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Antimicrobial Activity of Novel Triterpenoid Derivatives Isolated from Ethyl Acetate Extract of Cassia *fistula* Stem Bark: *In-vitro* and *In-silico* Analysis

Ireen Christopher[1,2], Aishwariya Sowntharrajan[1,2], Viji Murugesan[1,2], Indu Sabapathy[1,2], Vijayalakshmi Periyasamy[1,2], P Rajagopal[3], Rajalakshmi Manikkam[1,2,4*]

[1]*Department of Biotechnology and Bioinformatics, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India*

[2]*DBT-BIF Centre, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India*

[3]*Central Research Laboratory, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research (Deemed to be University), Chennai, 600095, India*

[4]*Department of Zoology, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India*

Abstract

Standard treatments for bacterial infections are becoming increasingly ineffective as antibiotic resistance grows worldwide. Due to the overuse of antibiotics, multidrug-resistant bacteria have emerged as a serious hazard and a major worldwide healthcare issue in the twenty-first century. Traditional approaches to creating novel antibacterial medications are insufficient to fulfil the existing pipeline, hence new tactics in the field of antibacterial discovery are being developed. Cassia fistula (C.fistula), a member of the Leguminosae family, naturally contains antibacterial properties. The plant is used to cure skin diseases, liver problems, tuberculose glands, and hematemesis, pruritus, leucoderma, and diabetes. As a result, effective antimicrobial treatment beyond antibiotics is critical. The Plants contain a wide range of secondary metabolites, including tannins, terpenoids, alkaloids, flavonoids, and glycosides, which have antibacterial characteristics. Terpenenes and terpenoids are effective against bacteria, fungus, viruses, and protozoa. Terpenes' mode of action involves lipophilic chemicals disrupting membranes. Adding a methyl group to increase the hydrophilicity of kaurene diterpenoids decreased their antibacterial efficacy significantly. In the study, antibacterial screening assay against S.aureus and K.pneumonia, a new chemical isolated from C.fistula's ethyl acetate extract demonstrated wider inhibitory zones than the positive control. The treated culture's genomic DNA profile remains unchanged after treatment with the new chemical. The new chemical suppressed protein synthesis, resulting in reduced protein content in treated cultures of both strains, confirming its bactericidal effect. Further immune-blot analysis is required to confirm the particular protein. Investigating a novel triterpenoid that reduces pharmaceutical drug load and resistance risk, as well as treatment costs, could offer promising therapeutic options for treating secondary urinary tract infections associated with diabetes.

Keywords: *Antibacterial Activity, C.fistula, K.pneumonia, S.aureus, Triterpenoids.*

Introduction

The health benefits of medicinal plants are immense, and they have bactericidal properties that combat bacterial infections [1]. The pharmaceutical industry produces a variety of novel antibiotics and microorganism-resistant

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****Corresponding Author: rajalakshmi@hcctrichy.ac.in.***

products. Genetically, bacteria can spread and develop resistance to synthetic medications used as therapeutic agents in the wild [2]. One of the most vital tools in the fight against bacterial illnesses is the antibiotic. Drug-resistant bacteria have emerged, which has made ordinary antibiotics less effective against some illnesses and more likely to cause harmful reactions. Native to India, the Amazon, and Sri Lanka, *Cassia fistula* (*C. fistula*) is a semi-wild Indian Labernum plant also referred to as the golden shower. The plants are extremely rich in a wide range of secondary metabolites that have been discovered in vitro to possess antibacterial agent qualities, including tannins, terpenoids, alkaloids, flavonoids, glycosides, etc. [3]. Three new compounds were identified from the ethyl acetate extract of *C. fistula* stem bark that had previously been exposed to a range of extraction techniques in our laboratory [4]. These triterpenoids were shown to have hypoglycemic action in diabetic Wistar rats caused by streptozotocin [5]. The promise of β-sitosterol as a therapeutic approach for treating oral cancer is highlighted by its cytotoxic effects on oral cancer cells and its regulation of apoptotic signals [6]. The medications could control secondary infections in addition to diabetes problems. We must look at the antibacterial activity of the new compounds on the microorganisms causing diabetes-related secondary infections because of the synergistic action of these drugs. According to studies by [7-10], terpenenes or terpenoids are effective against bacteria, fungi, viruses, and protozoa. H. pylori may be involved in aggravating dental caries, as evidenced by the strong correlation seen between its presence in deep carious lesions and increased caries severity [11]. Terpenes work by breaking down membranes by the action of lipophilic molecules [12]. Utilising plant extracts as antimicrobial agents is made possible in large part by the combination of phytochemicals' synergistic effects [13]. Alkaloids, tannins, saponins, anthraquinones, anthocyanides, flavonoids, glycosides, and terpenoids were found by phytochemical screening, suggesting that these phytoconstituents may be in charge of their anti-acne action [14]. Calotropin's promise as a treatment for oral cancer is demonstrated by the way it slows migration, causes apoptosis, and reduces cell proliferation in HSC-3 oral cancer cells [15]. Studies have been done on the in vitro antibacterial activity of ethanol extracts of *C. fistula*'s leaves and roots. *Sarcina lutea, Bacillus megaterium, Bacillus subtiIis, Streptococcus f3-haemolyticus, S. aureus, Salmonella typh*i, and *Shigella dysenteriae* are the five Gram-positive and nine Gram-negative bacteria. Testing was done on *Escherichia coli*, *Pseudomonas aeruginosa, K. pneumoniae, Shigella boydii, Shigella sonnei, Shigella flexneri*, and *Shigella shiga*. At a concentration of 30µg/disc, neither extract showed any action against the tested pathogens; however, at a concentration of 200µg/disc, there was moderate to good activity. The leaf extracts showed zones of inhibition ranging from 10 to 19 mm, with the biggest zone being against *Shigella dysenteriae* (19 mm). These extracts demonstrated somewhat better activity. Roots, on the other hand, revealed narrower inhibition zones (9–14 mm), suggesting a reduced sensitivity. According to [16], there is evidence that *C. fistula* possesses antibacterial properties that may be beneficial for treating microbial illnesses. The Mahakoshal region of central India's tribal people support the traditional usage of these plants to treat urinary tract infections [17]. The scientific and pharmaceutical communities have focused more attention on studies on the potential antimicrobial activity of substances derived from plants, an untapped source of antimicrobial chemotypes used in various traditional medical practises worldwide, as a result of the rising prevalence of drug-resistant pathogens.

Materials and Methods

Sample Collection

The fresh bark of *C. fistula* was collected from Kodaikanal, Tamil Nadu, India during summer. The species was recognized and verified in the herbarium of Department of Botany, Holy Cross College, Tiruchirappalli.

Isolation and Identification of Compounds

The collected plants were shade dried and were ground into a powder, and one kilogram of the powder was extracted using hexane, ethyl acetate, and methanol in a Soxhlet apparatus in that order. The extracts were then dried under low pressure in a rotary evaporator. The yields for the hexane, ethyl acetate, and methanol extracts were 17.8, 16.6, and 20.1 g, respectively. For later usage, the dry leftovers of the produced crude extracts were kept in storage at 4°C. The extract of ethyl acetate underwent further chromatography on a Merck 70-230 mesh, 400 g, 3.5 i.d. ×60 cm silica gel column. It was subsequently eluted using a continuous gradient that progressed from 100% hexane to 95% hexane and 5% ethyl acetate, ultimately reaching 10% ethyl acetate.

Anti-bacterial Activity

Both gram positive *Staphylococcus aureus* (*S. aureus*) (ATCC 4322) and gram-negative bacteria *Escherichia coli* (*E. coli*) (ATCC 3726) were obtained from Department of Microbiology, K.A.P. Vishwanathan Government Medical College, Tiruchirappalli, Tamil Nadu, India.

Preparation of Inoculum

Strains of *S. aureus* and *E. coli* were streaked on Muller Hinton Agar (MHA) medium, and the plates were then incubated at 37 °C for the entire night. Using a sterile inoculating loop, four or five isolated colonies were chosen after incubation, transferred, and thoroughly mixed in a tube of sterile Muller Hinton Broth (MHB). Then, the bacterial suspension was contrasted with the McFarland standards of 0.5. Before using, the turbidity standard needs to be shaken on a vortex mixer.

Well Diffusion Test

Using the well diffusion method, the compound's antibacterial activity was evaluated in vitro. Aseptic transfer of a 100 µl tiny aliquot of bacterial culture (*E. coli, S. aureus*) was performed to the MHA plates. Using a sterile L-rod, the culture was equally distributed across the agar plate's surface. Sterilized tips were used to cut wells, which were then filled with varying concentrations of 5, 10, 15, and 20 µg/ml extracted chemical. Tetracycline discs served as a favorable reference point. After that, the plates were incubated for 24 hours at 37 °C. Following incubation, each well's zone of clearance around the plates was checked. The zone of inhibition was identified by the diameters of each zone of clearing [18].

Minimum Inhibitory Concentrations (Mics)

The novel compound was synthesized at varied quantities (10, 15, and 20 µg/ml) and tetracycline (20 µg/ml) using serial dilutions in MHB medium. Following the inoculation of each tube with 160 µl of the respective bacterial strain, the final volume was increased to 200 µl using sterile MHB. As the growth and sterility controls, two blank Muller Hinton broth tubes were utilized, one with and one without a bacterial inoculation. For twenty-four hours, the tubes containing bacteria were incubated aerobically at 37 °C. After the incubation period, the tubes were checked for MICs by measuring the concentration of the first tube in the series, which included increasing concentrations of the novel compound and antibiotics and exhibited no signs of visible growth.

Determination of Minimum Bactericidal Concentrations (MBC)

Following MIC testing, the tubes were gently mixed by flushing them with a sterile pipette, and a 100 µl aliquot was removed. Each aliquot was then placed on a single antibiotic-free nutrient agar plate in a single streak down

the center of the plate, following the protocol [19]. The samples were allowed to absorb into the agar until the plate surface appeared dry (roughly 30 min), at which point the aliquot was spread out over the plate using lawning technique. The growth and sterility controls were sampled in the same way, and the MBC lawned plates were incubated for 24 hours at 37 °C. The novel compound's lowest concentrations that did not result in any bacterial growth on the solid medium were determined to be the extract's MBC values after the incubation time. This observation was compared to the MIC test tube, which after 48 hours of incubation showed no signs of growth.

Determination of Rate of Time-kill Assay

An assay was conducted utilizing a modified plating approach of Eliopoulos and Moellering et al. (2002) to determine the rate of bacterial death caused by the novel compound. At ½ MIC, MIC, and 2× MIC, the new drug was added to 10 ml MHB in sterile 50 ml conical flasks. There were two controls: MHB combined with the novel compound at the test doses without the test organisms and MHB without extract implanted with test organisms. The test and control bottles were inoculated with 10 ml volumes of inoculum at a density of roughly 105 CFU/ml. On an orbital shaker running at 120 rpm, the bottles were incubated at 37 °C. By plating out 25 µl of each of the dilutions, a 100 µl aliquot was taken out of the culture medium at 0, 1½, and 3 h intervals in order to calculate the CFU/ml using the plate count technique. Following a 24-hour incubation period at 37 °C, the total number of bacterial colonies was tallied and contrasted with the number of the culture control, which lacked the novel compound.

Cell Morphology Characterization Analysis

Fluorescent Microscopy

The culture was inoculated and after 18-24 h, the 20 µl of novel triterpenoid was added in 1 ml *S. aureus* bacterial cell culture. After 4 h, the sample was observed by the fluorescent microscopy [20].

Isolation of DNA from *S.aureus* Bacterial Culture

The liquid culture was added 20 µl of novel compound and after 4 h, centrifuged to 1 ml of culture and removed the media. Then the DNA of the sample was extracted by SDS/proteinase K method and EtBr-stained DNA bands were analysed using agarose gel electrophoresis under UV illumination [21].

RNA Isolation from *S. aureus* Bacterial Culture (GeniPureTM Kit)

Overnight grown (16-18 h) culture of bacteria (0.5-1.0 ml) in LB medium at 37 °C was added with 20 µl of novel triterpenoid and then the procedure of RNA isolation was done using GeniPureTM total RNA isolation kit.

Isolation and Estimation of Protein from S. aureus Bacterial Culture

5 ml of LB broth that had been inoculated with *S. aureus* was incubated at 37 °C for two hours. After that, the culture was combined with 20 µl of the novel compound, and the mixture was incubated for two hours at 37 °C. For five minutes, 100 µl of cultures treated and untreated with new compounds were centrifuged at 10,000 rpm. Following centrifugation, the pellet and supernatant were collected separately, and the samples' protein contents were measured using the Lowry et al. (1951) method [22]. Plotting the optical density on the Y-axis and the protein concentration on the X-axis resulted in a conventional graph. The standard graph was used to estimate the amount of protein in the sample.

Statistical Analysis

All data were expressed as mean ± SD for control and experimental groups. The data were analysed using Statistical Package for Social Sciences (SPSS) (Version 17.0). The results were considered statistically significant if the calculated 'p' value was less than 0.05 [23].

Molecular Docking Studies

PyRx (Autodock vina) version v0.8 program was used for the docking studies. Polar hydrogen was added to the ligand moieties, and the searching grid stretched over the desired target proteins. Included were Kollman charges and atomic solvation parameters. After the carbons and non-polar hydrogen atoms were combined, the polar hydrogen charges of the Gasteiger-type were modified in addition to the internal values of torsions. The ligand was flexible, and the target, which was thought to be a rigid body, was docked to the chemicals acquired by GC-MS. To facilitate blind docking, the search was expanded to include the entire receptor protein [25]. The Lamarckian Genetic Algorithm was used to conduct the search. Grid spacing of 0.375 Å was used to generate an electrostatic map and affinity maps for each of the existing atom types. Sorting the various complexes according to the anticipated binding energy allowed for the evaluation of the findings. After that, a cluster analysis was carried out using root mean square deviation values in relation to the initial geometry. The most reliable solution was determined to be the one with the lowest energy conformation of the most populous cluster.

Results

The molecular docking analysis of the antibacterial targets and the novel triterpenoids presented that, the *C.fistula* compound 2 showed promising interaction with increased number of hydrogen bond interactions and optimal binding affinity (fig.1-5). The ADMET properties (table 1) along with the Lipinski rule of five (table 2) supported the possible drugability of the compound (cassia 2) when compared with the other two (cassia 1 and 3).

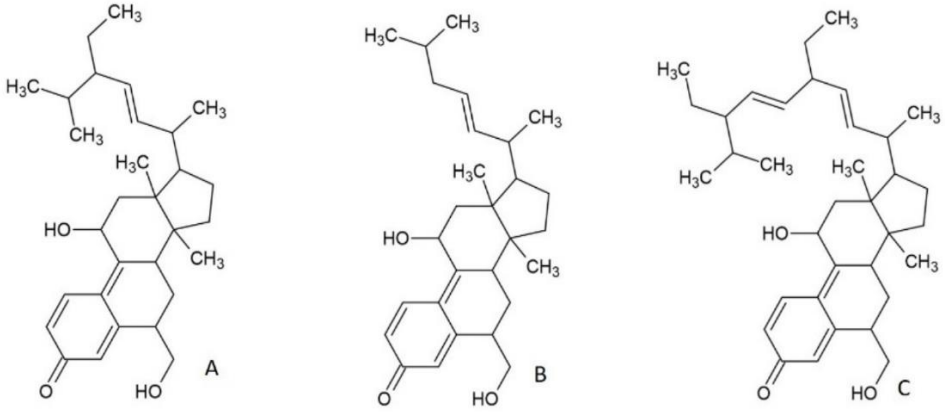

Figure 1. Chemical Structure of the Novel Triterpenoid Compounds (A) Cassia 1 (B) Cassia 2 and (c) Cassia 3 Drawn using Chemsketch Software

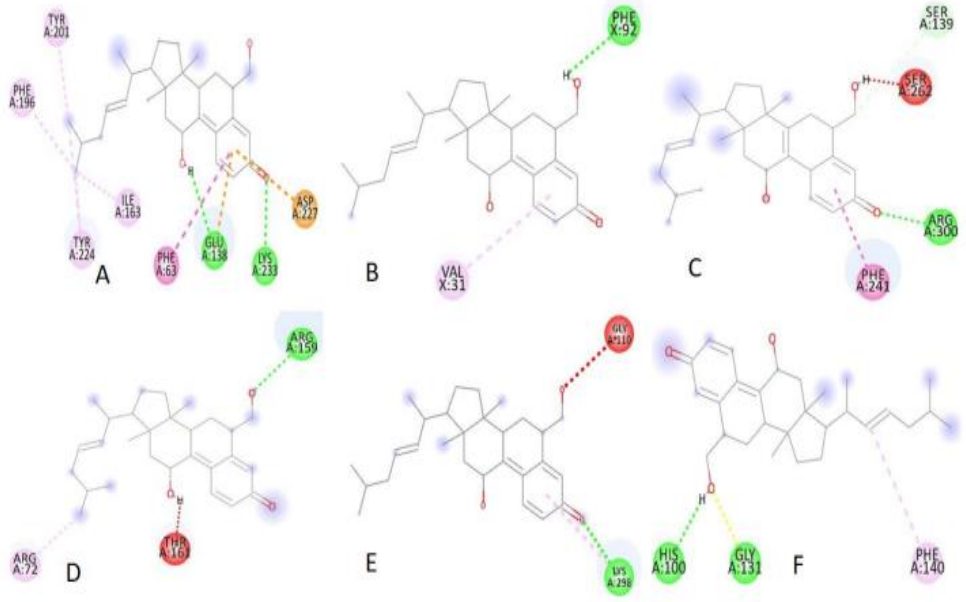

Figure 2. Molecular Interactions of the Triterpenoid Compound (Cassia 2) with (A) Autolysin, (B) Dihydrofolate Reductase, (C) Penicillin Binding Protein 4, (D) DNA Topoisomerase 4, (E) Gyrase, (F) Alaine Racemase

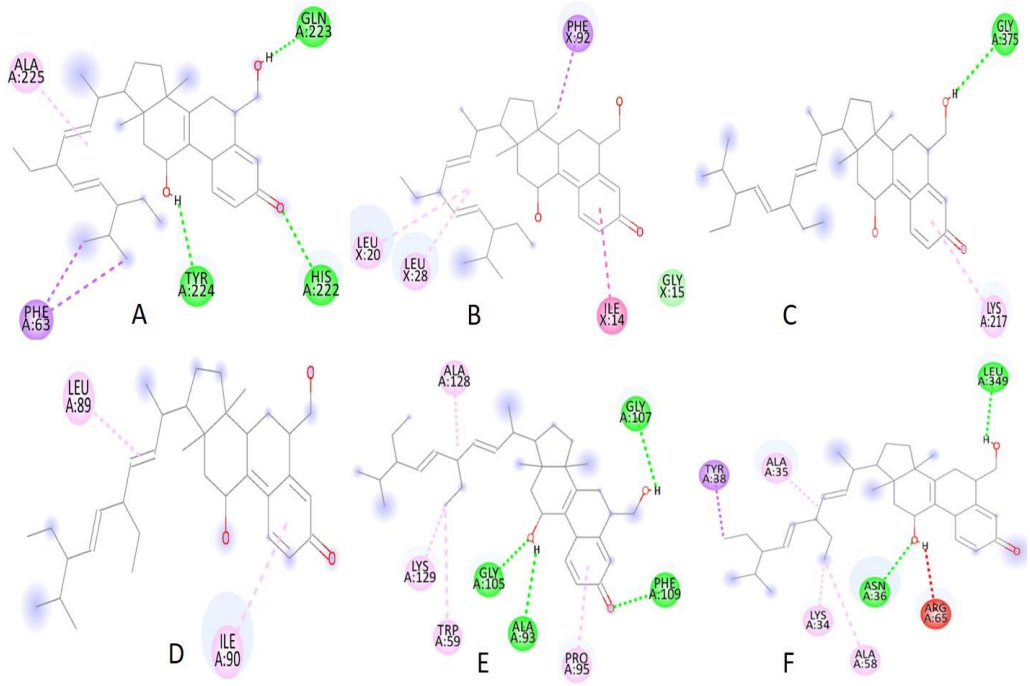

Figure 3. Molecular Interactions of the Triterpenoid Compound (Cassia 3) with (A) Autolysin, (B) Dihydrofolate Reductase, (C) Penicillin Binding Protein 4, (D) DNA Topoisomerase 4, (E) Gyrase, (F) Alaine Racemase

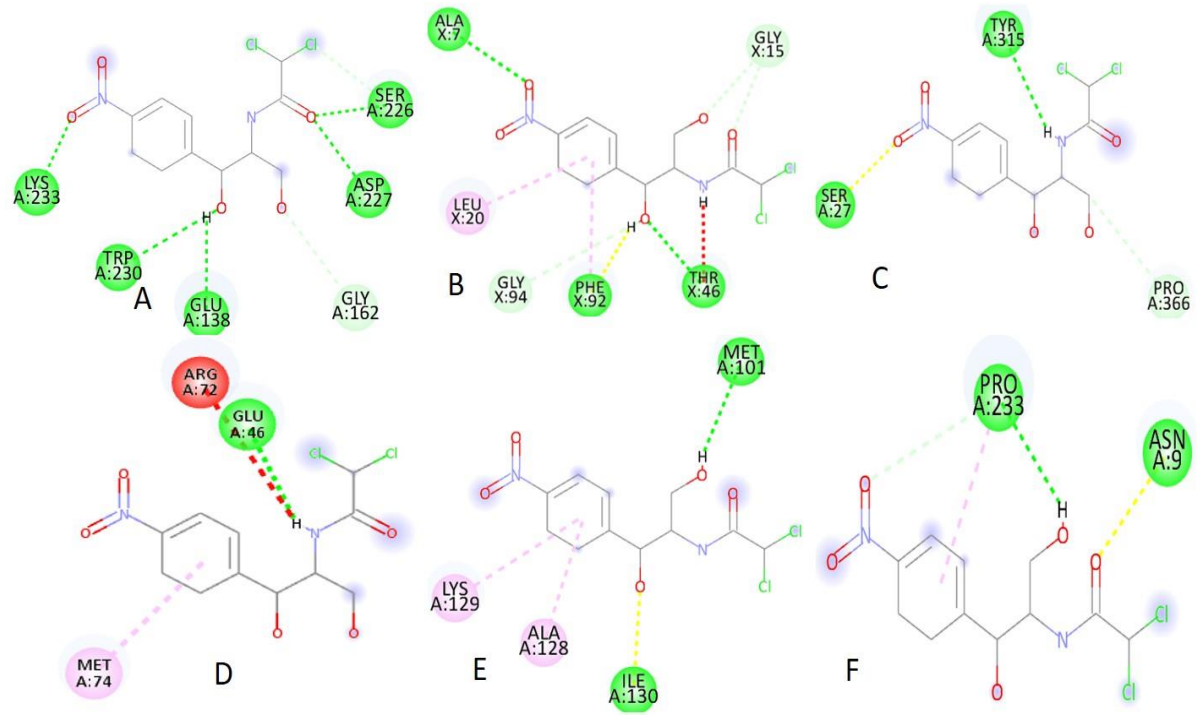

Figure 4. Molecular Interactions of the Tetracycline (Reference Drug) with (A) Autolysin, (B) Dihydrofolate Reductase, (C) Penicillin Binding Protein 4, (D) DNA Topoisomerase 4, (E) Gyrase, (F) Alaine Racemase

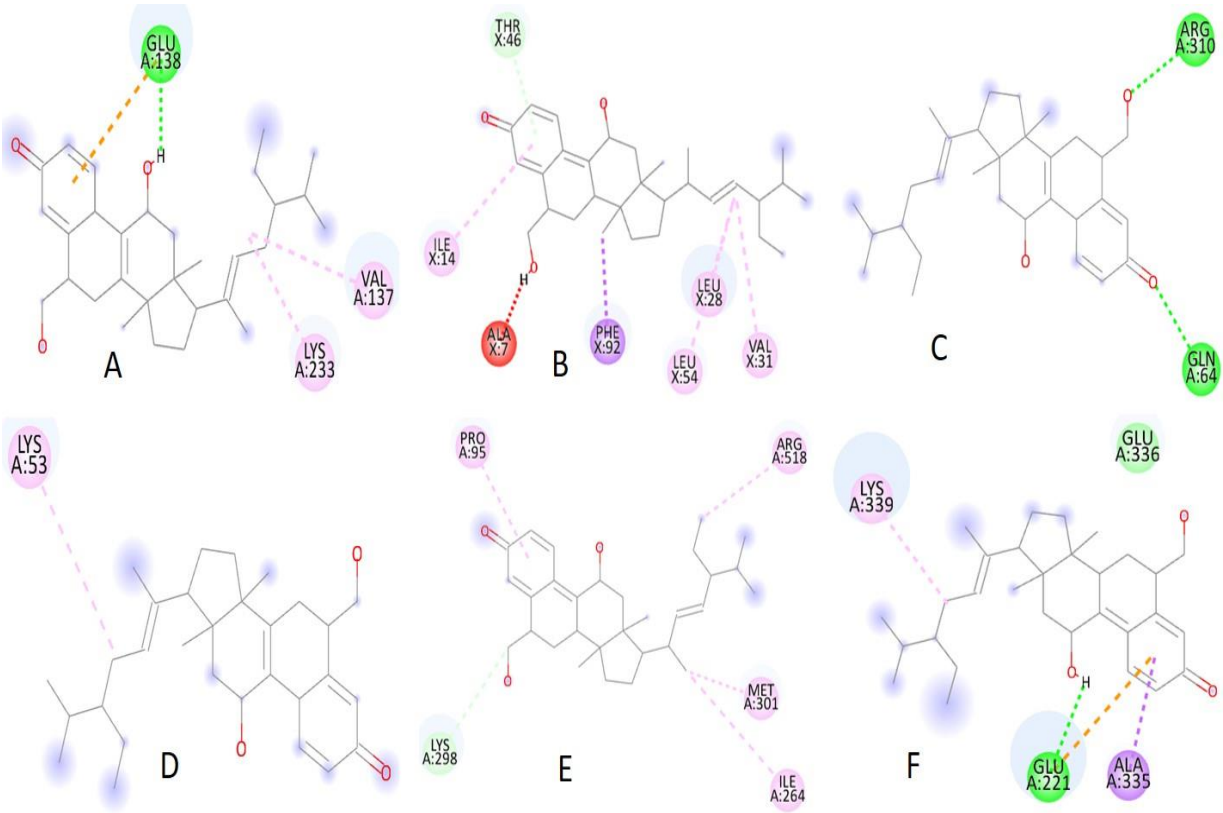

Figure 5. Molecular Interactions of the Triterpenoid Compound (Cassia 2) with (A) Autolysin, (B) Dihydrofolate Reductase, (C) Penicillin Binding Protein 4, (D) DNA Topoisomerase 4, (E) Gyrase, (F) Alaine Racemase

Table 1. ADMET Properties of the Novel Triterpenoid Compounds

Ligand Name	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p (skin permeation)
Cassia-1	High	No	No	No	No	No	No	Yes	-5.33 cm/s
Cassia-2	High	Yes	No	No	No	Yes	No	Yes	-5.77 cm/s
Cassia-3									
	Low	No	Yes	No	No	No	No	Yes	-4.46 cm/s

Table 2. Lipinski Rule of 5 for the Novel Triterpenoid Compounds

Ligand	Mol. Weight	LogP	# Rotatble bonds	# Acceptors	# Donors	Surface area
Cassia-1	452.67	6.04	6	3	2	57.53 Å²
Cassia-2	424.62	5.40	5	3	2	57.53 Å²
Cassia-3	520.79	7.62	9	3	2	57.53 Å²

The antibacterial activity of the novel triterpenoid isolated from *C. fistula* was evaluated by measuring the zone of inhibition in different concentrations (5, 10, 15 and 20 µg/ml) against two pathogenic bacterial strains and compared to the antibiotic standard tetracycline. The results of antibacterial activities are presented in table 3. The well diffusion assay indicated that the bacterial growth was inhibited by the novel compound in a dose dependent manner. The inhibition zones produced by various concentrations of the novel triterpenoid ranged 15mm to 20mm for *S. aureus* while the highest zone of inhibition was obtained at 20 µg/ml concentration.

Table 3. Antibacterial Activity of the Novel Triterpenoid from Ethyl Acetate Extract of C.fistula Against Bacterial Test Organisms

Microorganims	Zone of Inhibition of the Novel Triterpenoid in mm/ Conentraion in µg/ml				
	5 µL	**10 µL**	**15 µL**	**20 µL**	**Tetracyclin (standard drug)**
Staphylococcus aureus	4mm	10mm	12mm	14mm	20mm
Escherichia coli	-	-	-	-	15mm
Klebsilla pneumonia	-	-	-	-	13mm

(-) = no zone of inhibition

The degree of the antibacterial activity of the novel compound was assayed by serial two-fold dilution method while the bactericidal activity was assessed by time kill assay *in vitro*. The MIC and MBC values of the novel compound against *S. aureus* was observed very low (i.e., in the range of 20µg/ml), indicating bacteriostatic activity of the novel compound

(Table. 4). Data are presented in terms of the log10 CFU/ml change and are based on the conventional bactericidal activity standard, that is, a 3Log10 CFU/ml or greater reduction in the viable colony count. The greatest reduction in cell density was observed with the novel compound at 20 µg/ml concentration wherein the bacterial colonies were almost wiped out after incubating for 3 hours.

Table 4. Antibacterial Activity of the Novel Compound

Concentration of novel compound on the *S. aureus*	**MIC**	**MBC**	**MIC/MBC**	**Tetracycline-20µg/ml (MIC)**
10µg/ml	0.062±0.01	0.031±0.01	2	0.124±0.01
15µg/ml	0.099±0.01	0.044±0.01	2	0.254±0.01
20µg/ml	0.120±0.01	0.067±0.01	2	0.346±0.04

Each value represents mean ± SD of triplicates. Significance at: $p<0.05$

The micrographs of control and novel triterpenoid treated cultures of *S. aureus* is presented Fig. 6 & 7 which showed the effect of novel triterpenoid (20 µg/ml) damaging the bacterial cell wall at exponential phase table 5.

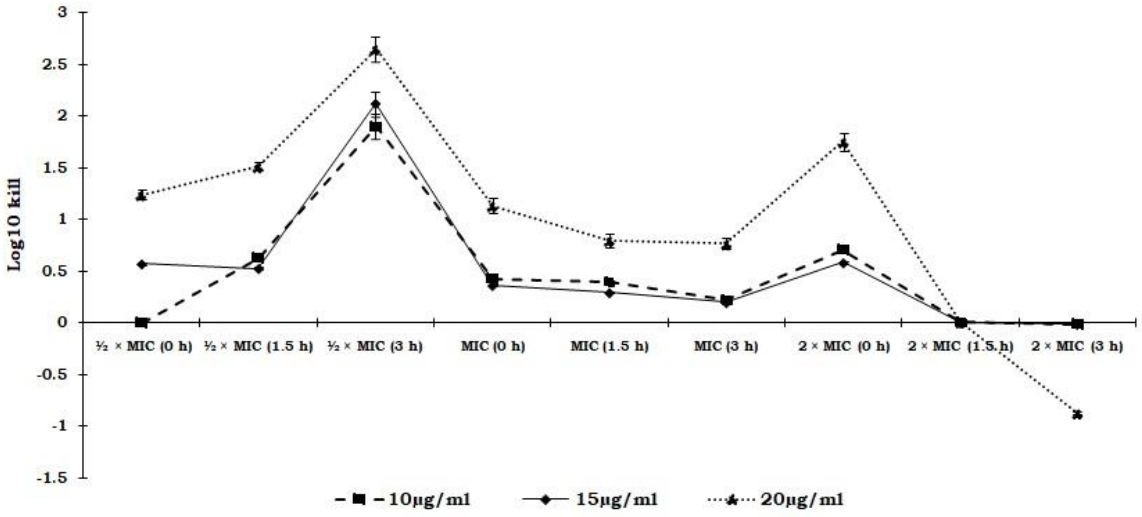

Figure 6. In-vitro Time Kill Assessment of the Novel Triterpenoid Compound

Table 5. In vitro Time Kill Assessment of the Novel Compound

Concentration of the Novel Compound	**Log10 kill ½ × MIC**			**Log10 kill × MIC**			**Log10 kill 2 × MIC**		
	0h	**1½ h**	**3h**	**0h**	**1½ h**	**3h**	**0h**	**1½ h**	**3h**
10µg- *S.aureus*	0.641 ±0.01	0.628 ±0.01	1.901 ±0.12	0.427 ±0.01	0.391 ±0.01	0.217 ±0.01	0.706 ±0.01	0.004 ±0.01	-0.018 ±0.01
15µg- *S.aureus*	0.571 ±0.01	0.527 ±0.01	2.120 ±0.12	0.363 ±0.01	0.294 ±0.01	0.198 ±0.01	0.582 ±0.01	0.003 ±0.01	-0.012 ±0.01
20µg- *S.aureus*	1.243 ±0.05	1.511 ±0.04	2.651 ±0.12	1.134 ±0.07	0.798 ±0.07	0.767 ±0.05	1.752 ±0.09	0.009 ±0.01	-0.872 ±0.01

Each value represents mean ± SD of triplicates. Significance at: $p<0.05$

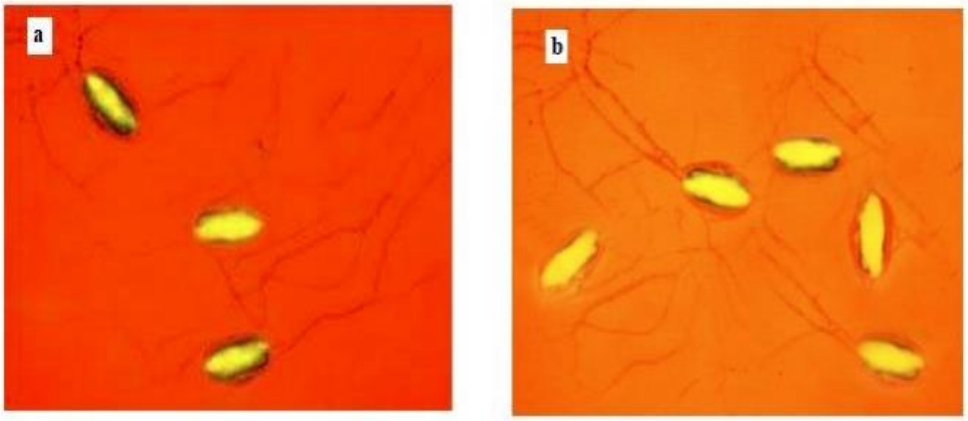

Figure 7. Morphological Characterization Analysis of Fluorescent Microscopy. a) Untreated Culture. b) Novel Triterpenoid Treated Culture

The effect of novel triterpenoid on the DNA and RNA expression were analysed and presented in Fig. 8. It demonstrated that the DNA band has no changes in the compound treated and untreated cultures. But there are transcription level effects of novel triterpenoid which is shown with changes in the RNA bands of compound treated and untreated culture.

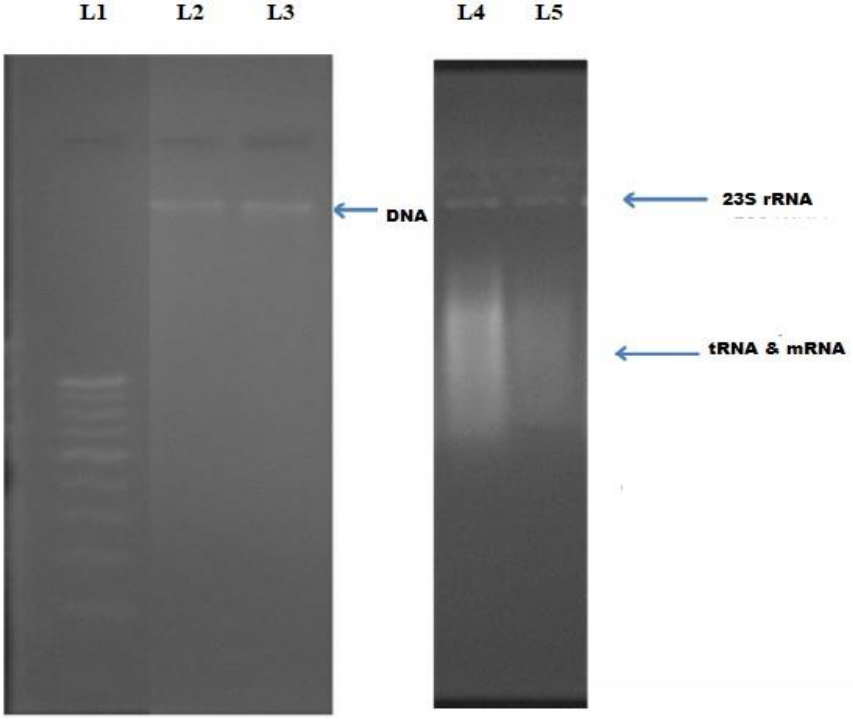

Figure 8. Genomic DNA and RNA Isolation from S.aureus Untreated and Treated. DNA: Lane (L): Low Range DNA Ladder (100kbp); Lane 1: UNTREATED Genomic DNA of S.aureus and Lane 2: Treated with Novel Triterpenoid of S.aureus. RNA: Lane 6: Untreated Total RNA of S.aureus and Lane 2: total RNA of S.aureus Treated with Novel Triterpenoid

The amount of protein isolated from the cultures is presented in table 7. It is very much clear that the protein content isolated from the novel compound treated cultures of are comparatively lesser than that of the positive control.

Table 6. The Protein Content of the Novel Compound Treated, Positive and Negative Control Cultures of S. aureus

Culture	Protein content (mg/dl)
20µg/ml of novel compound treated *S. aureus*	15.8±2.3[a]
Positive control	20.2±3.6
Negative control	37.7±3.6[a]

Each value represents mean ± SD of triplicates. Significance at: $p<0.05$ compared with positive control

Discussion

Medicinal plants are a rich source of antimicrobial agents [26]. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs [27]. Various genetic alterations in oral squamous cell carcinoma grades, offering information for better customized medication and treatment approaches [24]. When compared to oral leukoplakia and normal mucosa, salivary MMP9 levels were significantly greater in OSCC patients, indicating that it may be a symptom of malignant transformation [28]. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids etc. that are present in these plants [29]. In the present study, the antimicrobial screening assay of novel compound isolated from ethyl acetate extract of *C.fistula* gave relatively wide inhibition zones against the test strains compared with the positive control.

There is no change in the genomic DNA profile of the culture treated with the novel compound. This implies that the compound does not have impact on the DNA by means of fragmentation or DNA purine bases chelation. But the reduction in the tRNA and mRNA expression in the compound treated culture depicts that the compound could have ability to inhibit the expression of DNA at the transcriptional level. The reduction in the protein content of the novel compound treated cultures of both the strains shows that protein synthesis was inhibited by the novel compound that in turn confirms the bactericidal activity of the novel compound. Further immune blot analysis has to be done for the confirmation of the specific protein. The increasing incidence of multi-drug resistant bacteria is a major concern in different countries [30,31] and therefore, in recent years, there has been an emphasis on the need to find alternative antibacterial agents [33]. The promise of miRNAs as therapeutic targets and biomarkers for oral premalignant illnesses, highlighting their significance in early detection and individualized treatment plans [32].

In this study, multi-drug resistant clinical Gram-positive and Gram-negative were inhibited by the tested novel compound of *C.fistula*. *S. aureus* in particular was inhibited by novel compound at concentrations as low as 10-20µg/ml. The higher susceptibility of Gram-positive bacteria compared to Gram-negative bacteria ($p < 0.0001$) has been previously reported for other natural products [34-36, 38]. Leukoplakia and OSMF patients with high risk of malignant transformation may be identified using novel biomarkers such as plasma circulating exosomal miRNAs miRNA 21, miRNA 145, and miRNA 184 [37].

This differential sensitivity of Gram-positive and Gram-negative bacteria to the novel could be clearly explained the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components; this makes the cell wall impermeable to lipophilic solutes. The Gram-positive bacteria should be

more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier [39]. In the present study this view about the Gram-positive bacteria could be the reason for the damage in cell wall of the *S.aureus* culture treated with the novel triterpenoid.

A number of different antibiotics that prevent translation by binding to the 50S ribosomal subunit of bacterial cells has been shown to also prevent assembly of this subunit. Antibacterial agents affecting 30S a particle activity has not been examined extensively for effects on small subunit formation. The aminoglycoside antibiotics paromomycin and neomycin bind specifically to the 30S ribosomal subunit and inhibit translation. These drugs were examined in *S.aureus* cells to see whether they had a second inhibitory effect on 30S particle assembly. This is the second demonstration of 30S ribosomal subunit-specific antibiotics that prevent assembly of the small subunit [40]. Salivary 1-25dihydroxycholecalciferol and IL-17A levels were negatively correlated during orthodontic treatment, indicating that vitamin D supplementation may lengthen treatment duration with fewer tissue effects [41].

Bacterial protein synthesis is the target for several classes of established antibiotics. It has been reported describe the characterization of a novel translation inhibitor produced by the soil bacterium Flexibacter. The dipeptide antibiotic TAN1057 A/B was synthesized and designated GS7128. As reported previously, TAN1057 inhibits protein synthesis in both *E. coli* and *S.aureus*, leaving transcription unaffected. Cell-free translation systems from *E.coli* were used to further dissect the mechanism of translational inhibition. Binding of mRNA to ribosomes was unaffected by the drug, whereas the initiation reaction was reduced. Elongation of translation was completely inhibited by GS7128. Detailed analysis showed that the peptidyl transferase reaction was strongly inhibited, whereas tRNA binding to both A and P-site was unaffected. Selection and analysis of drug-resistant mutants of *S. aureus* suggests that drug uptake may be mediated by a dipeptide transport mechanism [40].

Generally, terpenes compounds are also referred to as isoprenoids and their derivatives containing additional elements, usually oxygen, are called terpenoids. The novel triterpenoid used in the present study also possesses a hydroxyl group. Antibacterial activity on Gram-positive and Gram-negative bacteria might have occurred through the interaction of the novel compound, a triterpenoid, with components of the bacterial cell wall, speculated to involve membrane disruption by the lipophilic compounds. This mechanism depicts the strong structure-function influence of the antibacterial potential of novel triterpenoid.

Conclusion

Our results suggest that further investigation into the activity of the novel compound is warranted, especially because their activity against strains of *S.aureus* suggests a broad, but potentially novel, antimicrobial cellular mechanism. The investigation of the novel compound which could minimize the pharmaceutical drug load and risk of resistance among patients, as well as reducing treatment costs, might also provide attractive therapeutic alternatives for treating secondary urinary tract infections to diabetes.

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