.2 g of dried material. The dried leaves were powdered and 9 g of this powder was subjected to methanol extraction using a Soxhlet apparatus (Borosil Technologies Ltd. Hinjewadi, Pune) at 30–40 °C for 48 hours. The resulting dark greenish residue was stored in sterile Eppendorf tubes and diluted with dimethyl sulfoxide (DMSO) for phytochemical and antibacterial analyses.

2.1 Test Microorganisms and Growth Media

The bacterial strains *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (MTCC 737), and *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MG1644 MTCC 1586) were chosen based on their clinical importance and were obtained from Dextrose Technology Pvt. Ltd. (Bengaluru, India) for antibacterial activity testing. The strains were grown and maintained in Luria–Bertani (LB) and brain heart infusion (BHI) media (HiMedia Pvt. Ltd. Mumbai, Maharashtra). The strains were freshly sub cultured prior to the experiments.

2.2 Qualitative Phytochemical Testing of the Leaf Extract

The methanol leaf extract of *C. fistula* was subjected to standard qualitative phytochemical tests to detect various secondary metabolites [23, 24]. Alkaloids were tested using Hager's reagent (SD Fine-Chem Mumbai, Maharashtra), while phenols and tannins were identified using ferric chloride (SD Fine-Chem Mumbai, Maharashtra). The presence of flavonoids was confirmed using the alkaline reagent (SD Fine-Chem Mumbai, Maharashtra) test, whereas the presence of saponins was confirmed via the foam test. The presence of glycosides was detected using Borntrager's reagent (Himed Kolhapur, Maharashtra), and steroids were tested by Salkowski's reagent (SRL Pvt. Ltd., Mumbai, Maharashtra).

2.3 Thin Layer Chromatography

TLC was used to identify the various phytocomponents present in the methanol leaf extract of *C. fistula*. The extracts were spotted onto silica gel TLC sheets (Merck Millipore, Bengaluru, Karnataka) using capillary tubes and developed in different solvent systems specific to the phytochemical class being analysed. The TLC sheets were placed in jars containing 10 mL of the respective solvent systems, and the mobile phase was then allowed to move through the adsorbent phase up to three-fourths of the sheet. The following solvent systems (for 10 ml) were used for the analysis:

- Alkaloids- 5.5 ml chloroform: 2.7 ml glacial acetic acid: 1 ml methanol: 680 µl distilled water
- Flavonoids- 7.2 ml ethyl acetate: 400 µl water: 1.3 ml methanol: 900 µl n-hexane
- Glycosides- 6.6 ml chloroform: 2.3 ml methanol: 0.9 ml ammonia solution
- Tannins- 5.9 ml chloroform: 3.18 ml methanol: 0.9 ml water
- Phenols- 6 ml chloroform: 4 ml ethyl acetate

The TLC sheets were exposed to UV light at 336 nm, which induced fluorescence and made the bands visible. The Rf values were calculated using the following formula: Rf = (distance travelled by the compound/distance travelled by the solvent front).

2.4 Antibacterial Activity

The agar well diffusion method was employed to evaluate the antibacterial activity of methanol leaf extract of *C. fistula* against clinically significant bacteria, including *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *E. coli*. The bacteria were inoculated using the spread plate technique on LB or BHI agar plates, and wells were created in the solidified agar. Varying concentrations (25–150 µL) of the extract dissolved in DMSO were added to the wells, and the plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured to evaluate the antibacterial efficacy of the extract. Ciprofloxacin (Cipla Ltd., Bengaluru, Karnataka) and ampicillin (Cipla Ltd., Bengaluru, Karnataka) were used as standard controls.

2.5 GC-MS Analysis

GC-MS analysis of methanol leaf extract of *C. fistula* was conducted using a Perkin Elmer Clarus 680 GC system paired with a Clarus SQ 8C mass spectrometer and Turbo Mass software (v6.1.1). Components were identified by comparing the obtained spectra with the NIST-2014 library. Chromatographic separation was carried out on HP-5MS column (30 m × 0.25 mm × 0.25 µm) using helium as the carrier gas at a constant flow rate of 2 mL/min. A 1 µL sample was injected in splitless mode with the injector at 280°C. The oven temperature program began at 100°C (2 min hold), ramped to 200°C at 10°C/min (3 min hold), then to 300°C at 25°C/min (10 min hold), with a total run time of 29 minutes. The mass spectrometer operated with electron impact ionization at 70 eV, with interface and source temperatures of 250°C and 230°C, respectively. Scanning was performed over a mass range of 40–600 Da with a scan duration of 0.2 s and interval of 0.1 s.

2.6 ADME Profiling and Ligand Selection

Based on the GC-MS analysis and a literature review, compounds with known antibacterial activity were identified and subjected to ADME profiling using SwissADME [Sci. Rep. (2017) 7:42717]. Key parameters such as solubility, lipophilicity (log Po/w), skin permeation (log Kp), and bioavailability score were evaluated to assess their drug-like properties. Promising candidates were selected as ligands for molecular docking studies to assess their binding affinity and potential efficacy against the target bacterial proteins.

2.7 Docking

The target protein structure was selected from the Protein Data Bank (PDB) and downloaded in the PDB format. Using Notepad++, water molecules labelled as "HETATM" were removed to prepare the protein for docking. The corresponding ligand molecule was identified, and its 3D structure in SDF format was downloaded from PubChem. Molecular docking was then performed using the CB-Dock2 tool to predict the binding affinity and interaction sites between the protein and the selected ligand.

3. RESULTS

3.1 Qualitative Phytochemical Testing

Table 1 presents the results of the qualitative phytochemical analysis of the leaf extract. The phytochemicals found in *C. fistula* leaf extracts included tannins, glycosides, phenols, alkaloids, flavonoids, and steroids.

Table 1: Qualitative phytochemical characteristics of *C. fistula* leaf extract

Phytochemicals	Test performed	Interference	Result
Alkaloids	Hager's reagent	Yellow precipitate	Present
Phenols	Ferric chloride	Bluish green colour	Present
Flavonoids	Alkaline reagent	Intense yellow colour, becomes colourless	Present
Tannins	Ferric chloride	Greenish-black precipitate	Present
Saponins	Foam	Formation of persistent foam	Absent
Glycosides	Borntrager's	Reddish brown colour	Present
Steroid	Salkowski's	Red colour produced in the lower chloroform layer	Present

3.2 Thin Layer Chromatography

The fluorescent bands indicated the presence of alkaloids, flavonoids, glycosides, tannins, and phenols. The Rf values of the phytochemicals found in the leaf extract of *C. fistula* are presented in Table 2.

Table 2: Rf values of the phytochemicals found in the leaf extract of *C. fistula*

Phytochemicals	Rf value
Alkaloids	0.98
Flavonoids	0.91
Glycosides	0.87
Tannins	0.97
Phenols	0.96

3.3 Antibacterial Activity

The antibacterial activities of both standard antibiotics and the methanol leaf extract of *C. fistula* were evaluated by measuring the zones of inhibition in millimetres, as presented in Tables 3. The bacterial culture plates showing inhibition zones in response to the antibiotics and leaf extract are shown in Figure 1 and 2, respectively. The extract demonstrated a concentration-dependent antibacterial effect, with inhibition zones ranging from 10 mm to 17 mm. Notably, *E. coli* showed the highest susceptibility, followed by *P. aeruginosa*, *S. aureus*, and *E. faecalis*. These findings indicate a positive correlation between the extract concentration and the antibacterial efficacy.

Table 3: Antibacterial activity of antibiotics and *C. fistula* leaf extract on the test microorganisms

Test microorganisms	Zone of inhibition (in mm)									
	Ampicillin (500 mg)		Ciprofloxacin (500 mg)		Methanol leaf extract of *C. fistula*					
	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL	75 µL	100 µL	125 µL	150 µL
E. faecalis	29	30	>35	>35	-	-	-	12	15	16
P. aeruginosa	30	35	>35	>35	-	-	13	14	15	17
S. aureus	20	25	30	35	-	-	-	10	12	13
E. coli	23	30	>35	>35	-	10	12	13	16	17

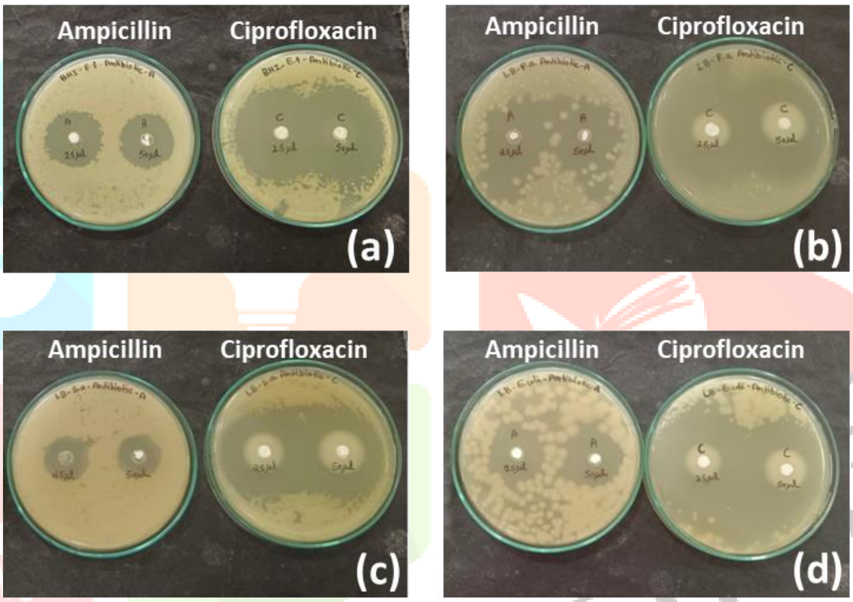

Figure 1: Antibacterial activity of antibiotics Ampicillin and Ciprofloxacin against a) *E. faecalis* b) *P. aeruginosa* c) *S. aureus* d) *E. coli*

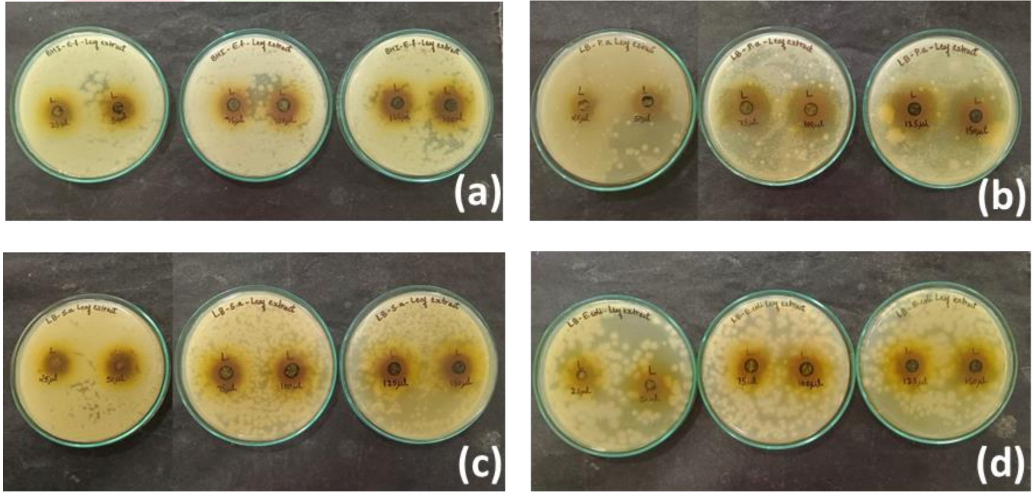

Figure 2: Antibacterial activity of *C. fistula* leaf extract against a) *E. faecalis* b) *P. aeruginosa* c) *S. aureus* d) *E. coli*

3.4 GC-MS Analysis

In the analysis with GC-MS, a total of 90 compounds were identified. A subsequent literature review revealed that 21 of these compounds exhibited antibacterial activity. The GC-MS total ion chromatogram (TIC) for the leaf sample "SAMPLE CF-DTPL O41," shown in Figure 3, displays retention times from 0 to 24 minutes on the x-axis and signal intensity on a normalized percentage scale on the y-axis. Notable peaks were observed at retention times such as 11.49, 14.24, 14.86–14.99, 15.90, 16.68, 16.80, 16.88, and

18.37 minutes, indicating the presence of various compounds. The most prominent peak at 16.68 minutes suggests that the corresponding compound is the most abundant compound, while the cluster of peaks between 14.86 and 14.99 minutes points to the presence of structurally similar compounds or isomers.

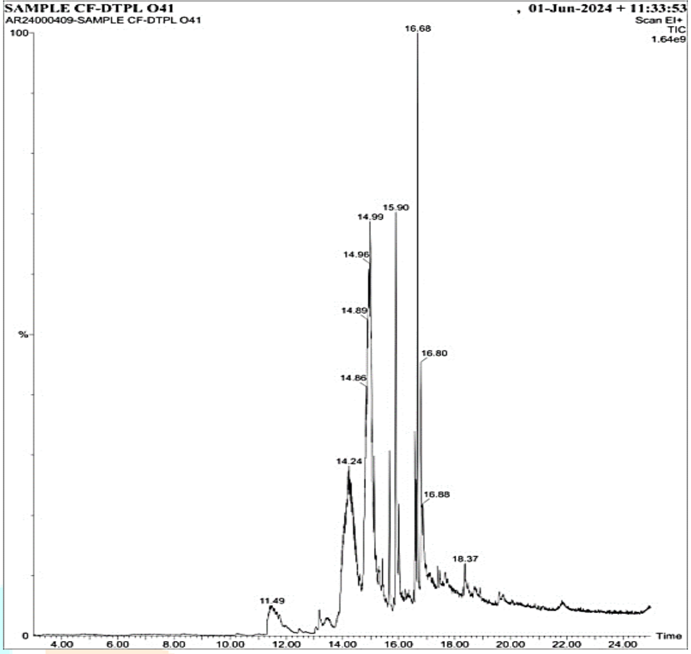

Figure 3: GC-MS TIC graph depicting peak level

3.5 ADME Profiling

The selected 21 antibacterial phytocompounds were evaluated using the SwissADME program for key ADME parameters, including water solubility, lipophilicity, skin permeation, and the bioavailability score. Based on this analysis, six compounds with favourable drug-like properties were selected as ligands for further study (Table 4).

Table 4: ADME profile of phytocompounds selected for docking studies

Phytocompound	Molecular formula	Molecular weight (g/mol)	Water solubility	Lipophilicity (log Po/w)	Skin permeation (log Kp)	Bioavailability Score
Myo-inositol,4-C-methyl	$C_7H_{14}O_6$	194.18	Highly soluble	-2.48	-9.98 cm/s	0.55
Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.42	Moderately soluble	5.07	-3.07 cm/s	0.55
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40	Moderately soluble	4.84	-3.07 cm/s	0.85
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Moderately soluble	4.45	-3.35 cm/s	0.85
7-hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	268.43	Moderately soluble	5.22	-3.41 cm/s	0.55
Thymol, TBDMS derivative	$C_{16}H_{34}OSi$	264.48	Moderately soluble	4.68	-3.53 cm/s	0.55

3.6 Molecular Docking

The molecular docking study evaluated the binding affinities of six ligands myo-inositol 4-C-methyl, tetradecanoic acid 12-methyl-methyl ester, pentadecanoic acid, tetradecanoic acid, 7-hexadecenoic acid methyl ester (Z)-, and the thymol TBDMS derivative against key bacterial proteins, including DNA gyrase subunit B, elongation factor P, and UDP-N-acetylglucosamine 1-carboxyvinyltransferase from *E. coli*, *S. aureus*, *E. faecalis*, *and P. aeruginosa*. The interactions were assessed using docking scores (Table 5) to explore their potential antibacterial activity.

Myo-inositol 4-C-methyl showed strong binding affinities with various proteins, with scores ranging from -4.4 to -6.4, its best docking scores were -6.4 with elongation factor P in *E. coli* and -6.2 with UDP-N-acetylglucosamine 1-carboxyvinyltransferase in *E. coli*, -6.0 with elongation factor P, and UDP-N-acetylglucosamine 1-carboxyvinyltransferase in *E. faecalis*, followed by -5.5 with UDP-N-acetylglucosamine 1-carboxyvinyltransferase in *S. aureus*, -5.4 with elongation factor P in *P. aeruginosa*, and -5.2 with DNA gyrase subunit B in *S. aureus* (Figure 4).

The thymol TBDMS derivative generally exhibited negative docking scores, ranging from -2.2 to -4.6, implying moderate binding affinity. Tetradecanoic acid 12-methyl-methyl ester showed both negative and positive scores, ranging from -0.21 to 1.91, suggesting weak and non-existent binding strength. Pentadecanoic acid, tetradecanoic acid, and 7-hexadecenoic acid methyl ester presents yielded positive docking scores ranging from 1.53 to 2.81, 0.23 to 2.73, and 0.03 to 2.30, respectively, suggesting non-existing binding strength.

Table 5: Molecular docking score for the protein–ligand complexes

Proteins (PDB ID)		Docking score					
		Ligands (PubChem CID)					
		Myo-Inositol, 4-C-methyl- (244581)	Tetradecanoic acid, 12-methyl-, methyl ester (21206)	Pentadecanoic acid (13849)	Tetradecanoic acid (11005)	7-Hexadecenoicacid, methyl ester, (Z)- (14029831)	Thymol, TBDMS Derivative (610042)
DNA gyrase subunit B	*E. coli* (1AJ6)	-4.7	1.9	2.8	2.7	2.3	-2.4
	S. aureus (2XCO)	-5.2	1.1	1.5	1.1	0.9	-2.9
	E. faecalis (4GEE)	-4.4	NIL	3.0	NIL	NIL	-2.7
	P. aeruginosa (3OYY)	-4.7	1.1	NIL	NIL	NIL	-3.1
Elongation factor P	*E. coli* (1AJ6)	-6.4	1.5	2.2	1.9	1.2	-2.2
	S. aureus (2XCO)	-4.8	NIL	NIL	NIL	NIL	-2.7
	E. faecalis (4GEE)	-6.0	0.9	2.5	2.0	0.2	-2.2
	P. aeruginosa (3OYY)	-5.4	1.8	2.5	2.5	1.8	-2.3
UDP-N-acetylglucosamine 1-carboxyvinyl transferase	*E. coli* (1AJ6)	-6.2	1.5	NIL	2.0	1.6	-3.3
	S. aureus (2XCO)	-5.5	-0.2	1.6	0.2	0.3	-3.9
	E. faecalis	-6.0	NIL	2.1	NIL	NIL	-4.6

	(4GEE)						
	P. aeruginosa (3OYY)	-4.4	1.4	1.7	2.5	1.1	-2.7

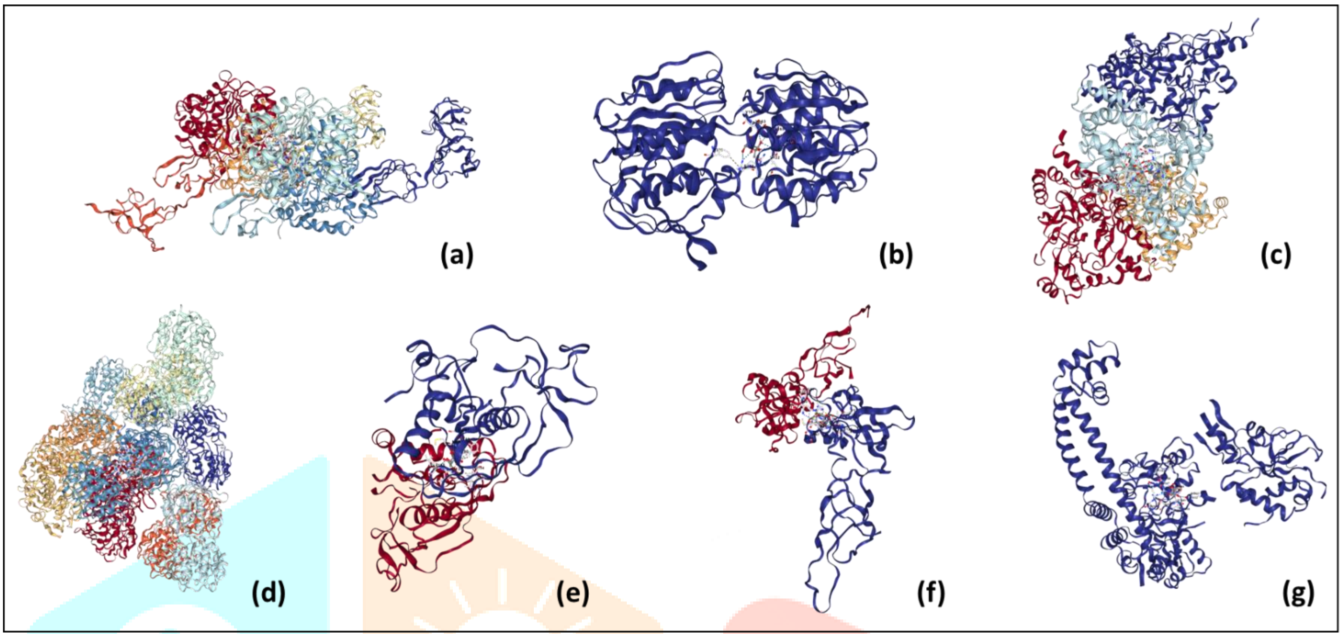

Figure 4: Molecular docking of myo-inositol 4-C-methyl with a) elongation factor P in *E. coli* b) UDP-N-acetylglucosamine 1-carboxyvinyltransferase in *E. coli* c) elongation factor P in *E. faecalis* d) UDP-N-acetylglucosamine 1-carboxyvinyltransferase in *E. faecalis* e) UDP-N-acetylglucosamine 1-carboxyvinyltransferase in *S. aureus* f) elongation factor P in *P. aeruginosa* g) DNA gyrase subunit B in *S. aureus*

4. DISCUSSION

Plants are a rich source of therapeutic agents, with secondary metabolites like glycosides, alkaloids, phenols, flavonoids, tannins, and steroids known for their antimicrobial action through mechanisms such as membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis [6, 12, 19, 20]. In this study, the qualitative phytochemical screening reported the presence of these compounds, corroborating earlier findings by Panda et al. [9] and Parekh and Chanda [10]. Presence of these metabolites was also confirmed by TLC analysis that gave different Rf values, which denoted the polarity and possible variety of the compounds.

This study reflects on the current challenge of antimicrobial resistance that poses the problem of resistance to traditional antibiotics. Since resistance among pathogenic organisms like *E. faecalis*, *S. aureus*, *E. coli* and *P. aeruginosa* has been growing increasing, identification of phytochemicals targeting specific bacteria proteins is a crucial step in discovering new antimicrobial agents [1, 2, 3]. The concentration-dependent antibacterial activity observed in methanol leaf extracts of *C. fistula*, with inhibition zone of 10–17 mm, supports its efficacy against both Gram-positive and Gram-negative bacteria. These findings corroborate earlier works by Bhalodia and Shukla [11] and Patel and Vyas [13]. The current study however extends upon the published literature by integrating experimental assays with a bioinformatics based approach.

Based on GC-MS analysis, 90 phytocompounds were identified, of which 21 have reported antimicrobial activity. These bioactive compounds exhibit chemical diversity with potential to target various microbial pathways. While the findings are consistent with those of Kulkarni et al. [16], however variations in compound profiles may be attributed to differences in extraction methods and plant parts used. The most abundant compound, observed at a retention time of 16.68 minutes, suggests notable pharmacological significance. The novelty of this work is the incorporation of ADME profiling and molecular docking to prioritize compounds based on the desirable drug like character and target specificity. To enhance the pharmacological relevance of these findings, the 21 antibacterial compounds were subjected to ADME profiling. This computational screening led to the selection of six compounds with favourable pharmacokinetic properties, thereby narrowing down the candidates suitable for further drug development. Among these, myo-Inositol 4-C-methyl demonstrated the highest binding affinity in molecular docking with key bacterial proteins, particularly elongation factor P and UDP-N-acetylglucosamine 1-carboxyvinyltransferase. Such proteins play a fundamental part in protein synthesis and cell wall biosynthesis, two of the key pathways in the survival and growth of bacteria [20, 21]. The significance of this study lies in the fact that elongation factor P is a relatively unexplored antimicrobial target in plant based research and identification of myo-Inositol 4-C-methyl as a potential lead compound. Unlike widely investigated phytoconstituents such as rhein, emodin, quercetin, and kaempferol [18, 19], myo-Inositol 4-C-methyl's bioactivity is less reported, thus providing a new domain for targeted drug discovery.

Although previous studies by Patel and Vyas [13], Bhalodia and Shukla [11], and Panda et al. [9] examined the antibacterial properties of *C. fistula* using conventional in vitro assays, the present study introduces a novel integrative approach involving an experimental assay with phytochemical screening, TLC and GC-MS coupled with computational approaches such as ADME profiling and molecular docking, to provide deeper insight into the pharmacokinetics and protein-binding potential of its bioactive compounds. In comparison to Kulkarni et al. [16], where a different chemical profile was identified using GC-MS, the present study highlights the critical impact of extraction methods on chemical diversity and biological activity. This emphasizes the importance of optimizing extraction protocols to align with desired therapeutic objectives. Such a multifaceted approach not only aids in identifying antibacterial compounds but also provides mechanistic insights into their effects, thereby advancing beyond the descriptive character of previous studies.

This study aligns with global efforts to develop alternative antimicrobials to combat multi-drug resistant bacteria [1, 2, and 3]. It highlights the potential of *C. fistula* as a source of selective, potent agents that target specific bacterial proteins, supported by computational validation. However, further research is essential, including in vivo studies to assess efficacy and safety, clinical trials to evaluate therapeutic potential and synergy with existing antibiotics, and further mechanistic studies are also required to fully comprehend the antibacterial activities and respective mechanisms of action of these drugs so as to facilitate successful optimization of drug development. Additionally, investigating the biodistribution and metabolism of key compounds in animal models is necessary to establish their safety profile. Given our findings and previous research, optimizing extraction methods remains crucial, as different techniques can yield varying compound profiles and antibacterial activities.

5. CONCLUSION

The present study underscores the potential application of *C. fistula* leaf extracts as novel antibacterial compounds. By combining experimental assays and bioinformatics analyses, the study has identified multiple compounds extracted from *C. fistula*, particularly myo-inositol 4-C-methyl, that exhibit strong antibacterial activity against pathogenic bacteria. This finding validates the traditional use of *C. fistula* in ethno medicine and may also facilitate the development of new antibacterial agents to address the growing challenge of antibiotic resistance. This study's findings highlight the importance of integrating traditional knowledge with modern scientific techniques to uncover new therapeutic avenues. Future research directions include thorough *in vivo* studies to corroborate the efficacy and safety of these extracts as well as clinical trials to establish their potential as alternatives or adjuncts to existing antibiotic regimens. Moreover, evaluating the synergistic effects of these compounds with conventional antibiotics could help identify novel treatment strategies against multidrug-resistant bacterial infections. Thus, this study not only contributes valuable information to the field of natural product research but also sets the stage for further investigations that could lead to significant advancements in the fight against bacterial resistance.

6. ACKNOWLEDGMENT

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