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GC-MS profiling, Beta-lactamase Inhibition Assay, and Molecular Docking Studies on Selected Medicinal Plants

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Abstract

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. There is a need to explore sustainable, innovative, and safe natural therapeutic methods in preventing and managing AMR infections. Beta-lactamase secretion by bacteria is one of the main resistant mechanisms bacterial enzymes use to resist antibiotics. Hence, this study investigated the beta-lactamase inhibition potential of Salacia nitida and Rauvolfia vomitoria root extracts. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on the root extracts of S. nitida and R. vomitoria. Molecular docking was performed to determine the binding affinity and energy between class A beta-lactamase and selected bioactive compounds. Results from the beta-lactamase inhibition assay showed that S. nitida and R. vomitoria root extracts had 65.87% and 69.89% inhibitory activity, respectively, against the beta-lactamase enzyme, suggesting that these plant extracts have the potential to be used as a beta-lactamase inhibitor in combination with beta-lactam antibiotics. The GCMS results revealed 10 bioactive compounds in S. nitida and 28 compounds in R. vomitoria. Molecular docking results showed favourable hydrogen bonds and Van der waal interactions between beta-lactamase and selected bioactive compounds. The findings suggest that these plant extracts possess significant beta-lactamase inhibition activity and this can further lend pharmacological support to their folklore uses as antibiotic agents.

***Keywords:* Antimicrobial activity, Binding affinity, Binding energy, Beta-lactamase, Molecular docking, Phytoconstituents**

Abbreviations: CID- Compound Identification number, GCMS- Gas Chromatography Mass Spectrophotometry, PDB- Protein Data Bank, AMR- Anti Microbial Resistance

Statement of Novelty

The novelty of this research resides in the Computational Techniques applied to carry out molecular docking analysis on the bioactive compounds identified from the plant extracts, and their effects on the beta-lactamase enzyme as shown in the results.

INTRODUCTION

The relationship between humans and microorganisms has been ongoing for as long as science has been available. We sometimes refer to the microbial cells in the human body as bacteria (Sanders *et al*, 2019). It's interesting to note that microbes coexist peacefully with humans and carry out crucial tasks necessary for our survival. The bacteria, viruses, eukaryotes, and archaea that live both within and outside of our bodies make up the human microbiome. These organisms influence human physiology in healthy and diseased states, helping improve or impair immunological and metabolic processes (Ogunrinola *et al.*, 2020). Medicinal plants have also made Many plant-derived therapeutic compounds available to modern medicine (Evans, 2000). As a result, these plants are used medicinally to treat a variety of illnesses and diseases (Okorondu *et al.*, 2006; Suresh *et al.*, 2008). Bacterial infections are continually developing resistance to conventional antibiotic agents.

Beta-lactamase secretion by bacteria is one of the main resistant mechanisms used by bacterial enzymes to resist antibiotics and develop resistance. Bacteria often form biofilms, or colonies, on surfaces during infections. These biofilms protect bacteria from antimicrobials and the human immune system while also encouraging cooperation and communication among the bacteria. Antimicrobial resistance (AMR) develops quickly and frequently within the course of a single infection. It happens when bacteria exchange resistance-granting genes with one another or when a bacterium develops resistance due to genetic changes inside its own genome. Even in the absence of antimicrobial resistance, these characteristics make treating biofilm infections challenging (Fitzgerald, 2019; Ogunrinola *et al.*, 2020; Culyba and Van Tyne, 2021). Since the dawn of time, medicinal plants have been used to cure a wide range of illnesses, including bacterial infections. The basic active ingredients used for treating various ailments are accumulated in the roots of plants (Ugbogu *et al.*, 2021; Elekwa *et al.*, 2017). Herbal medicine has demonstrated great potential for therapeutic benefits in modern medicine. Nigeria is endowed with many plants that can be used for medicinal purposes, ***Salacia nitida*** (Benth.) N.E.Br and ***Rauvolfia vomitoria*** (Afzel.) are examples of such plants. Their roots are used as components in concoctions in the treatment of bacterial infections. Despite the use of these plants for such purposes, there is little information on the bioactive composition and antibacterial activity of ***S. nitida*** and ***R. vomitoria***. This work is therefore aimed at documenting the bioactive compositions, assessing the beta-lactamase inhibitory effects, and evaluating the interactions between the bioactive compounds and beta-lactamase enzyme using molecular docking techniques on ***S. nitida*** and ***R. vomitoria*** in a bid to determine its efficacy as potent antibiotic agents or otherwise.

MATERIALS AND METHODS

Plant Preparation: The roots of *S. nitida* and *R. vomitoria* underwent a meticulous preparation process. After harvesting, they were washed and cut into pieces of about 15mm in sizes, sun-dried for two weeks (14 days), and 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, 20 mL of water was added. The mixture was allowed to stand for 24 hours and then filtered. The filtration process's liquid content was subjected to phytochemical screening and GC-MS analysis.

GC-MS Analysis: In accordance with the protocol described by Ugbogu *et al.* (2024) and Ukpai *et al.* (2024), the GC-MS analysis of *S. nitida* and *R. vomitoria* root extracts was carried out by soaking 50 g of ground root extract of *S. nitida* and *R. vomitoria* were soaked in 350 mL of 98% methanol for 24 hours. The crude extract was produced using a rotary evaporator to filter and condense the mixture, and then analyzed by GC-MS using a Buck M910 GC in high electron ionization mode (70 eV). The GC-MS system measured 30 m in length, 250 µm widths and 0.25 µm thicknesses. The carrier gas was ultra-pure helium flowing at a 1.0 mL/min rate. A holding period of around 10 minutes was permitted, and an expansion rate of 3oC/min was applied to the underlying temperature, which was fixed between 50 and 150°C. After that, the temperature was increased by 10 degrees Celsius every minute to 300 degrees Celsius. One microliter of the prepared sample was measured using the splitless mode. MS was performed for 30 min with a scan range of 50-550 m/z in order to complete the scanning. The constituents were identified and characterized by comparing the spectra of the chemical constituents in r.v root extracts with those in the NIST (National Institute of Standards and Technology) collection.

Beta-Lactamase Inhibition Assay: The highly conjugated beta-lactam antibiotics' hydrolysis causes an increase in absorbance at 482 nm, which can be used to determine spectrophotometrically how many antibiotics, like nitrocefin, are hydrolyzed per unit of time. Using the technique outlined by Viswanatha *et al.* (2008), twelve (12) crude plant extracts were examined for their capacity to prevent the hydrolysis of nitrocefin (Ox oid, Ltd.) by beta-lactamase at a single concentration (5 mg/ml).

Molecular docking analysis: The refined crystal structure of beta-lactamase from *Staphylococcus aureus* with PDB code 3BLM, was downloaded from RCSB protein data bank and the 3D structure of ligand compounds; 1-Cyclohexylnonene, Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-, Butylated hydroxytolene and 5-Eicosene with CID codes 5364533, 281840, 31404 and 5364600 respectively were downloaded from PubChem database in sdf format. Amoxicillin, a standard antibiotic drug with Drug Bank Accession Number: DB01060 and PubChem Compound CID: 33613, and Clavulanic Acid, a known beta-lactamase inhibitor with CID 5280980 were also downloaded from PubChem database in sdf format.

Protein Receptor

Refined crystal structure of beta-lactamase from Staphylococcus aureus, PDB 3BLM.

Ligand Compounds

1-Cyclohexylnonene, CID 5364533 (Present in *R. vomitoria*)
Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis- (Present in *R. vomitoria*)
Butylated hydroxytolene, CID 31404 (Present in *S. nitida*)
5-Eicosene, CID 5364600 (Present in *S. nitida*)
Clavulanic Acid, CID 5280980 (Beta-lacatamase inhibitor)
Amoxicillin, CID 33613 (Antibiotic drug)

Using the Biovia Discovery Studio program, the crystal structure of beta-lactamase (PDB 3BLM) was ready for docking by eliminating unnecessary chains, water molecules, and co-crystal ligands (Biovia, 2019). The docking study was conducted using CB-Dock2 software (Liu and Cao, 2024) and Autodock Vina (Trott and Olson, 2010) to determine binding pockets and analyze interactions, binding energy, and binding affinity between protein receptors and ligands. Biovia Discovery Studio software was utilized to generate both 2D and 3D representations of the docking analysis outcomes.

RESULTS

Table 1: Bioactive components from *Salacia nitida* root extract

S/N	RT	MOLECULAR FORMULAR	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

GC-MS Analysis results in Table 1 show ten bioactive compounds present in root extracts of *Salacia nitida*.

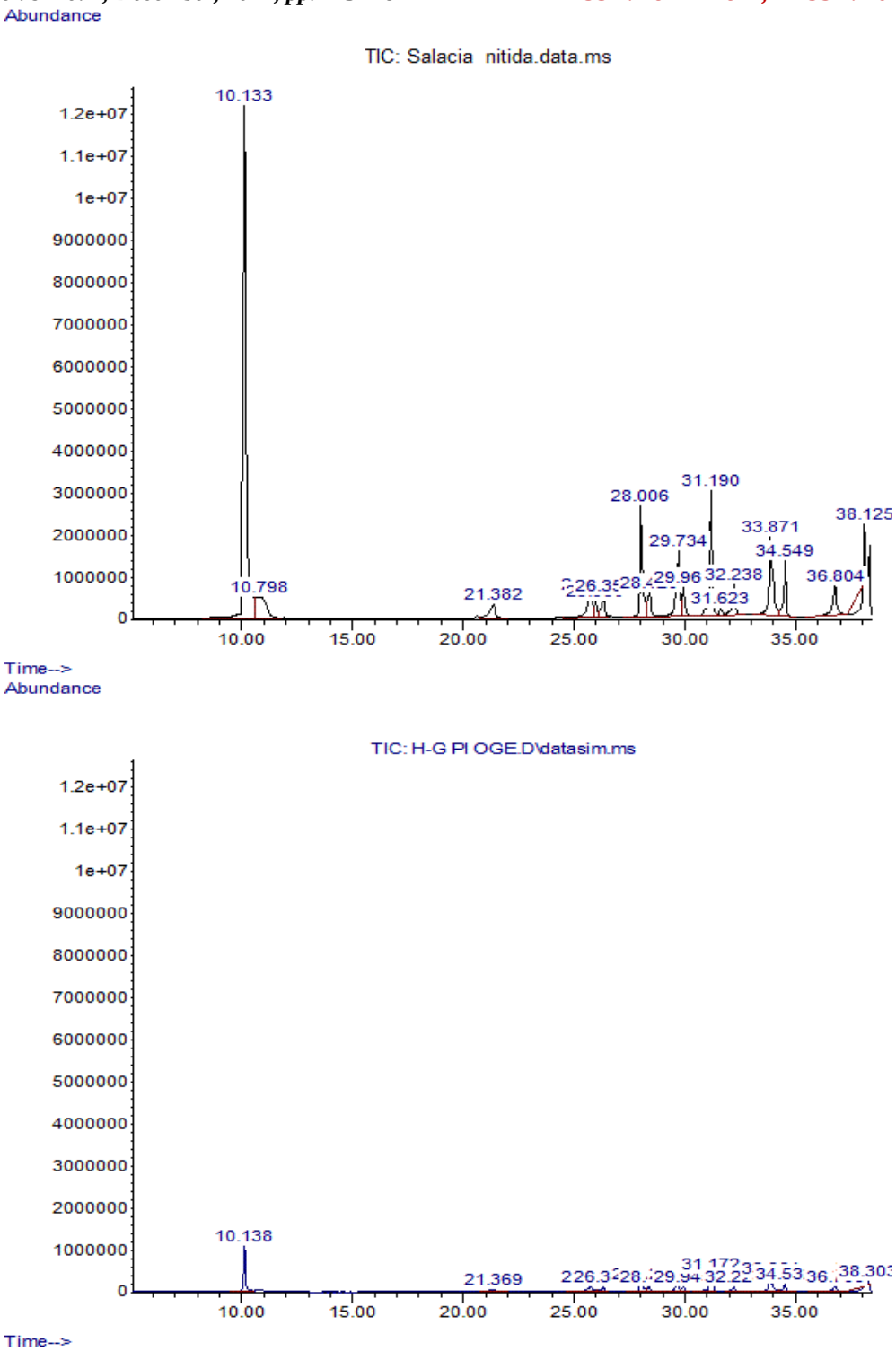

Figure 1: Mass Chromatogram of *S. nitida* root extract

Table 2: Bioactive components from *Rauvolfia vomitoria* root extract

S/N	RT	MOLECULAR FORMULAR	NAME OF COMPOUND	MOLECULAR WEIGHT	PEAK AREA (%)
1	8.000	$C_{22}H_{40}$	Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-	304.6	0.18
2	8.732	C_9H_{18}	Cyclooctane, methyl-	126.24	0.43
3	11.859	$C_{20}H_{40}O_2$	Acetic acid n-octadecyl ester	312.5	0.08
4	16.273	$C_{21}H_{35}F_7O_2$	Heptadecyl heptafluorobutyrate	452.5	0.33
5	16.715	$C_{16}H_{30}O$	7-Hexadecenal, (Z)-	238.41	0.33
6	17.016	$C_{19}H_{38}O$	Disparlure	282.5	0.16
7	17.782	$C_{18}H_{36}$	1-Octadecene	252.5	1.52
8	18.276	$C_{16}H_{29}Cl_3O_2$	Trichloroacetic acid, tetradecyl ester	359.8	0.45
9	18.858	$C_{20}H_{40}$	3-Eicosene, (E)-	280.5	0.42
10	19.071	$C_{15}H_{28}$	Cyclohexane, 1-(cyclohexylmethyl)-4-ethyl-, trans-	208.38	0.31
11	19.324	$C_{15}H_{30}O_2$	Oxirane, [(dodecyloxy)methyl]-	242.4	0.83
12	19.835	$C_{26}H_{37}F_{15}O_2$	Pentadecafluorooctanoic acid, octadecyl ester	666.5	0.44
13	19.974	$C_{34}H_{68}O_2$	Heptadecanoic acid, heptadecyl ester	508.9	0.31
14	20.367	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	270.5	14.61
15	21.108	$C_{19}H_{31}F_7O_2$	Heptafluorobutyric acid, pentadecyl ester	424.4	0.68
16	21.253	$C_{18}H_{34}O_2$	cis-Vaccenic acid	282.5	0.93
17	21.386	$C_{18}H_{35}BrO_2$	2- Bromopropionic acid, pentadecyl ester	363.4	1.28
18	21.809	$C_{18}H_{36}$	1-Octadecene	252.5	6.41
19	22.292	$C_{20}H_{40}$	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	280.5	2.20
20	22.554	$C_{18}H_{35}ClO_2$	Acetic acid, chloro-, hexadecyl ester	318.9	1.76
21	19.071	$C_{15}H_{28}$	1-Cyclohexylnonene	208.38	0.31
22	23.194	$C_{16}H_{27}F_3O_2$	(Z)-Tetradec-11-en-1-yl 2,2,2-trifluoroacetate	308.38	2.31
23	23.427	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester	294.5	21.45
24	23.565	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	296.5	33.38
25	24.165	$C_{19}H_{38}O_2$	Methyl stearate	298.5	3.70
26	25.486	$C_{18}H_{29}F_7O_2$	Heptafluorobutyric acid, n-tetradecyl ester	410.4	0.22
27	34.202	$C_{18}H_{32}O$	9,17-Octadecadienal, (Z)-	264.4	1.03
28	35.083	$C_{30}H_{50}$	Squalene	410.7	0.33

RT = Retention time

GC-MS Analysis results in Table 2, shows the presence of twenty eight bioactive compounds present in root extracts of *Rauvolfia vomitoria*.

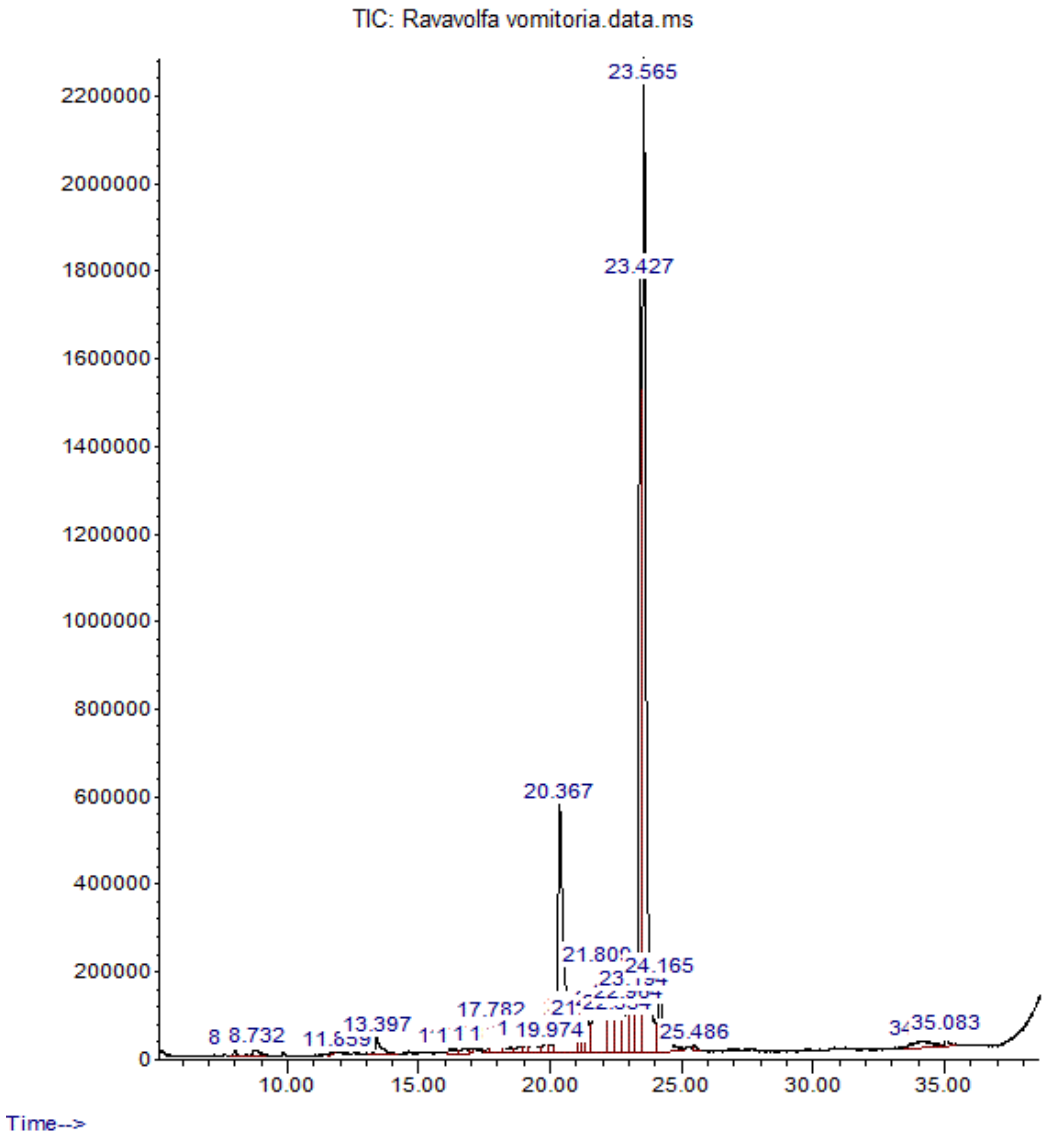

Figure 2: Mass Chromatogram of *R. vomitoria* root extract

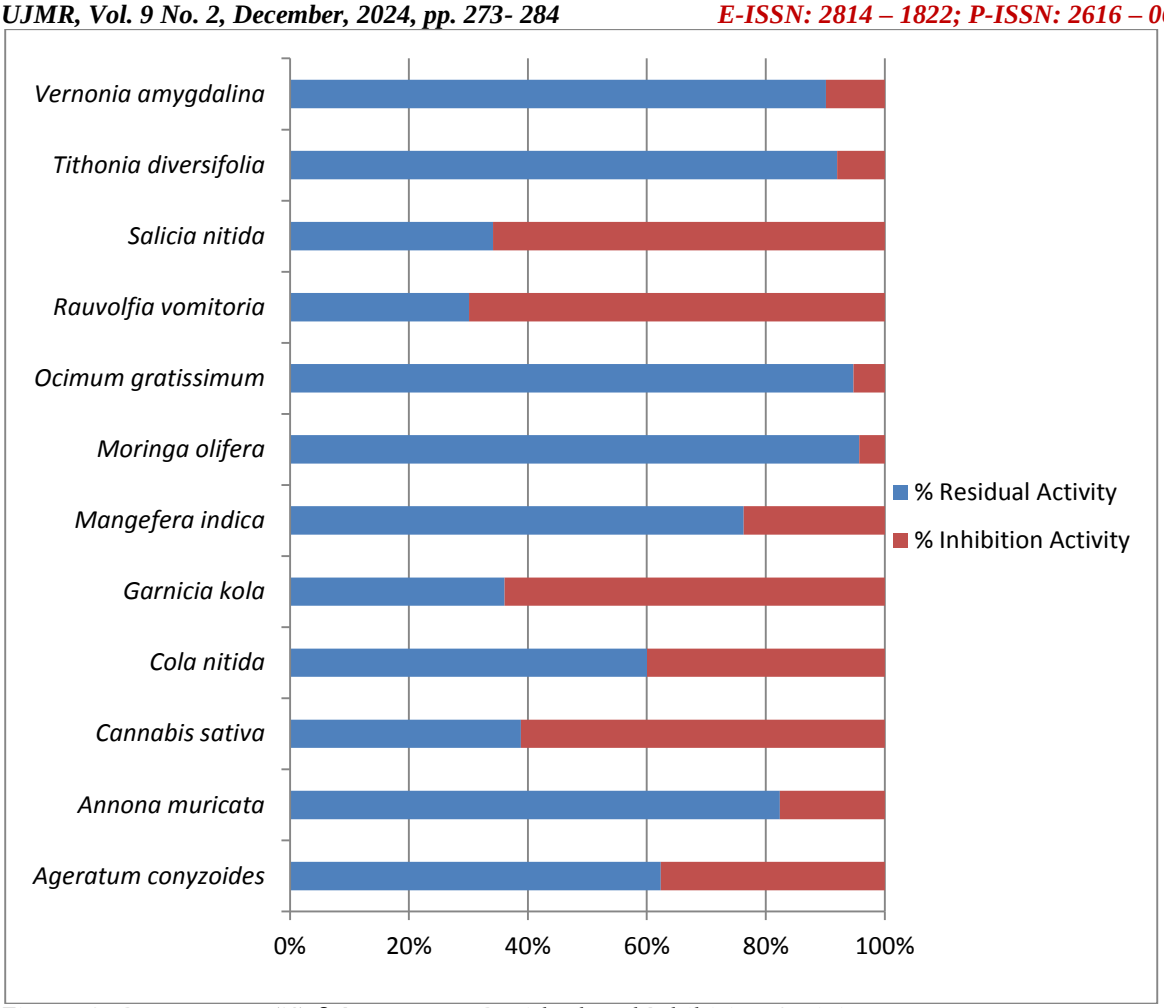

Figure 3: Percentage (%) ß-Lactamase Residual and Inhibition Activity

The root extracts of *Rauvolfia vomitoria* showed the highest inhibition activity against the enzyme with a percentage inhibition of 69.89% followed by the root extracts of *Slicia nitida* with a percentage inhibition of 65.87%, *Garnicia kola* and *Cannabis sativa* leaf extracts also had high percentage inhibition value of 63.90% and 61.20% respectively. While the leaf extracts of *Tithonia diversifolia*, *Ocimum gratissimum*, and *Moringa oleifera* showed the lowest inhibition activity against beta-lactamase enzyme with a percentage inhibition value of 8.02%, 5.23%, and 4.30%, respectively.

Table 3: Represents the binding complex between Beta-lactamase (PDB 3BLM) and ligand compounds Amoxicillin (CID 33613), Butylated hydroxytolene (CID 31404), 1-Cyclohexylnonene (CID 5364533), Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis- (CID 281840), Clavulanic Acid (CID 5280980) and 5-Eicosene (CID: 5364600) showing binding enegry and hydrogen bonds at different binding sites.

BS	CID 33613		CID 31404		CID 5364533		CID 281840		CID 5280980		CID 5364600	
	BE (kcal/ mol)	H B	BE (kcal/ mol)	H B	BE (kcal/ mol)	H B	BE (kcal/ mol)	H B	BE (kcal/ mol)	H B	BE (kcal/ mol)	H B
C1	-5.7	3	-4.9	1	-4.5	0	-4.9	0	-5.0	5	-3.3	0
C2	-5.6	2	-5.1	1	-4.2	0	-4.8	0	-5.0	4	-3.6	0
C3	-7.3	6	-5.8	0	-5.0	0	-6.0	0	-5.9	6	-4.2	0
C4	-5.3	3	-4.4	0	-4.0	0	-5.1	0	-4.3	3	-4.0	0
C5	-5.8	5	-4.9	0	-4.9	0	-5.3	0	-4.6	2	-3.7	0

KEY:
BS: Binding Site
BE: Binding Energy
HB: Hydrogen Bond

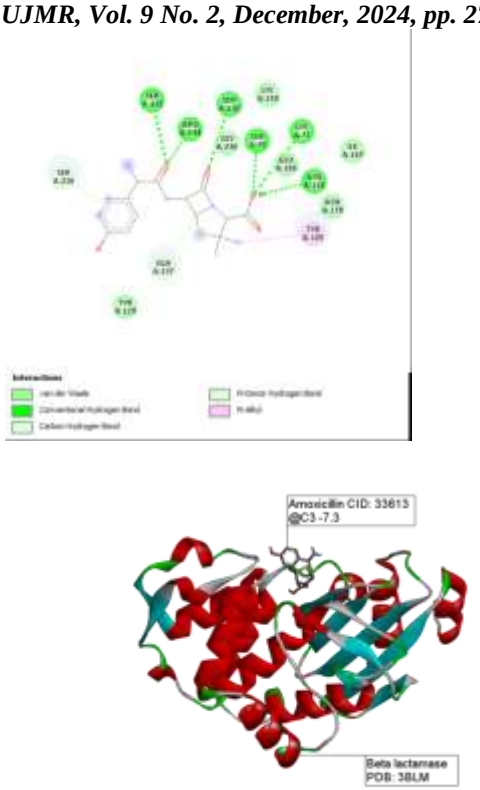

Figure 4: 2D and 3D Image Representations of Beta-lactamase (PDB 3BLM) and ligand compound Amoxicillin (CID 33613) at positions C3 showing binding affinity and bonds.

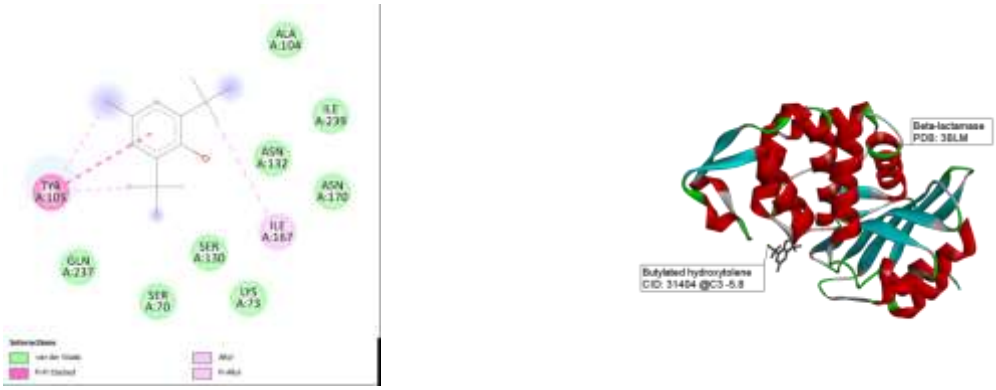

Figure 5: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 31404) at binding site C3 showing binding affinity and bonds.

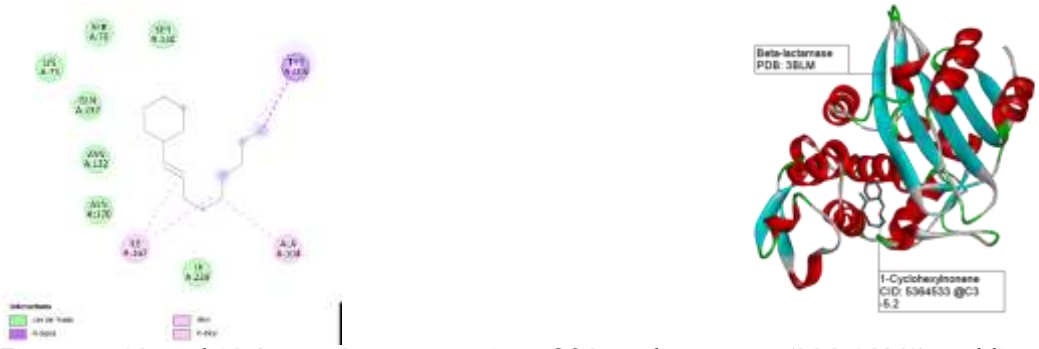

Figure 6: 2D and 3D Image Representations Of Beta-lactamase (PDB 3BLM) and ligand compound 1-Cyclohexylnonene (CID 5364533) at positions C3 showing binding affinity and bonds.

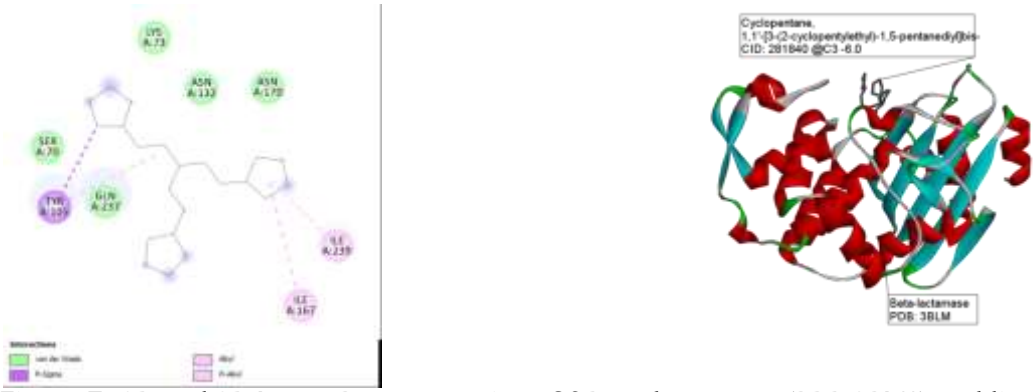

Figure 7: 2D and 3D Image Representations Of Beta-lactamase (PDB 3BLM) and ligand compound Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis- (CID 281840) at position C3 showing binding affinity and bonds.

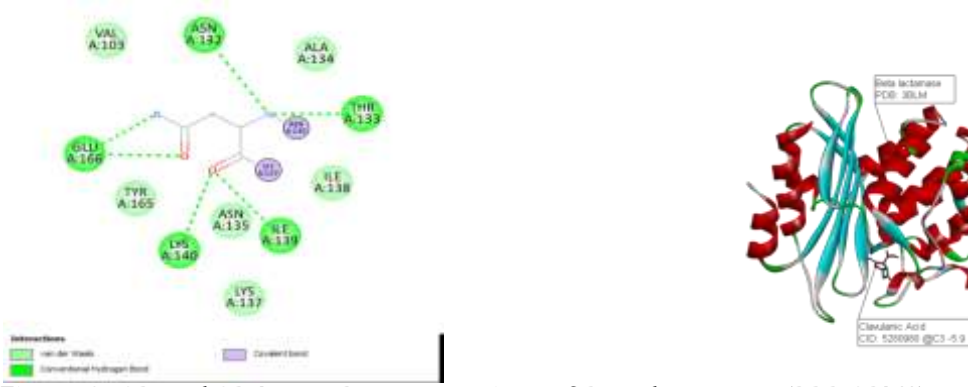

Figure 8: 2D and 3D Image Representations of Beta-lactamase (PDB 3BLM) and ligand compound Clavulanic Acid (CID 5280980) at positions C3 showing binding affinity and bonds.

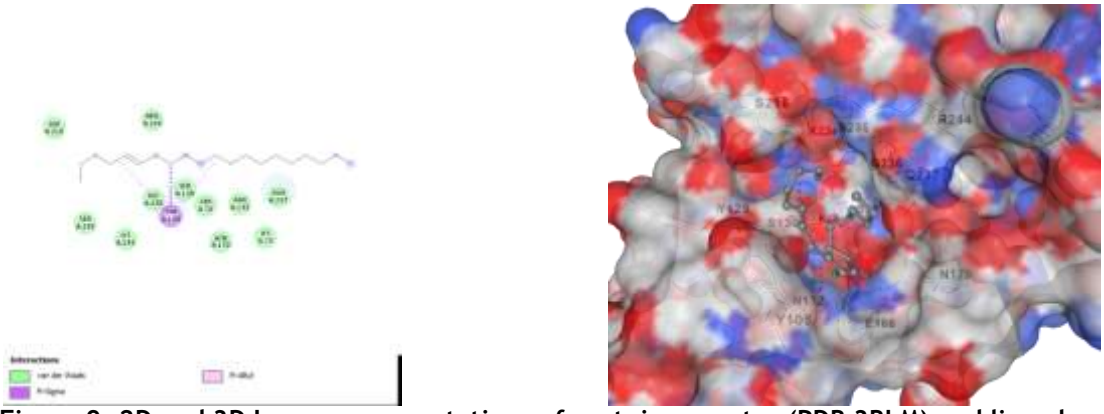

Figure 9: 2D and 3D Image representations of protein receptor (PDB 3BLM) and ligand compound (CID 5364600) at binding site denoted as C3 showing binding affinity and bonds.

DISCUSSION

The GC-MS techniques have demonstrated efficacy and increased reliability in the identification of chemical substances found in plants (Okereke *et al.*, 2017). The GC-MS analysis of *Rauvolfia vomitoria* and *Salacia nitida* root extracts indicates the presence of phytochemical constituents that could contribute to the medicinal quality of the plants. The results from GC-MS analysis of *Rauvolfia vomitoria* and *Salacia nitida* root extracts showed the presence of ten and twenty-eight bioactive compounds listed in (Tables 1 and 2), with their chemical formulas and molecular weights as well as the retention time, the chromatograph showing peaks of the compounds are shown in (Figures 1 and 2). Some of these chemical compounds are considered crucial for both the defense mechanism of plants and the treatment of various illnesses.

They have already been found and isolated from other kinds of medicinal plants (Olasehinde *et al.*, 2022). Among the identified compounds, 5-eicosene, had been isolated by Lulamba *et al.*, (2021) and was shown to possess antimicrobial potentials. The compounds that had anti-inflammatory and anti-oxidant properties included; 9,12-Octadecanoic acid, 9-Octadecenoic acid (Z)-, 9-Octadecenoic acid and 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester (Ekweogu *et al*, 2024; Kooltheat *et al.*, 2023).

A vast reservoir of antibacterial compounds and sensitizers against resistant microorganisms can be found in plants. Many different secondary metabolites resistant to pathogenic invasions and environmental stress are found in plants. Terpenoids, alkaloids, flavonoids, polyphenols, coumarins, and fatty acids are the several categories these substances fall into (Shin *et al.*, 2018). "Antibiotic adjuvants" and "antibiotic potentiators" are other terms for antibiotic sensitizers. While they don't have much or any antibiotic activity, they can strengthen a medication's antimicrobial effects when taken with antibiotics. This approach has the benefit of combating antibiotic resistance from the drug discovery perspective (Wright, 2016). Results from the beta-lactamase inhibition assay in (Figure 3) showed that *Rauvolfia vomitoria and Salacia nitida* ethanol root extracts had 69.89% and 65.87% inhibitory activity, respectively, against the beta-lactamase enzyme, suggesting that these plant extracts have the potential to be used as a beta-lactamase inhibitor in combination with beta-lactam antibiotics. Furthermore, a review by Li *et al.* (2023) reported that the most common enzyme inhibitors in plants are ß-lactamase inhibitors and also listed numerous cases where plant extracts have been successfully used as antibiotic sensitizers.

Molecular docking is a computational method for predicting ligand binding affinities to receptor proteins. It uses computer-based techniques to predict the ligand-receptor complex. Sampling the ligand and applying a scoring function are the two primary processes in the docking process. Considering the binding mode of the ligand, sampling algorithms assist in determining the most energetically advantageous conformations of the ligand within the protein's active site. A score algorithm is then used to order these confirmations. The binding affinity of ligands is assessed, facilitating the determination of which ligand rotation and structure is most beneficial with respect to the receptor protein (Agu *et al*, 2023). It entails predicting the interactions between a target biomolecule, such as an enzyme, and a tiny molecule, which is frequently a prospective drug. This approach investigates the ligand's spatial and energetic compatibility with the receptor's active site, assisting in the development of new drug candidates, refining current molecules, and comprehending the intricate interactions between medications and receptors. A precise scoring function is essential to differentiate high-affinity ligands from low-affinity ones and find prospective drug candidates for validation in studies (Chaudhary and Tyagi, 2024). In order to distinguish active small molecules from inactive ones, the scoring function of protein-ligand interactions is utilized to identify the "native" binding position of a ligand on the protein and to predict the binding affinity. In computational docking of molecules and structure-based drug discovery, scoring functions are frequently utilized (Yan and Wang, 2016). The results from this study as shown in (Table 3), investigating possible interactions between Class A Beta-lactamase as receptor protein (PDB 3BLM) and the active ingredients Cyclohexylnonene (CID 5364533) and Cyclopentane (CID 281840) present in *Rauvolfia vomitoria* and Butylated hydroxytolene (CID 31404), and 5-Eicosene (CID 5364600), present in *Salacia 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 or beta-lactamase inhibitors. The highest binding affinity for plant constituents was observed in the binding site identified at C3 with a binding energy of -6.0 for Cyclopentane, -5.8 for Butylated hydroxytolene, and -4.2 for 5-Eicosene, while Amoxicillin and Clavulanic acid showed binding affinity values of -7.3 and -5.9, also at C3 binding site. Interestingly, [Table 3 and Figures 4-9(4, 5, 6, 7, 8, 9)] meticulously show the binding energies at different positions, offering a quantitative measure of interaction strength, with reported values ranging from -3.3 to -7.3 kcal/mol, and depict 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 the molecular interactions, deducing the implications for antibacterial and/or beta-lactamase inhibition properties is possible. 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 binding affinity between the ligand and protein receptor increases with decreasing binding energy (Kollman *et al.*, 2000). Observation of consistent binding energy across the 5 binding sites is shown in Table 3. 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).

CONCLUSION

The GC-MS examination of root extracts of *Rauwolfia vomitoria* and *Salacia nitida* in this study showed that the plant extracts contain lots of phytoconstituents, which are pharmacologically important, thus, the study has ascertained the presence of bioactive phytoconstituents in root extracts of these plants. The findings also suggest that these extracts are potential beta-lactamase inhibitors. The molecular docking analysis gave an insight into the potential inhibitory interactions between beta-lactamase and bioactive compounds present in the root extracts. Furthermore, the root extracts contain a number of biologically active substances which are responsible for numerous pharmacological actions. Finally, the study recommends further investigations to pinpoint the active ingredients in question that may have therapeutic effects in the root extracts.

Authors' Contributions

"Conceptualization, IWU., OSC., and NCI.; methodology, EGE.; validation, OOS., NCI. and ACF; formal analysis, AC.; investigation, IWU; resources, IWU.; data curation, NCI; writing—original draft preparation, IWU.; writing—review and editing, ACF.; supervision, OSC., and NCI; project administration, AC.; funding acquisition, IWU. All authors have read and agreed to the published version of the manuscript.

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