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Original Research Article

Antibacterial, Antioxidant Activities, GC-MS Analysis and Docking Studies of *Guiera senigalensis* (L.) Ethanol Leaves Extract

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ABSTRACT

The increment of Antimicrobial irrational use leads to the exacerbation of infections. The present study is designed to evaluate the antibacterial, and antioxidant activities of *Guiera senigalensis* ethanolic leaves extract and to determine the components of the extract for docking studies. *G. senigalensis* extract was used against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, and *Pseudomonas aeruginosa* ATCC27853. The antimicrobial and antioxidant activities and active ingredients were determined using standard methods, computational studies were conducted via Maestro v12.8 of Schrodinger2020. Antibacterial activity of the crude extract showed that *S. aureus* was the most sensitive strain with MIC and MBC (12.5 and 25) mg/ml respectively followed by *P. aeruginosa* MIC and MBC (25 and 50) mg/ml. While *E. coli* was found to be resistant. In the DPPH radical scavenging assay of the extract and quercetin at concentrations (250, 125, 50, 10, and 5) µg/ml. The radical scavenging activity was 72.5%, 63.5%, 50.5%, 45.5%, and 31.3% for the extract, compared to quercetin which gave 89.7%, 85.8%, 62.1%, 55.5%, and 45% respectively. The GC-MS analysis of the total constituents revealed that 9H-Xanthene-9-one, 1,3,5,6-tetramethoxy- (28.81%), followed by phytol (22.74%), n-Hexadecanoic acid (11.3%) and 1,2,3-Benzenetriol (9.13) as major components. The docking studies of these major constituents support the antibacterial activity as they exhibited high binding affinity to UPPS targeted enzyme. Finally, this research provided useful information on the therapeutic potential of *G. senegalensis*. The major constituents could be considered potential antibacterial agents against *S. aureus* and recommended to be evaluated against MDR Bacterial strains.

Keywords: *Guiera senigalensis*, Antibacterial, Antioxidant, GC-MS, *In-silico* .

Introduction

Infectious diseases are the second most important cause of morbidity and mortality globally.[1] Regarding Antibiotics in the past present and future, and as found in the literature over 100 years, where the discovery of penicillin by Alexander Fleming in 1928 started the golden era of natural product antibiotics discovery that peaked in the mid-1950s.[2] After that, a gradual decline in antibiotics discovery and rising in Antibiotics resistance in different pathogenic organisms lead to the current crisis of Antimicrobial resistance.[2,3] Nevertheless, the emergence of multidrug-resistant (MDR) bacteria demands innovations in the evolution of new antimicrobial agents to combat the superbug.[3]

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The future of Antibiotics discovery may look bright as drug delivery and nanotechnologies are used to manipulate the resistant gene to discover new natural products with various bioactivities.[2]

The increment of inappropriate use of Antimicrobial agents including self-medication leads to exacerbation of infections,[4,5] with a specific high prevalence of MDR infections of bacterial pathogens.[6] Especially the increasing of MDR Gram-negative bacteria lead to significant health problems globally, since the wide use of broad-spectrum Antimcrobial agents such as Penicillins, Fluoroquinolones, Aminoglycosides, and *β*-lactams, such as; Carbapenems, Monobactam, and Cephalosporins; which usually fail to combat the MDR Gram-negative bacteria,[7] that include *E. coli* and *P. aeruginosa* the most prevalent respiratory pathogen in immunocompromised patients.[4] Moreover, the treatment of severe infections is caused by Gram-positive bacteria and since the 1980s the threat of resistant strains has been increased with life-threatening consequences.[8] The MDR bacteria genetically overcome the available antibiotics stress, generating challenges for the treatment of both Hospital and community-acquired infections.[6]

Previous studies stated extensive information about some medicinal plants which possess antibacterial activity that has been scientifically studied and popularly used in folk medicine.[9-14] Whereas, there phytochemical constituents with antibacterial activities and therapeutic

value in the treatment of bacterial infections were though investigated. [15, 16]

G. senegalensis used traditionally for the treatment and or management of many diseases which include diabetes infectious diseases, jaundice, arthritis, fever, diarrhea, gastrointestinal and respiratory diseases, and cancer.[17,18] The leaves of *G. senegalensis* were found to possess many bioactive compounds which include antioxidants in high content of polyphenols, tannins, flavonoids, glycosides, and essential oils.[19, 20]

Computer-aided drug design (CADD) is an effective tool for discovering promising treatment candidates. It has become an essential component of drug design due to its ability to quicken medication development by utilizing current knowledge and theories on receptor-ligand interactions. [21]

This study is designed to bridge the existing research gaps in the field of folk medicine, and to make recommendations built on existing knowledge of benefits derived from a medicinal plant. The research was designed to investigate the antibacterial and antioxidant activities, phytochemical analysis, and docking studies of *G. senegalensis* alcoholic leaves extract.

Materials and Methods

Plant materials

Collection of the plant material and preparation of the extract

The leaves of *G. senegalensis* which are locally known as Gubeish were collected in June 2022 from Al-nihood Cty, Western Kordofan State, Sudan. Then authenticated by Prof. Alhadi Mohamed Mohamed Ahmed Ali, with the voucher number: (G 1) deposited in the Herbarium unit of the Medicinal and Aromatic Plants Research Center (MAPRC) at the University of Gezira, Sudan After thoroughly washing, the leaves were dried at room temperature. 50 g of the powdered *G. senegalensis* leaves were extracted by maceration using ethanol 96% (500 ml) at room temperature for 3 consecutive days with intermittent shaking. The plant extract was filtered through a Whatman No. 1 filter paper using a Bukhnur funnel vacuum. The filtrate was collected and evaporated using a rotary evaporator at 60ºC under reduced pressure to produce dry extract. [22]

Determination of the extract Yield

The evaporated dried extract on a dry weight basis (crude extract) was calculated by the following equation:

$$(X = \frac{N}{N0}\ X100\%$$

Where N0: the weight of the dry plant material loaded for extraction
N: the weight of the extract after the solvent evaporation. [22]

Chemicals and reagents

The Antibiotics used for this study include Ceftriaxone 30 MCG, Item No SD065-5CT, Cat HIMEDIA*, Meropenem 10 MCG, Item NO SD727-5CT, Cat HIMEDIA*, Vancomycin 30 MCG, Item No SD045-5CT, Cat HIMEDIA*. All solvents used for this experiment including Ethanol (99.9%) and Methanol (99.9%) were of high purity (Research lab, India). The purified distilled water was prepared in the Quality control laboratory, Faculty of Pharmacy, University of Gezira, Sudan. All other chemicals were of analytical grade.

Equipment and instruments

The sensitive balance (BOECO, Germany), the water bath (Scott Science, U.K), the autoclave (Modle: YX-280A, Volume: 18 L, Pressure: 0.14MPa-0.16MPa), Incubator (BACTERIOLOGICAL INCUBATOR i-therm Al-7741) and the Hot air oven (ELECTROTHERMAL Thermostat Dryer Model: G2X-DH-300 BS, Power: 1200 W, Voltage: 220, V: 50 Hz, Date: JUL 2011).

Bacterial strains

Standard strains of Gram-positive Bacteria *S. aureus* ATCC 25923, Gram-negative *E. coli* ATCC 25922 and, *P. aeruginosa* ATCC 27853 were obtained from the Medical laboratory and blood transfusion safety services administration, General directorate of curative medicine, Ministry of Health, Khartoum, Sudan (March 2022). The bacterial pathogens were chosen based on the *WHO* recommendation for the priority pathogens according to their antibiotic resistance to encourage research and development of new antibiotics. [23]

Preparation of bacterial inoculums

The 24 h old culture of Bacterial standard strains was emulsified in sterile nutrient broth media.

In vitro antimicrobial screening

The antimicrobial screening was performed by using the agar well diffusion method and as mentioned by Manilal, [24] with few modifications were considered, where sterile Mueller Hinton's agar media (HIMEDIA) was dispensed into sterile Petri dishes and uniformly, seeded with 100 µl of a suspension containing 1.5×10^8 CFU/ml of appropriate standard strains of Gram-Positive Bacteria *S. aureus* ATCC 25923, Gram-negative *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 using a swab stick.[19] The inoculums were previously refreshed from overnight cultures by the direct colony method. Where a single colony was picked up directly from the plate with a sterile wire loop and suspended into the sterile nutrient broth. The turbidity of the suspension to be inoculated was equivalent to 0.5 McFarland's standard solution. After that, the tested organisms were uniformly swabbed over the surface of Mueller-Hinton agar. Then punched with the back for sterile blue tips of a graduated pipette to form 7 mm diameter wells, which were filled with the 100 µl of the appropriate extract of a known concentration (50 mg/ml and 25 mg/ml) separately in correspondence to the stock solution 50 mg/ml that prepared by dissolving (500 mg of the dried crude extract into 10 ml of 75% methanol in distilled water) and solvent blank (Methanol 75% in distilled water) used as negative control separately where the positive control used standard Antibiotics disk placed on the surface of the medium.[24] Vancomycin was used for *S. aureus* ATCC 25923, Ceftriaxone for *E. coli* ATCC 25922, and Meropenem for *P. aeruginosa* ATCC 27853. The plates were then incubated at 37ºC overnight. After incubation, the zone of the growth inhibition was measured in millimeters (mm). Each experiment was performed in triplicate. to validate the findings statistically. [24]

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antimicrobial activities of *G. senigalensis* were further investigated by micro-dilution method to determine the minimum inhibitory concentration (MIC) where the method approved by Parvekar,[3] Cheng, [6] Hussain, [25] Carrol, [26] was adopted with few modifications,[3,6,25,26] For this purpose, stock solutions of *G. senigalensis* extract 50 mg/ml, Mueller-Hinton broth, and Bacterial strain suspension equivalent to 0.5 MacFarland's standard solution were prepared following standard methods. [6] Serial dilution of (50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml) was made in the tubes containing the broth media except for the last one which inoculated with 100 µl of the solvent to be considered as the negative control and the all different tubes were inoculated with 100 µl of the bacterial suspension. Subsequently, all concentrations that showed no change in color were transferred onto nutrient agar and incubated at 37°C overnight, also the concentration exposed a turbidity was subculture in the agar plate as a confirmatory test, the lowest concentration with no growth of bacteria was considered as MBC.[27] The MBC was determined as the lowest concentration that eliminate 99.9% of the initial bacterial population.[3]

Evaluation of antioxidant characteristics

DPPH assay

DPPH radical scavenging activity of *G. senigalensis* leaves extract was measured following previously reported methods with some modification.[29] Sample stock solutions (0.1 g/100ml) were diluted to final concentrations of 250, 125, 50, 10, and 5 µg/ml in methanol. 1.0 ml of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in methanol solution was added to a 2.5 ml solution of the different concentrations of the extracts and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below:

$$(Antioxidant\ Activity\ (inhibition\ \%) = \frac{AC - AS}{AS} \times 100\%$$

where AC: The absorbance of a control solution,
AS: The absorbance of standard or sample solution.
Each sample and standard were measured in three replications.

Methanol (1.0 ml) plus plant extract solutions (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as a control. Stock solution (1 mg/ml) of Quercetin was diluted to final concentrations of 250, 125, 50, 10, and 5 µg/ml in methanol and used as a positive control.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis
The ethanolic extract of *G. senegalensis* leaves was analyzed for its chemical composition using GC-MS systems. The GC-MS analysis was performed on Shimadzu (GC\MS 2010) Helium was used as carrier gas and the separation was achieved using a column (TG-SQG-15 ms×0.25 mm×0.25 µm). The starting oven temperature was programmed at 60°C with an increasing temperature10°C until reached 280°C. The crude extract was injected with split mode. Mass spectra were taken at 70eV; fragments from 40 to 1000 Dalton. The final confirmation of constituents was made by computer matching the mass spectra peaks with the Wily and National Institute Standard and Technology (NIST) Libraries mass spectra database (Biomedical Research 2017).

In silico design
Molecular docking
The mechanism of anti-bacterial drugs generally includes wall synthesis inhibition, protein synthesis inhibition, DNA gyrase inhibition of nucleic acid synthesis, and anti-metabolism. The antibiotics inhibit these routes by interacting with specific cell proteins which are responsible for specific activity.[25,29]
The steps involved in the docking process are as follows:
Preparation of ligands and proteins
The structure of Undecaprenyl diphosphate synthase (UPPS) of *S. aureus* (PDB ID: 4H8E) from RCSB PDB. Using the protein preparation wizard of Schrodinger software, water molecules and ligands already present in the proteins were removed; hydrogen atoms were added and saved in PDB format. The role of (UPPS in the biosynthesis of the cell wall of *S. aureus* is very significant. UPPS is important since it is vital for the formation of peptidoglycan. Also, UPPS is not present in humans and is additional merit for the development of good antibacterial agents. The binding site was determined around the pre-existing ligand. Molecular docking calculations were carried out with the aid of the Glide of Schrodinger 2020 and MM-GBSA binding free energy of the protein—ligands were calculated using the Prime software.

Data organization and statistical analysis
The Data was organized and tabulated by using Microsoft Word 2016 and Microsoft Excel 2016. The experiments were carried out in triplicates and the average of the clear zone of inhibition and standard deviations (SD) were obtained as mean and standard deviation (M±SD).

Results and Discussion

The extractive value/ yield from G. senegalensis leaves obtained by maceration in alcohol was found to score (38.94%). Ethanol was used as a solvent for its ability to extract a vast range of compounds of different polarities Saha. [30] The high yield obtained indicated that alcohol (96%) is capable to extract phenolic compounds in which *G. senegalensis* is rich.
As shown in Table 1, the Antibacterial activity of *Guiera senigalensis* ethanolic extract (50 mg/ml) was evaluated against three standard strains: *S. aureus ATCC 25923*, *E.* coli *ATCC 25922 and P. aeruginosa* ATCC 27853 by well diffusion method. The results revealed that *S. aureus ATCC 25923 and P. aeruginosa* ATCC 27853 were sensitive with a diameter of (20.00 ± 0.50 mm and 27.33 ± 1.52 mm) respectively in similarity to the sensitivity reported by, [31] the positive control for *P. aeruginosa was (*Meropenem 10mcg which exposed 29.33 ± 1.15 mm) (Table 1) also on the sensitive rage reported in CLSI. [31] On the other hand, *G. senigalensis* ethanolic extract was devoid of such activity against *E. coli ATCC 25922 which showed only* 11.50 ± 0.86 mm *compared to the positive control Ceftriaxone*. The sensitive strain was further tested by reducing the concentration of the extract to 25 mg/ml. The result showed that both *S. aureus* and *P. aeruginosa* strains were sensitive with a diameter of 17.83 ± 1.15 mm and 27.33 ± 0.76. Nevertheless, the *determination of MIC* (50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml) conducted by tube dilution technique and the MBC by agar diffusion, present that the MIC *of G. senigalensis* extract against the standard strain of *S. aureus* was 12.5 mg/ml and for *P. aeruginosa* was 25 mg/ml. The above-mentioned findings vitrified the uses of *G. senegalensis* for the treatment of wound infections, and respiratory diseases. Also, the results harmonized with the study presented that the leaves of *G. senegalensis* are used traditionally for pulmonary and respiratory problems.[19]
The DPPH radical scavenging assay of *G. senigalensis* leaves extract showed dose-dependent activity with EC50: 7.74 µg/ml (Table 2), compared to that of the standard Quercetin. Results coincide with those reported earlier by Parvez,[21] and confirmed the good antibacterial potential of *G. senegalensis* extract.[20]

Table 1: Antimicrobial Susceptibility of *Guiera senigalensis* Ethanol Extract dissolved in Methanol 75% against different Bacterial Strains vs suitable Antibiotics

Bacterial strain	Mean Zone of inhibition (mm) ±SD				
	***G. senigalensis* Extract Conc.**	**Mean Zone of inhibition (mm) ±SD**	**Susceptibility S/R**	**Antibiotics (+ve control)**	**Mean Zone of inhibition (mm) ±SD**
S. aureus ATCC 25923	50 mg/ml	20.00 ± 0.50	S	Vancomycin 30 mcg	21.33 ± 6.11
E. coli ATCC 25922	50 mg/ml	11.50 ± 0.86	R	Ceftriaxone 30 mcg	29.00 ± 2.00
P. aeruginosa ATCC 27853	50 mg/ml	27.33 ± 1.52	S	Meropenem 10 mcg	29.33 ± 1.15
S. aureus ATCC 25923	25 mg/ml	17.83 ± 1.15	S	Vancomycin 30 mcg	17.66 ± 1.52
P. aeruginosa ATCC 27853	25 mg/ml	27.33 ± 0.76	S	Meropenem 10 mcg	31.33 ± 0.57

*S: Sensitive R: Resistance
*N. B: Mean Zone of Inhibition in CLSI (mm): *S. aureus* ATCC 25923=17-21, *E. coli* ATCC 25922= 29-35, *P. aeruginosa* ATCC 27853 = 27-33

The chemical composition of *G. senegalensis* leaves extract was analyzed by using GC-MS systems. The separation of the total constituents revealed that 9H-Xanthene-9-one, 1,3,5,6-tetramethoxy- (28.81), phytol (22.74%), n-Hexadecanoic acid (11.3%) and 1,2,3-Benzenetriol (9.13%), were the major constituents of 71.98% abundance (Table 3). These identified components in *G. senegalensis* leaves extract are known to possess high antioxidant activity, [19, 32] as well as antibacterial effects.[17,18,33,34] The presence of the Xanthene derivative which known as bioactive with antibacterial activity according to Souza. 35 Phytol has antibacterial activity proved by Sánchez. [33] and n-Hexadecanoic acid as major components which is in the same line with the previous study carried out by Sánchez. [33] n-Hexadecanoic acid was proven to have bioactivities including antimicrobial and antioxidant activities.[36] Benzenetriol and its derivatives possessed antibacterial activity as the study conducted by Cavalca. [34]

The docking study presented that phytol, 1,2,3-Benzenetriol, Hexadecanoic acid, and Xanthen-9-one, 1,3,5,6-tetramethoxy- respectively were comparable to the docking score of the reference undecaprenyl diphosphate synthase (Table 4). The MM-GBSA binding free energy of the same components is higher than the reference as shown by Phytol, Hexadecanoic acid, and Xanthen-9-one, 1,3,5,6-tetramethoxy- respectively while 1,2,3-Benzenetriol was slightly lower than the reference (Table 5) (Figure 1). Phytol and Hexadecanoic acid made a hydrogen bond with ALA69 (Alanine) where 1,2,3-Benzenetriol made a hydrogen bond with ALA142 and the later compound made a hydrogen bond also with ASN144 (Figure. 1).

Table 2: Radical scavenging activity of *Guiera senegalensis* and Standard (Quercetin)

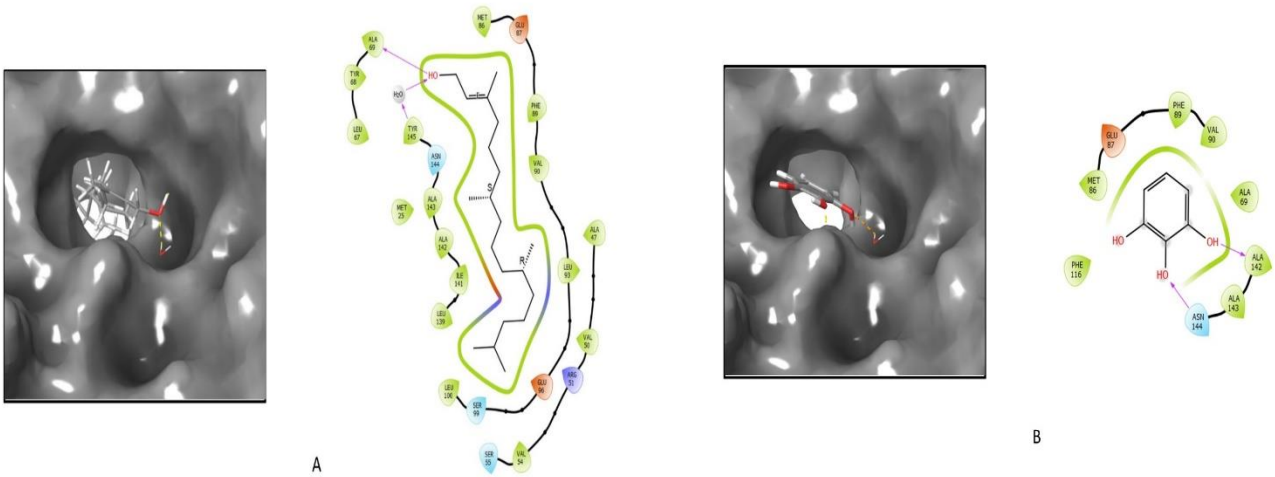
Concentration	Scavenging activity (%)	
	Guiera senegalensis leaves Extract	Standard (Quercetin)
250 µg/ml	72.5%	89.7%
125 µg/ml	63.5%	85.8%
50 µg/ml	50.5%	62.1%
10 µg/ml	45.5%	55.5%
5 µg/ml	31.3%	45%

Table 3: Gas Chromatography-Mass Spectrometry (GC-MS) Analysis *of Guiera senegalensis* leaves extract major constituents

Peak	***Guiera senegalensis* leaves extract**			
	R. Time	**Area**	**Area %**	**Name**
17	11.216	2122576	9.13	1,2,3-Benzenetriol
24	18.363	2633741	11.3	n-Hexadecanoic acid
26	19.911	5285299	22.74	Phytol
31	24.299	2306927	9.93	9H-Xanthene-9-one, 1,3,5,6-tetramethoxy-
33	24.361	4387405	18.88	9H-Xanthene-9-one, 1,3,5,6-tetramethoxy-

Table 4: Docking scores and MM-GBSA binding energy of 4 phytochemicals and the reference with undecaprenyl diphosphate synthase

No.	Scavenging activity (%)		
	Component	**Docking score *(kcal/mol)***	**MMGBSA dG Bind *(kcal/mol)***
1	Phytol	-6.196	-47.58
2	1,2,3-Benzenetriol	-5.99	-22.05
3	Hexadecanoic acid	-5.014	-46.79
4	Xanthen-9-one, 1,3,5,6-tetramethoxy-	-4.292	-30.69
5	Reference	-7.106	-24.1



A B

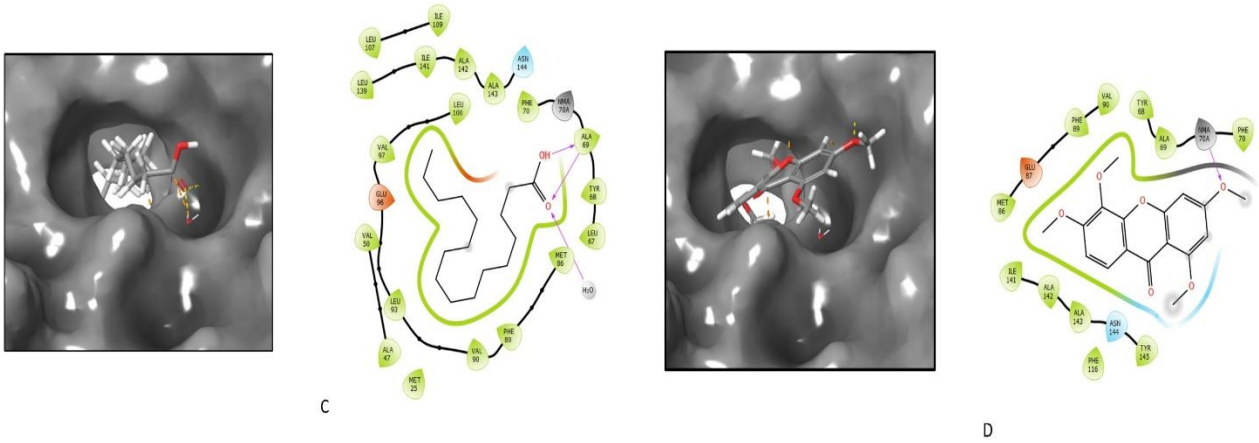


Figure 1: Two-dimensional (2D) and three-dimensional interactions (3D) of the top-ranked compounds 1-4 with the active site of undecaprenyl diphosphate synthase (UPPS) of *S. aureus* (PDB ID: 4H8E) after Glide docking. The hydrogen-bond interactions with residues are represented by a purple dashed arrow directed toward the electron donor. The pi-pi interactions are represented by a green line.
A. compound 1 B. compound 2 C. compound 3. D. compound 4

Conclusion

The study on *G. senegalensis* revealed that the ethanolic extract exhibits prominent antioxidant and antibacterial activities. The presence of 9H-Xanthene-9-one, 1,3,5,6-tetramethoxy-, Phytol, and Hexadecanoic acid are the major components that could be considered potential antibacterial agents. *In silico* and *in vitro* tests on the antibacterial activities, of *G. senegalensis* ethanolic extract confirmed that *S. aureus* is the most susceptible Bacterium to the extracts among the three tested bacterial strains.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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