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RESEARCH ARTICLE OPEN ACCESS

Novel Phytosterol with Antimicrobial Potential of *Digera muricata* Mart. from Chittorgarh Region, India

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Abstract

***Digera muricata* Mart, a plant having therapeutic characteristics that has been utilised traditionally, belongs to the Amaranthaceae family, and a promising source of specific natural products utilized as antioxidant, prophylactic, antimicrobial, anthelmintic, anti-diabetic, and allelopathic agent. In the present study, a biologically active phytosterol was isolated from *Digera muricata* Mart. The isolated compound was characterized by 13C, 1H NMR, FTIR, and HRMS. Characterization of the isolate was done by antimicrobial assay, and molecular docking. The antimicrobial potential of the isolated phytosterol (50 µl) against *Streptococcus pyogenes* was found to be maximum (ZOI-20.0 ± 1.0), followed by *Streptococcus agalactiae* (ZOI-11.3±1.5), *Candida albicans* (ZOI- 09.0 ± 1.0), *Klebsiella pneumonia* (ZOI-8.6 ± 1.5) and *Escherichia coli* (ZOI-8.6 ± 1.5). The molecular docking results indicate that the phytosterol binds to the receptor 1AI9 at the 32th and 58th positions; 1KZN receptor at the 76th position, the 5L3J receptor at the 46th (ASN) and 136th (ARG) position; 7WIJ receptor at the 419th (ARG) and 582th (ASP) and 585th (ASN) positions.**

Keywords: Antimicrobial, Antifungal, Phytosterol, *Digera muricata*, Molecular Docking

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INTRODUCTION

Attention has been drawn to natural products based on plants as sustaining medicinal and health ailments due to the cost-effective treatment of chemically synthesized drugs, their toxicities in the sustainability of individual medical aid and development, and the exploitation of plant-mediated new medicines. Some reports on recent investigations and economic expenses, medicinal plants will ultimately continue to have a crucial role in developing as a health aid. These bioactive compounds based on therapeutic plants have a essential role in medical treatment and play a twin role in the innovation of such drugs as they are the backbone for designing such medicine, a natural blueprint for creating novel molecules or; natural products engaged in combating many diseases.[1] Therefore, these medicinal plants are acquired for the isolation of such compounds to gain knowledge about their underlying mode of action with reduced toxicity and cost-effective features.[2] The enhanced risk and toxicities of chemotherapeutics and the occurrence of multiple drug resistance mutants in pathogenic bacteria forced us to search for such novel drugs having antimicrobial potential.[2,3]

About experimental plant

As part of the family Amaranthaceae, *Digera muricata* Mart (*D. muricata*) grows in the wilderness and is a consumable plant. It is found across India, especially Rajasthan, Maharashtra and Andhra Pradesh.[4] *D. muricata* have many names, including Cancalisoppu, Latamouri or Gungutiya, Latmahuria, Lesua, False Amaranth, Latmahuria, Kunanjara, and Aranya. *D. muricata* is an annual medicinal plant growing 20-70 cm tall with long, narrowed, and pointed leaf stalks. Flowers grow to 30 cm in height and are spike-like racemes.[5] There is typically a greenish-white colour in fruit, with white flowers mixed with pink, carmine, or red. August and September are the months when flowers bloom. The tender shoots and foliage of *D. muricata* are utilised both medicinally and as a vegetable in the local community for the treatment of gastrointestinal diseases, diabetes, constipation, and bowel issues. Urinary discharges can be treated with flowers and seeds. The application of leaf paste prevented the local production of pus. The mother is given a boiled root infusion after childbirth for induced lactation.[6] *D. muricata* is also used as an expectorant and mild astringent.[7,8]

D. muricata is regarded as a laxative and a cooling astringent to the bowels in Ayurveda. Fungal infections were treated with the extract of the leaves of the plant.[9] People's health is directly benefited by phytochemicals found in traditional foods.[10] A study of *D. muricata* treatment provides evidence that it has antioxidant properties, is protective against CCl_4-induced toxicity, and may help treat diseases caused by free radicals.[11] Significant antiproliferative activity against MG-63 cell lines has been shown by the ethanolic leaf extract of *D. muricata*.[12] Recently, two compounds with herbicidal properties were isolated from *D. muricata*.[13] During the growing season, the roots of *D. muricata* contain sugars and phenols, while the leaves contain proteins, starches, and fats. In addition, the leaf extract has antimicrobial properties against *Escherichia coli* and *Fusarium oxysporum*. Kidney stones can be treated with leaf extract.[14] For a variety of male hormones in rats, CCl_4 disturbances were reversed by *D. muricata*.[15] By use of this herb as a substitute for secondary infertility.[16] *D. muricata* has a well-documented history of being an effective antibacterial.[17] The study was intended to extract, isolate and structurally characterize bioactive compound and its antimicrobial potential.

MATERIALS AND METHODS

Collection and verification of plant

The herb was gathered from Rajasthan's Chittorgarh district. The collected plant material was identified and deposited in Botanical Survey of India, Jodhpur, Rajasthan.

Isolation of compound using column chromatography

Column packing

To create an admixture, 16 g of ethyl acetate extract was combined with silica gel (60-120 mesh). The admixture combined with hexane was placed onto a column with a 2.4-inch diameter. Hexane was used as the starting solvent, followed by ethyl acetate to elute the column.

Fractionation of the extract

60 to 120 mesh size activated Silica gel used to fill the glass column. The extract was added to the column and gradually eluted by using 1000 ml of hexane of the ratio 100:0 v/v, 1200 ml of hexane: ethyl acetate mixture in the proportion of 95:5 v/v, 900 ml of hexane:ethylacetate in the proportion of 90:10 v/v, 900 ml of hexane:ethylacetate in the proportion of 85:15 v/v and 800 ml of hexane:ethylacetate in the ratio of 80:20, 400 ml hexane:ethylacetate in the proportion of 75:25 v/v, 800 ml of hexane:ethylacetate in the proportion of 70:30 v/v, 200 ml of ethyl acetate in the proportion of 0:100 v/v.

Thin-layer chromatography (TLC)

Concentrated fraction was placed onto the 20X20 cm activated silica gel TLC plates. The plates were created using various hexane:ethyl acetate ratios, including 90:10, 75:25, 50:50, and 25:75. The plate was subjected to vanillin and iodine fumes in order to identify any spots. A single spot denoted a pure compound with excellent purity and yield was selected for spectral analyses, and structures will be clarified.

Structure elucidation

High-Resolution Mass Spectrometry (HRMS) and Nuclear magnetic resonance (NMR) was exploits to draw the structures of the isolated compound. Thermo Scientific's Evolution 160 spectrophotometer was used to record UV spectra, while Perkin-Elmer's FTIR model 1600 spectrophotometer was used to record IR spectra. Tetramethylsilane (TMS) served as the internal standard while spectra of NMR were captured in $CDCl_3$ using a Bruker AMX 500 spectrometer at 500 MHz. Relative to the TMS internal standard, chemical shifts are expressed in ppm, and scalar coupling constants are described in Hz (J).

Antimicrobial susceptibility test

Growth conditions of microorganisms

In the current investigation, test microorganisms included *Streptococcus agalactiae* (ATCC13813), *Escherichia coli* (MTCC730), *Klebsiella pneumoniae* (MTCC432), and *Streptococcus pyogenes* (MTCC1924). In nutrient broth, the bacterial strains were resurrected. Each bacterium received a fresh inoculum for the antibacterial test, which was performed at 37°C for 24 hours. The nutrient broth was then added to the cell suspension to modify the turbidity until it was close to the 1.5×10^8 CFU/ml McFarland 0.5 standard.

In the current investigation, *Candida albicans* (MTCC7315) was utilised as a test fungus. It was kept at 4°C on potato dextrose agar (PDA) slants before usage.

Antibacterial susceptibility assay

The conventional disc diffusion technique was used to test the antibiotic sensitivity of bacterial strains to two antibiotics with potencies of 10 µg per disc, including gentamycin, and tetracycline.[18] Using the disc diffusion technique, the antibacterial test of isolated compound was conducted. Swabs of the test microorganisms were diluted to a concentration of 100 µl of diluted inoculum ($1.5X10^8$ CFU/ml) and added to plates of sterile Mueller Hinton agar (pH 7.2). 1 mg of isolated compound dissolved in DMSO used as stock. From stock 40 µl was added to the 4 mm diameter agar plate well. Pure DMSO used as negative control.

Antifungal susceptibility assay

Candida albicans inoculum was suspended in 5 ml of potato dextrose broth and cultured at 37°C for a day. The experiment was used to evaluate the antifungal activity using disc diffusion principle.[18] The inoculum was applied to the PDA medium with a glass spreader aseptically. Separately, 2.5 mm radius paper discs soaked with 40 µl from the stock was put on the infected plates' surface and kept at 37°C in the incubator for 24 hours. The diameter of the inhibitory zone was used to gauge the antifungal activity. Dimethyl sulfoxide (DMSO) was used as a negative control, and fluconazole served as the positive control. All experiments were run in triplicate.

Minimum inhibitory concentration

The Clinical and Laboratory Standards Institute (CLSI) recommendations and Eloff's microtitre broth dilution procedure[19] were followed to ascertain the isolated compound's Minimum Inhibitory Concentration (MIC). Initially,

isolated compound was prepared by dissolving it in dimethylsulphoxide (DMSO) to achieve a final concentration of 1 mg/ml.

A bacterial suspension with around $5X10^5$ CFU/mL was produced using a 24-hour culture plate and 100 µl of this suspension was introduce into every well of the microplates. For each strain, sterile environment and growth was controlled. The microtiter plates were incubated for 24 hours at 37°C for bacterial species and 48 hours for fungal strains. Following incubation, 40 µl of a 0.4 mg/ml solution of iodonitrotetrazolium chloride was added to every well as a microbial growth indicator. The plates were then incubated at 37°C for 30 min for bacteria and 24 h for fungi, and MIC values were visually determined. The minimum inhibitory concentration was noted as having the least amount of extract with no visible growth, while the MIC value was identified as the concentration completely inhibiting bacterial/ yeast growth (the first clear well).

To assess microorganism sensitivity, A negative control test utilizing only DMSO was also conducted and positive control experiments were performed for bacterial strains, gentamycin was used at a starting concentration of 0.10 mg/ml in sterile water, and for fungal strains, fluconazole was used at a starting concentration of 0.10 mg/ml in DMSO and water. Final concentrations for these experiments ranged from 0.1 mg/ml to 0.001 mg/ml.

Molecular docking study

The aim is to explain the behavior of small molecules within the binding site of target proteins. Employing the molecular docking technique, this investigation seeks to comprehend essential biological processes by reproducing the atomic-level interaction with a tiny chemical and a protein.[20] Using AutoDock, phytosterol was docked towards the binding site of the proteins. By setting the scaling factor for the nonpolar atoms to 0.8, the flexibility of the proteins was controlled. Default conditions were set for all other parameters. Docking scores were used to measure the binding affinity of protein/ligand complexes.

Receptor information

a) 1AI9: *Candida albicans* (Yeast)
Protein - Dihydrofolate reductase
Gene - DFR1
AA - 192

b) 1KZN: *Escherichia coli* (strain K12)
Protein - DNA gyrase subunit B
Gene – gyrB
AA – 804

c) 5L3J: *Escherichia coli*
Protein- DNA Gyrase b in complex with benzothiazole-based inhibitor
Gene- gyrB, Z5190, ECs4634
AA- 378

d) 7WIJ – *Macrophomina phaseolina* (strain MS6) (Charcoal rot fungus)
Protein - geranylgeranyl diphosphate synthase
AA – 698

RESULTS

Collection and verification of Plant

Collected herb was identified as *D. muricata* and deposited in Botanical Survey of India, Jodhpur, Rajasthan.

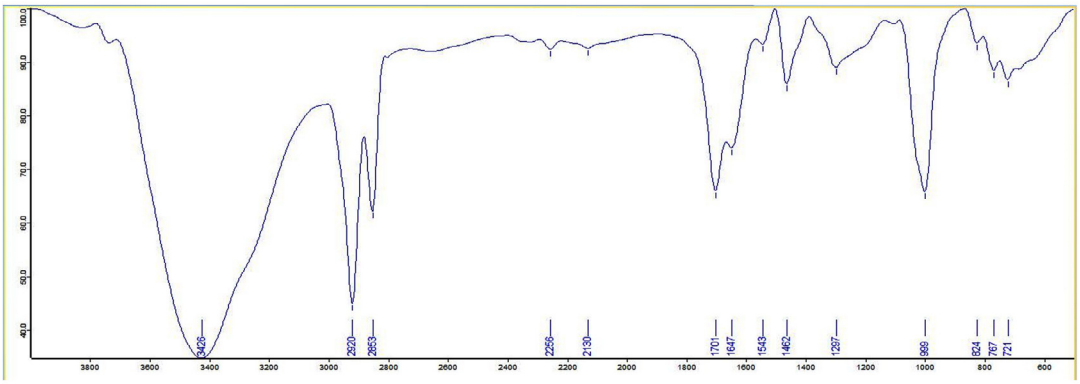

Figure 1. FTIR spectrum of the isolated compound

Isolation of compound using column chromatography

Fractions were collected from column chromatography and concentrated by the rotary evaporator. A pure portion was collected and used for further characterisation at the Hexane:Ethyl acetate (70:30) concentration.

Thin-layer chromatography (TLC)

Isolated pure compounds showing good purity and good yield was taken for spectrum studies and used for structure elucidation.

Characterisation of the isolated compound

The IR spectrum exhibited a band for H-bonded OH (3246 cm^{-1}), COOH (1701 cm^{-1}) and

Table 1. Antimicrobial activity of the isolated compound

Microorganism	Compound (50 µl) ZOI (mm) ± SD)	Compound (25 µl) ZOI (mm) ± SD)	Tetracycline (mm) ± SD)	Gentamycin (mm) ± SD)
Streptococcus agalactiae	11.3 ± 1.5	09.6 ± 0.7	14.1 ± 0.7	14.2 ± 0.6
Streptococcus pyogenes	20.0 ± 1.0	05.8 ± 0.7	11.4 ± 0.4	14.3 ± 0.7
Escherichia coli	08.0 ± 1.0	14.7 ± 0.6	14.2 ± 0.7	14.9 ± 0.1
Klebsiella pneumoniae	8.6 ± 1.5	20.6 ± 0.7	19.2 ± 0.6	19.2 ± 0.7
Candida albicans	09.0 ± 1.0	10.8 ± 0.6	-	-

Table 2. Minimum inhibitory concentration (MIC) of the isolated compound

Microorganism	Compound	Gentamycin	Fluconazole
	MIC values (mg/ml)		
Streptococcus agalactiae	10	2	NA
Streptococcus pyogenes	8	4	NA
Escherichia coli	12	1	NA
Klebsiella pneumoniae	16	0.5	NA
Candida albicans	20	NA	6

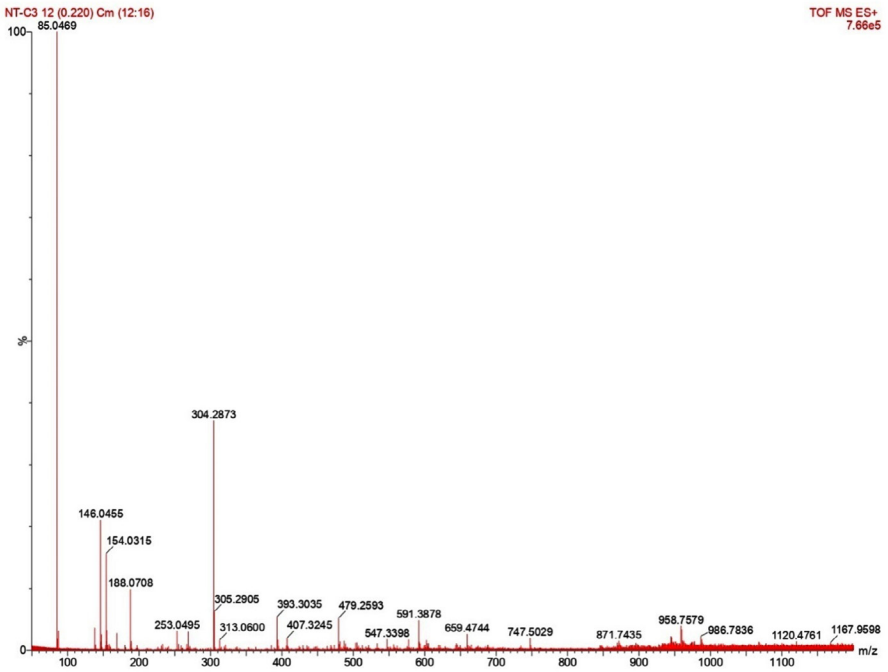

Figure 2. HRMS of the isolated compound

asymmetric stretching (2920 cm^{-1}), C-H stretching (2853 cm^{-1}), CH_2 scissoring (1462 cm^{-1}) and C-O stretching (999 cm^{-1}), C-H in plane and out of plane bending for benzene ring (824-721 cm^{-1}) (Figure 1). The isolated compound's HRMS, C-13, and proton NMR are presented in Figures 2, 3, and 4, respectively.

1H NMR (500 MHz, DMSO–d6) δ: 0.74–0.92 (s, 6H, $-CH_3$), 1.12-1.63 (m, 14H, $-CH_2$ of ring), 2.11-2.18 (t, 3H, -CH), 2.45 (s, 2H, $-CH_2$- of open chain), 3.22-3.30 (s, 6H,$-OCH_3$), 3.64-3.81 (m, 1H,-CHOH), 4.11-4.16 (d, 1H,-C-OH), 5.17-5.25 (t, 1H, =CH-), 11.91 (1H, s, -COOH) (Figure 5). HRMS; calculated for $C_{24}H_{38}O_5$, 407 (M+ + H). The ^{1}H NMR spectrum revealed the presence of a COOH (11.905 ppm), two methoxy groups (3.308 ppm), secondary methylene (2.45 ppm), one hydrogen directly attached to unsaturated group (5.254 ppm), one hydrogen attached to an electronegative oxygen atom (3.308 ppm) and three hydrogens attached to cyclic group (2.11 ppm) (Figure 4). Figure 5 shows the structure of the isolated compound.

Antimicrobial Susceptibility test

Antimicrobial activity

The antimicrobial potential of the isolated compound (50 µl) against *Streptococcus pyogenes* was found maximum (ZOI-20.0 ± 1.0) followed by *Streptococcus agalactiae* (ZOI-11.3 ± 1.5)*, Candida albicans* (ZOI-09.0 ± 1.0) *Klebsiella pneumoniae* (ZOI-8.6 ± 1.5) and *Escherichia coli* (ZOI-8.6 ± 1.5) as compared to tetracycline and gentamycin (Table 1).

MIC of the isolated compound

MIC outcomes for the isolated compounds and fractions are depicted in Table 2. The isolated compound exhibits higher antibacterial potential against *Streptococcus pyogenes* with MIC of 8 mg/ml as compared to the gentamycin *i.e.,* 4 mg/ml. MIC for *Candida albicans* found to be 20 mg/ml as compared to the fluconazole i.e., 6 mg/ml.

Molecular docking

1AI9_C3

The results indicate that the isolated phytosterol binds to the receptor 1AI9 at the

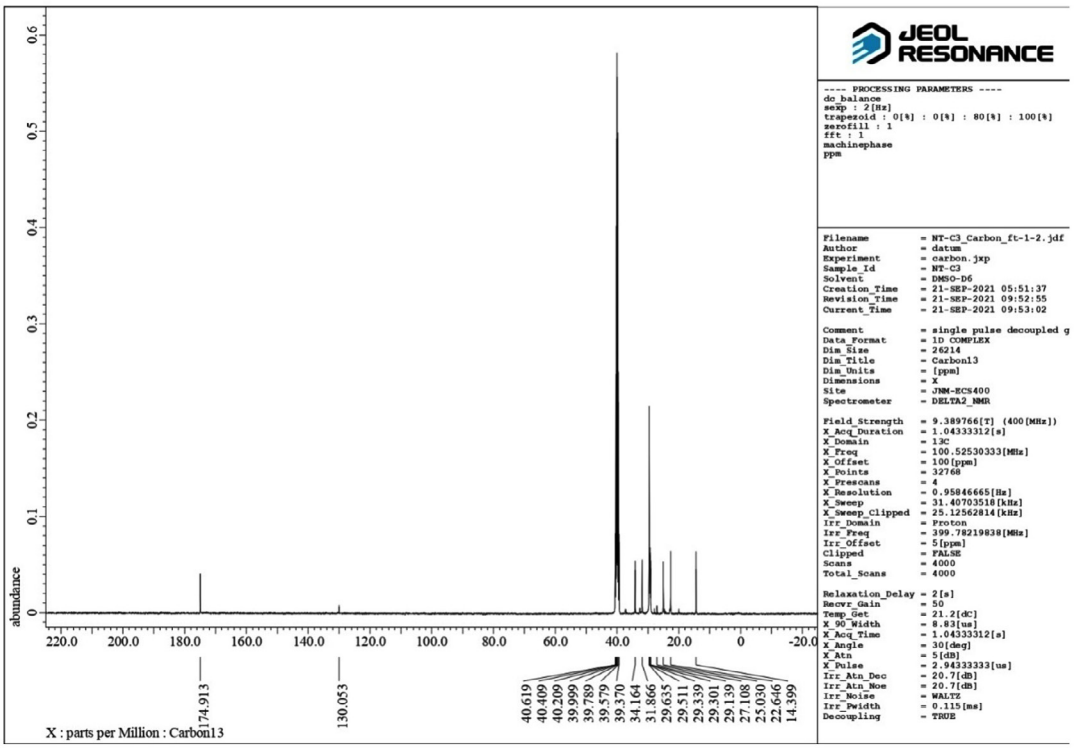

Figure 3. C-13 NMR of the isolated compound

32nd and 58th position, respectively. GLU at 32th position makes a substrate binding site, while THR at 58th position makes a binding site for NADP+. Both the amino acid forms a helix structure in the protein. The isolated molecule interacts with the side chains of both amino acids, with a docking score of -5.869. The docking score indicates a good interaction between the molecule and the receptor, while gentamycin and streptomycin bind more strongly to the receptor, with docking score of -7.15 and -9.338, respectively (Table 3, Figure 6).

1KZN_C3

The results indicate that the isolated phytosterol binds to the receptor 1KZN at the 76th position. ARG at 76th position makes an ATPase domain region. Two different oxygen atoms

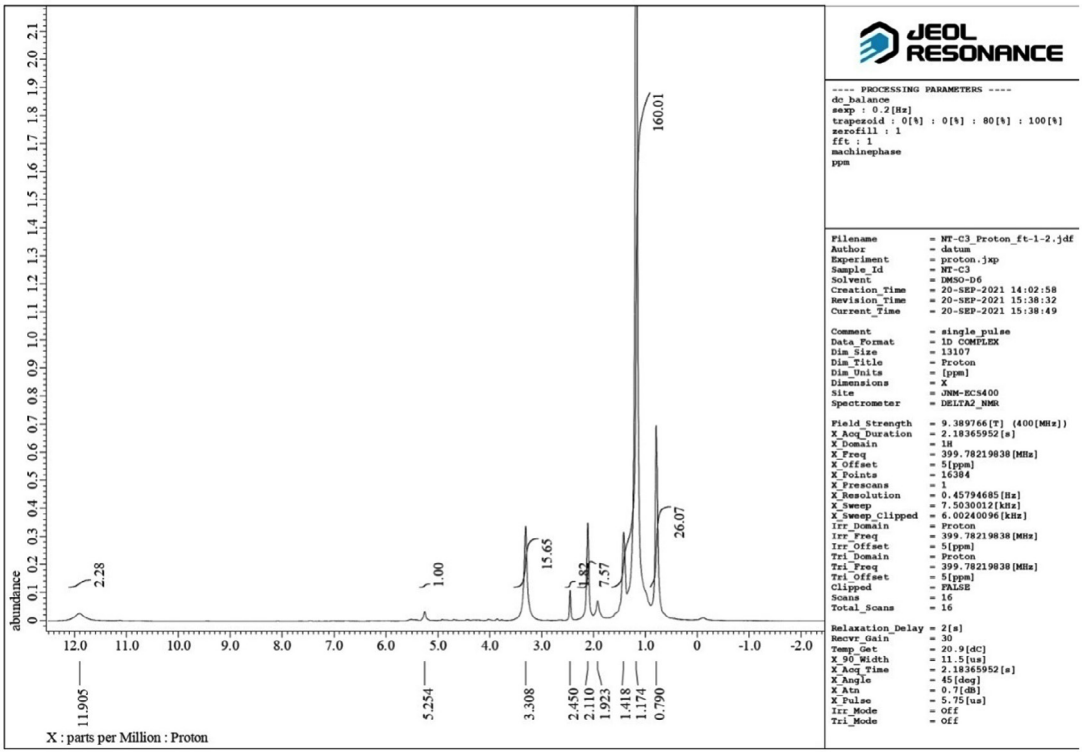

Figure 4. Proton NMR of the isolated compound

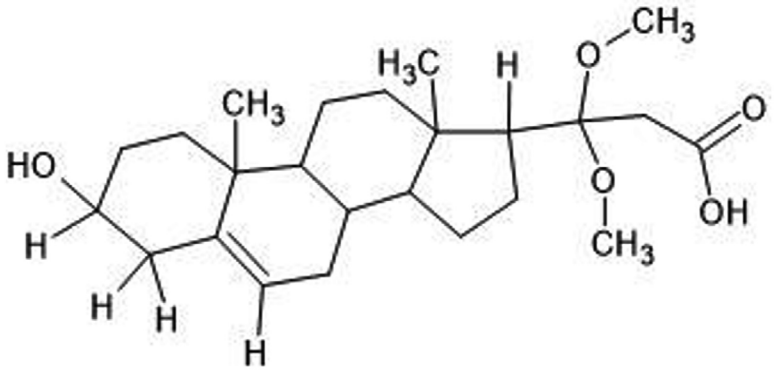

Figure 5. Structure of isolated phytosterol: 3-(2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3,3-dimethoxypropanoic acid

Table 3. Observation of docking

No.	Gene	Electron Donor	Electron Acceptor	AA Residue Position	Role of AA in Protein Feature	Secondary structure of protein	AA Residue	Binding site	Docking Score
1	1AI9_C3	OH		32	Binding site (substrate)	Helix	GLU	Side chain	-5.869
			O-	58	Binding site (NADP+)	Helix	THR	Side chain	
	1AI9_Gentamycin	NH		61	-	Helix	SER	Side chain	-7.15
			O	115	Binding site (NADP+)	Helix	ALA	Back bone	
			O	115	Binding site (NADP+)	Helix	ALA	Back bone	
		NH		118	Binding site (substrate)	Helix	TYR	Side chain	
	1AI9_Streptomycin	OH		11	Binding site (NADP+)	Beta strand	ALA	Back bone	-9.338
			OH	11	Binding site (NADP+)	Beta strand	ALA	Back bone	
		NH		19	Binding site (NADP+)	Beta strand	ILE	Back bone	
		NH		112	Binding site (substrate)	-	ILE	Back bone	
		OH		112	Binding site (substrate)	-	ILE	Back bone	
			OH	115	Binding site (NADP+)	Helix	ALA	Back bone	
2	1KZN_C3		O	76	ATPase domain				
	-	ARG	Side chain	-3.95					
			O-	76	-	-	ARG	Side chain	
	1KZN_Gentamycin	NH		46	Binding site (ADP)	Helix	ASN	Back bone	-3.068
		NH		50	-	Helix	GLU	Side chain	
	1KZN_Streptomycin	OH		46	Binding site (ADP)	Helix	ASN	Side chain	-7.039
		OH		46	Binding site (ADP)	Helix	ASN	Side chain	
		OH		46	Binding site (ADP)	Helix	ASN	Side chain	
		OH		90	-	Helix	ILE	Side chain	
			OH	96	-	Helix	ALA	Back bone	
			OH	120	Binding site (ATP)	Helix	VAL	Back bone	
3	5L3J_C3	OH		46			ASN	Side chain	-3.627
			O	136			ARG	Side chain	
		O (Covenant bond)		136			ARG	Side chain	
	5L3J_Gentamycin	NH		46			ASN	Side chain	-6.238
		NH		50			GLU	Side chain	
		OH		50			GLU	Side chain	
		NH		73			ASP	Side chain	

Table 3. Cont...

No.	Gene	Electron Donor	Electron Acceptor	AA Residue Position Feature	Role of AA in Protein	Secondary of protein Residue	AA structure	Binding site	Docking Score
	5L3J_Streptomycin	OH		46			ASN	Side chain	-6.282
		OH		46			ASN	Side chain	
		OH		50			GLU	Side chain	
		NH		97			VAL	Back bone	
		NH		97			VAL	Back bone	
4	7WIJ_C3	O- (Covalent bond)		419			ARG	Side chain	-4.507
		OH		582			ASP	Side chain	
			OH	585			ASN	Side chain	
	7WIJ_Gentamycin	OH		451			SER	Side chain	-5.052
			OH	517			GLN	Side chain	
			O	542			LYS	Side chain	
			O	578			GLN	Side chain	
			O	578			GLN	Side chain	
		NH		581			ASP	Side chain	
	7WIJ_Streptomycin	OH		451			SER	Side chain	-8.896
			OH	517			GLN	Side chain	
		OH		520			ASP	Side chain	
		NH		542			LYS	Side chain	
		NH		578			GLN	Side chain	
		OH		578			GLN	Side chain	
		NH		581			ASP	Side chain	
		NH		581			ASP	Side chain	

*More docking score indicates a more binding affinity with the receptor molecule

in the isolated molecule interact with the side chains of both amino acids, with a docking score of -3.95, which is more than the docking score of gentamycin (-3.068) and less than the docking score of streptomycin (-7.039). More docking scores indicate more binding affinity with the receptor (Table 3, Figure 7).

5L3J_C3

The results indicate that the isolated phytosterol binds to the receptor 5L3J at the 46th (ASN) and 136th (ARG) positions, respectively. The isolated molecule interacts with the amino acid's side chains, with a docking score of -3.627. The docking score of the isolated compound is

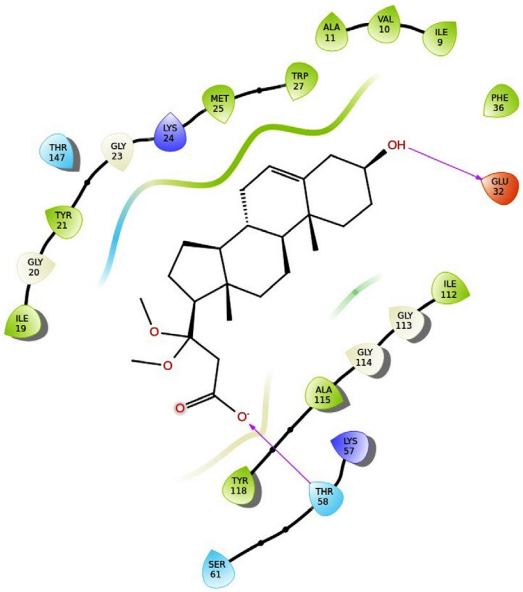

Figure 6. Isolated phytosterol binds to the receptor 1AI9 at the 32nd and 58th position

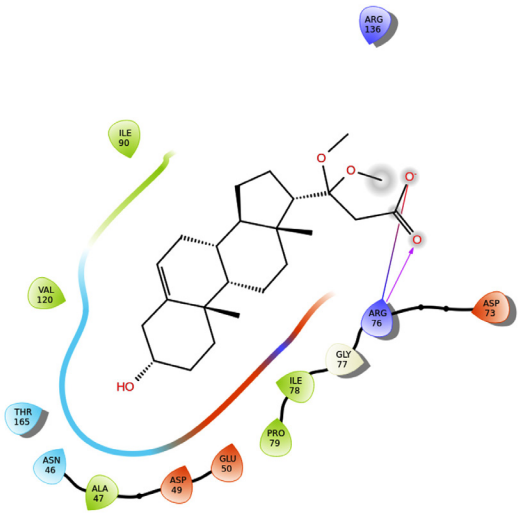

Figure 7. Phytosterol binds to the receptor 1KZN at the 76th position

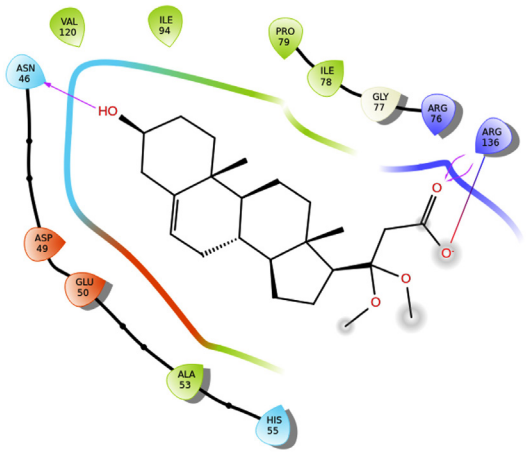

Figure 8. Phytosterol binds to the receptor 5L3J at the 46th (ASN) and 136th (ARG) positions

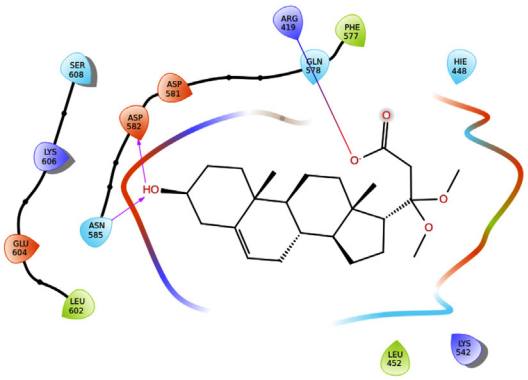

Figure 9. Phytosterol binds to the receptor 7WIJ at the 419th (ARG) and 582th (ASP) and 585th (ASN) positions

less than gentamycin (-6.238) and streptomycin (-4.507). Besides, all three molecules compound, Gentamycin, and Streptomycin interacts with the 5L3J rectors at the same position 46th amino acid residue ASN but the binding affinity of compound is less (Table 3, Figure 8).

7WIJ_C3

The results indicate that the isolated phytosterol binds to the receptor 7WIJ at the 419th (ARG) and 582th (ASP) and 585th (ASN) positions, respectively. The isolated molecule interacts with the side chains of all three amino acid, with a docking score of -4.507. The docking score of compound is less than the docking score of gentamycin (-5.052) and streptomycin (-8.896) (Table 3, Figure 9).

DISCUSSION

Digera muricata Mart. is a plant with a long history of traditional medicinal use in various cultures. Recently, there has been growing interest in exploring its bioactive compounds for potential therapeutic applications. One such compound that has garnered attention is a novel phytosterol found in *Digera muricata* Mart., which exhibits promising antimicrobial properties. The antimicrobial potential of phytosterols extracted from the leaf of *Annona squamosa*, *Adenocalymma alliaceum*, and *Amaranthus tricolor* was examined. These phytosterol was isolated, purified, characterized, and assessed for their antibacterial effects on both gram-positive bacteria, like *Staphylococcus aureus*, *Staphylococcus albus*, and *Streptococcus viridians*, and gram-negative bacteria, including *Escherichia coli*, *Pseudomonas pyocyanea*, and *Klebsiella*.[21] The study by Monu *et al*. in 2008 also investigated the antimicrobial potential of phytosterols in milk.[22]

Furthermore, a bacteriostatic experiment involving phytosterols derived from pumpkin seeds revealed significant antagonistic actions against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Salmonella*. Complete inhibition of bacterial at a dosage of 3.0 mg/mL, proliferation was seen.[23] Numerous phytochemicals contained in *D. muricata* have been shown to have potent anti-inflammatory and powerful free radical scavenging effects.[24] *D. arvensis* (a synonym of *D. muricata*) demonstrates notable antibacterial activity against *Ralstonia solanacearum* (previously known as *Pseudomonas solanacearum*), with a mean zone of inhibition measuring 6.14 mm.[25]

Present evidence from studies demonstrating the antimicrobial activity of the novel phytosterol. The discovery of a novel phytosterol with antimicrobial potential in *Digera muricata* Mart. opens up exciting possibilities for harnessing its health-promoting effects. The antimicrobial properties of this phytosterol hold promise for addressing oxidative stress-related conditions, and further research may unveil its full therapeutic potential. Integrating this compound into functional foods and pharmaceuticals could offer innovative strategies for promoting health and preventing diseases associated with oxidative damage. However, ongoing exploration is necessary to fully recognize its mechanisms of action, assess safety, and improve its applications for human health.

CONCLUSION

The isolated compound was characterized by 13C, 1H NMR, FTIR, and HRMS and subjected to antimicrobial assay, and molecular docking study. The antimicrobial potential of the isolated phytosterol against *Streptococcus pyogenes* was found to be maximum (ZOI-20.0 ± 1.0). The molecular docking results indicate that the phytosterol binds to the receptor 1AI9 at the 32th and 58th positions; 1KZN receptor at the 76th position, the 5L3J receptor at the 46th (ASN) and 136th (ARG) position; 7WIJ receptor at the 419th (ARG) and 582th (ASP) and 585th (ASN) positions. This study showed that isolated phytosterols might exploits as potential antibacterial agents.

The novel phytosterol isolated from *Digera muricata* Mart. represents a promising avenue for the development of antimicrobial agents. Its unique properties and demonstrated efficacy against various microorganisms make it a valuable candidate for further exploration. However, more research is required to know its mechanisms, assess its safety, and optimize its potential applications in clinical settings. The discovery of this novel phytosterol opens up new possibilities for combating microbial infections and addressing the challenges of antibiotic resistance

in modern medicine. Propose future research directions, including further investigations into the specific molecular pathways affected by the phytosterol and optimization of delivery methods.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SM conceptualized and designed the study. NT, AKS, HN, RD, AS performed analysis. ZZ performed data interpretation. MIA and MM wrote the manuscript. All authors read and approved the final manuscript for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Jain SK. Dictionary of Indian folk medicine and ethnobotany. 1991.
2. Bozovic M, Mladenovic M, Ragno R. Chemical composition and antimicrobial activity of essential oils. *Front Pharmacol.* 2023;14:1120756. doi: 10.3389/fphar.2023.1120756
3. Rolnik A, Stochmal A, Beata O. The *In vitro* antiplatelet activities of plant extracts from the Asteraceae family. *Biomed Pharmacother.* 2022;149:112809. doi: 10.1016/j.biopha.2022.112809
4. Aravindhan V, Rajendran A. Diversity of invasive plant species in boluvampatti forest range, the southern Western Ghats, India. *Am-Eurasian J Agric Environ Sci*. 2014;14:724-731.
5. Jagatha G, Senthilkumar N. Evaluation of anti-diabetic activity of methanol extract of *Digera muricata* (l) mart in alloxan induced diabetic rats. *Int J Pharm Sci Res*. 2011;2(6):1525-1529. doi: 10.13040/IJPSR.0975-8232.2(6).1525-29
6. Miah MM, Das P, Mridha SA, Kuddus MR, Rashid MA. Bioactivities of *Digera muricata* (L.) Mart. Available in Bangladesh. *Dhaka Univ J Pharm Sci*. 2017;16(2):251-254. doi: 10.3329/dujps.v16i2.35264
7. Parrotta JA. Healing plants of peninsular India. CABI Publishing, Wallingford, UK. 2001. doi: 10.1079/9780851995014.0000
8. Shah A, Marwat SK, Gohar F, et al. Ethnobotanical study of medicinal plants of semi-tribal area of Makerwal and Gulla Khel (lying between Khyber Pakhtunkhwa and Punjab Provinces), Pakistan. *Am J Plant Sci.* 2013;4:98-116. doi: 10.4236/ajps.2013.41015
9. Chandra M, Mahesh NM. Antifungal activity of medicinal plant extracts against seed-borne pathogenic fungi, Acta Biol Indica, 2013;2:481-483. http:// bioscipubl.com/journals/abi/pdf/481-483.pdf
10. Palozza P, Krinsky, NI. β-Carotene and α-tocopherol are synergistic antioxidants. *Arch Biochem Biophys*. 1992;297(1):184-187. doi: 10.1016/0003-9861(92)90658-J
11. Usmani S, Hussain A, Farooqui AHA. Pharmacognostical and phytochemical analysis of *Digera muricata* Linn. growing as a weed in fields of Uttar Pradesh region of India. *Int J Pharm Sci*. 2013;5(suppl 1):142-145.
12. Selvi VP, Roy A, Jagadeswaran D. Anticancer Property of *Digera muricata* Leaf Extract: An *In vitro* Study. *Cureus.* 2023;15(11):e49276. doi: 10.7759/cureus.49276
13. Akbar M, Raza A, Khalil T, Yasin NA, Nazir Y, Ahmad A. Isolation of herbicidal compounds, quercetin and β-caryophyllene, from *Digera muricata*. *Arab J Chem.* 2023;16(5):104653. doi: 10.1016/j.arabjc.2023.104653
14. Sharma N, Tanwer BS, Vijayvergia R. Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. *Int J Pharmtech Res.* 2011;3(1):110-113.
15. Khan MR, Ahmed D. Protective effects of *Digera muricata* (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. *Food Chem Toxicol.* 2009;47(6):1393-1399. doi: 10.1016/j.fct.2009.03.020
16. Chettleborough J, Lumeta J, Magesa S. Community use of non-timber forest products: A case study from the Kilombero Valley Frontier Tanzania. Kilombero Valley Integrated Environmental Management Programme. The Society for Environmental Exploration, UK & The University of Dar es Salaam. 2000. https://www.easternarc.or.tz/wp-content/uploads/2019/12/FT-KV-NTFP-report.pdf
17. Mathad P, Mety SS. Phytochemical and Antimicrobial Activity of *Digera Muricata* (L.) Mart. *E J Chem*. 2010;7(1):275-280. doi: 10.1155/2010/509254
18. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am J Clin Pathol.* 1966;45(4):493-496. doi: 10.1093/ajcp/45.4_ts.493
19. Eloff J. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med.* 1998;64(8):711-713. doi: 10.1055/s-2006-957563
20. McConkey BJ, Sobolev V, Edelman M. The performance of current methods in ligand-protein docking. *Curr Sci.* 2002;83(7):845-856.
21. Sharm RK. Phytosterols: Wide-spectrum antibacterial agents. *Bioorganic Chem.* 1993;21(1):49-60. doi: 10.1006/bioo.1993.1007

22. Monu E, Blank G, Holley R, Zawistowski J. Phytosterol effects on milk and yogurt microflora. *J Food Sci.* 2008;73(3):M121-M126. doi: 10.1111/j.1750-3841.2008.00668.x
23. Xu YQ, Pang LP, Qi HJ, Yang Y, Yang YL. Antioxidant activity and antibacterial effect of phytosterol from pumpkin seeds. Acad. *Periodical Farm Prod. Process.* 2012;5:14-16.
24. Ravi S, Duraisamy P, Krishnan M, Martin LC, Manikandan B, Ramar M. Sitosterol-rich Digera muricata against 7-ketocholesterol and lipopolysaccharide-mediated atherogenic responses by modulating NF-B/iNOS signalling pathway in macrophages. *3 Biotech.* 2023;13(10):331. doi: 10.1007/s13205-023-03741-6
25. Taran SNU, Ali SA, Haq NU, et al. Antioxidant and antimicrobial activities, proximate analysis and nutrient composition of eight selected edible weeds of Peshawar region. *J Xi'an Shiyou Univ, Nat Sci.* 2022;18(9):517-545.