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GC-MS and *In-Silico* Assessment of Orange Peel Phyto-compounds as Antimicrobial and Drug-Like Agents

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ABSTRACT

Plants are valuable sources for creating new medicines because they contain many natural compounds that can have positive health effects due to their secondary metabolites. This study investigates the *in silico* antimicrobial activity of methanol extract of orange peels (*Citrus sinensis*) against strains of bacteria and fungi. Gas Chromatography–Mass Spectrometry (GC-MS) was employed to analyze the methanolic extract, known for its rich bioactive constituents, with the aim of identifying possible antimicrobial compounds. The analysis revealed eighteen compounds. *In silico* experiments through molecular docking using PyRx software showed different binding scores of the compounds with the selected proteins: *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Compound No. 10 had the best docking score and favorable features compared to the control drugs (ketoconazole, ciprofloxacin, and nalidixic acid). Additionally, this outperforming compound demonstrated favorable ADME properties, confirming its potential as a drug for treating infections caused by these organisms. In conclusion, methanol extract of the orange peel possess bioactive compounds that could be used to develop promising antimicrobial drugs.

Keywords: Orange peels, Molecular Docking, Phyto-compounds, *In-silico*, Ligands, Proteins

1. INTRODUCTION

Since ancient times, humans have recognised many plants' ability to heal infections due to their secondary metabolites (Attah *et al*., 2020). A recent study has shown that these chemicals are also efficient antibacterial agents against human infections (Umashankar, 2020). Over the last decade, there has been a lot of study on phytochemicals and their capacity to kill bacteria, particularly resistant strains of both Gram-negative and Gram-positive bacteria (Jubair *et al*., 2021). Antimicrobial resistance is becoming a severe worldwide concern owing to bacterial and fungal strains (Abushaheen *et al*., 2020). The World Health Organization predicted that antibiotic resistance will become the primary cause of death. Obtaining a novel therapeutic substance through these computational methods will bypass the

resistance posed by pathogens. Plants with medicinal value had been known for treatment. These are gifts of nature with different physiological conditions (Oligie *et al*., 2023).

The *Citrus sinensis* is one of the most widely produced fruit trees in the world, especially in African conditions (Singh *et al*., 2021). It is a member of the Rutaceae family. The local names of the *Citrus species* in Hausa and Yoruba are "Leemun" and "Osan," respectively. The English name is sweet orange (Oligie *et al*., 2023). The plant may reach a height of six meters and has large, round, glossy, evergreen leaves with delicate wings on the petioles (Oligie *et al*., 2023). According to Olakunle and Titilayo (2018), categorically, orange peel is a byproduct of fruit that possesses many bioactive compounds. The insecticidal properties of several varieties of orange peel and seed extract have been studied against many types of insects because of their secondary metabolites (Ukoroije and Otayor, 2020). Oligie *et al*. (2023) reported the antimicrobial property of orange peel against *E. coli* and *S. aureus. Candida albicans and Salmonella typhi,* which vindicate this present study.

Many compounds used to treat infectious illnesses are either naturally occurring or partly synthetically changed to boost their efficiency, such as using natural product engineering to generate potent antibiotics. Therefore, it is critical to evaluate natural materials for potential treatment methods against infectious diseases. Plant-derived compounds, in particular, have shown promise for treating bacterial and fungal infections (Guevara-Lora *et al*., 2020).

The initial stage in drug research is determining which illness to target (Deore *et al*., 2019). Scientists may concentrate on specific enzymes or receptors that may play a role in the disease's pathogenesis by learning about its biochemical pathways or genetic components.

Rational drug design is primarily reliant on molecular docking, a computer process made possible by tools like AutoDock Vina. Molecular docking is used to predict the binding orientation of a small molecule (ligand) to the active site of a protein, resulting in a stable protein-ligand complex (Bhagyashri *et al*., 2023). Hence, the approach is very essential in identifying antimicrobial agents (Jakhar *et al*., 2020). Despite the established antibacterial properties of citrus peels, there has been little investigation into the identification and *in silico* assessment of specific bioactive components in orange peel extracts for targeted medication development. As a result, the goal of this research is to identify prospective antimicrobial medication candidates using molecular docking of phytocompounds found by GC-MS analysis of an orange peel of methanol extract.

2. MATERIALS AND METHODS

Collection and preparation of orange peels

Orange peels were collected from orange sellers in Bosso Local Government, Minna, Niger State, and brought to the Department of Microbiology; they were rinsed with clean water and dried for three days at room temperature. The dried orange peels were pulverized into powder with the aid of an electric blender for extraction.

Plant materials and extraction

A 100 g amount of the powdered peels was immersed in 600 mL of methanol. The mixtures were agitated every 6 hours while being left to stand at room temperature (28ºC) for seven days. The Whatman (No.1) filter paper was used to filter the extract after being sieved through muslin cloth (Zode and Chakole, 2020). To create a greasy mass, the extracts were concentrated by heating them to 50°C in a water bath. Once the greasy mass was formed, it was utilized as the final material for the extractions of methanol. It was then placed into screw-cap bottles, labeled, and kept in a refrigerator between 2 and 5°C until needed.

The Analysis of GC-MS

The analysis of orange peel of methanol extract was performed at NARICT in Zaria, Nigeria, using GC-MS (Model QP 2010 PLUS, Shimadzu, Japan). The system includes a 30-meter-long VF-5ms fused silica capillary column with a diameter of 0.25 mm and a film thickness of 0.25 µm. The column oven temperature was set from 80°C to 280°C at 2°C/min. The sample components were ionized using electron impact (EI) at energy of 70 eV. The temperature of the injector was set at 250°C, and one of the detectors was set to 200°C. The carrier gas used was helium (99.9995% purity), with a set flow rate of 1.5 mL/min. The mass range of 40–1000 m/z was scanned at a rate of 3.0 scans per second. A Hamilton syringe was used to manually inject one microliter (1.0 µl) of the extract samples into the GC-MS for total ion chromatography (TIC) using the split injection method. The total running duration of the GC-MS is 27 minutes. The relative proportion of each extract's contents was reported as a percentage using peak area normalization. GC-MS combines two

analytical methods to provide a single approach for studying the combination of chemical compounds. Gas chromatography separates the mixture's components, and mass spectroscopy examines each of them individually.

Identification of components

The National Institute of Standards and Technology (NIST) database, which has more than 62,000 patterns, was used to determine the mass spectrum of the GC-MS. The NIST database and the Fatty Acid Methyl Esters Library version 1.0 (FAME library) were used to interpret the mass spectrum GC-MS after the spectrum of the unknown component was compared to the spectrum of known components kept in the NIST Library. The discovered components in the extract were matched using sources. The features of component test materials, such as molecular weights and retention time, were determined, and the results were tabulated. The known and unknown components' spectra were contrasted (Abdulsalami *et al*., 2022).

Molecular Docking of the Ligands against Proteins (microbial enzymes)

Three protein targets based on the past study were selected, namely 14-α sterol demethylase of *Candida albicans,* DNA gyrase of *Staphylococcus aureus,* and DNA gyrase of *Escherichia coli* (Eakin *et al*., 2012; Narramore *et al*., 2019; VL *et al*., 2022; Ejidike *et al*., 2024).

The X-ray diffraction structures of these proteins were taken from the RCSB Protein Data Bank in PDB ID: 5FSA, 3U2D, and 6F86, respectively, in PDB format. The ligands were the phytoconstituents of the methanol extract of the orange peels, identified through GC-MS analysis in this study. Before the docking study, protein preparation was conducted using Discovery Studio 2021 Client, which involved removing water molecules, unwanted residues, and other inhibitors (ligands) that were already co-crystallized with the proteins; finally, energy minimization was performed using Swiss Viewer for protein data.

The structures of phytoconstituents (ligands) were prepared by downloading them from the PubChem database in 3D conformer as an SDF file format. All the ligands were then converted to the Protein Data Bank (PDB) file format with 2021 Discovery Studio Client. Three standard drugs used as controls, namely ketoconazole for fungal, ciprofloxacin for *S. aureus,* and nalidixic acid for *E. coli,* were selected for comparison of docking scores. After the assignment of the Kollmann and Gasteiger charges to protein in the PyRx software where it was loaded, it was converted to the PDBQT file format, followed by loading ligands. The energy of the loading was also converted to the PDBQT file format.

Subsequently, the small molecules and protein were prepared for simulation, and a grid box appeared on the protein structure interface to define the binding site dimensions: center x = 195.5400, center y = -2.2838, and center z = 38.7426 for 5FSA; center x = 61.710, center y = 28.3355, and center z = 64.399 for 3U2D; and center x = 0.1821, center y = 2.6511, and center z = 24.1660. Grid dimensions were assigned, and then Vina in the PyRx program was used to perform molecular docking. Once the procedure was completed, each of the ligands produced nine poses and matching docking scores. Using Discovery Studio 2021 Client, the docking contacts of the ligand that performed best compared to standard drugs were displayed in two dimensions (2D) (VL *et al.,* 2022).

Determination of Druggability, pharmacokinetics, and toxicity

The canonical SMILES strings of the best docked bioactive compounds were pasted in the Swiss ADME web server (www.swissadme.ch), and the pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/prediction) to evaluate its druggability, Pharmacokinetics and toxicity. Lipinski's Ro5 was used. According to this rule, when there are more than five H-bond donors, ten H-bond acceptors, the molecular weight is more than 500, and the computed Log P (Log P) is more than five. If any compound had two or more Ro5 violations would have low solubility and/or poor permeability (Benet *et al*., 2016). The Boiled-Egg model, available on SwissADME was used to estimating gastrointestinal (GI) absorption and brain penetration (BBB) of the best docked molecule and control drugs. It's based on a visual representation, resembling an egg, where the yolk signifies brain penetration and the white represents GI absorption.

3. RESULTS

Analysis of GC-MS of the methanol extract of orange peels

A total of 18 phytocompounds were identified in the GC-MS analysis of the methanol extract of orange peels. Compounds were distinguished based on their peak number, retention time and area abundance as shown in Table 1 and the chromatogram of the GC-MS which showed different peak corresponding to the compound per each peak was shown in Figure 1. Ethyl beta -d-riboside

exhibited the highest relative abundance (21.65%), suggesting it is significant component of the extract followed by 9,12-Octadecadienoyl chloride, (Z, Z)- (14.23%) and 4-Hepten-3-one, 4-methyl- (13.89%).

Table 1: GC-MS Analysis of Methanol extract of orange peels

Peak	Retention time	IUPAC Name of the compounds	Area (%)	Molecular formula	Molecular weight
1	3.658	2-butoxyethanol	1.92	$C_6H_{14}O_2$	118
2	4.527	(2-methyl-1-nitropropan-2-yl) acetate	4.17	$C_6H_{11}NO_4$	161
3	6.103	4-hydroxy-2,5-dimethylfuran-3-one	1.36	$C_6H_8O_3$	128
4	6.534	1-(1-methylcyclopropyl)ethanone	1.66	$C_6H_{10}O$	98
5	7.335	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	5.64	$C_6H_8O_4$	144
6	8.371	methyl carbamimidate;hydrochloride	4.03	$C_2H_6N_2O$	74
7	8.851	(*E*)-4-methylhept-4-en-3-one	13.89	$C_8H_{14}O$	126
8	9.383	4-ethenyl-2-methoxyphenol	4.41	$C_9H_{10}O_2$	150
9	9.882	2-methyl-1,4-dihydroimidazol-5-one	2.36	$C_4H_6N_2O$	98
10	2.626	9,11-bis(4-hydroxyphenyl)-8-oxa-12,13,15,17-tetrazatetracyclo[8.7.0.0^{2,7}.0^{12,16}]heptadeca-1(10),2,4,6,13,15-hexaen-4-ol	1.33	$C_{24}H_{18}N_4O_4$	428
11	15.100	2-ethoxy-5-(hydroxymethyl)oxolane-3,4-diol	21.65	$C_7H_{14}O_5$	178
12	18.690	hexadecanoic acid	2.63	$C_{16}H_{32}O2$	256
13	21.117	(9Z,12Z)-octadeca-9,12-dien-1-ol	0.55	$C_{18}H_{34}O$	266
14	21.409	(Z)-octadec-9-enoic acid	10.97	$C_{18}H_{34}O_2$	282
15	22.833	1-fluorodecane	1.16	$C_{10}H_{21}F$	160
16	24.643	undec-10-enal	2.14	$C_{11}H_{20}O$	168
17	25.250	2,3-dihydroxypropyl hexadecanoate	5.91	$C_{19}H_{38}O_4$	330
18	27.164	(9Z,12Z)-octadeca-9,12-dienoyl chloride	14.23	$C_{18}H_{31}ClO$	298

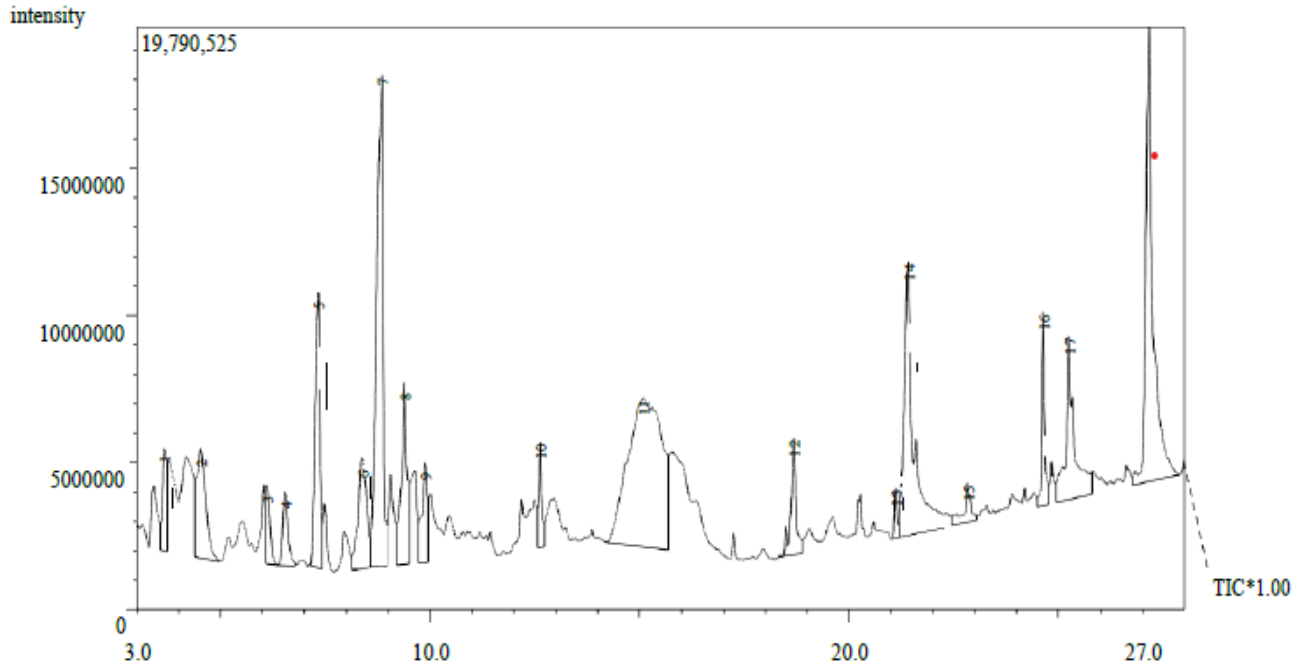


Figure 1: Chromatogram of GC-MS Analysis of Methanol Extract of Orange Peel.

Molecular Docking Studies

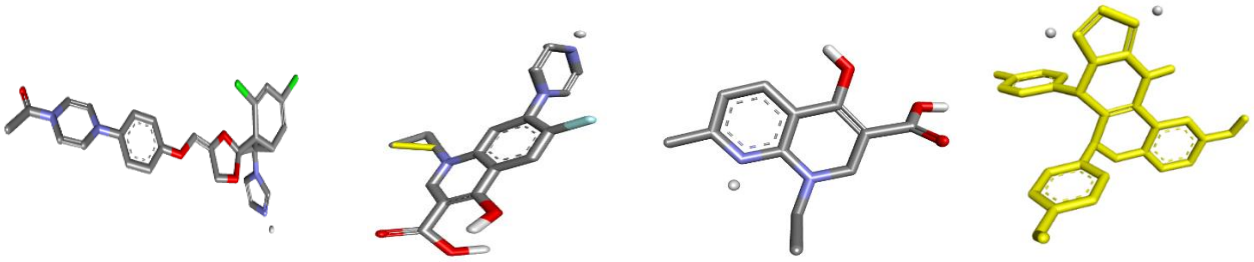
The docking of eighteen bioactive compounds in methanol extract of orange peel, as per the GC-MS report, was done using PyRx software. The bioactive compounds were docked against the following proteins: 14-α sterol demethylase (5FSA) of *Candida albicans*, DNA gyrase (3U2D) of *Staphylococcus aureus,* and DNA gyrase (6F86) of *Escherichia coli*. Among all the bioactive compounds, compound No. 10 showed the best docking scores compared to the rest of the seventeen. The binding affinity score of compound No. 10 was higher than ciprofloxacin (-6.1) and nalidixic acid (-6.5) for gram-positive and gram-negative bacteria, while ketoconazole (-11.5) was higher than compound No. 10, which was summarized in Table 2.

The controlled ligands (ketoconazole, ciprofloxacin, nalidixic acid) and the best docked ligand were represented in Figure 2. The 2d visualization of 5FSA and ketoconazole showed 25 interactions with amino acids residues without hydrogen bond as indicated in Figure 3 while the 2d visualization of compound No. 10 with 5FSA showed 16 interactions with amino acids residues and one conventional hydrogen bond shown in Figure 4. The 2d visualization of 6F86 and nalidixic acid in Figure 5 showed 13 interactions with amino acids residues without hydrogen bond while compound No. 10 in Figure 6 showed 8 interactions with amino acids, 2 conventional hydrogen bonds and one carbon hydrogen bond and lastly, the 2d visualization of 3U2D and ciprofloxacin in Figure 7 showed 12 interactions with amino acid residues, 2 conventional hydrogen bonds while compound No. 10 had 16 interactions, 1 convectional hydrogen bond and 2 pi-donor hydrogen bonds as shown in Figure 8.The interacting residues were depicted by a ball and stick model, the ligands were represented by different colours.

Table 2: PDB ID, Organisms, Target proteins, docking score of standard drugs andphytochemicals

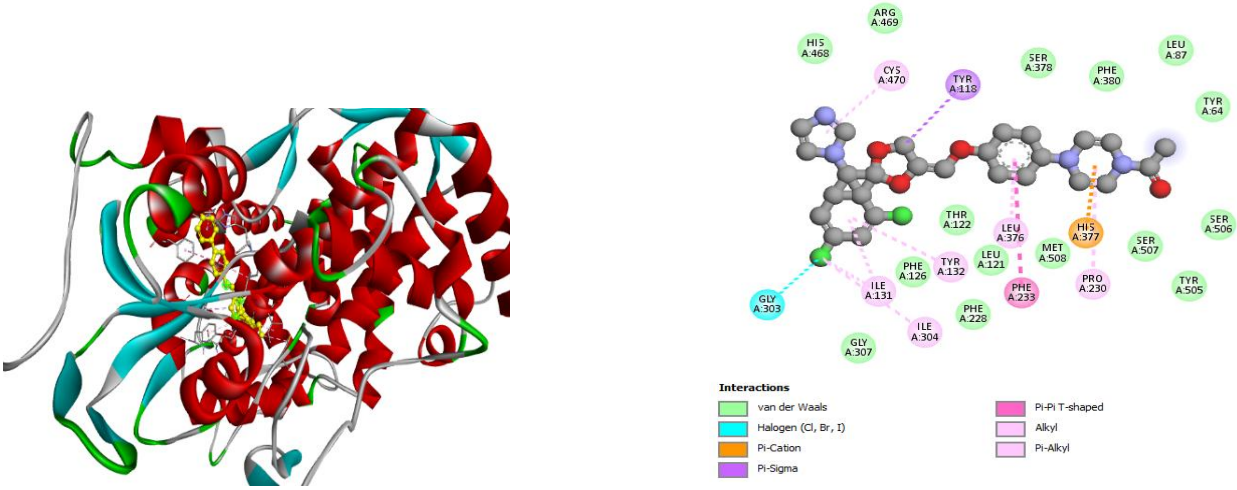
Protein Data Bank ID		***5FSA***	**3U2D**	**6F86**
Organisms		*C. albicans*	*S.aureus*	*E. coli*
Target proteins		*14-α Sterol demethylase*	DNA gyrase	DNA gyrase
Standard drugs and docking scores (kcal/mol)				
Ketoconazole		-11.5	NA	NA
Ciprofloxacin		NA	-6.1	NA
Nalidixic acid		-6.NA	NA	-6.5
Orange peels phyto-compounds' docking scores (kcal/mol)	**PN**	-4.2	-3.4	-4.4
2-butoxyethanol	1			
(2-methyl-1-nitropropan-2-yl) acetate	2	-5.2	-3.9	-4.1
4-hydroxy-2,5-dimethylfuran-3-one	3	-4.9	-3.9	-4.9
1-(1-methylcyclopropyl) ethenone	4	-4.5	-3.5	-3.9
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	5	-5	-4.3	-5.1
methyl carbamimidate;hydrochloride	6	-3.7	-2.8	-3.4
(*E*)-4-methylhept-4-en-3-one	7	-5.1	-4.1	-5
4-ethenyl-2-methoxyphenol	8	-5.9	-4.3	-5.2
2-methyl-1,4-dihydroimidazol-5-one	9	-4.4	-3.6	-4.6
9,11-bis(4-hydroxyphenyl)-8-oxa-12,13,15,17-tetrazatetracyclo[8.7.0.0^{2,7}.0^{12,16}]heptadeca-1(10),2,4,6,13,15-hexaen-4-ol	10	-9.7	-8.5	-7.9
2-ethoxy-5-(hydroxymethyl)oxolane-3,4-diol	11	-5.3	-4.2	-4.7
hexadecanoic acid	12	-6.3	-4.9	-4
(9Z,12Z)-octadeca-9,12-dien-1-ol	13	-6.7	-5.2	-4.8
(Z)-octadec-9-enoic acid	14	-6.7	-5.1	-4.3
1-fluorodecane	15	-5	-3.9	-4.9
undec-10-enal	16	-5.4	-4	-4.6
2,3-dihydroxypropyl hexadecanoate	17	-6.8	-5.1	-5.1
(9Z,12Z)-octadeca-9,12-dienoyl chloride	18	-6.4	-5.5	-4.6

Keys: NA = Not applicable, PN = Peak Number



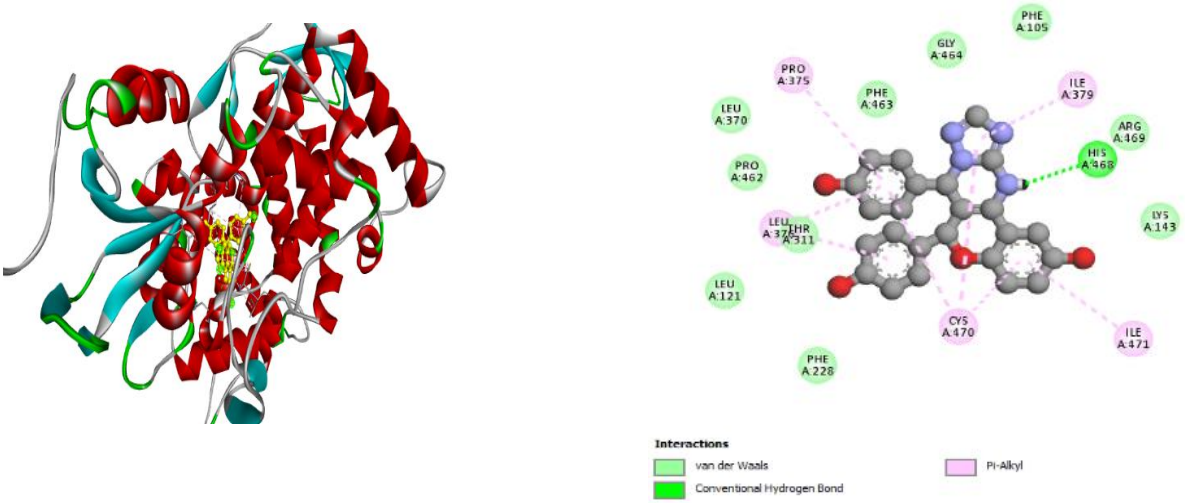
(a) Keteconazole **(b)** Ciprofloxacin **(c)** Nalidixic acid **(d)** No. 10

Figure 2: The 2D of controlled drugs and the best docked compound (No. 10)



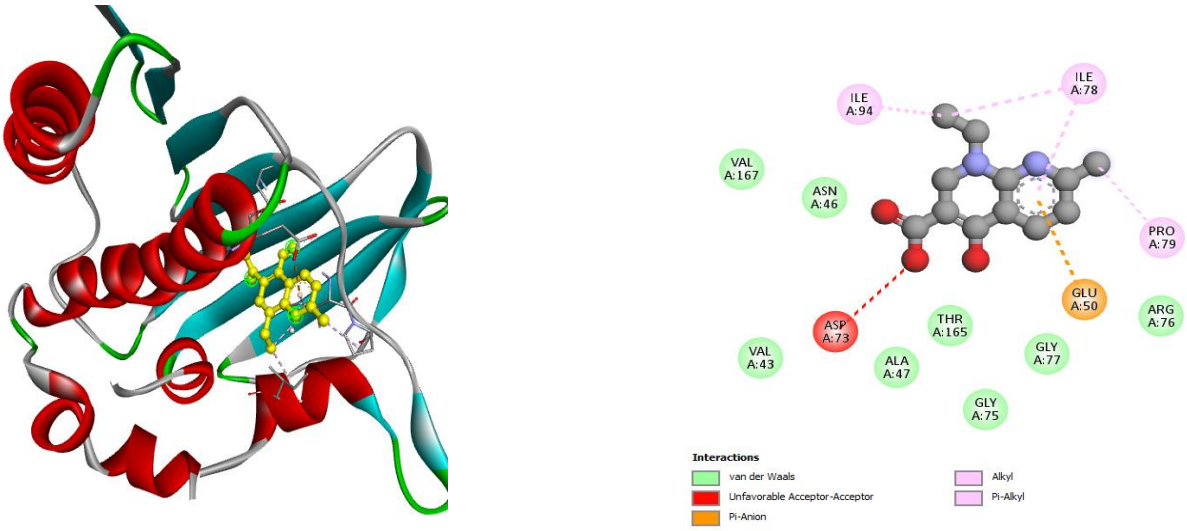
(a) 5FSA and Ketoconazole 3d interaction **(b)** 5FSA and Ketoconazole 2d interaction

Figure 3: The 3D and 2D visualization of docking analysis of ketoconazole molecular interaction with 5FSA



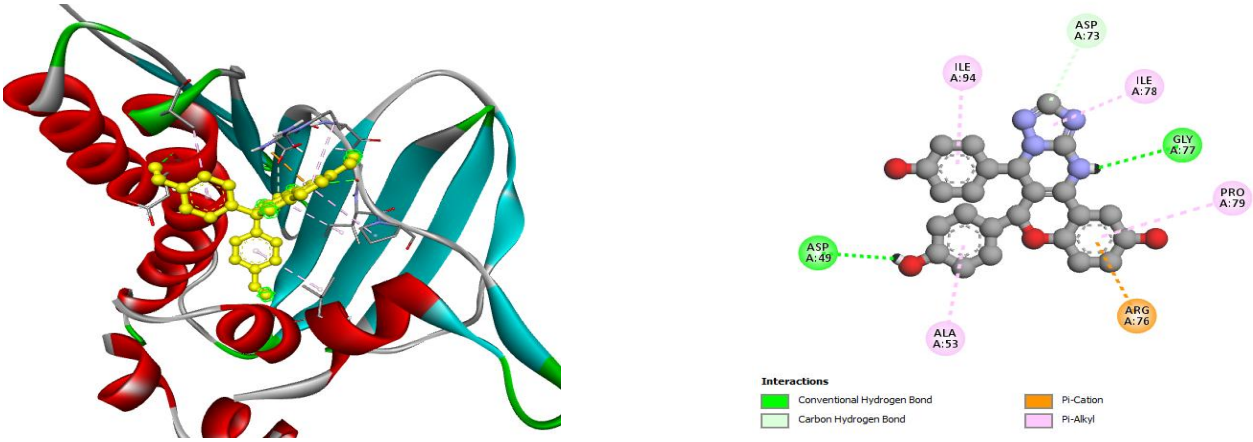
(a) 5FSA and No.10 3d interaction **(b)** 5FSA and No 10 2d interaction

Figure 4: The 3D and 2D visualization of docking analysis of No. 10 molecular interaction with 5FSA



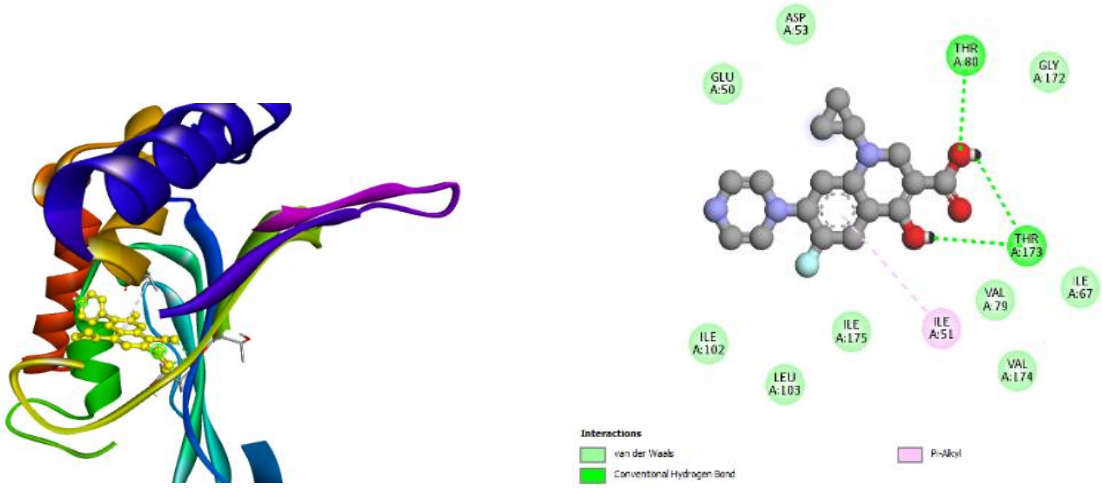
(a) 6F86and nalidixic acid 3d interaction **(b)** 6F86 and nalidixic acid 2d interaction

Figure 5: The 3D and 2D visualization of docking analysis of nalidixic acid molecular interaction with 6F86



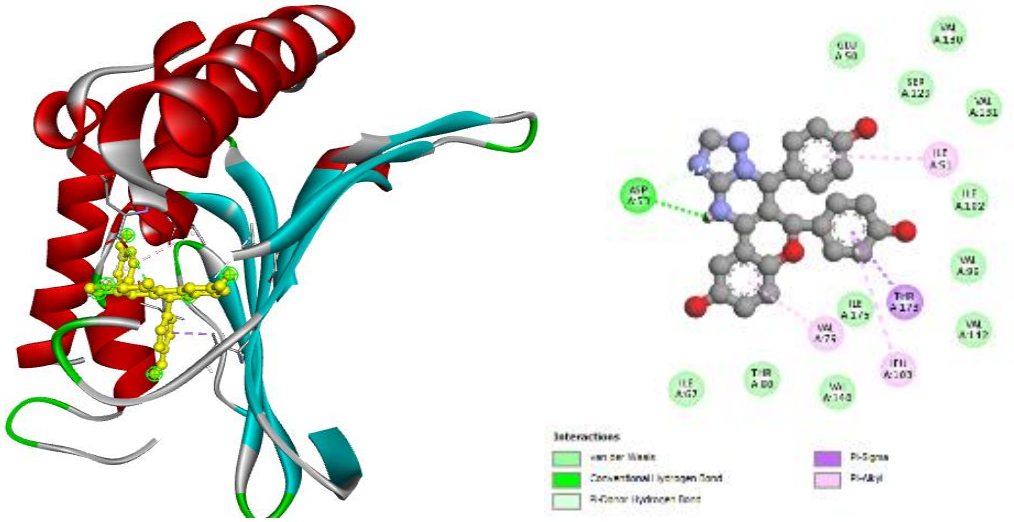
(a) 6F86 and no 10 3d interaction **(b)** 6F86 and no 10 2d interaction

Figure 6: The 3D and 2D visualization of docking analysis of No.10 molecular interaction with 6F86



(a) 3U2D and ciprofloxacin 3d interaction **(b)** 3U2D and ciprofloxacin 2d interaction

Figure 7: The 3D and 2D visualization of docking analysis of ciprofloxacin molecular interaction with 3U2D



(a) 3U2D and No. 10 3d interaction **(b)** 3U2D and No. 10 2d interaction

Figure 8: The 3D and 2D visualization of docking analysis of No. 10 molecular interaction with 3U2D

Druggability, pharmacokinetics and toxicity

The drug-likeness of the best-docked phyto-compounds (No. 10) and the standard drugs was evaluated based on pharmacokinetics ADMET (Absorption, Distribution, Metabolism, and Excretion and Toxicity). The above parameters are very important for drug-destined oral administration. The parameter tests were LogP values, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, hERG1 and hERG2 inhibition, hepatotoxicity, the Ames test, number of hydrogen bond acceptors, molecular weight, and number of hydrogen bond donors.

Both the standard drugs and phytocompound No. 10 complied with Lipinski's Rule of Five. GI absorption was high; BBB permeability was positive only for ketoconazole. All compounds except ketoconazole showed negative results for hERG1 and hERG2 inhibition, while ketoconazole tested positive specifically for hERG2 inhibition. Hepatotoxicity was observed only in the standard drugs, while the Ames test returned positive results for compound number 10 and ketoconazole, as shown in Table 3.

Table 3: Drug-likeness, pharmacokinetics and toxicity of compound number 10 and the standard drugs

Parameters	No 10	Ketoconazole	Ciprofloxacin	Nalidixic acid
Molecular weight	426.4	531.2	331.3	389.2
No of H-acceptors	2	7	5	4
No of H-donors	8	0	2	1
LogP value	3.95	4	1.6	1.4
GI absorption	High	High	High	High
BBB permeant	No	Yes	No	No
hERG1inhibitor	No	No	No	No
hERG2 inhibitor	No	Yes	No	No
Hepatoxicity	No	Yes	Yes	Yes
Ames Test	Yes	Yes	No	No

4. DISCUSSION

Plant-derived phyto-compounds have been reported as a source of novel candidates for therapeutic substances against microbial diseases (Paul *et al*., 2021). Mankar *et al*. (2016) and Anwar *et al*. (2023) also reported antimicrobial activity of orange peel. This study focuses on *in silico* antimicrobial activity using the molecular docking method of the identified compounds. Molecular docking is a computer-assisted method for discovering new drugs that has grown as better technologies have been developed to find drugs made

from phytochemicals found in different medicinal plants. Molecular docking serves as an efficient and cost-effective method for the development and testing of pharmaceuticals. (Sharma *et al*., 2023)

Molecular docking scores indicated how well each compound binds to the target proteins, which is crucial for assessing their potential as therapeutic agents. Compound No. 10 (9,11-bis(4-hydroxyphenyl)-8-oxa-12,13,15,17-tetrazatetracyclo[8.7.0.02,7.012,16] heptadeca-1(10),2,4,6,13,15-hexaen-4-ol) exhibited the best docking scores among all tested phyto-compounds of orange peel extract as follows: -9.5, -8.5, and -7.9 for 5FSA, 3U2D, and 6F86, respectively. These scores were significantly better than those of the standard drugs (ciprofloxacin and nalidixic acid). Madriwala *et al*. (2022) reported similar docking scores for ciprofloxacin against DNA ligase (3PN1) and ketoconazole against sterol demethylase (5FSA) with binding affinities of -8.9 and -10.2, respectively. The difference may be a result of different grid coordinates. VL *et al*. (2022) reported a -10.6 kcal/mol binding affinity for ketoconazole and 14-$\boldsymbol{\alpha}$ Sterol Demethylase (PDB ID: 5FSA).

A higher docking score means that the medication interacts better with its target protein, which could lead to better treatment results, making it important for predicting how effective a drug will be compared to standard treatments (García-Ortegón *et al*., 2022*).* According to reports of Choi and Lee (2021), using docking scores in conjunction with machine learning models can expedite the design of new drug-like compounds with desirable features.

Molecular docking assessment using a scoring function helps to select the best-scored ligand with the protein, which increases the chance of drug discovery (Alov *et al*., 2022). The most important interactions between the docked ligand and the protein, including hydrophobic, electrostatic, and hydrogen bond interactions (Agu *et al*., 2023). Hydrogen-bonding (HB) interaction governs the stability of the host-guest complex that is established (Vaidyanathan *et al*., 2023). The 2D interaction diagrams provide a detailed view of how Compound No. 10 interacts with the target proteins, highlighting the specific binding sites and interactions that contribute to its high docking scores. The ligand-protein complex visualizations help to understand the molecular basis of the compound's binding affinity. There were no hydrogen interactions between 5FSA and the standard drug ketoconazole, or between 6F86 and the standard drug nalidixic acid; however, compound No. 10 formed a hydrogen bond with both 5FSA and 6F86. The absence of hydrogen bonds in ligand-protein binding sites may compromise the stability of these inhibitors. Kenny (2022) reported that hydrogen bonding stabilizes the three-dimensional structures of therapeutic targets, including proteins and RNA.

The 2D interaction of 3U2D and the standard drug, ciprofloxacin, has 2 conventional hydrogen bonds with 12 amino acid residue interactions, while compound No. 10 had 16 amino acid residue interactions and 3 hydrogen bonds. Compound No. 10 may be more stable as an inhibitor of DNA gyrase in *S. aureus* than the control standard drug, ciprofloxacin (Zochedh *et al*., 2023).

The drug-like qualities of compound No. 10 and the control drugs, such as ketoconazole, ciprofloxacin, and nalidixic acid, were evaluated by ADMET (Kumar et al., 2024; Nyamba *et al*., 2024; Chikowe *et al*., 2024; Pentu *et al*., 2025). All test compounds, including phytocompound No. 10, conformed to Lipinski's Rule of Five, indicating favorable oral bioavailability. High GI absorption was predicted across all compounds, suggesting excellent potential for intestinal uptake. However, only ketoconazole managed to cross the blood-brain barrier, indicating its potential to impact the central nervous system—a crucial consideration if the drug is intended to target the CNS.

All compound tests were negative for blocking hERG1 and hERG2, except for ketoconazole, which specifically blocks hERG2. The prediction of hepatotoxicity indicated no harm for compound No. 10; however, all control drugs predicted positive for hepatotoxicity, meaning that they cause liver damage. The Ames test (Thomas *et al*., 2024) predicted that compound No. 10 and ketoconazole were positive for mutagenic potential, which may risk genetic damage.

5. CONCLUSION

Our study explores the computational antimicrobial potential of the bioactive compounds from orange peel extract using molecular docking of GC-MS-identified compounds. Among the eighteen bioactive compounds, compound peak number 10 had the best docking score to selected bacterial proteins, better than the control drugs (nalidixic acid and ciprofloxacin). The compound forms strong interactions with hydrogen bonds, especially with DNA gyrase. The analysis of toxicity and ADME showed that the compound had druggable qualities such as high oral absorption, lack of hepatotoxicity, and no hERG inhibition. Overall, compound No. 10 is a promising antibacterial candidate with favorable pharmacokinetic and safety profiles. However, a positive Ames test suggests possible mutagenicity, requiring further investigation. Therefore, methanol extract of orange peels contains a bioactive compound that could be used to formulate a powerful antimicrobial drug.

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Ethical Approval

Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interests, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

All data associated with this study will be available based on the reasonable request to corresponding author.

REFERENCES

1. Abdulsalami H, Daudu OAY, Adabara NU, Hamzah RU. Antisalmonellal Activity and GC-MS Analysis of *Piliostigma thonningii* leaf extract. AROC in Natural Products Research 2022; 2(2):1-9
2. Abushaheen MA, Fatani AJ, Alosaimi M, Mansy W, George M, Acharya S, Rathod S, Divakar DD, Jhugroo C, Vellappally S, Khan AA. Antimicrobial resistance, mechanisms and its clinical significance. Disease-a-month 2020; 66(6):100971.
3. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, Ugwuja EI, Aja PM. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Scientific reports 2023; 13(1):13398.
4. Alov P, Pajeva I, Tsakovska I, Pencheva T. October. Comparison of Docking Scoring Functions by InterCriteria Analysis on a Set of Protein Targets Related to Alzheimer and Parkinson Diseases. In The International Symposium on Bioinformatics and Biomedicine 2022; 98-110). Cham: Springer Nature Switzerland.
5. Anwar T, Qureshi H, Fatima A, Sattar K, Albasher G, Kamal A, Ayaz A. Zaman W. Citrus sinensis peel oil extraction and evaluation as an antibacterial and antifungal agent. Microorganisms 2023;11(7):1662.
6. Attah F, Otalu O, Ukwubile CA, Onoruoyiza OV. Edisha GD; Use of medicinal plants as antimicrobial agents: a review of the Successes Story. International Journal of Medicinal Plants and Natural Products 2020; 6(3):28-33.
7. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the Rule of 5 and drugability. Advanced drug delivery reviews 2016;101:89-98.
8. Bhagyashri JW, Sandip NW, Vivekanda AC, Merve Y, Mithun R., Johra K, Sampath C, Aniket AG, Vishnu SN, Design, docking, MD simulation and in-silco ADMET prediction studies of novel indolebased benzamides targeting estrogen receptor alfa positive for effective breast cancer therapy. Pharmacia 2023; 70(2):307-316.
9. Choi J, Lee J. V-dock: fast generation of novel drug-like molecules using machine-learning-based docking score and molecular optimization. International journal of molecular sciences 2021;22(21):11635.
10. Deore AB, Dhumane JR., Wagh R, Sonawane R. The stages of drug discovery and development process. Asian Journal of Pharmaceutical Research and Development 2019;7(6):62-67.
11. Eakin AE, Green O, Hales N, Walkup GK, Bist S, Singh A, Mullen G, Bryant J, Embrey K, Gao N, Breeze A. Pyrrolamide DNA gyrase inhibitors: fragment-based nuclear magnetic resonance screening to identify antibacterial agents. Antimicrobial agents and chemotherapy, 2012;56(3):1240-1246.

12. Ejidike IP, Direm A, Parlak C, Bamigboye MO, Oluade O, Adetunji JB, Ata A, Eze MO, Hollett JW, Clayton HS. Cadmium oxide nanoparticles from new organometallic Cd (II)-Schiff base complex and in vitro biological potentials: dual S. aureus and E. coli DNA gyrase inhibition by the precursors via in silico binding modes' study. Research on Chemical Intermediates 2024;50(6):2763-2791.
13. García-Ortegón M, Simm GN, Tripp AJ, Hernández-Lobato JM, Bender A, Bacallado S. DOCKSTRING: easy molecular docking yields better benchmarks for ligand design. Journal of chemical information and modeling, 2022;62(15):3486-3502
14. Guevara-Lora I, Bras G, Karkowska-Kuleta J, González-González M, Ceballos K, Sidlo W, Rapala-Kozik M. Plant-derived substances in the fight against infections caused by Candida species. International Journal of molecular sciences 2020; 21(17):6131.
15. Jakhar R, Dangi M, Khichi A. Chhillar AK. Relevance of molecular docking studies in drug designing. Current Bioinformatics 2020;15(4):270-278.
16. Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A. Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria (MDR). Evidence-Based Complementary and Alternative Medicine 2021(1):3663315.
17. Kenny PW. Hydrogen-bond donors in drug design. Journal of Medicinal Chemistry 2022; 65(21):14261-14275.
18. Kumar B, Devi J, Dubey A, Tufail N, Khurana D. Thiosemicarbazone ligands-based transition metal complexes: a multifaceted investigation of antituberculosis, anti-inflammatory, antibacterial, antifungal activities, and molecular docking, density functional theory, molecular electrostatic potential, absorption, distribution, metabolism, excretion, and toxicity studies. Applied Organometallic Chemistry 2024; 38(3):e7345.
19. Madriwala B, Jays J, Sai GC. Molecular docking and computational pharmacokinetic study of some novel coumarin–benzothiazole schiff's base for antimicrobial activity. International Journal of Pharmacy and Pharmaceutical Sciences 2022; 14(8):16-21.
20. Mankar CD, Shah MU, Doshi YS, Bajaj M, Kevadia V, Vinod R. Evaluation of antimicrobial activity of orange peel extract against oral biofilm forming organisms: an in vitro microbial study and scanning electron microscopic assessment. International Journal of Basic & Clinical Pharmacology 2016; 5(5):1917-1923.
21. Narramore S, Stevenson CE, Maxwell A, Lawson DM, Fishwick CW. New insights into the binding mode of pyridine-3-carboxamide inhibitors of E. coli DNA gyrase. Bioorganic & medicinal chemistry 2019; 27(16):3546-3550.
22. Nyamba I, Sombie CB, Yabre M, Zime-Diawara H, Yameogo J, Ouedraogo S, Lechanteur A, Semde R, Evrard B. Pharmaceutical approaches for enhancing solubility and oral bioavailability of poorly soluble drugs. European Journal of Pharmaceutics and Biopharmaceutics 2024;204: 114513.
23. Olakunle OO, Titilayo AA. Activity and characterization of antifungal compounds from the peel of sweet orange Citrus sinenesis fruits. Journal of Crop Protection 2018;7(3):327-335.
24. Oligie MHA, Eduwuirofo LO, Iyekekpolor RM, Oghomwenrhiere O, Ejimadu CM, Obasuyi EI, Archibong UD, Michael OT, Oghomwen RO, Iyekowa O, Phyto-Constituents, GC-MS Analysis and Antifungal Activity of Methanol Extract of Sweet Orange Peels. NIPES-Journal of Science and Technology Research, 2023;5(2):50-57.
25. Paul GK, Mahmud S, Hasan MM, Zaman S, Uddin M S, Saleh M A. Biochemical and in silico study of leaf and bark extracts from *Aphanamixis polystachya* against common pathogenic bacteria. Saudi journal of biological sciences 2021; 28(11):6592-6605.
26. Pentu N, Azhakesan A, Kumar PK. Insilico molecular docking and ADME/T studies of flavonol compounds against selected proteins involved in inflammation mechanism. Journal of Applied Pharmaceutical Research 2025;13(1): 95-111.
27. Sharma R, Kaur G, Bansal P, Chawla V, Gupta V. Bioinformatics Paradigms in Drug Discovery and Drug Development. Current Topics in Medicinal Chemistry 2023; 23(7): 57.
28. Singh J, Sharma V, Pandey K, Ahmed S, Kaur M, Sidhu GS. Horticultural classification of citrus cultivars. Citrus research, development and biotechnology 2021:1-24. doi: 10.5772/intechopen.96243
29. Thomas DN, Wills JW, Tracey H, Baldwin SJ, Burman M, Williams AN, Harte DS, Buckley RA, Lynch AM. Ames test study designs for nitrosamine mutagenicity testing: qualitative and quantitative analysis of key assay parameters. Mutagenesis 2024; 39(2):78-95.
30. Ukoroije RB, Otayor RA. Review on the bio-insecticidal properties of some plant secondary metabolites: types, formulations, modes of action, advantages and limitations. Asian Journal of Research in Zoology 2020;3(4):27-60.
31. Umashankar DD. Plant secondary metabolites as potential usage in regenerative medicine. J. Phytopharmacol 2020; 9(4):270-273.
32. Vaidyanathan R, Sreedevi SM, Ravichandran K, Vinod SM, Krishnan YH, Babu, LK, Parthiban PS, Basker L, Perumal T, Rajaraman V, Arumugam G. Molecular docking approach on

the binding stability of derivatives of phenolic acids (DPAs) with Human Serum Albumin (HSA): hydrogen-bonding versus hydrophobic interactions or combined influences?. JCIS Open 2023; 12:100096.

33. VL AB, Madriwala B, Jeevitha L, Mathew MS, Abhilash S, Singh A, Shwetha K. Development, characterization and in vitro antifungal evaluation of topical formulation of Tridax procumbens L. Leaf extract. Journal of Pharmacognosy and Phytochemistry 2022;11(2):289-295.
34. Zochedh A, Shunmuganarayanan A, Sultan AB. Conformational fidelity and hydrogen bond associability of L-histidine with sulfamate anion studied through XRD, quantum chemical, spectroscopic and molecular docking simulation as a cdk-4 inhibitor against retinoblastoma. Journal of Molecular Structure 2023; 1274: 134402.
35. Zode RD, Chakole CM. Formulation and Evaluation of Antibacterial Gel Using Leaf Extract of *Andrographis Paniculata*."Indo American Journal of Pharmaceutical Research 2020; 10(6): 2231-687.