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Original Research Article

Bio-active composition, antibacterial activity and molecular docking studies of *Salicia nitida* (Benth.) root extracts

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Abstract

This study identifies the bioactive compounds of know pharmacological importance present in the root extracts of *Salicia nitida.* A computational analysis was conducted to analyze the potential antibacterial bioactive component, Butylated hydroxytolene (Compound Identification number CID: 31404) and 5-Eicosene (Compound Identification number (CID): 5364600), identified from Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis, against class A beta-lactamase enzyme (Protein Data Bank (PDB): 3BLM) to identify its inhibition potentials. Finally, we also investigated the antibacterial potentials of *S. nitida*. Standard qualitative screening methods were employed to access phytochemical constituents present in the root extracts of *S. nitida*. Gas Chromatography-Mass Spectrophotometry analysis was used to identify the bioactive components present in root extracts of *S. nitida*. The disc diffusion method with slight modifications was adopted to test for the susceptibility of the selected test bacteria to the root extracts of *S. nitida*. Molecular docking was utilized to predict the binding affinity and energy of the bioactive components, butylated hydroxytolene and 5-eicosene, as ligand compound with class A beta-lactamase enzyme as protein receptor. The phytochemical analysis showed that saponins, tannins, alkaloids, flavonoids and phenols are present while glycosides are absent in root extracts of *S. nitida*. Gas Chromatography-Mass Spectrophotometry analysis of root extracts of *S. nitida* revealed the presence of ten chemical compounds. The highest retention time (RT) recorded for *S. nitida* was in 9-Octadecenoic acid (36.804) and the least in Butylated Hydroxytoluene (10.13). The peak area and the eluted chemical compounds found in the GCMS analysis are shown in the chromatogram. Molecular docking simulations revealed valuable insights into the potential

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interactions between Class A beta-lactamase enzyme (PDB: 3BLM) with Butylated hydroxytoluene (CID: 31404) and 5-Eicosene (CID: 5364600). The binding energies reported provide a quantitative measure of the interaction strength at various positions. The results from the anti-bacterial assay showed that *Salicia nitida* root extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* with inhibitory zone diameters range of 17.00 - 19.50 mm and 20.50 - 28.50 mm for the standard antibiotic agents. The phytochemical and GC-MS findings suggest that *Salicia nitida* root extracts contain bioactive constituents which are known to exhibit physiological activities. More so, stable complexes were formed between Class A beta-lactamase enzyme (PDB: 3BLM), Butylated hydroxytoluene (CID: 31404) and 5-Eicosene (CID: 5364600), at different positions, indicating strong and favorable interactions. However, it is essential to note that these results are based on computational predictions, emphasizing the importance of experimental validation to confirm the actual nature and strength of the interaction. The root extracts also exhibited antibiotic potentials as evidenced by the results from the anti-bacterial assay.

Biological: Biochemistry.
Keywords: *Molecular docking, Salicia nitida, beta-lactamase, antibacterial agents, phytochemical compounds*

Introduction

Bacteria overwhelmingly outnumber eukaryotes and archaea in the human body. Many bacteria can interact with higher organisms in different ways and the outcome of these interactions can either be advantageous or disadvantageous to the host (Fuchs *et al*, 2010). Infectious diseases caused by bacteria are constantly inundating rural areas in developing countries and attempts to manage these ailments with conventional drugs have posed mankind with a lot of health consequences. Moreover, bacterial infections are continually developing resistance to conventional antibiotic agents, thereby prompting the search for bioactive compounds from plant parts that would serve as lead molecules in the discovery and development of new drugs. Over-dependence on drugs as sole remedies for treating diseases has led to the malfunction of the important organs of the body such as the liver, the kidney, the eyes, the lungs and the brain. Beta -lactamase secretion by bacteria is one of the main resistance mechanisms used by bacterial enzymes to fight antibiotics and develop drug resistance. Thereby, encouraging the use of medicinal plants in the treatment of infectious diseases (Okereke *et al.*, 2017). The use of medicinal plants for pharmaceutical benefits in the treatment of various diseases such as bacterial infections is as old as the origin of mankind. The basic active ingredients used for treating various ailments are accumulated in the roots of plants (Ugbogu *et al.*, 2021; Elekwa *et al.*, 2017). *Salicia nitida* (Igbo: Enyimocha) is an example of a plant, which is widely distributed in tropical regions and has been used for thousands of years in traditional medicine for the treatment of several ailments including malaria, rheumatism, asthma, fever, menorrhagia, diabetes and skin diseases. *S. nitida* (Benth.) N.E.Br. is a woody climber and a liana of 3-30 m long, and 6 cm diameter widely distributed in Cameroon, Congo, Gabon, Liberia, Ivory Coast, Sierra Leone, Nigeria, Ghana and the Democratic Republic of Congo rained forests. It is also distributed in Sri Lanka, South-West India, Thailand, the Philippines, Java and South Africa. The roots of *S. nitida* are used in the southeastern part of Nigeria for the treatment of malaria and typhoid fever (Mba'ning *et al.*, 2019). Despite the use of the plant for such purposes, there is little information on the antibacterial efficacy. This work is therefore aimed at documenting the bioactive compositions, accessing bacterial susceptibility and analyzing potential interactions between bioactive compounds present

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in *S. nitida* and Class A beta- lactamase enzyme, in a bid to determine its usefulness and suitability

as potent antibiotic agents or otherwise.

Materials and Methods

Plant preparation: The roots of *S. nitida* underwent a meticulous preparation process. After harvesting, they were washed and cut into pieces of about 15mm in size and then sun-dried for two weeks (14 days) and subsequently ground into powdered form using a mechanical grinder. The methanol extraction method was employed for the extraction process. For every 20g of the powdered preparation, 20ml of water was added. The mixture was allowed to stand for 24 hours and then filtered. The liquid content from the filtration process was subjected to phytochemical screening and GC-MS analysis.

Phytochemical screening

Test for Saponins: About 5 g of dry extract was mixed with 5ml of distilled and was vigorously shaken. A foam-like substance known as froth was formed. About 3 drops of olive oil were then added to the froth. We observed the formation of emulsion.

Test for Tannins: About 3 ml of the plant extract was dispensed in a test tube, and 3ml of 10% alcoholic ferric chloride ($FeCl_3$) solution was added to the test tube. The formation of a dark blue colour compound was observed.

Test for Alkaloids: Exactly 3 ml of the plant extract was dispensed in a test tube, and 3 ml of Wagner's reagent (1.27g of iodine and 2g of potassium iodide to 100ml of distilled water) was added to the test tube. Formation of reddish-brown color precipitate was observed.

Test for Flavonoids: Exactly 3 ml of the plant extract was dispensed in a test tube, few drops of 1% dilute ammonia solution (NH_4OH) were added to the test tube, followed by the addition of few drops of conc. H_2SO_4 solution. The formation of a yellow colour was observed.

Test for Phenols: Exactly 3 ml of the plant extract was dispensed in a test tube, a few drops of 5% ferric chloride solution were added to the test tube and the formation of a greenish colour was observed.

Test for Cardiac glycosides: Exactly 0.5 ml of the plant extract was dispensed in a test tube, and 1 ml of glacial acetic acid (CH_3COOH) containing a drop of ferric chloride solution was added to the test tube. The mixture was carefully added to 1ml of concentrated sulphuric acid (H_2SO_4) in another test tube such that the conc. H_2SO_4 is directly beneath the mixture. No colour change was observed.

GC-MS Analysis: The identification of main the phytoconstituents of *Salicia nitida* root extract was carried out using methanol as the extraction solvent. The extract was subjected to the Gas chromatography – mass spectrometric (GC-MS) method described by Akpojotor and Ebomoyi (2021), using an Agilent 7890A equipped with mass spectrometer MS 5975C (Agilent Technologies, CA, U.S.A). The capillary column used was DB-5MS with a column thickness of 0.25µm; internal diameter of 0.32mm; and length of 30m (J&W Scientific, CA, U.S.A). Carrier gas was helium with a flow rate of 1ml/min. The temperature programme was set as follows; initial temperature 80°C held for 2 minutes at 10°C per minutes to 240°C held for 6 min, a total run time of 22 minutes. About 2 ml of extract was loaded into the vial and 2µL of the extract was injected via the injector port into the GC-MS. Inside the GC-MS, the sample is heated and turned into

vapour (gas). The vapour was then blown by an inert gas (helium) from the mobile phase into the column unit. As the gaseous form of the sample travels through the column, the different phytoconstituents travelling with different speeds due to their different mass and vitality get to the detector unit of the GC at different retention times (RTs) and then pass through the mass spectrometry system operated in electron ionization mode with selected ion monitoring. The RTs, mass and spectra are then displayed on the monitor and then compared and matched against those of authentic standard spectra using a computer search in NIST LIBRARY 2015.

Molecular docking analysis: The refined crystal structure of beta-lactamase from *Staphylococcus aureus* with PDB code 3BLM, was downloaded from the RCSB protein data bank and the 3D structure of Ligand compound; Butylated hydroxytoluene and 5-Eicosene, with CID code 31404 and 5364600 respectively, were downloaded from PubChem database in sdf format.

Protein Receptor: Refined crystal structure of beta-lactamase from *Staphylococcus aureus*.
PDB Code: 3BLM

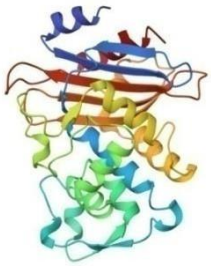


Figure 1: 3D diagram of crystal structure class a beta-lactamase from *staphylococcus aureus*. Pdb code: 3blm

Ligand Compound: Butylated hydroxytoluene (Compound CID: 31404)

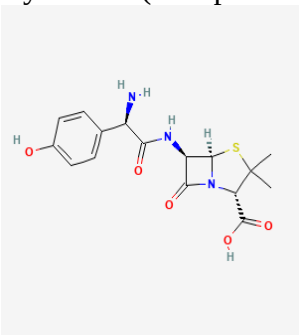


Figure 2.1: 2D diagram of CID 31404

Ligand Compound: 5-Eicosene (Compound CID: 5364600)

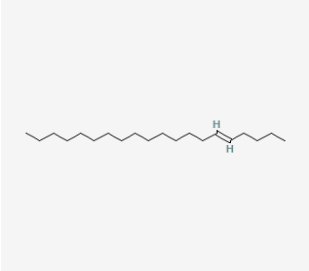


Figure 2.3: 2D diagram of CID 5364600

The crystal structure of Class A Beta-lactamase (PDB 3BLM) was prepared for docking by removing water molecules and co-crystal ligands using Biovia Discovery Studio software (Biovia, 2019). The CB-Dock2 software (Liu and Cao 2024), was used for the docking analysis toexamine the interactions, binding affinity and binding energy between Butylated hydroxytolene (CID 31404) and Class A Beta-lactamase (PDB 3BLM) as well as between 5-Eicosene (CID: 5364600) and Class A Beta-lactamase (PDB 3BLM). The results of the docking analysis were also perused in 3D and 2D images, using Biovia Discovery Studio software.

Experimental design: The disc diffusion method with slight modifications was adopted to test for the susceptibility of the selected test bacteria to the extracts. The study involved a selection of local clinical culture isolates of *Staphylococcus aureus* and *Escherichia coli*. These isolates were collected from the Faculty of Medical Laboratory Science, Abia State University Uturu. Overnight cultures of the selected bacteria isolates were used. An already solidified sensitivity test medium on Petri dishes were seeded with the test organisms using sterile swab stick (spread plate method). After which sensitivity disc of about 6mm in size already loaded with the plant extracts were placed on the Petri dishes. The plates were incubated at 37^0C for 24 hours and the zones of inhibition were measured in millimeters using transparent meter rule. Antibiotic sensitivity discs were used as a positive control, the test organisms were also prepared using the spread plate method, while methanol and normal saline served as negative control. Each antibacterial assay was carried out in triplicate.

Results

Phytochemical constituents of plant materials are generally required for the discovery of novel drugs. The phytochemical analysis as represented in Table 1 shows that saponins, tannins, alkaloids, flavonoids and phenols were present while glycosides were absent.

Table 1: Phytochemical Composition of *Salicia nitida* root extract

Phytochemicals	***S. nitida***
Saponins	+
Tannins	+
Alkaloids	+
Flavonoids	+
Phenols	+
Glycosides	-

KEY:
+ = present
- = not present

Table 2: Major components from *Salicia nitida* methanolic root extract

S/N	RT	Molecular Formula	Name of Compound	Molecular Weight (g/mol)	Peak Area (%)
1	10.133	$C_{15}H_{24}O$	Butylated Hydroxytoluene	223.37	39.42
2	26.358	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid	280.4	2.29
3	26.358	$C_{18}H_{32}O_2$	Linoelaidic acid	280.4	2.29
4	25.980	$C_{23}H_{48}$	Tricosane	324.6	1.05
5	29.734	$C_{31}H_{64}$	Hentriacontane	436.8	6.52
6	29.961	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	356.5	1.99
7	31.190	$C_{20}H_{40}$	5-Eicosene	280.5	8.79
8	32.238	$C_{17}H_{32}O_2$	8-Pentadecen-1-ol acetate	268.4	2.81
9	33.871	$C_{20}H_{38}O_3$	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	326.5	8.74
10	36.804	$C_{18}H_{34}O_2$	9-Octadecenoic acid	282.5	3.27

RT = Retention time

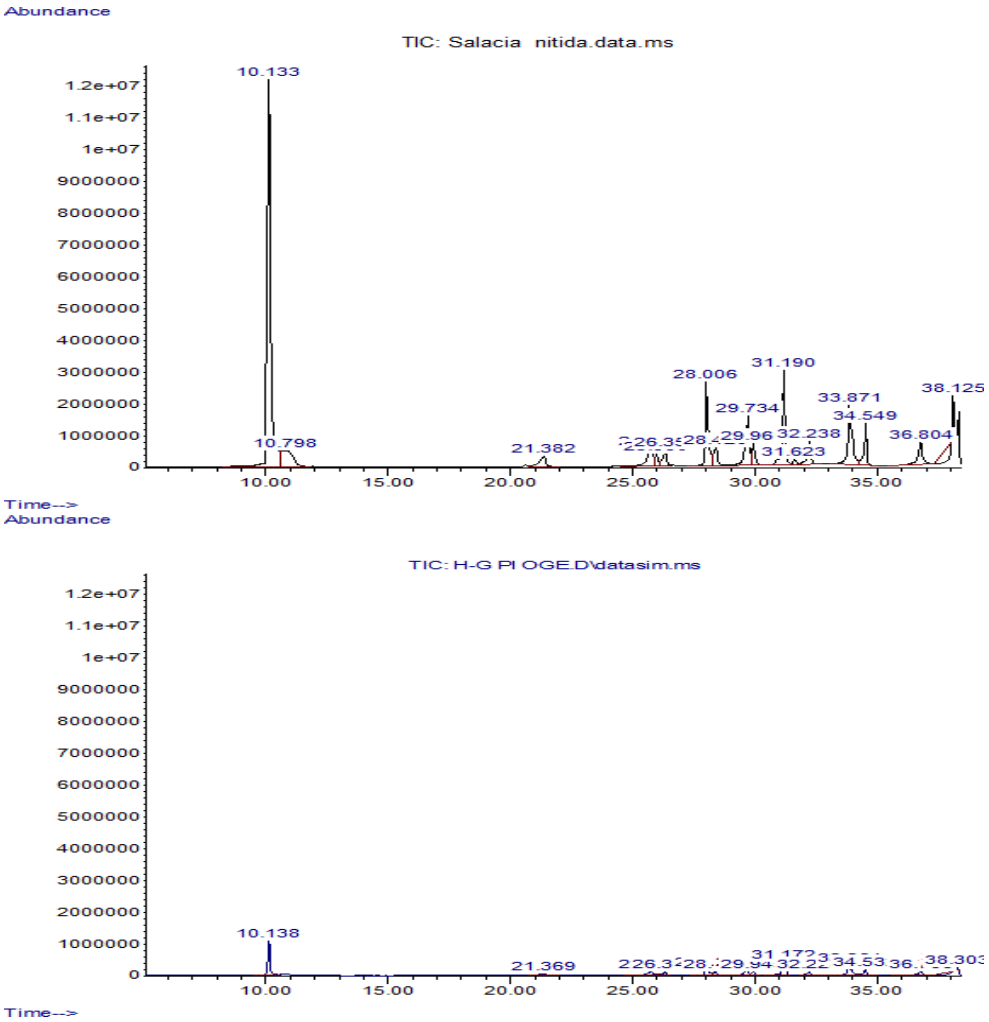


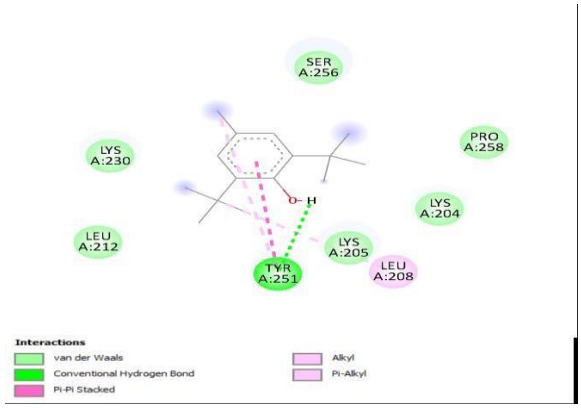
Figure 3: Mass Chromatogram of *S. nitida* methanolic root extract

Table 3: Represents the binding complex between Beta-lactamase (PDB 3BLM) and ligand compound Butylated hydroxytolene (CID 31404), showing vina score, Vander waals, hydrogen, and Alkyl/Pi-Alkyl bonds at different binding positions.

Positions	VS	HB	Vander waals	Alkyl/Pi-Alkyl
C1	-4.9	1	6	1
C2	-5.1	1	7	1
C3	-5.8	0	8	2
C4	-4.4	0	5	2
C5	-4.9	0	7	2

KEY:
VS: Vina Score (Representing binding energy Kcal/Mol)
HB: Hydrogen Bond

FIGURE 3: 2D and 3D Image Representations of Beta-lactamase (PDB 3BLM) and ligand compound Butylated hydroxytolene (CID 31404) at positions C1-C5 showing binding affinity and bonds at each binding site.



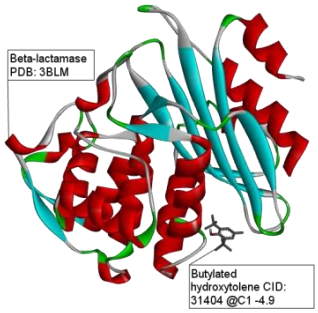
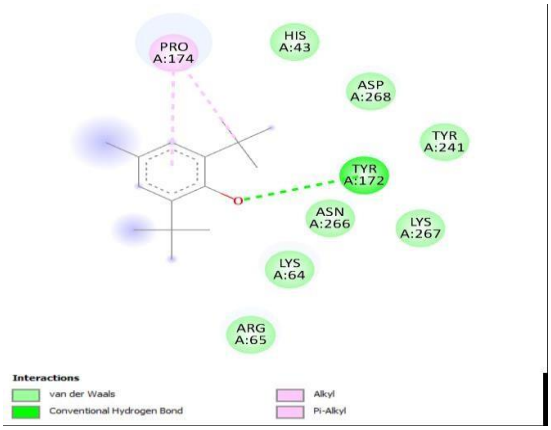


Figure 3.1: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 31404) at binding site denotaed as C1.



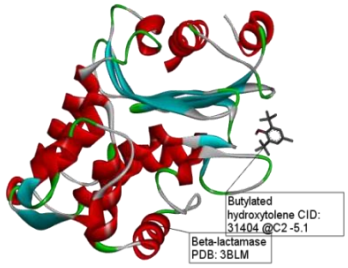


Figure 3.2: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 31404) at binding site denoted as C2.

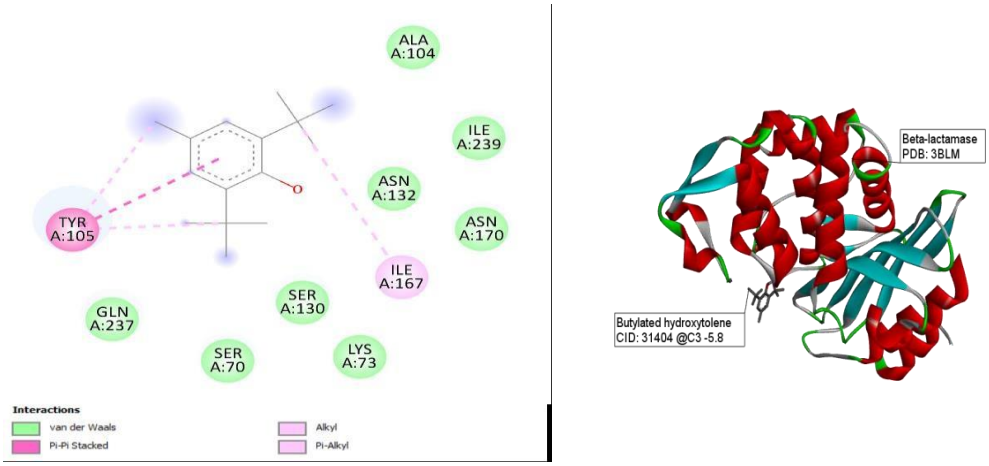


Figure 3.3: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 31404) at binding site denoted as C3.

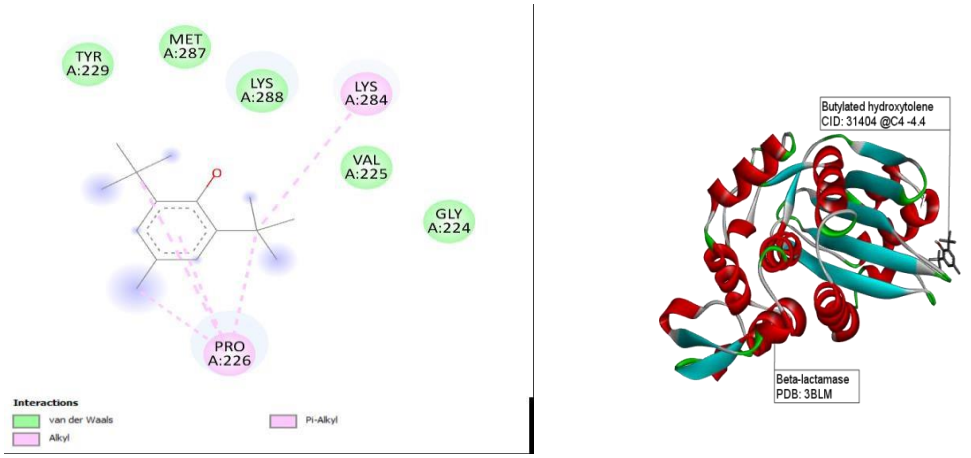


Figure 3.4: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 31404) at binding site denoted as C4.

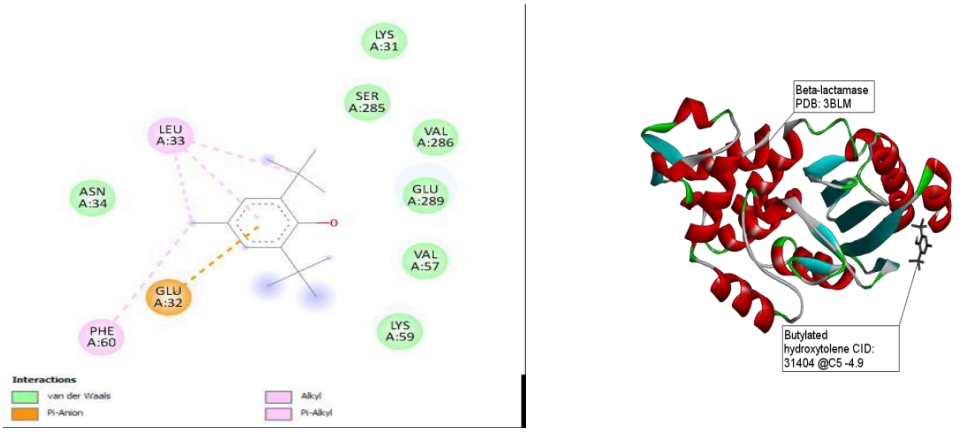


Figure 3.5: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 31404) at binding site denoted as C5.

Molecular docking is a computational method employed to anticipate how molecules might bind to each other, predicting the stability of the resulting complex. The binding energies reported reflect the strength of the interaction at various positions.

Table 4: Represents the binding complex between Beta-lactamase (PDB 3BLM) and ligand compound 5-Eicosene (CID 5364600), showing the vina score, Vander waals, hydrogen, and Alkyl/Pi-Alkyl bonds at different binding positions.

Positions	VS	HB	Vander waals	Alkyl/Pi-Alkyl
C1	-3.3	0	2	6
C2	-3.6	0	7	8
C3	-4.2	0	11	3
C4	-4.0	0	7	7
C5	-3.7	0	7	3

KEY:
VS: Vina Score (Representing binding energy Kcal/Mol)
HB: Hydrogen Bond

FIGURE 4: 2D and 3D Image Representations of Beta-lactamase (PDB 3BLM) and ligand compound 5-Eicosene (CID 5364600) at positions C1-C5 showing the binding affinity and bonds at each binding site.

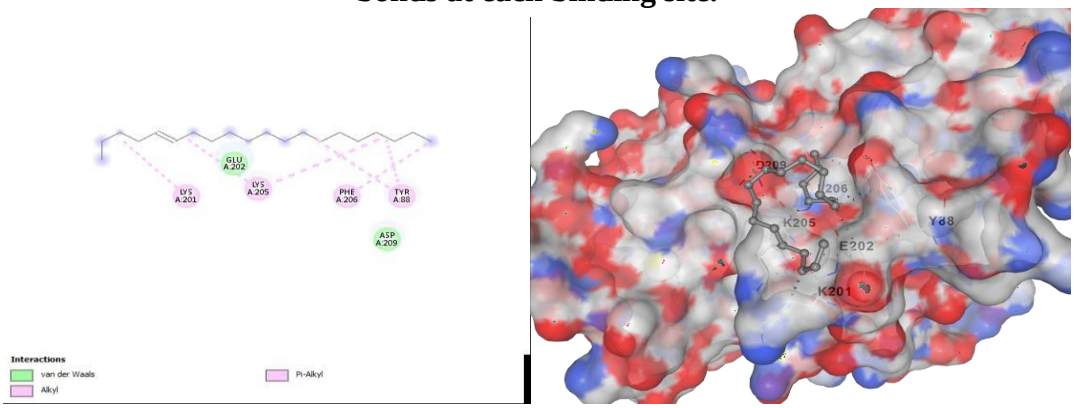


Figure 4.1: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 5364600) at binding site denoted as C1.

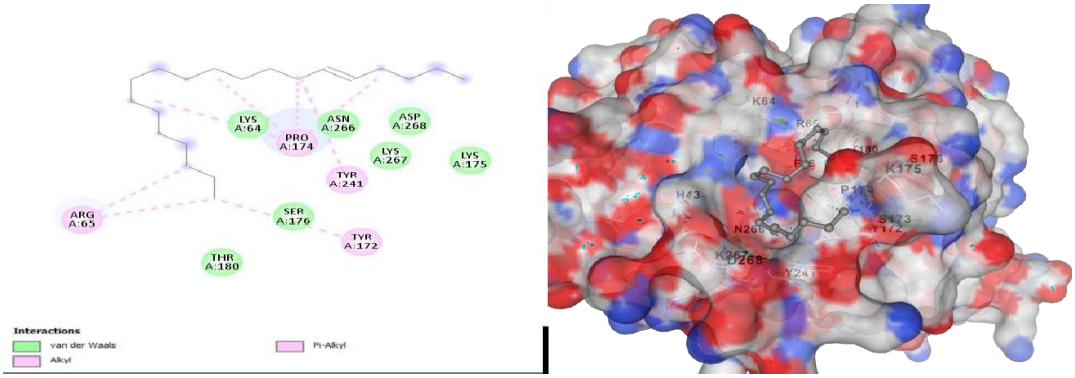


Figure 4.2: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 5364600) at binding site denoted as C2.

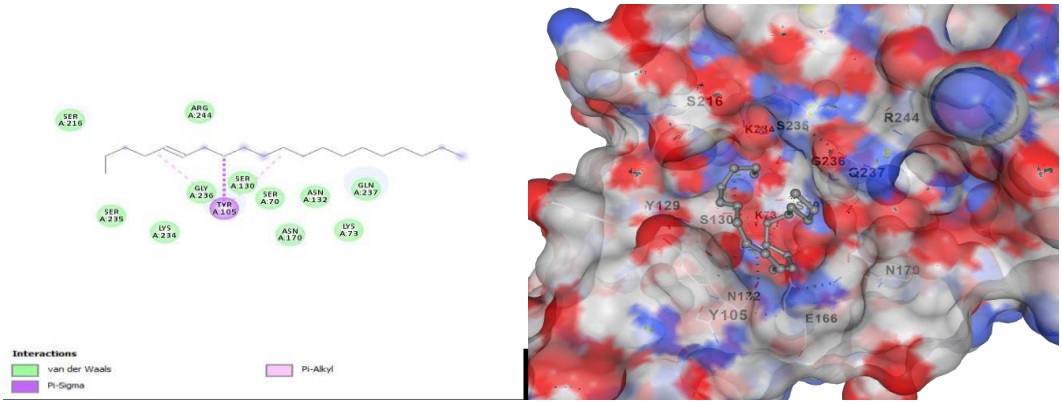


Figure 4.3: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 5364600) at binding site denoted as C3.

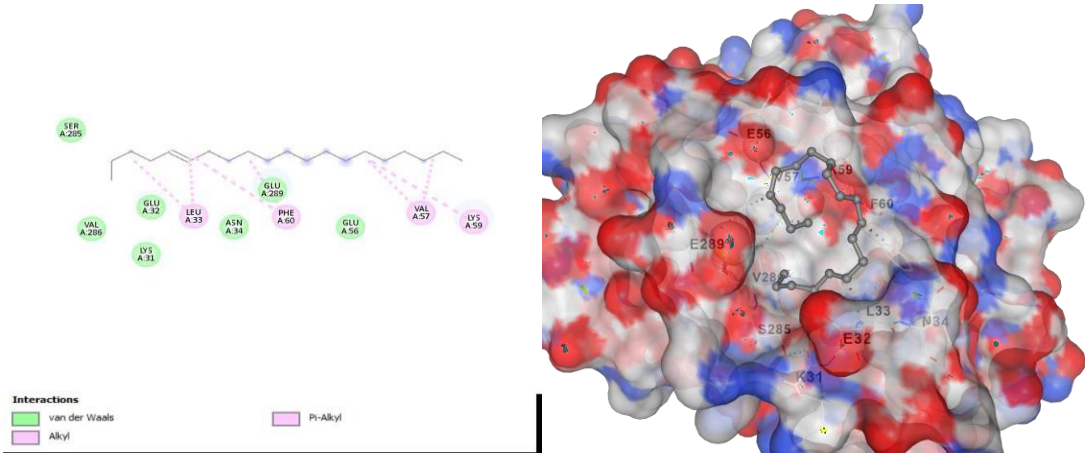


Figure 4.4: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 5364600) at binding site denoted as C4.

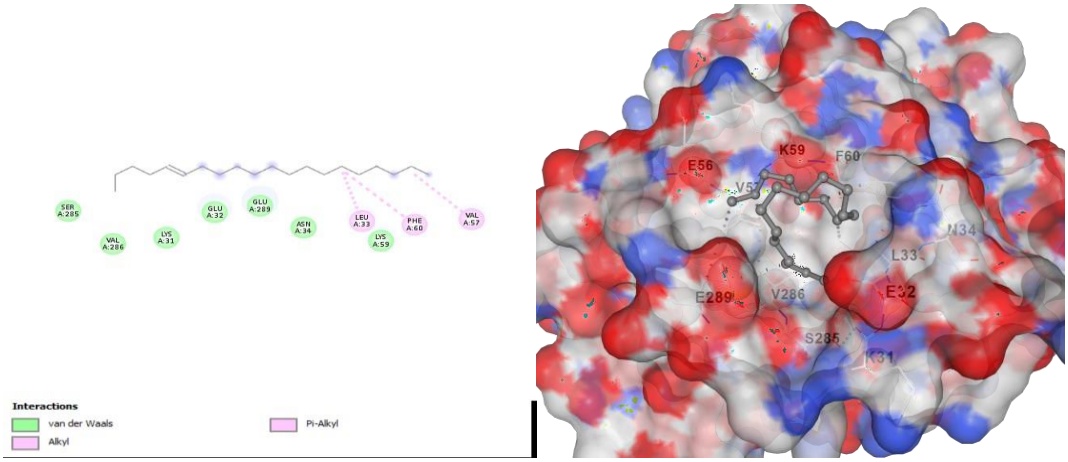


Figure 4.5: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 5364600) at binding site denoted as C5.

Table 5: Susceptibility profile of selected bacteria isolates on the extracts and standard antibiotic agents

Test Microorganisms	*S. ni* (mm)	+C (mm)	-C (mm)	AMX (mm)	CPX (mm)	CN (mm)	S (mm)	AU (mm)	LEV (mm)
Staphylococcus aureus	19.70 ± 0.39	0	0	24.00 ± 0.39	25.50 ± 0.94	23.00 ± 0.60	27.00 ± 0.30	22.50 ± 0.50	23.00 ± 0.84
Escherichia coli	17.00 ± 0.25	0	0	21.00 ± 0.47	26.00 ± 0.56	21.50 ± 0.52	28.50 ± 0.42	20.50 ± 0.50	18.00 ± 0.20

KEY:
S. ni = *Salicia nitida*
+C= Positive control
-C =Negative control
AMX = Amoxicillin
CPX = Ciprofloxacin
CN = Gentamycin
S = Streptomycin
AU = Augmentin
LEV = Levofloxacin
mm = millimeter

The results from the anti-bacterial assay showed that the extracts inhibited the growth of all test bacteria with inhibitory zone diameters range of 17.02 - 19.70 mm and 20.50 - 28.50 mm for the standard known antibiotic agents.

Discussion

Numerous phyto components and bioactive compounds have been shown to be abundant in plants used for antibacterial purposes (Lewis and Ausubel, 2006). Phytochemical screening is a qualitative test used to detect the presence of secondary metabolites in plant materials, and it is based on either formation of color and/or precipitate. In accordance with the findings of Nwiloh *et al.*, (2016), the phytochemical screening results in Table 1 indicate the presence of saponins, tannins, alkaloids, flavonoids, and phenols but the absence of glycosides. Several researchers (Ekweogu *et al.*, 2024; Ajayi *et al*., 2021; Huaib *et al*., 2008; Akubugwo *et al*., 2008; Okorondu *et al*., 2006), have also emphasized the medicinal advantages of these phytochemical bioactive substances, identifying them as potent anti-inflammatory, antioxidant, anti-bacterial, anti-malaria, and wound-healing agents.

The GC-MS analysis of extracts from *Salicia nitida* roots revealed the presence of ten bioactive compounds, which are listed in Table 2. The chromatograph displaying the compound's peaks is displayed in Figure 3, along with the compounds' chemical formulae, molecular weights, and retention times. It is thought that some of these chemical components, which have been found and isolated from other medicinal plant species in the past, are crucial to the defense mechanisms of plants and to the treatment of a variety of illnesses (Olasehinde *et al.*, 2022). Lulamba *et al.*, (2021) isolated 5-eicosene, one of the chemicals found, and showed that it possessed antimicrobial properties. Among the substances with anti-inflammatory and antioxidant qualities were 2-hydroxyethyl ester, 9,12-octadecanoic acid, 9-octadecenoic acid (Z)-, and 9-octadecenoic acid (Ekweogu *et al*, 2024; Kooltheat *et al.*, 2023).

Bacteria interestingly develop resistance against potent antibiotic agents by synthesizing a class of enzymes referred to as beta-lactamases, which enzymatically inhibit the actions of beta-lactam

antibiotics. The alarming rise of microbial resistance to antibiotics has severely limited the efficacy of current treatment options. The prevalence of β-lactamase enzymes is a significant contributor to the emergence of antibiotic resistance. There are four classes of β-lactamases: A, B, C, and D. Class B is the metallo-β-lactamase, while the rest are serine β-lactamases (Arer and Kar, 2023). Molecular docking has become an increasingly important tool for drug discovery (Meng *et al.*, 2011). Although it was originally developed to help understand the mechanisms of molecular recognition between small (ligand compounds) and large molecules (protein receptors), the uses and applications of docking in drug discovery have heavily changed over the last few years (Pinzi and Rastelli, 2019). Molecular docking studies revealed results as shown in (Tables 3 and 4), investigating possible interactions between Class A Beta-lactamase as receptor protein (PDB 3BLM) and the active ingredients Butylated hydroxytolene (CID 31404), and 5-Eicosene (CID 5364600),) isolated from *Salicia nitida* root extracts as ligand compounds. The focus is on understanding their binding modes and binding affinities to enable us to determine possible potential effects as antibacterial agents. The highest binding affinity was observed in the binding site identified at C3 with the binding energy of -5.8 for Butylated hydroxytoluene and -4.2 for 5-Eicosene. Table 3 and 4 meticulously show the binding energies at different positions, offering a quantitative measure of interaction strength, with reported values ranging from -3.3 to -5.8 kcal/mol. At the same time, Figures 3 and 4, depicts two and three-dimensional images showing these interactions, the different types of bonds as well as surrounding residue amino acids. Although the study concentrates on molecular interactions, it is possible to deduce the implications for antibacterial properties. A strong and stable binding between the ligand compound and the receptor protein could suggest a possible role in regulating antibacterial activity. The relevance of binding energy scores is based on the analysis that lower values represent more stable interactions. The lower the binding energy, the higher the binding affinity between ligand and protein receptor (Kollman *et al*., 2000). We observed consistent binding energy across the 5 binding sites shown in Tables 3 and 4. The potential complementarity of ligand compounds to binding sites of protein receptors is accessed for strong and consistent interactions across various positions, supporting the assertion of a stable binding pattern (Kitchen *et al.*, 2004). While the focus remains on molecular interactions, implications for potential antibacterial properties are evident. The different interactions between the ligand compound and the receptor protein suggest a potential role in modulating antibacterial activity (Leach *et al.*,2006). Acknowledging the computational nature of the study, the need for experimental validation is emphasized to strengthen the conclusions.

The root extracts of *Salicia nitida* were tested for their antibacterial potencies against *E. coli* (gram-negative bacteria) and *S. aureus* (gram-positive bacteria). The results are depicted in (Table 5). The root extracts of *S. nitida* exhibited statistically significant antibacterial activity against the two local bacteria isolates when compared to the standard antibiotic agents. The results from the anti-bacterial assay showed that the extracts inhibited the growth of all test bacteria with inhibitory zone diameters range of 17.02 - 19.70 mm and 20.50 - 28.50 mm for the standard known antibiotic agents. The results are in collaboration with works done by Mba'ning *et al.,* (2019) and Nwiloh *et al.,* (2016) who both recorded *Salicia nitida* as a potent antibiotic agent. However, records from (Chukwujekwu *et al.,* 2005; Anyanwu and Okoye, 2017), listed numerous medicinal plants used as anti-microbials in Nigeria but *Salicia nitida* was not mentioned. Interestingly, it has been suggested that weakly active phytochemicals can be combined synergistically to produce new antibacterial treatments with increased pharmaceutical potency (Lewis and Ausburn, 2006).

Conclusion

The results of the present study on the phytochemical screening and GC-MS analysis of *S. nitida* root extracts showed that the plant extract contained some pharmacologically important phytoconstituents. This plant part could represent a potential source of lead molecules with pharmacological activities for the development of new novel pharmaceutical antibiotic products. Also, the presence of compounds with biological activities justifies the traditional use of *S. nitida* root extracts for the treatment of bacterial infections.

Molecular docking analysis highlighted interesting interactions between Class A beta-lactamase enzymes and active compounds present in root extracts of *Salicia nitida*, suggesting a possible modulatory role as an antibiotic agent at the molecular levels. The synergy effects of multiple active compounds may also pose a possible inhibitory effect against beta-lactamase enzymes. The anti-bacterial analysis supported the use of *Salicia nitida* root extracts as an antibiotic agent. However, further studies into the isolation and identification of the individual bioactive compounds responsible for their therapeutic activity and the elucidation of their mechanism(s) of action are needed.

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