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Antimicrobial Potential of *Salvadora persica* Stem Extract Against Dental Plaque-Associated Microorganisms: An Integrated *In vitro* and Molecular Docking Study

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ABSTRACT

The increasing prevalence of antimicrobial resistance and oral biofilm-associated diseases has intensified the search for alternative therapeutic agents from medicinal plants. Salvadora persica L. (miswak) has long been employed in traditional oral hygiene owing to its antimicrobial properties; however, the molecular mechanisms underlying its antibacterial activity remain incompletely understood. This study investigated the antimicrobial activity of the methanolic stem extract of S. persica against selected oral pathogens and explored the inhibitory potential of its phytochemical constituents against bacterial DNA gyrase using molecular docking. Methanolic stem extract of S. persica was prepared by cold maceration and qualitatively screened for phytochemical constituents. Antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Aspergillus niger was evaluated using the agar well diffusion assay, while the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) were determined using standard microbiological methods. Identified phytochemicals were subsequently subjected to molecular docking against Escherichia coli DNA gyrase subunits A and B (PDB ID: 3NUH), using levofloxacin as the reference inhibitor. Protein–ligand interactions were analyzed using Discovery Studio Visualizer and PyMOL. Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, anthraquinones, phenols, and cardiac glycosides. The extract exhibited concentration-dependent antimicrobial activity against all tested organisms, with the highest inhibition observed against S. aureus (22.1 mm), followed by A. niger (18.6 mm) and E. coli (15.4 mm) at 100 mg/mL. The MIC and MBC values against the bacterial isolates were 12.5 mg/mL and 25 mg/mL, respectively, while the MFC against A. niger was 50 mg/mL. Molecular docking identified the compound with PubChem CID 135580681 as the most promising ligand, exhibiting stronger binding affinities toward DNA gyrase

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subunit A (−8.0 kcal/mol) and subunit B (−7.6 kcal/mol) than the reference inhibitor levofloxacin (−6.1 and −6.8 kcal/mol, respectively). Interaction analysis demonstrated that the compound formed favorable hydrogen-bonding, hydrophobic, and electrostatic interactions with key active-site residues involved in enzyme inhibition. The methanolic stem extract of Salvadora persica possesses significant antimicrobial activity against selected oral pathogens and contains bioactive phytochemicals with promising inhibitory activity against bacterial DNA gyrase. These findings provide mechanistic support for the traditional use of S. persica in oral healthcare and identify PubChem CID 135580681 as a potential lead compound for the development of novel therapeutics targeting dental plaque-associated bacterial infections. However, further toxicity evaluation, molecular dynamics simulations, and experimental validation may be needed to further confirm this data.

INTRODUCTION

Dental plaques (DPs) are complex biofilms that form on the surfaces of salivary teeth. Bacterial adherence to salivary components and adsorption to the tooth surface are two variables that influence the genesis of DP (Khalil *et al.,* 2019). Gingivitis, the most prevalent form of periodontal disease, is caused by plaque and the activity of bacteria that create biofilms along the gingival boundary. Gingivitis is an inflammation of the gingival tissue that causes gums redness, swelling, and bleeding. Untreated or inadequately controlled gingivitis can cause bone and tissue damage. Illness and tissue damage resulted from a combination of bacterial activity in subgingival biofilms and human responses to them (Khalil *et al.,* 2019).

Porphyromonas gingivalis, *Prevotella intermedia*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Capnocytophaga sp.* have all been related to the development of dental caries by producing decalcification and, eventually, decay (Khalil *et al.,* 2019) Plants are used for decoration, food, medicine, and cosmetics (Upahi *et al.,* 2021; Yahaya *et al.,* 2025). Because of their potential health advantages, medicinal herbs have become a key component of alternative medicine all over the world (Kang-Ju *et al.,* 2016; Bawa *et al.,* 2022; Upahi *et al.,* 2026). Salvadora (toothbrush tree/miswak), a member of the Salvadoraceae family, is one of the most important of the 182 plant species used as chewing sticks. Many Asian, Middle Eastern, and African countries have employed the plant's roots, twigs, and stems for dental hygiene (Goyal *et al.,* 2011).

A significant step in the development of DP and the progression to tooth decay and periodontal disorders, according to Palombo (2011) and Omer *et al*. (2011), is the reduction of microbial pathogen adherence to the tooth surface. This study, therefore, aimed to evaluate the antimicrobial potential of aqueous and methanol extract of *Salvadora persica* as well as determine the possible mechanism involved.

MATERIAL AND METHODS

Sample Collection and Preparation

Dried stems of *Salvadora persica* (*aswaki*) were bought from local Yoruba and Hausa herb sellers at the Sheik Gumi Market (Central Market) Kaduna State, Nigeria. The chewing stick samples were prepared and washed under running tap water to remove dirt. The samples were air-dried for two days to prevent distortion in the composition of the chewing stick active component. The dry samples were thoroughly crushed into a fine powder using a mixer grinder. The powder was stored in air tight aseptic containers (28ºC±2) for subsequent use.

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Preparation of Extracts

The maceration process described by Adekunle and Odukoya (2006) was slightly modified to produce the methanolic extracts. The dried stem was grounded to fine powder. A 250 ml reagent vial was used to soak about 60 g of the powder in 180 ml of 95% methanol before closing. The powdered chewing sticks were allowed to stand for 7 days in a dark, tightly-sealed bottle while being frequently shaken, enabling complete extraction of the active components. Following that, Whatman No. 1 filter paper was used to filter the liquids while the plant macerate was evaporated to dryness at a temperature of 40 °C and reduced pressure prior to being kept in the freezer at 4^{o}C for further use.

Phytochemical Analysis of Chewing Stick Extracts

A qualitative screening of the phytochemical components of the chewing sticks was performed using the method proposed by Harborne (1991) to check for the presence of glycosides, alkaloids, saponin, tannins, flavonoids anthocyanin, anthraquinone, and phlobatannin.

Preparation of Media and Test Organisms

Clinical isolates of *Staphylococcus aureus*, *Aspergillus niger*, and *Escherichia coli* were obtained from the microbiology lab at the Applied Biology Department of Kaduna Polytechnic, Kaduna State, Nigeria using the media generation method described by Habamu *et al*. (2010).

Authentication of Test Organisms and Preparation of Nutrient Broth

Isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* isolates were employed to characterize bacteria through observation of their behavious when subcultured on nutrient agar and how they responded to the gram staining reaction. Biochemical tests for motility, citrate, and methyl red, as well as positive confirmatory tests, were also performed on the test organisms. The employed nutritious broth was prepared using the Habamu *et al*. (2010) technique.

Preparation of Different Concentration of Extracts

The technique described by Perez *et al*. (2001) was used to create different extract concentrations. The extract concentrations of 1000 mg/ml, 50 mg/ml, 25 mg/ml, and 12 mg/ml were generated, and 1 g of each extract was dissolved in 10 ml of sterile distilled water to achieve a concentration of 100 mg/ml. This also served as a stock solution from which the other concentrations, each of which were prepared up to 10ml and labelled, were created.

Preparation and Standardization of Inoculum

The overnight culture in nutrient broth of each previously obtained bacterium isolate was used to prepare inocula by dilution with sterile saline. Following the protocol indicated by Habamu *et al*. (2010), 10ml of the solution was split among three (3) clean test tubes and autoclaved at 121^{o}C for 15 minutes in each test tube. After the sterile saline had cooled, 0.1ml of the overnight broth culture of *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* was added before they were placed into another test tube containing sterile normal saline which were used as the reference inoculum for the antibacterial and antifungal assays.

Determination of Antibacterial and Antifungal Activity Using Agar Well Diffusion Method.

The agar well diffusion method developed by Habamu *et al*. (2010) was employed to evaluate the antibacterial activity of the extracts against the test species. Nutrient agar was prepared and allowed to firm up in 15 cm sterile disposable petri dishes. Before flooding these plates with diluted, standardized overnight cultures, a loopful of bacterium or fungus inocula was collected and streaked across the entire surface of the dried agar. On each plate, wells of 6mm diameter were produced in triplicate with sterile pipettes, each containing 0.1ml of diluted extract concentrations (100mg/ml, 50mg/ml, and 25mg/ml). A popular antibiotic, ciprofloxacin, was used as a positive control at 1 mg/ml. A different plant used sterile distilled water as the negative control.

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The millimeter rule was employed to determine the diameters of the zones of inhibition after 24 hours of incubation at 37°C. On triplicate plates, Perez *et al*. computed and recorded the means of the zones of inhibition for each organism at each concentration of the extract (Perez *et al*. (2001) which is also the method employed in this study.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal-fungicidal Concentration

The minimum inhibitory concentration of the extract against each organism were determined. A nourishing broth with double the strength was created. Then, 1 ml of double strength extract was put into four test tubes labelled 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively, for each organism. The aforementioned concentration was blended 1:1 with nutritional broth, and each test tube received 0.1 ml of the standard suspension of test tube organisms.

The test tubes were then incubated for 24 hours at 37 degrees Celsius for fungus and 25-27 degrees Celsius (ambient temperature) for bacteria. A tube containing broth and inoculum serves as a negative control for comparison, whereas tubes carrying broth and extract serve as positive controls. The growth (turbidity solution) or visible growth was defined as the minimal inhibitory concentration while the Minimum Bactericidal/Fungicidal Concentration was calculated using the Vinothilkumar *et al*. (2010) method.

***In-silico* Modelling of *Salvadora persica* Compounds**

The crystal structures of DNA Gyrase subunits A and B (PDB ID: 3NUH) were obtained from the RCSB Protein Databank (http://www.rcsb.org). The water molecules and co-crystal ligands from the initial protein crystal were removed. The 2D structures of the chemicals isolated from *Salvadora persica* extract, as determined by gas chromatography-mass spectrometry, as well as levofloxacin (a standard inhibitor) were retrieved from the PubChem database and converted to pdbqt file with the proper torsion values using Autodock vina while the compounds were molecularly docked (Trott and Oslon, 2010). The Root Mean Square Deviation (RMSD) and Affinity Energy were used to determine the best pose and interaction sites respectively (Trott and Oslon, 2010). The comparative protein-ligand interactions between the *Salvadora persica* extract hit compounds and the standard (levofloxacin) were also visualized using Discovery Studio Visualizer version 21.1.0.20298 and Schrodinger PyMol® (TM) version 2.5.1 (version 16).

RESULTS

Extraction Yield, Qualitative and Quantitative Phytochemical Screening

Methanol was used as the solvent to extract 20% of Salvadora persica. According to Table 1, phytochemicals such as anthraquinones, flavonoids, terpenoids, saponins, alkaloids, tannins, phenols, and cardiac glycosides were discovered.

Table 1: Phytochemical Composition of Methanolic Stem Extract of *Salvadora persica*

Constituents	Results
Flavonoids	+ve
Saponins	+ve
Cardiac glycosides	+ve
Alkaloids	+ve
Anthraquinone	+ve
Tannins	+ve
Terpenoids	+ve
Phenols	+ve

Key: +ve=positive and -ve =negative

Antimicrobial Activity of Salvadora Extract Against the Test Organisms

The extract exhibited high activity against staphylococcus aureus, with zones of inhibition of 22.1 at 100 mg/ml, 15.4 at 100 mg/ml for *Escherichia coli*, and 18.6 at 100 mg/ml for *Aspergillus niger*. At the lowest concentration, Escherichia coli 8.4 at 12.5mg/ml, *Staphylococcus*

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aureus 11.0 at 12.5mg/ml, and *Aspergillus niger* 6.2 were all inhibited (Table 2).

Table 2: Antimicrobial Activity of Salvadora Extract Against the Test Organisms

Test organisms	Concentration(mg/ml)			
	100%	50 %	25%	12.5%
Staphylococcus aureus	22.1	19.0	14.7	11.0
Escherichia coli	15.4	11.0	10.08	8.3
Aspergillus niger	18.6	9.0	7.3	6.2

Table 3: The Minimum bactericidal concentration (MBC) of *Salvadora persica* Methanolic Stem Extract Against the Bacteria Isolate

Bacteria isolates	Concentration (m/ml)			
	100	50	25	12.5
Staphylococcus aureus	-	+	+	+
Escherichia coli	-	+	+	+

Key: + = turbidity observed - = No turbidity observed

All concentrations except 100 showed turbidity (Table 3).

The Minimum Fungicidal Concentration (MFC) of *Salvadora persica* Methanolic Stem Extract Against the Fungi Isolate

Table 4 shows that the methanolic extract of *Salvadora persica* has good antifungal activity. The *Salvadora persica* and methanol extract exhibited significantly higher antibacterial properties, while there was no antibacterial effect in the control group.

Table 4: The Minimum Fungicidal Concentration (MFC) of *Salvadora persica* Methanolic Stem Extract Against the Fungi Isolate

Fungi isolates	Concentration (mg/ml)			
	100	50	25	12.5
Aspergillus niger	-	-	+	+

Key: + = turbidity observed - = No turbidity observed

The Minimum Inhibitory Concentration (MIC) of *Salvadora persica* Methanolic Stem Extract Against the Bacteria Isolate

The extract displayed a significant minimum inhibitory concentration at 50 and 100 mg/ml against the isolates of *Staphylococcus aureus* and *Escherichia coli* (as shown in Table 3.5).

Table 3.5: The Minimum Inhibitory Concentration (MIC) of *Salvadora persica* Methanolic Stem Extract Against the Bacteria Isolate

Bacteria isolates	Concentration (mg/ml)			
	100	50	25	12.5
Staphylococcus aureus	-	-	+	+

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Bacteria isolates	Concentration (mg/ml)			
	100	50	25	12.5
Escherichia coli	-	-	+	+

Key: + = Growth observed - = No Growth observed

The Minimum Inhibitory Concentration (MIC) of *Salvadora persica* Methanolic Stem Extract Against the Fungi Isolate

Table 3.6: The Minimum Inhibitory Concentration (MIC) of *Salvadora persica* Methanolic Stem Extract Against the Fungi Isolate.

All concentrations, except 12.5 mg/ml, of the extract exhibited significant inhibitory activity against the isolate of *Aspergillus niger* (Table 3.6).

Fungal Isolates	Concentration (mg/ml)			
	100	50	25	12.5
Aspergillus niger	-	-	-	+

Key: + = Growth observed - = No Growth observed

Binding Affinity Results of Modelled Compounds of *Salvadora persica* Methanolic Stem Extract Against DNA Gyrase Subunit A, as well as their Interactions

Binding Affinity of Modelled Compounds of *Salvadora persica* Methanolic Stem Extract Against DNA Gyrase Subunit A

The compound with PubChem ID: 135580681, humulene, caryophyllene, beta-Selinene, as well as 899374-61-9 exhibited comparatively higher binding affinity than levofloxacin (standard inhibitor) (-6.1 Kcal/mol) on DNA gyrase subunit A, with compound with PubChem ID: 135580681 (-8.0 Kcal/mol) displaying the highest binding affinity value (Figure 1).

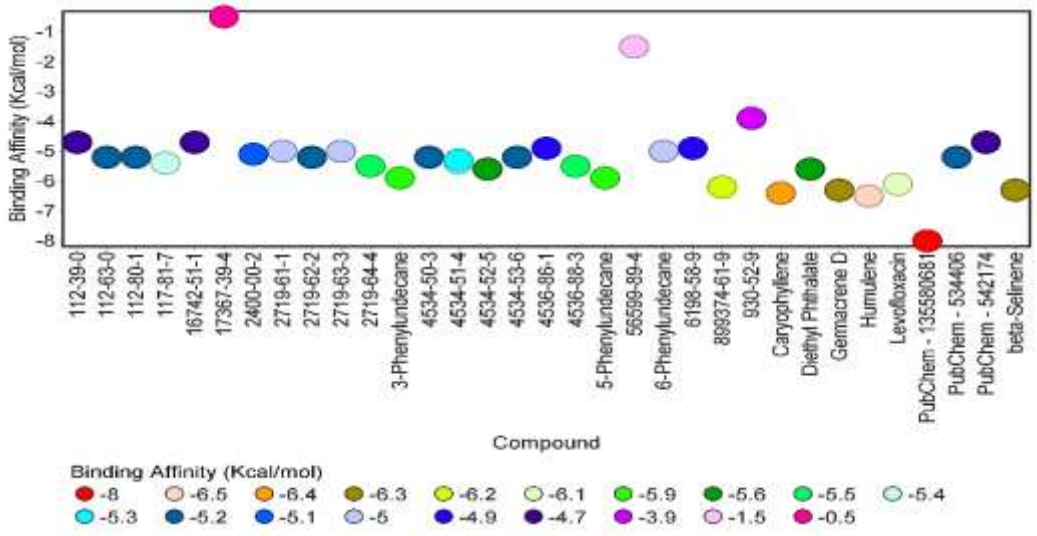

Figure 1: Binding Affinity Results of Identified Compounds of *Salvadora persica* Methanolic Stem Extract Against DNA Gyrase Subunit A

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Comparative Interactions of PubChem – 135580681 (Hit Compound) and Levofloxacin (Standard Inhibitor) on DNA Gyrase Subunit A

Levofloxacin binds with tryptophan 59 (at a distance of 2.41 Å) and aspartic acid 137 (at a distance of 2.37 Å) of DNA gyrase subunit A pocket through the conventional hydrogen bonding; alanine 136 through carbon-hydrogen bonding (at a distance of 3.45 Å); glutamic acid 139 (at a distance of 3.43 Å) through the ionic halogen bonding, as well as the active hydrophobic histidine 132 through the hydrophobic Pi-cation bond (at a distance of 4.78 Å) (Figure 2).

The compound with PubChem ID: 135580681 binds with histidine 132 through carbon-hydrogen bond (at a distance of 3.52 Å); active asparagine 53 (at a distance of 2.54 Å); alanine 136 through the hydrophobic Pi-alkyl bond (at an average distance of 7.11 Å) which are in common with the binding pattern of levofloxacin, in addition to binding to side residues leucine 138 (at a distance of 3.17 Å), as well as tyrosine 50 through the hydrophobic Pi-Pi T-shaped interactions (at a distance of 5.37 Å) (Figure 3).

Figure 2: Pocket View (A), Pocket Hydrophobicity View (B), and Two-Dimensional (2D) View (C) of Interaction Between Levofloxacin and DNA Gyrase Subunit A

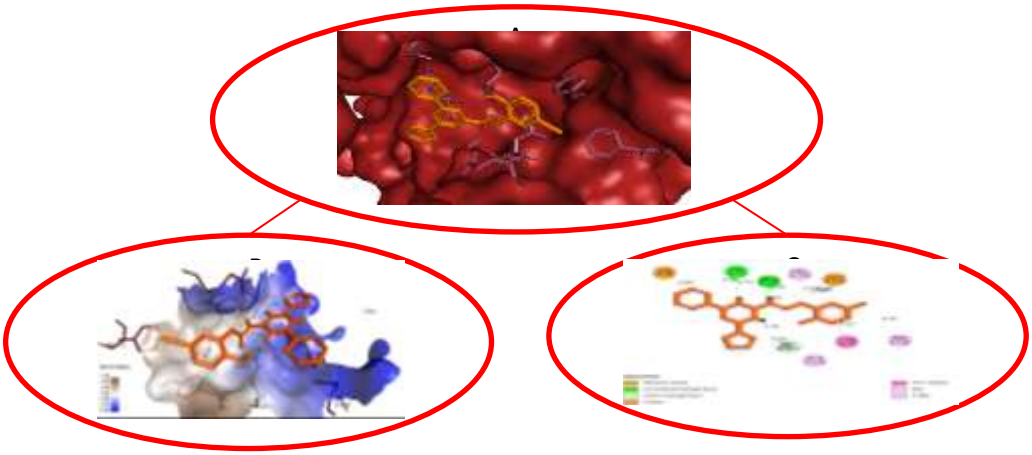

Figure 3: Pocket View (A), Pocket Hydrophobicity View (B), and Two-Dimensional (2D) View (C) of Interaction Between PubChem - 135580681 and DNA Gyrase Subunit A

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Binding Affinity Results of Modelled Compounds of *Salvadora persica* Methanolic Stem Extract Against *Escherichia coli* K-12 DNA Gyrase Subunit B, as well as their Interactions

Binding Affinities of Modelled Compounds of *Salvadora persica* Methanolic Stem Extract Against DNA Gyrase Subunit B

All the compounds exhibited comparatively higher binding affinity than levofloxacin (standard inhibitor) (-6.8 Kcal/mol) on DNA gyrase subunit A, except the compound with PubChem ID: 135580681 (-7.6 Kcal/mol) which displayed comparatively higher binding affinity value than the standard.

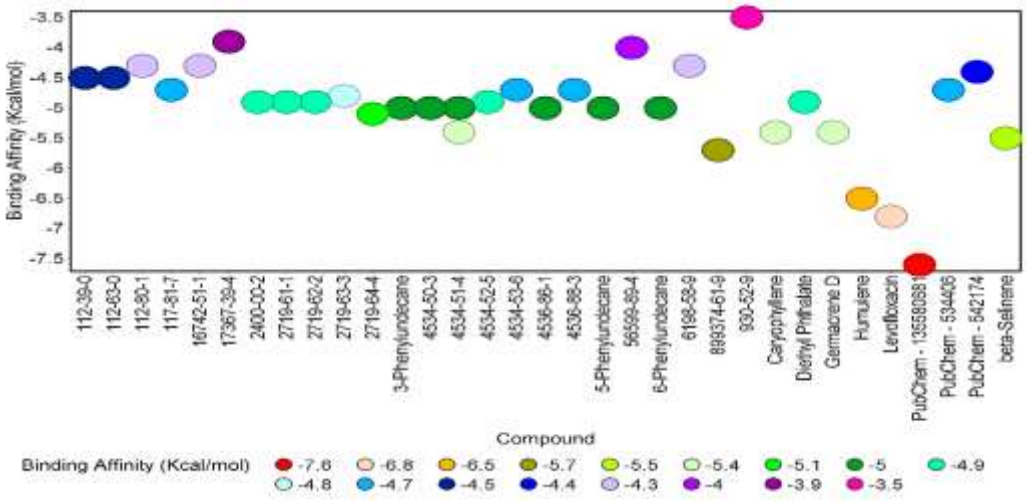

Figure 4: Binding Affinity Results of Identified Compounds of *Salvadora persica* Methanolic Stem Extract Against DNA Gyrase Subunit B

Comparative Interactions of PubChem – 135580681 (Hit Compound) and Levofloxacin (Standard Inhibitor) on DNA Gyrase Subunit B

Levofloxacin binds with leucine 534 and alanine 499 through the alky and Pi-sigma bond at the distance of 3.50 Å and 3.74 Å respectively; binds to proline 532 and lysine 740 through carbon-hydrogen bond at the distance of 4.02 Å and 4.56 Å, as well as the hydrophobic arginine 738 through Pi-cation interaction at the distance of 4.16 Å (Figure 5).

Compound with PubChem ID: 135580681 binds (in common with levofloxacin) to leucine 534 and alanine 499 through alkyl bond at the distance of 4.28 Å and 4.64 Å respectively; proline 532 through Pi-alkyl interaction (at a distance of 4.47 Å); active aspartic acid 548 through the ionic salt bridge (at an average distance of 2.83 Å), in addition to the interaction with the side chain residue glutamine 531 through carbon-hydrogen bond (at a distance of 2.66 Å). (Figure 6).

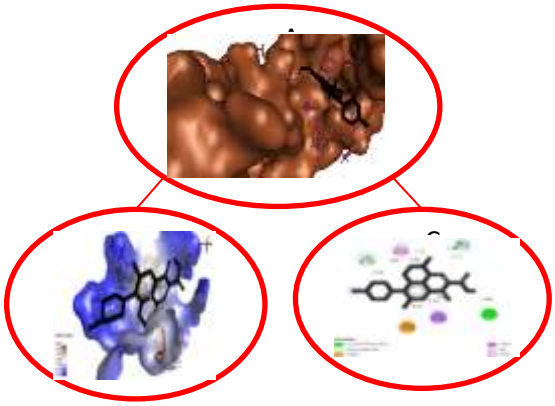

Figure 5: Pocket View (A), Pocket Hydrophobicity View (B), and Two-Dimensional (2D) View (C) of Interaction Between Levofloxacin and DNA Gyrase Subunit B

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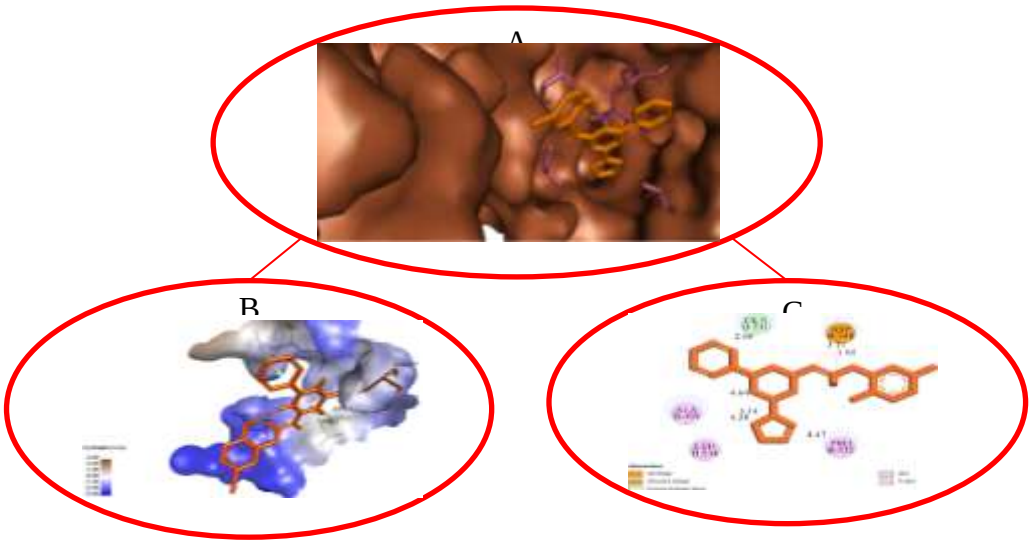

Figure 6: Pocket View (A), Pocket Hydrophobicity View (B), and Two-Dimensional (2D) View (C) of Interaction Between PubChem – 135580681 and DNA Gyrase Subunit B

DISCUSSION OF FINDINGS

Several studies have claimed that the *Salvadora persica* chewing stick possessed high medicinal efficacies due to its bioactive components (Adekunle and Odukoya, 2006). The current study found that the extract of *Salvadora persica*, a tropical Nigerian chewing stick, showed antifungal and antibacterial properties on the test organisms. *Salvadora persica* had a high inhibitory activity against the tested bacterium species because it displayed the greatest antibacterial inhibition. The studies confirmed methanol's potency and usefulness as a solvent. This is consistent with the findings of Kareem *et al*. (2008), who discovered that plant-active compounds are more soluble in organic solvents. The greater potency of the methanol extract may be due to the alcohol's capacity to dissolve compounds (Majorie, 1999).

The phytochemical components of chewing stick extract, such as glycosides, alkaloids, saponin, tannins, flavonoids anthocyanin and anthraquinone, may be responsible for the reported antifungal and antibacterial inhibitory effects. This is also compatible with the studies detailed by Cowan (1999). The main reason Salvadora persica is utilized for dental cleaning in tropical Nigeria to prevent dental cavities, gingivitis, and plaque is due of its antibacterial action (Kareem et al., 2012, Iyanda-Joel et al., 2019). The findings revealed that extracts from the two chewing sticks exhibited a Minimum Inhibitory activity (MIC) at various dosages. The extract has significant antibacterial activity against *Staphylococcus aureus*, with zones of inhibition of 22.1 at 100 mg/ml, 15.4 at 100 mg/ml for E. coli, and 18.6 at 100 mg/ml for *Aspergillus niger*. *Aspergillus niger* 6.2, *Staphylococcus aureus* 11.0, and *Escherichia coli* 8.4 were all inhibited at the lowest concentration.

The tannin in chewing sticks may have also been responsible for the substantial antibacterial action observed in this study (Kareem et al., 2012, Iweala et al., 2023). According to Hagerman and Butler (1981) and Iyanda-Joel et al (2015), tannins can create irreversible compounds with proline-rich proteins, limiting the synthesis of cell wall proteins. This feature may help to explain how chewing stick extract function. However, the *In silico* modeling of the identified compounds of the chewing stick extract revealed the aromatic nitrogen-rich compound with PubChem ID: 135580681 as likely responsible for the mechanistic inhibition

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associated with the antibacterial effect of *Salvadora persica* ethanolic against dental plagues observed in this study, while the mode of inhibition is similar to the pan inhibitory effects of quinolone-derived levofloxacin against both DNA gyrase subunits A and B. The structure name of the compound with PubChem ID: 135580681 is 2-[(5-Iodosalicylidene) hydrazino]-4-morpholino-6-(1-pyrrolidinyl)-1,3,5-triazine.

CONCLUSION

This study, therefore, has revealed the antifungal and antibacterial effect of *Salvadora persica* methanolic stem extract in *in vivo* model of orodental hygiene, as well as the mechanistic inhibitory activity of its constituting 2-[(5-Iodosalicylidene) hydrazino]-4-morpholino-6-(1-pyrrolidinyl)-1,3,5-triazine against both the subunit A and B of *Escherichia coli* K-12 DNA gyrase (a major component microbial composition of dental plague) through *in silico* simulations. These findings also evidently support the view that chewing sticks may serve as an effective natural alternative source of antibacterial and antifungal agents against different oral diseases, along with additional interproximal cleaning aides. Toxicological studies are recommended to ascertain the toxicity status of the medicinal plant, and there is a need for further evaluation of 2-[(5-Iodosalicylidene) hydrazino]-4-morpholino-6-(1-pyrrolidinyl)-1,3,5-triazine in both pre-clinical and clinical studies for the development of natural, cost-effective, clinically safe, and highly potent anti-dental plague agent.

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