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Biochemical and in silico study of leaf extract from *Rumex dentatus* against *Staphylococcus aureus*

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ABSTRACT

Rumex dentatus is a medicinal plant with a variety of bioactive components that can be used to treat infections and multidrug-resistant (MDR) microbes. Infections caused by MDR microbes may pose a real threat and the available antibiotics can no longer be used to control or kill those bacteria. Thus, the study aimed to ascertain the antibacterial activity of *Rumex dentatus* leaf extract and identify a potent compound that can be used as a drug developmental agent may against *Staphylococcus aureus*. Disc diffusion method, mortality assay of brine shrimp, and DPPH free radical scavenging assay were carried out for in vitro analysis. Standard computational tools and servers such as Pymol, PyRx, and Discovery Studio were used for carrying out the in-silico studies. *Staphylococcus aureus* was isolated from the infected area of eczema sufferers and identified through morphological, biochemical, and 16SrRNA sequence analysis. *Rumex dentatus* leaf extract was diluted with methanol and it showed the maximum zone of inhibition (14.33 ± 0.68 mm) after applying it against *Staphylococcus aureus* at the dose of 150µg/disc. Furthermore, the extract revealed remarkable antioxidant activity and the death rate of brine shrimp and leaf extract concentration were positively connected. Finding pharmacological targets with a high affinity for the FosB (4nb2) protein, which controls antibiotic resistance in *Staphylococcus aureus*, was done via molecular docking. 1-alpha-18O-1,25-dihydroxycholecalciferol (CID-5280453), androstan-3-ol, 9-methyl-(3 beta,5 alpha) (CID-22215820), phenylacetaldehyde (CID-998), and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester (CID-62603) substances had binding energies of -6.8 Kcal/mol, -6.6 Kcal/mol, -5.6 kcal/mol, and -5.5 kcal/mol, respectively. As an organic source of medicines, *Rumex dentatus* leaf extract could be used to combat the antimicrobial-resistant pathogen.

INTRODUCTION

Eczema, often known as dermatitis, is a group of diseases that are irritating, inflammatory, and cause skin rashes [1]. Atopic dermatitis (AD) is the most common type of eczema, characterized by dry, inflamed, and itchy skin, and it refers to a group of disorders caused by allergic reactions, including asthma and hay fever. AD has been more common in recent decades, with 10-30% of children in affluent nations suffering [2]. Atopic eczema can be caused by a variety of infectious agents and microorganisms such as *Staphylococcus aureus*, *Candida albicans*. Although candida, a yeast infection spread on weepy and heated skin, can cause eczema, the common bacterium *Staphylococcus aureus* is primarily blamed for the condition. The isolated strain *Staphylococcus aureus*, which thrives on moist and damaged skin, causes atopic eczema. As the infection originates in the bloodstream, *Staphylococcus aureus* infection can spread by contact with fluids into the blisters located on the affected skin [3]. Bacteria can genetically change themselves to become antibiotic-resistant. The main issue has been identified as people's overuse of antibiotics, which causes bacteria to become

resistant to antibiotics, resulting in an unclear future. Multidrug-resistant (MDR) organisms that are resistant to multiple antibiotics or antifungals are difficult to treat, and therefore can causes serious illness or even death. It is a better strategy to control the usage of antibiotics by producing natural drugs that aim to provide patients with the appropriate antimicrobial agents [4]. Plant extracts were used to obtain antimicrobial compounds, as there are around 120 plant species belonging to 28 distinct families [5] that contain various phytochemicals and are excellent sources of natural antioxidants for public health. Infectious bacteria species pose a multitude of contagious diseases [6] as a consequence of antibiotic and drug resistance [7]. *S. aureus* bacteria are the most common microbes that cause a variety of diseases in humans. It is critical in the treatment of bacteremia and necrotizing enterocolitis, as well as osteoarthritis, respiratory infections, pleuropulmonary infections, and device-related diseases [8]. Different medicinal plant extracts offer effective antibacterial properties against bacteria and fungi that cause a variety of skin diseases. Flavonoids and anthraquinones have been identified as important chemical components of this species in several studies [9]. It includes several pharmaceutically important substances that can help with a variety of conditions, including infection [10]. *Rumex dentatus* leaves were shown to have refrigerant characteristics [11]. *Rumex dentatus* extracts have potential antibacterial, antifungal, cytotoxic, anticancer, and allopathic properties [12]. These leaves also contain several important bioactive chemicals, including palmitic acid, hexestrol, phenylacetaldehyde, and vitamin C. According to World Health Organization (WHO), a wide range of plant extracts might produce a variety of pharmaceuticals., including medicines with effective healing properties [13]. Secondary metabolites, such as phenolic compounds found in essential oils and tannins [14], are plant extracts containing diverse types of phytochemicals that resist microorganisms. According to the documentary, *Staphylococcus aureus* was susceptible to 81 extracts found in 58 plants [15]. Due to its numerous uses in the analysis of molecular recognition events such binding energetics, molecular interactions, and induced conformational changes, molecular docking is one of the most widely utilized structure-based drug design methodologies. Using bioactive small-molecule libraries is a cutting-edge approach to drug development. The specific chemical space occupied by ligands known to interact with a particular target is represented by these libraries. The goal of ligand-protein docking is to predict the predominant binding modes of a ligand with a protein of known three-dimensional structure.

The purpose of this research is to investigate the antimicrobial property of *Rumex dentatus* leaf extracts in the laboratory and in silico approach to determine the potential compound of the *Rumex dentatus* leaf extract, which can be used in the development of drugs against *Staphylococcus aureus*. One of the causes of atopic dermatitis is infectious microorganisms. Antibiotics are urgently needed to combat such microorganisms since antibiotic resistance is making the situation worse. Therefore, if we can stop the protein that causes atopic dermatitis, it will aid in the fight against the condition. *Rumex dentatus* plant extracts' antagonistic properties against the bacterium were also investigated.

MATERIALS AND METHODS

The following materials and methods were used for the completion of this research work.

Bacterial isolation and identification

The sample was taken from blisters that had formed in the infected area of eczema sufferers. The procedure was carried out using the swabbing technique, which involved rubbing a sterile dry cotton-tip swab on the blister of an infected skin location. The inoculated cotton tip was then placed in a conical flask or beaker containing saline water. After collecting the sample, it was serially diluted. Due to growing bacteria overnight in the mineral salt (MS) medium at 37 °C the diluted solution was mixed with the media. The colony was selected depending on the shape, and size of the single colony, and the streaking method was used for producing pure culture due to further use. For studying the characteristics of the bacteria morphological and biochemical tests were carried out such as gram staining test, mobility test, methyl red test, hemolysis, pigment test, eosin methylene blue agar test, urease test, catalase test, methyl red test, mannitol test, starch hydrolysis test, triple sugar iron test, and citrate test.

Evaluation of 16S rRNA gene sequencing

The Genomic DNA of the pure bacterial isolate was extracted. Here, universal reverse and forward primers, namely, 1492R (5′- GGTTACCTTGTTACGACTT-3′) and 27F (- 5′-AGAGTTTGATCCTGGCTCAG-3′-) were used. After the completion of PCR, the amplified PCR products were sent for 16S rRNA sequencing through a commercial sequencing service of Invent Technologies Ltd. Dhaka, Bangladesh. The 16S rRNA genes of the isolate were sequenced for checking the homology matched to other sequence data in the gene database server utilizing the Basic Local Alignment Search Tool (BLAST)(www.ncbi.nlm.nih.gov/Blast) [16].

Plant sample collection

Rumex dentatus leaves were collected from various locations in the Rajshahi University (Bangladesh) area, and the individuals placed in a sterilized zipper paunch were microbes free, and then the zipper paunch was taken to the Microbiology Lab in the Department of Genetic Engineering and Biotechnology University of Rajshahi. The plant was identified (Voucher no. 47) by Dr. A.H.M. Mahbubur Rahman, Professor, Department of Botany, University of Rajshahi, Bangladesh

Preparation of extracts

The sample of plant leaves was obtained and dried with the help of air at room temperature in the research lab for one week. A blender was used to grind the leaves. Mariswamy *et al.* [17] described a technique for preparing methanolic extracts in which 50 g of the specimen was diluted with 250 mL of methanolic solvent extract and positioned in an orbital shaker for shaking for five days. For clearing the unwanted particle from the sample filtered paper was used before being accumulated in an open jar for drying and the extract was stored at 4 ºC for further use.

Antibacterial activity test

The antibacterial activity of the isolate the test was carried out through the protocol explained by Nostro *et al.* [18]. For performing the test 6 mm discs of Whatman paper were used. 1 ml methanol was added to 0.05 gm extract for preparing the stock solution. The disinfected discs were then immersed in diluted extracts at 50, l00, 150, and 200

μg/ml concentrations. Finally, each agar plate was seeded with $3.5x10^8$ CFU/mL of bacteria, and the medicated discs were positioned with a sterile loop on each plate. As a regulation, gentamicin was used. The plates were then managed to keep at 37 °C overnight for observation. The presence of distinct zones indicated that the growth of microorganisms was not rapid. The zones in the vicinity of the discs were measured and recorded.

Cytotoxicity assay

A cytotoxic test determines whether a substance is hazardous to cells. It is a frequently used method for determining the hazardous level of a naturally occurring plant extract or any other compound. Meyer *et al.* [19] described a cytotoxicity test that was done on brine shrimp (*Artemia salina*) nauplii.1 ml methanol was added to 0.05 gm extract for preparing the stock solution. In a tank, brine shrimp were generated at 25 °C temperature. In test tubes, extract concentration levels of 50, 100, 150, and 200 μg/mL were managed to make. After that, 10 live *Artemia salina* nauplii were placed in each test tube and left at room temperature for 24 h. Finally, the LC50 values of methanolic leaf extracts were calculated and recorded.

Antioxidant activity test

The antioxidant potential of the leaf extracts was determined using the DPPH (2,2-Diphenyl-1-Pi cryl-Hydrazyl-Hydrate) free radical scavenging assay with BHT (Butylated Hydroxy Toluene) as a control [20]. BHT concentration levels varied from 50, 100, and 150 μg/ml. Each test tube obtained 1.5 mL of 0.1 mM DPPH solution. Finally, by adding methanol to all the test tubes, the total volume was increased to 2.5ml. The test tubes were then retained in the dark room for 30 minutes time duration to complete the interaction. Finally, using a spectrophotometer and BHT as a control, absorbance at 517 nm was measured [21]. The scavenging portion was sorted out using the equation narrated by Mahmud *et al.* [22] and the IC50 was determined by calculating by projecting the results on a linear scatter graph in a graph pad prism.

Antibiotic sensitivity test

The Kirby-Bauer disc diffusion model was used to calculate the antibacterial drug responsiveness and resistance pattern of the bacterial isolate [23]. The isolated bacterial strain was cultivated and incubated at 37 °C for the antibiotic's susceptibility test. The LB agar medium was prepared and commercially available, and antibiotics that were regularly prescribed (gentamycin, fosfomycillin, amoxicillin, ciprofloxacin, cefuroxime) were inserted in the center of the plates and incubated at 37 °C. The zones on the plate were noticed and measured using an mm scale after such a nighttime incubation period.

Molecular docking study

Ligand preparation

For docking analysis ligands were determined according *to Rumex dentatus* GC-MS compounds, a total of 17 *Rumex dentatus* GC-MS compounds were chosen as docking ligands [24]. The literature following a conscientious examination of the literature, the chemical substances of *Rumex dentatus* [25, 26] were reprocessed from the PubChem database of chemical molecules and their activities against biological assays [27].

Employing a Merck molecular force field (MMFF94), the substances were extracted in three dimensions as well as confined to minimize the objective function.

Protein preparation

The protein data library provided the crystalline structure of the *S. aureus* protein (PDB ID: 4nb2). Using Pymol software (version 2.4.0) and Discovery Studio software, the protein structure was washed, and heteroatoms were eliminated (version 4.5.0). In Swiss PDB Viewer software (version 4.1), the washed protein was energy reduced and streamlined using the Groningen molecular Simulation (GROMOS) 431B force field. The Ramachandran plot analysis was utilized to determine the quality geometry of the protein structure.

Molecular docking

With the help of AutoDock Vina software package (version 1.1.2) molecular docking analysis was carried out for appreciating the binding kinetics of substances from *Rumex dentatus* and the target protein [28, 29]. For converting the protein structure into molecules, the software had been used and after applying it ligand molecules became converted into PDBQT layout. Ligand-protein interactions can be defined with the help of Lamarckian genetic algorithms. Binding free energy and docking directions within a 2.0 Root-Mean-Square Deviation (RMSD) range were approved to alternate the molecules, which is considered a key advantage. A range of 10, 20 modes, and an exhaustiveness of 8 was used to complete molecular docking. In Pymol [30] and Discovery Studio [31], the autodock structures were eventually examined for non-bonded contacts.

RESULTS

Morphological and biochemical tests of isolates

The sample was collected from eczema-affected skin and then plated onto Mannitol Salt agar media which is selective for pathogenic bacteria. In the staining test, the isolated bacteria showed purple color indicating gram-positive spherical-shaped bacteria and motile. Biochemical tests indicated that the isolate was negative in the MacConkey test, TSI test, Bismuth sulfate agar (BSA) test, EMB agar test, and positive for Methyl red test, Catalase test, Mannitol test, Starch hydrolysis test, Urea hydrolysis test, and citrate test .In hemolysis and pigment tests on blood agar, it showed often-creamy gold pigment by producing various types of hemolysins (alpha, beta, gamma, and delta) (data are not shown).

Species identification of the isolate

Figure 1A illustrated that the length of 16S rRNA of the isolate was approximately 1500bp. After sequencing and editing the gene, the sequence was compared to other organism 16S rRNA gene sequences which had been uploaded to the Gene dataset of NCBI. Relating to substance numerous sequences were manifested for the isolate. *Staphylococcus aureus* formed a 99 percent identity with the 16S rRNA gene sequencing of the isolate bacteria (Figure 1B).

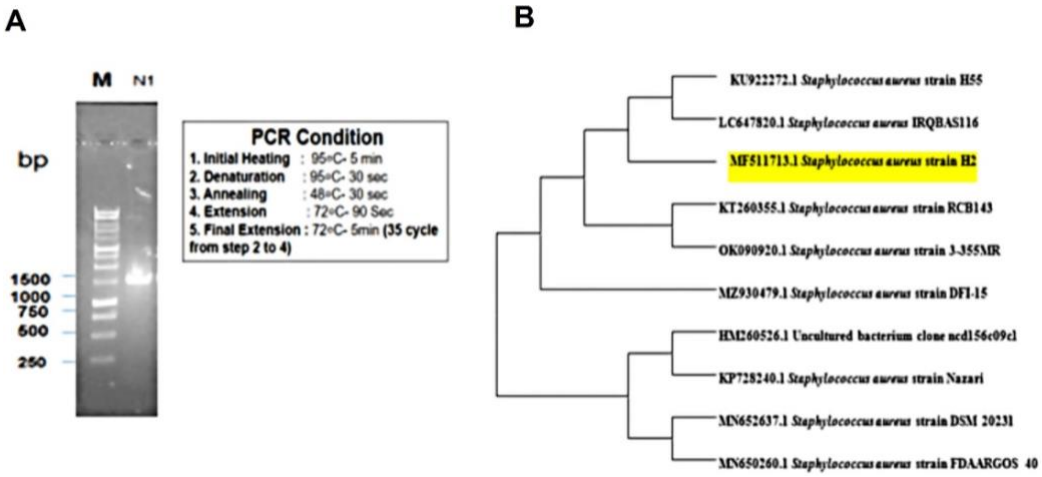

Figure 1. Molecular identification of the isolate. Here, A) describes the profile of 27F and 1492R generated 16SrRNA from the isolate, M: denotes 1kb DNA ladder (marker). B) indicates the phylogenetic position of *Staphylococcus aureus*. Staphylococcus aureus produced 99% identity with the 16S rRNA gene sequencing of the isolated bacterium.

Effect of *Rumex dentatus* leaf extract against activity of *Staphylococcus aureus*

Figure 2A and 2B illustrates *Rumex dentatus* antibacterial activity. Methanolic extraction of *Rumex dentatus* leaf showed 12.52 ± 0.48 mm, 13.12 ± 0.53 mm, 14.33 ± 0.68 mm, 14.18 ± 0.54 mm inhibition zones against *Staphylococcus aureus* at a concentration of 50, 100, 150 and 200µg/disc, respectively.

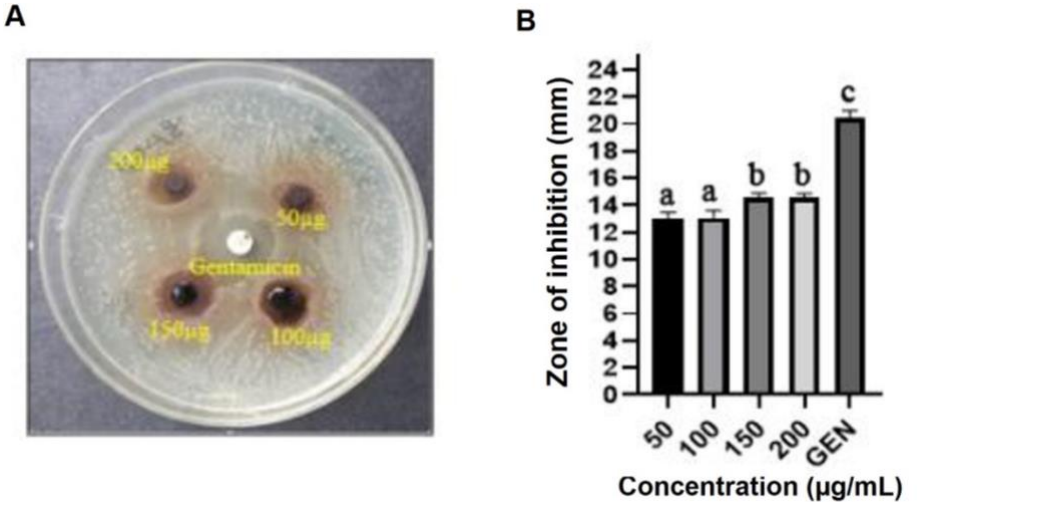

Figure 2. A) Antibacterial activity of *Rumex dentatus* leaf extract showed 12.52 ± 0.48 mm, 13.12 ± 0.53 mm, 14.33 ± 0.68 mm, 14.18 ± 0.54 mm inhibition zones against *Staphylococcus aureus* at 50, 100, 150 and 200µg/disc, respectively. B) Different letters indicate significant differences between mean ± SD of replications (n = 3) at a $p < 0.05$ significance level.

Effect of methanolic extract of *Rumex dentatus* leaf on cytotoxic activity

The LC50 values of methanolic *Rumex dentatus* leaf extracts were 296.57 ± 4.08 µg/mL. These findings suggest a strong positive correlation between the density of leaf extract

and the fatal rate of brine shrimp, with a maximum mortality percentage at 200 µg/mL dose compared to other dosages and on the other hand a lower or minimum mortality percentage at 100 µg/mL dose (Figure 3).

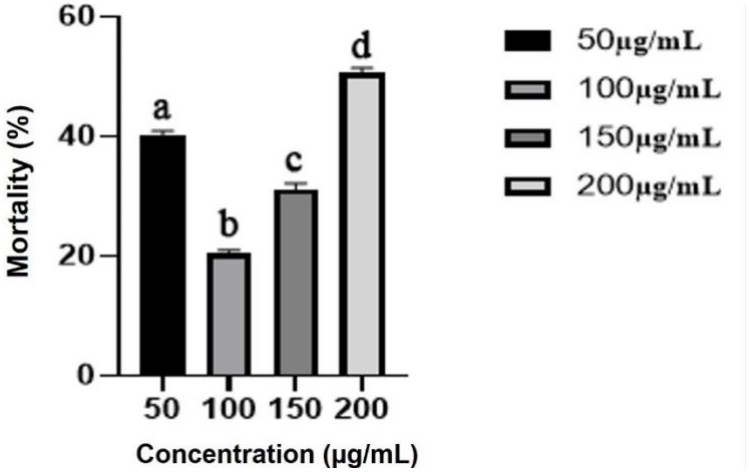

Figure 3. Cytotoxic activity of *Rumex dentatus* plant leaves extract against brine shrimp. These graphs demonstrate a positive connection between leaf extract concentration and brine shrimp mortality, with a high mortality percentage at 200 µg/mL, especially in comparison to other recommended doses, and a low survival percentage at 100 µg/mL. Different letters indicate significant differences between mean ± SD of replications (n = 3) at a $p < 0.05$ significance level.

Effect of *Rumex dentatus* leaf extract on antioxidant activity

At 150 µg/mL concentration, *Rumex dentatus* leaf extract had 56.21 % DPPH free radical scavenging activity, whereas BHT control activity was 83.46 % (Figure 4A). Leaf extract and BHT had IC50 values of 139 ± 3.14 µg/mL and 54 ± 2.01 µg/mL, respectively (Figure 4B). These outcomes show that perhaps the leaf extract had modestly potent antioxidant activity, especially in comparison to the BHT control.

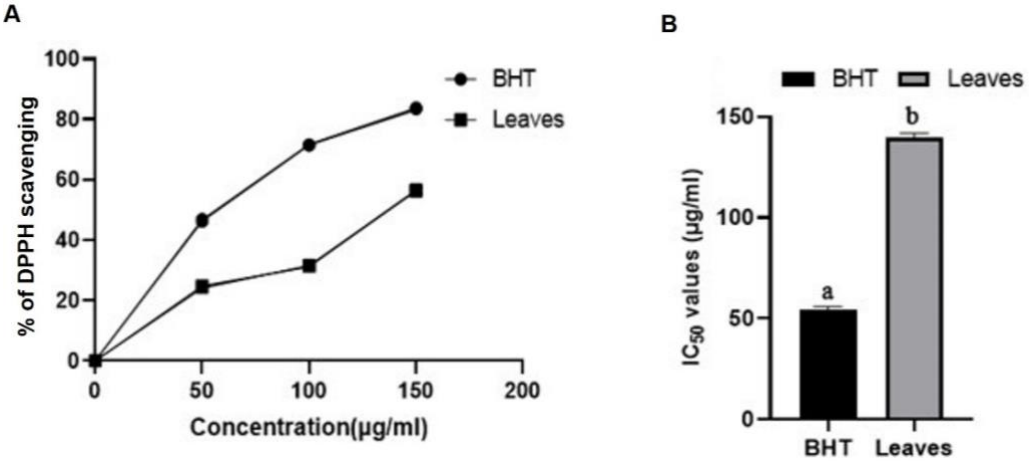

Figure 4. Antioxidant activity of *Rumex dentatus* plant leaves extract. A) DPPH scavenging activity leaf extract from *Rumex dentatus*, and B) IC_{50} value of leaf extract from *Rumex dentatus*. Different letters indicate significant differences between mean ± SD of replications (n = 3) at a $p < 0.05$ significance level.

Resistance pattern of the isolate

Staphylococcus aureus antibiotic responsiveness was evaluated utilizing five antibiotic disks: amoxicillin, gentamycin, ciprofloxacin, cefuroxime, and fosfomycin. Utilizing nutrient agar media, the standard disc diffusion model was used to narrate the trends of responsiveness and resistance of isolated bacterial cultures to five different antibiotics. Findings showed that *Staphylococcus aureus* was resistant to ampicillin, cefuroxime, and fosfomycin but responsive to gentamicin and ciprofloxacin (Figure 5A). Figure 5B illustrates the results of antibiotic sensitivity assessment and antibiotic inhibition against the bacterium.

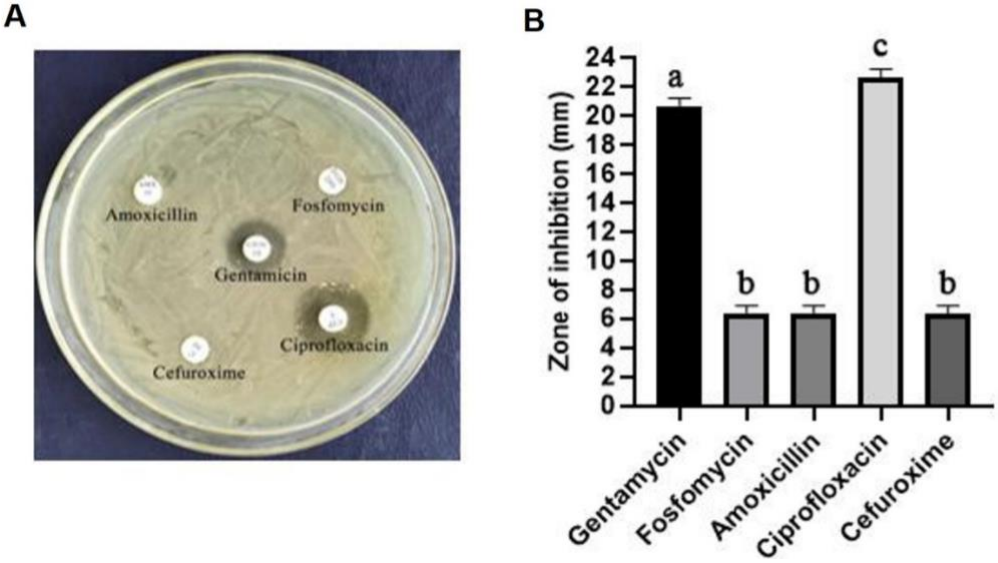

Figure 5. Antibiotic sensitivity test of the isolate, where A) indicates that the isolate was susceptible to gentamycin and ciprofloxacin, and resistant to amoxicillin, cefuroxime and fosfomycin, and B) indicates that the inhibitory activity of five antibiotics against isolate. Different letters indicate significant differences between mean ± SD of replications (n = 3) at a $p < 0.05$ significance level.

Ligand-protein interaction analysis

Molecular docking analyses were carried out for determining the interconnection among the bond and identifying the major molecules with a stronger relationship with the FosB (4nb2) protein complex. All 17 compounds from GC-MS analysis of methanolic leaf extracts of *Rumex dentatus* were used for molecular docking. Among them, only four phytochemicals from *Rumex dentatus* were scanned for the lesser binding score in this study. CID- 5280453, CID- 22215820, CID- 998, and CID- 62603 were assigned as D1, D2, D3, and D4, respectively. D1 and D2 compounds had a stronger affinity than other *Rumex dentatus* chemicals. -6.8 Kcal/mol and -6.6 Kcal/mol were the binding affinity score levels of D1 and D2 substances respectively (Table 1).

The D1 from *Rumex dentatus* interacted with the FosB protein and formed two hydrogen bonds (Figure 6 and Table 2) at Phe62, Thr65, alkyl bonds at Ile8, Ile55, Ile67, and pi-alkyl bonds at His112, His112, Phe10, and Tyr64, Phe62(3) positions. The D2 and 4nb2 protein complex formed one pi sigma bond at Trp46, alkyl bonds at Leu31 (2), Leu32(2), and pi-alkyl bond at Tyr39(2), and Trp46(2) (Figure 6**)**. However, D3 had a binding score of -5.6 kcal/mol and had four pi-alkyl bonds at Ala43, Leu26, Leu42, and Leu116 residues (Figure 7**)**. The D4 and 4nb2 protein complex was formed one pi sigma bond at Ile8, two pi-alkyl bonds at His112, Phe10 and one alkyl bond at Cys9 (Figure 7).

Table 1. Molecular docking score between *Rumex dentatus* plant compounds and selected protein.

Compounds	Docking score
Phenylacetaldehyde	-5.6
8-Methyl-alpha-ionone	-5.0
Propanoic acid, 2-methyl-3-[4-t-butyl] phenyl	-5.2
Morpholine,4-(1-cyclopenten-1-yl)-1-morpholino-1-cyclopentene	-5.3
Phenol, 2-methyl-5-(1-methylethyl)-carvacrol	-4.8
Urs-12-en-28-oic acid, 3-hydroxy-methyl ester, (3beta)	-4.6
Phenol, nonyl-nonylphenol	-5.4
Hexestrol	-5.3
Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo [4.1.0] hept-1-yl)-propenyl ester	-4.7
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester	-5.5
Palmitic acid	-4.2
(E,1'RS,2'RS,3'SR)-4-(2',3'-epoxy-2',6',6'-trimethylcyclohexyl)-3-methyl-3-buten-2-one	-5.1
Androstan-3-ol, 9-methyl-(3 beta,5 alpha)	-6.6
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-4.4
1-Alpha-18O-1,25-dihydroxycholecalciferol	-6.8
Ergosta-7,22-dien-3-ol, (3 beta,22E)	-4.1

Table 2. Non-covalent connections of compounds with specific proteins as well as their binding connections.

CID	Amino Acid Residues	Bond type	Distance (Å)	Docking score (Kcal/mol)
5280453	Phe62	H	4.57	-6.8Kcal/mol
	Thr65	H	4.66	
	Ile8	A	5.51	
	Ile55	A	5.51	
	Ile67	A	5.25	
	His112	PA	6.30	
	His112	PA	6.42	
	Phe10	PA	5.39	
	Tyr64	PA	5.07	
	Phe62	PA	3.52	
	Phe62	PA	4.91	
	Phe62	PA	5.56	
	Leu32	A	5.68	-6.6Kcal/mol
22215820	Leu32	A	5.98	
	Trp46	PS	4.52	
	Trp46	PA	5.46	
	Trp46	PA	5.04	
	Leu31	A	5.98	
	Leu31	A	4.76	
	Tyr39	PA	6.29	
	Tyr39	PA	4.26	
998	Leu26	PA	5.70	-5.6Kcal/mol
	Leu42	PA	5.36	
	Ala43	PA	4.90	
	Leu116	PA	6.55	
	Ile8	PS	4.58	-5.5Kcal/mol
62603	His112	PA	5.88	
	Phe10	PA	4.58	
	Cys9	A	5.47	

Where, H, A, PA, and PS symbolize hydrogen, alkyl, Pi-Alkyl, and Pi-sigma relations, respectively. The relationship distance is calculated in Å.

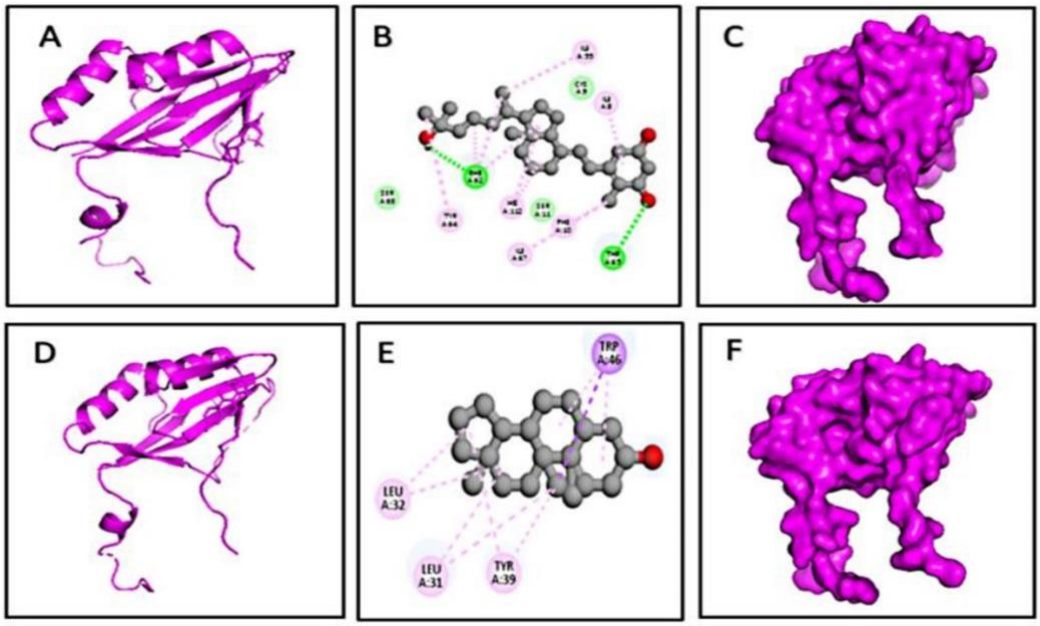

Figure 6. Molecular docking of the protein FosB (4nb2) and plant chemicals. Here, A, B, and C represent the Cartoon view, 2D view, and surface view of the CID-5280453 and FosB (4nb2) protein complex, respectively, and D, E, and F represent the Cartoon view, 2D view, and surface view of the CID-22215820 and FosB (4nb2) protein complex.

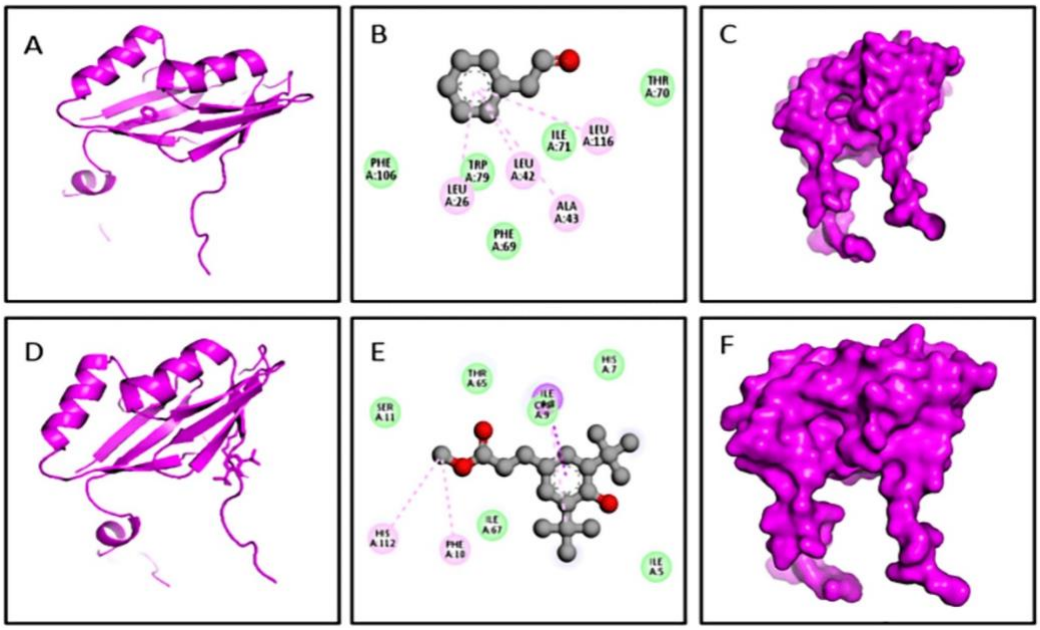

Figure 7. Molecular docking of the protein FosB (4nb2) and plant chemicals. A, B, and C represent the Cartoon view, 2D view, and surface view of the CID- 998 and FosB (4nb2) protein complex, respectively, and D, E, and F represent the Cartoon view, 2D view, and surface view of the CID- 62603 and FosB (4nb2) protein complex.

DISCUSSION

To serve as a defense against insects, fungi, and diseases as well as a treatment for human illnesses, medicinal plants manufacture hundreds of different chemical substances or compounds [32, 33]. The WHO has identified resistance to antibiotics as a national healthcare ultimatum to security which necessitates action from all levels of

the government and community [34]. Plant-based phytochemicals have newly sparked considerable attention as a possible cause of novel pharmacological and therapeutic applications against bacterial infections [35,36]. Phytochemical analysis of plant extracts revealed the presence of parts known to illustrate herbal and biological activities [37]. Phytochemicals such as phenolic acids, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, as well as alkaloids were discovered in leaf extracts. The phenolic compounds are among the most numerous and widespread groups of phytochemical constituents [38]. They have bioactivity such as anticarcinogen, anti-apoptosis, antiatherosclerosis, antiaging, anti-inflammation, as well as angiogenic [39]. Chromosomal DNA and 16S rRNA gene sequencing of the isolate was carried out for molecular identification and the isolated strains showed 99% similarities with *Staphylococcus aureus.* In this study, the leaf extracts of *Rumex dentatus* were found effective against *Staphylococcus aureus* and showed significant inhibition zone ranging between 11-14.5 mm. The antimicrobial activity, fungicidal, cytotoxic, antimutagenic, and medicinal prospects of *Rumex dentatus* leave extracts were evaluated. *Rumex dentatus* extracts were proven to be effective against a variety of infectious strains, with zones of inhibition ranging from 10 to 13 mm [12]. *Rumex dentatus* leaves extracts were screened on *Staphylococcus aureus* bacterial strains and indicated significant antibacterial activity [40]. The brine shrimp analysis can be used as a distinct probe for the pharmacological consequences of plant extracts [19]. The effective relationship between leaf extract density and the fatal rate of brine shrimp mortality was determined through the LC50 values of methanolic *Rumex dentatus* leaf extracts. According to Elzaawely *et al., Rumex dentatus* holds potential antioxidant activities that are correlated with its significant levels of phenolic compounds [41, 36]. Hence, they could be exploited as a natural source of antioxidants in the food industry. Numerous research has been done to determine the antioxidant effects of medicinal herbs that are rich in phenolics [42, 43].

These substances were compared to butylated hydroxyl toluene mostly based on how well they were able to neutralize DPPH free radicals (BHT), which was done to assess how effective an antioxidant they were. This plant's extracts scavenged free radicals in a dose-dependent process, correlating with the findings of Siddhuraju *et al.* [44] and the antioxidant activity also contain in the other plant metabolites such as flavonoids, tannins, catechins, and other phenolics. The highest superoxide anion radical scavenging activity of *Rumex dentatus* methanol extract agrees with the findings of Jayasri *et al.*, who found methanol to be the best superoxide radical scavenger at higher doses of plant extract [45].

Docking study evaluation is a reputable method for drug development [46]. By using molecular docking, we may clarify crucial metabolic processes while analyzing the behavior of small substances at the binding sites of target proteins [47,48,49]. Numerous studies can be undertaken as a result of the effort to find an inhibitor that is effective for the target proteins, and these studies are crucial for the development of computer modeling as well as for figuring out the dynamics of the ligand-protein complexes [50, 51]. Additionally, according to Ying *et al.,* docking calculations can be utilized to separate successful applicant composites from a range of ligand studies [52] since targeted proteins can be repressed by arresting the active site of proteins [53]. In order to find effective inhibitors from a variety of datasets, molecular docking and molecular dynamics analysis might be quite helpful. To investigate the binding interaction and find lead molecules *Rumex dentatus* with a greater affinity for the crystalline structure of the *S. aureus* protein's receptor-binding domain (PDB ID: 4nb2), molecular docking analysis was conducted in this case. Additionally, this study improves our comprehension of the relationship between ligand-protein and target binding affinity. *Staphylococcus aureus*, according to Bearman et al., is a leading cause of chronic

mortality throughout the world [54, 55]. Antibiotic-adjusting enzymes in *S. aureus* naturally attain resistance to a wide range of antimicrobial properties, resulting in a multi-drug-resistant bacterium [56]. The fosfomycin-inactivating enzyme which is known as FosB and depends on Mn2+ mainly found in *S. aureus*, and it play important role in adding either L-cysteine (L-Cys) or bacillithiol (BSH) to the antibiotic, and that's why the new compound is free of any bacteriostatic materials [57]. According to Kumar *et al., Rumex dentatus* is an affluent source of polyphenols and amino acids [58, 59]. The complexes of 1-Alpha-18O-1,25-dihydroxycholecalciferol and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester had non-bonded connections with the active points SER 11 and multiple connections all around effective points of the protein target, that could be liable for the suppression of the target molecule. These chemicals were found to form hydrogen bonds, alkyl bonds, pi-alkyl bonds, and pi sigma bonds. The active site of the target receptor established numerous linkages, implying that they could inhibit and disrupt the actions of these amino acids [60, 61]. Here, 1-Alpha-18O-1,25-dihydroxycholecalciferol; Androstan-3-ol, 9-methyl-(3 beta,5 alpha); phenylacetaldehyde; and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester compounds from *Rumex dentatus* showed the most effective result against FosB (4nb2) protein. As a result, compounds derived from *Rumex dentatus* plants may block the FosB protein's receptor domain. However, for ensuring the stabilization properties MD simulation is needed. Moreover, Wet-lab synthesis and more in vivo studies are needed to corroborate these findings.

CONCLUSION

MDR microbes seem to be a greater threat to human health and social assistance. Healthcare providers struggle to treat pathogen infections, along with those caused by *Staphylococcus aureus*. Plants have largely replaced synthetic drugs as an organic resource of health aid alleviation for their less reaction, and higher particularity. In the treatment of bacterial infections that are resistant to antibiotics, *Rumex dentatus* extracts may suppress some strains. In this research, *Staphylococcus aureus* was isolated from eczema sufferers and confirmed through morphological, biochemical, and molecular techniques. The strain was resistant to Penicillin, Ampicillin, Amoxicillin, and Cefuroxime. *Rumex dentatus* leaves extract in methanolic concentrations was used to control the identified strain and it demonstrated potent antibacterial activity. Interconnection between extract concentration and brine shrimp fatality is dependent on the cytotoxicity, indicating that at 200 µg/mL, brine shrimp mortality was greater than at other therapeutic doses, and the antioxidant test results showed that when it compared to the BHT control the leaf extract showed remarkable antioxidant properties. In molecular docking studies, the maximum target binding attractions for the strains were obtained for 1-Alpha-18O-1,25-dihydroxycholecalciferol, Androstan-3-ol, 9-methyl-(3 beta,5 alpha), Phenylacetaldehyde, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester, and this research could help future researchers to find an effective treatment for *Staphylococcus aureus* infections. More research on the safety characteristics of potential *Rumex dentatus* ingredients is required.

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AUTHOR CONTRIBUTIONS

MMZ: Experiment, conceptualizations, validations, data analysis; MMM: Experiment, conceptualizations, SI: Experiments, writing, editing, MMJ: Writing, data curation; JB: Writing, data curation; SB: validations, data analysis; AI: Data curation, data analysis; SZ: Review, editing; MAS: Review, editing, supervision; MSU: Conceptualization, editing, supervision, revision.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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