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Research Article

Phytochemical Screening and Molecular Targets Binding Ability of Selected Bioactive Compounds in the Fruit Extract of *Tetrapleura tetraptera*

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Abstract

In addition to the highly valued sweet and aromatic fragrance of *Tetrapleura tetraptera* fruit, it is used in ethnomedicine to treat and manage a wide range of diseased conditions. In this study, the methanol extract of the fruits of *Tetrapleura tetraptera* was analyzed by Gas Chromatography - Mass Spectrometry (GC-MS). Twenty-six different phytochemical compounds and their percentages have been characterized including: 3,4-Dihydroxyphenyl(2,3-trans-Catechin) (3.10%), 9-Octadecenal (3.96%), hexadecane (3.16%), pinene (4.80%), 1-methyl-4-cyclohexene (7.45%), humulene (3.60%), carbonic acid (3.34%), ferulic acid (4.03%), 5,6-dihydroxy-7-Oglucuronide flavone (22.46%), 2,4-Di-tert-butylphenol (8.08%) and oleic acid (10.99%). Molecular docking was done using the Maestro Schrodinger suite and glide tool which uses a genetic algorithm to optimize the placement of ligands within a receptor binding site, and employs an empirical scoring function to evaluate and rank the binding affinity between a ligand and a receptor. Molecular docking showed that bioactive compounds in the fruit extract; Naringenin and Hesperidin exhibit strong binding affinities to receptors of interest; AMP-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS), which are diabetes and hypertension-related target proteins; facilitating insightful structural assessments and predictive analyses. The interaction network of Naringenin and Metformin with AMPK residues showed that Naringenin exhibits greater interaction stability, stronger binding affinity and lower binding energy compared to Metformin, suggesting a potentially higher interaction with the protein target. The interaction network of Hesperidin and Nitroglycerine with eNOS amino acid residues also showed that Hesperidin forms a greater number of stabilizing interactions with more diverse interaction network compared to Nitroglycerine, indicating a stronger and more stable interaction which may contribute to its stronger binding affinity. These findings present *T. tetraptera* as a promising reservoir of active pharmaceutical ingredients warranting further exploration for novel therapeutic avenues and gives credence to the use of *T. tetraptera* in herbal medicine for the treatment of various diseases.

Keywords

Phytocompounds, Gas Chromatography-mass Spectrometry, Molecular Docking, *T. tetraptera*, Diabetes, Hypertension

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1. Introduction

The use of plants as medicine has long existed and is widely documented in records in ancient China, India and Egypt [1]. The World Health Organization (WHO) estimates that 65-80% of the population in developing countries relies on herbal medicines as a primary source of treatment [2].

Modern-day use of traditional medicine is also rapidly increasing in many countries, including Nigeria, where the vast biodiversity of the country is a potential source of new and more affordable treatments for numerous conditions [3].

Since many wild plants have tremendous therapeutic potential, mainly as traditional and pharmacopoeia drugs, many of the world's population still depend on traditional medicine because of the scarcity and high cost of orthodox medicine [4, 5].

The interest in ethnomedicine started when the indigenous peoples and researchers discovered that wild plants serve as indispensable constituents of the human diet and, more importantly, as a treatment for various disease conditions and ailments [6-12].

Medicinal plants have also provided modern medicine with numerous plant-derived therapeutic agents, and lead compounds with pharmacological or biological activity are likely to be therapeutically beneficial [13]. These lead compounds may, however, have a suboptimal structure that requires modification to fit better to the target. Thus, in drug discovery and design, the chemical structure of lead compounds from medicinal plants serves as starting materials for chemical modification to improve potency, selectivity or pharmacokinetic parameters [14]. This modification and optimization increase the drug-like properties of lead compounds, which can then undergo clinical trials. Plant-derived active compounds can also be screened through molecular modelling during rational drug design for their ability to inhibit (antagonist) or stimulate (agonist) receptors of interest, as well as determine their specificity and selectivity for them [14].

Consequently, traditional societies and ethnic nationalities have, over the years, employed medicinal plants in ethnomedicine for the treatment of various diseases without any scientific knowledge of the physiologically active ingredients responsible for the plant's medicinal and pharmacological potentials [15-18]. Phytotherapy, which is also a source of lead compounds for drug discovery [19], has made outstanding achievements in the attenuation of sickle-cell anaemia, dementia, epilepsy, arthritis, hypertension, Alzheimer's and Parkinson's diseases, malaria, stroke, diabetes and many others [20, 21]. The availability of medicinal products from living things, predominantly plants, has interested the pharmacological biochemist for decades. Phytochemicals or secondary metabolites are chemical compounds that occur naturally in plants and are derived from them. The phytochemicals that are effective in disease prevention and treatment include alkaloids, flavonoids, tannins, saponins, phytosterols, phenols, polyphenols, cardiac glycosides, terpenoids, anthraquinones, carotenoids and many others [1]. The phytochemical studies of medicinal plants have provided some biochemical basis for their ethnopharmacological uses in treating, managing, and preventing various diseases and disorders [22-25].

Historically, drugs were discovered by identifying the active ingredients from traditional remedies or serendipitous discovery, as with penicillin [26, 27].

Tetrapleura tetraptera is a species of the pea family endemic to West Tropical Africa. It is a deciduous flowering plant of the Fabaceae-Mimosoid clade family, which grows luxuriantly in the rainforest regions of West and Central Africa. The height and girth can reach 20-25 metres and 1.2-3 metres, respectively. The leaves are sessile (have no stalk), glabrous or minutely hairy. The stem bark is greyish-brown, thin and partially smooth. It has a unique four-wing fruit consisting of a woody shell, fleshy pulp, and small brownish-black seeds with a distinct flavour. The fruits appear green when tender, but the mature dark purplish-brown fruit ripens from September to December. The tree is commonly harvested from the wild for its edible fruit and medicinal applications [28]. *Tetrapleura tetraptera*'s sweet and aromatic fragrance is highly valued; it is used in ethnomedicine to treat and manage a wide range of diseased conditions. The fruit reportedly has anti-arthritic, anti-inflammatory, antihypertensive and antidiabetic properties. It is used to treat cardiovascular disorders, hypertension, convulsions and epilepsy, gastrointestinal disorders, malaria and other fevers, diabetes, asthma and chest pain, low body immunity, flu and colds, reproductive disorders, cancer of the breast and uterus, skin disorders for newly born babies, wounds and burns, back pain and general body pains and weakness, and dental disorders [28]. It is believed to be rich in antioxidants, vitamins, minerals, essential oils, sugars and phytochemicals [29].

Figure 1. *Tetrapleura tetraptera fruits collected from Nunya Isuikwuato, Nigeria (2024).*

Molecular docking is a technique that predicts the preferred orientation, affinity, and interaction of a ligand in the binding site of a target protein to form a stable complex [30]. It is a bioinformatic modelling strategy critical in drug development, structural biology, and biomolecular interaction research. The associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates and lipids play a central role in signal transduction, and the relative orientation of the two interacting partners may affect the type of signal produced, such as agonism or antagonism. Therefore, knowledge of the preferred orientation acquired by docking may be used to predict the strength of association or binding affinity between two molecules and the type of signal produced. Characterization of this binding behaviour plays an important role in the rational design of drugs and in elucidating fundamental biochemical processes [31, 32]. The docking process examines the ligand's spatial and energetic compatibility with the protein receptor's active site, thus assisting in discovering new drug candidates, refining and modifying existing compounds, and understanding the intricate interactions between drugs and receptors. The research thus focuses on computationally simulating the molecular recognition process, which would describe the "best fit" orientation of a ligand that binds to a particular protein of interest. During the docking process, the ligand and the protein adjust their conformation to achieve an overall "best fit," and this conformational adjustment, which results in the overall binding, is referred to as "induced fit" [33].

2. Materials and Methods

2.1. Chemicals and Reagents

All the solvents used were of analytical grade and were procured from Merck, Germany.

2.1.1. Collection and Authentication of Plant Material

Tetrapleura tetraptera fruits were harvested from Avuvu-Nunya, Isuikwuato Local Government Area of Abia State, South Eastern Nigeria, on December 29, 2023. The plant material was identified and authenticated on January 2, 2024, by Dr. Frank Akanwa, a Taxonomist in the Department of Plant Science and Biotechnology, Abia State University, Uturu, Abia State, Nigeria.

2.1.2. Preparation of *Tetrapleura tetraptera* Fruits Extract

The dried fruits were shade-dried for 15 days then chopped into small pieces, and blended into a pulp using a mechanically driven Arthur Miller Machine. The blended plant sample (500g) was successfully extracted with 2 L of methanol (8hrs/3 times/30°C). The extract was concentrated under reduced pressure, and the supernatant fruit (12.75g) extract was decanted after the solvent had been completely removed. The extract was centrifuged at 10,000 rpm for 20 minutes, and the clear supernatant was subjected to systematic GC-MS analysis.

2.2. GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

GC-MS analysis of the extract was carried out using SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm x 50 m) and the conditions were as follows: temperature programming from 60-280 ℃ held at 60 ℃ for 1 minute and at 160 ℃ for 2 minutes (rate 10 ℃/min), at 220 ℃ for 3 minutes (rate 10 ℃) and finally at 280 ℃ for another 2 minutes (rate 10 ℃), injection temperature 220 ℃. GC-MS (Gas chromatography-mass spectrometry) analysis was conducted using GC-MS-QP 2010 Plus Shimazu Japan with column oven temperature of 60 ℃, injection mode was split, flow control mode was linear velocity, carrier gas pressure was 100.2 Kpa, total flow was 6.2 mL /min, column flow was 1.62 mL /min, linear velocity was 46.3 cm/sec, purge flow was 3.0 mL /min and split ratio was 1.0. Also, the ion source temperature was 200 ℃, interface temperature was 250 ℃, solvent cut time was 2.5 min, detector gain was 0.00 KV, detector gain mode was relative, and the threshold was 1000. For the mass spec, the start time was 3.0 min, end time was 28.0 min, event time was 0.5 sec, scan speed was 1250, and start m/z was 50 while end m/z was 600. The scan range was 1364-2918. The mass spectrum was also equipped with a computer-fed mass spectra data bank. Hermle Z 233 M-Z centrifuge Germany was used. The components of the methanolic extract of T. tetraptera fruit were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing the mass spectra of the peaks and those from the literature [34, 35].

2.3. Molecular Docking

Molecular docking was carried out using the Maestro Schrodinger suite. The Maestro Schrodinger Glide tool uses a genetic algorithm to optimize the placement of ligands within a receptor binding site. It employs an empirical scoring function to evaluate and rank the binding affinity between a ligand and a receptor.

Procedure: The proteins AMP-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS) were retrieved in 3D crystal structures from the Protein Data Bank (PDB) - Research Collaboratory for Structural Bioinformatics (RCSB), in their PDB file formats, and processed using the protein pre-processing module in Schrodinger Maestro, version 12.8 [36].

The ligands Metformin and Nitroglycerin and selected phytocompounds Naringenin and Hesperidin were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov, Retrieved November 2024) in their Structural Data File (SDF) formats, reflecting their diverse PubChem CIDs. Preparation

was conducted using the LigPrep tool within the Schrodinger suite [36]. Receptor grids, essential for ligand binding, were generated using the Glide application in Maestro 12.8 and molecular docking was performed using the Glide's ligand docking module [37].

Post-docking, the binding stability of protein-ligand complexes was assessed by calculating the relative binding free energy (ΛG bind) utilizing the MM/GBSA method via the Prime Module in Maestro Schrodinger [38]. The binding free energy was calculated using the equation:

$$\Lambda G_{bind} = G_{complex} X -- (G_{protein} + G_{ligand})$$

3. Results and Discussion

The chromatogram of the methanol extract of *Tetrapleura tetraptera* fruit revealed the presence of 26 compounds in the extract (Figure 1). The molecular formulae, percentage composition, molecular masses and nature of these compounds are shown in Table 1. The compounds comprise butyl9-octadecenoate (1.48%), 3,4-Dihydroxyphenyl (2,3-trans-Catechin) (3.10%), 9-Octadecenal (3.96%), hexadecenoic acid (1.45%), benzene (1.78%), 4,5,6,7,8-polymethoxyflavone (2.42%), 2-Ethyl-1-dedecanol (1.89%), 1-octylsulfinyloctane (n-Octyl Sulfoxide) (2.45%), 4,5,7-trihydroxyflavonone (2.48%), hexadecane (3.16%), pinene (4.80%), hesperitin,7-O-rutinoside (1.34%), 1-methyl-4-cyclohexene (7.45%), humulene (3.60%), carbonic acid (3.34%), ferulic acid (4.03%), 3,5,4-trihydroxy-trans-stilbene (1.57%), 1-4-methanonaphthalene (0.84%), 5,6-dihydroxy-7-O-glucuronide flavone (22.46%), terpinene (1.31%), 3,7-Dimethyloct-6-en-1-ol (0.67%), naphthalene (0.68%), 2,4-Di-tert-butylphenol (8.08%), cyclopentadecanone (0.26%), 4-Hydroxy-3-Methoxy (2.05%) and oleic acid (10.99%). The major constituents in the fruit extract of T. tetraptera include 5,6-dihydroxy-7-Oglucuronide flavone (22.46%), oleic acid (10.99%), 2,4-Di-tert-butylphenol (8.08%) and 1-methyl-4-cyclohexene (7.45%).

Table 1. *GC - MS analysis result of Tetrapleura tetrapterae fruit extract.*

Chromatogram peak	Compound name	Molecular Formula	Molecular Weight (g/mol)	Retention Time (mins)	Peak Area (%)	Nature of Compound
1	Butyl 9-octadecenoate	$C_{22}H_{42}O_2$	338.58	5.25	1.48	fatty acid ester
2	3,4-Dihydroxyphenyl (Epicatechin)	$C_{15}H_{14}O_6$	290.27	5.74	3.10	Polyphenolic flavonoid
3	9-Octadecenal	$C_{18}H_{34}O$	266.46	6.89	3.96	Fatty Aldehyde
4	Hexadecenoic acid	$C_{16}H_{32}O_2$	256.43	7.14	1.45	Fatty acid
5	Benzene	C_6H_6	78.11	7.78	1.78	Aromatic compound
6	4,5,6,7,8-Polymethoxy flavone	$C_{20}H_{20}O_7$	372.37	8.34	2.42	Flavonoid
7	2-Ethyl-1-dedecanol	$C_{14}H_{30}O$	214.38	8.52	1.89	Aliphatic alcohol
8	1-octylsulfinyloctane	$C_{16}H_{34}OS$	274.51	8.61	2.45	Organosulfur compound
9	4,5,7-Trihydroxyflavonone	$C_{15}H_{12}O_5$	272.26	8.63	2.48	Polyphenol
10	Hexadecane	$C_{16}H_{34}$	226.45	8.89	3.16	Alkane hydrocarbon
11	Pinene	$C_{10}H_{16}$	136.24	9.10	4.80	Terpene
12	Hesperitin,7-O-rutinoside	$C_{28}H_{34}O_{15}$	610.56	9.12	1.34	Flavonoid
13	1-methyl-4-cyclohexene (Limonene)	$C_{10}H_{16}$	136.24	9.43	7.45	Monoterpene
14	Humulene	$C_{15}H_{24}$	204.36	9.74	3.60	Terpene
15	Carbonic acid	H_2CO_3	62.03	10.16	3.34	Weak acid
16	Ferulic acid	$C_{10}H_{10}O_4$	194.18	10.27	4.03	Phenolic compound
17	3,5,4-Trihydroxy-trans-Stilbene (Resveratrol)	$C_{14}H_{12}O_3$	228.24	12.38	1.57	Polyphenol
18	1-4-Methanonaphthalene	$C_{11}H_{10}O_2$	174.20	15.87	0.84	Aromatic compound
19	5,6-dihydroxy-7-O-glucuronide (baicalin)	$C_{21}H_{18}O_{11}$	446.36	16.53	22.46	Flavone glycoside

Chromato-gram peak	Compound name	Molecular Formula	Molecular Weight (g/mol)	Retention Time (mins)	Peak Area (%)	Nature of Compound
20	Terpinene	$C_{10}H_{16}$	136.24	18.28	1.31	Monoterpene
21	3,7-Dimethyloct-en-1-ol (citronellol)	$C_{10}H_{20}O$	156.27	19.36	0.67	Monoterpenoid
22	Naphthalene	$C_{10}H_8$	128.17	19.42	0.68	Aromatic compound
23	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.33	22.13	8.08	Phenol
24	Cyclopentadecanone	$C_{15}H_{28}O$	224.38	25.96	0.26	Ketone
25	4-Hydroxy-3-Methoxy	$C_8H_8O_4$	168.14	30.70	2.05	Methoxy-benzoic acid
26	Oleic acid	$C_{18}H_{34}O_2$	282.46	31.03	10.99	Fatty acid

Table 1 shows the chemical constituents of T. tetraptera methanol fruit extract revealed by GC-MS analysis. The components were identified by matching the chromatogram peaks with computer Willey MS libraries and confirmed by comparing the mass spectra of the peaks and those from the literature [34].

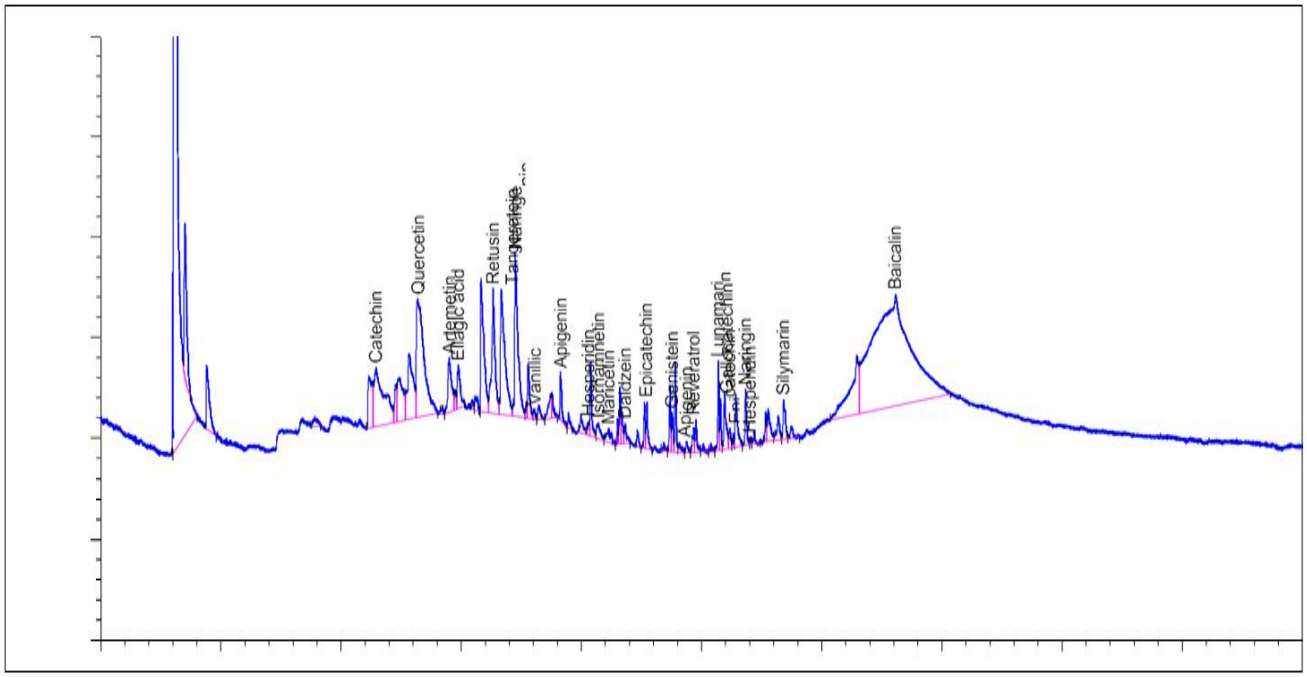

Figure 2. *GC-FID chromatogram of methanol extract of Tetrapleura tetraptera.*

Molecular Docking Analysis

Table 2. *Binding Interactions of Naringenin and Metformin with AMPK.*

Compound Names (CID) AMP-Protein Kinase (6BX6)	Binding Affinity (kcal/mol)	Hydrogen Bond Score	Binding Free Energy (kcal/mol)
NARINGENIN (439246)	-6.192	-1.442	-32.43
METFORMIN (4091)	-2.15	-1.072	-11.15

Table 2 shows the binding affinity and binding free energy analysis of Naringenin (CID: 439246) and Metformin (PubChem CID: 4091) with AMP-activated protein kinase (AMPK, PDB: 6BX6). Key parameters include binding affinity (kcal/mol), hydrogen bond score, and binding free energy (kcal/mol), which collectively indicate the strength and stability of each compound's interaction with the protein target. Naringenin exhibits a stronger binding affinity and lower binding free energy than Metformin, suggesting potentially higher interaction stability with AMPK.

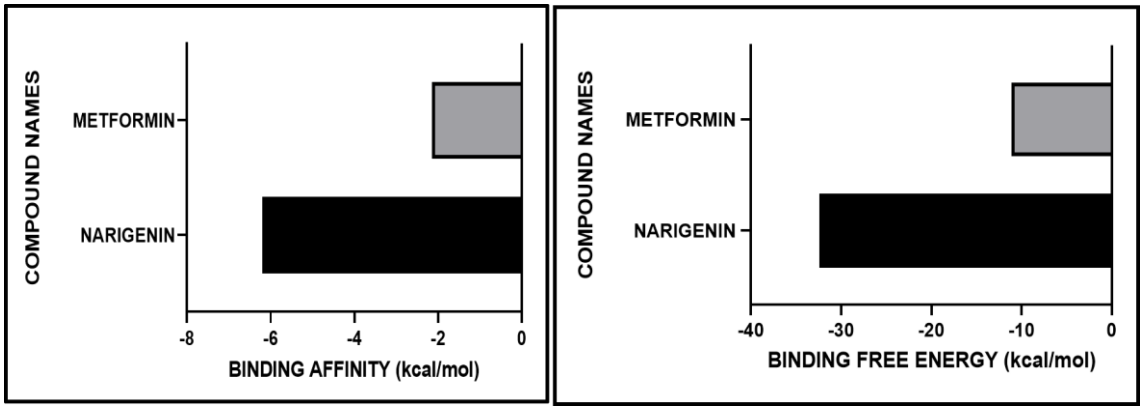

Figure 3. *Comparative Binding Affinity and Binding Free Energy of Naringenin and Metformin with AMPK (PDB: 6BX6).*

Figure 3 shows two bar graphs of the binding affinity (left) and binding free energy (right) of Naringenin and Metformin when docked with AMPK (PDB: 6BX6). The negative binding affinity values indicate the strength of molecular interaction, with Naringenin (-6.192 kcal/mol) exhibiting a higher binding affinity than Metformin (-2.15 kcal/mol). Similarly, the binding free energy values (right graph) suggest that Naringenin (-32.43 kcal/mol) forms a more stable interaction with AMPK compared to Metformin (-11.15 kcal/mol). The black and grey bars distinguish the respective compounds.

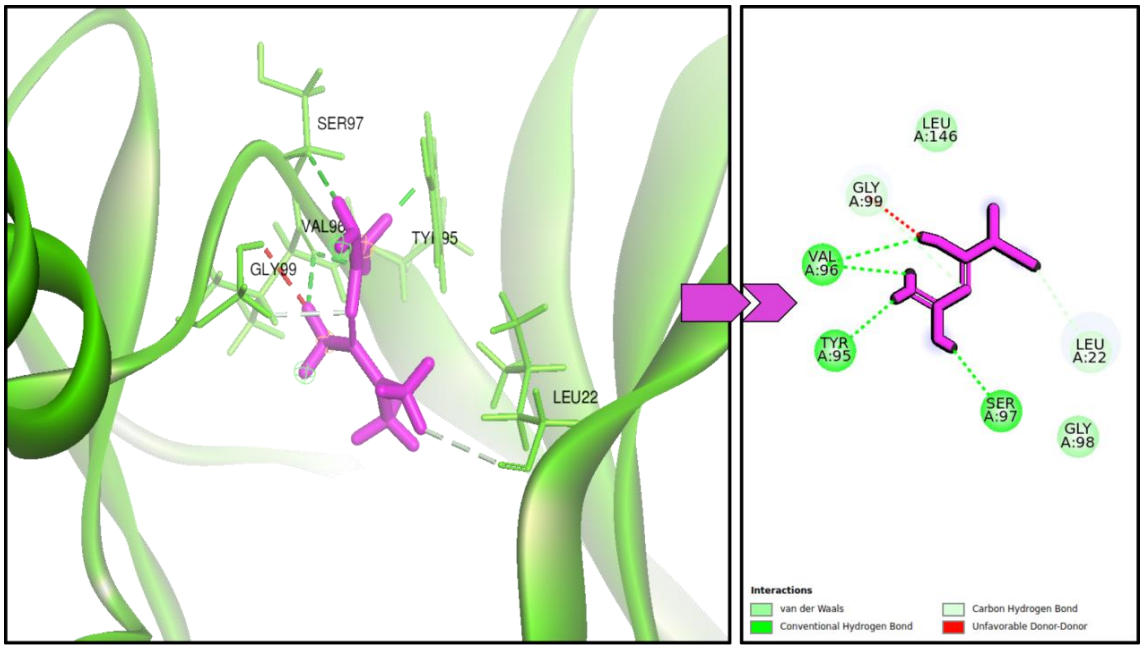

Figure 4. *3D and 2D Molecular Docking Interaction of Metformin with AMPK.*

The left panel shows the 3D visualization of Metformin (magenta ligand) docked within the AMPK active site, highlighting key binding interactions with amino acid residues. The right panel shows the 2D interaction map, showing hydrogen bonds (green dashed lines), carbon-hydrogen bonds (grey), and an unfavourable donor-donor interaction (red).

Notable interacting residues include VAL96, TYR95, SER97, GLY99, and LEU22, contributing to the compound's stability in the binding pocket.

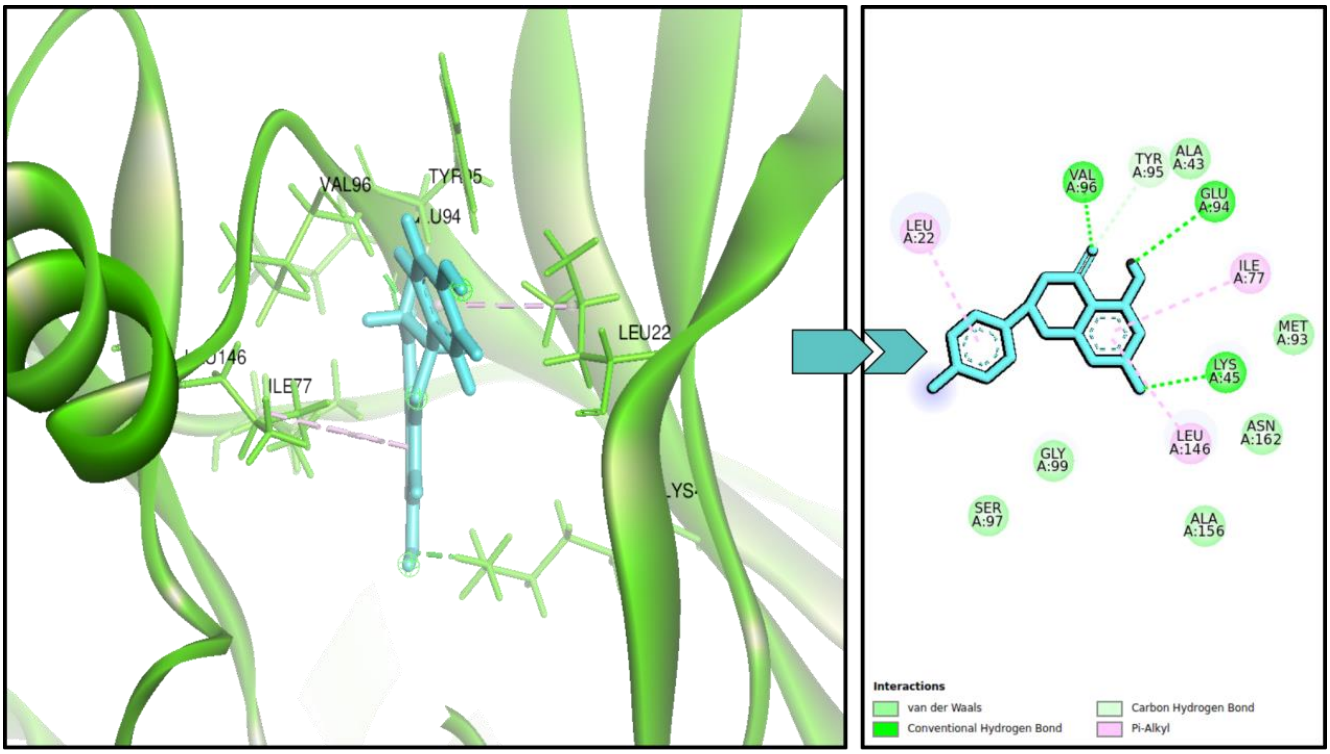

Figure 5. *3D and 2D Molecular Docking Interaction of Naringenin with AMPK.*

The left panel shows the 3D molecular docking visualization of Naringenin (cyan ligand) within the AMPK binding site, illustrating the spatial orientation of the ligand relative to the protein. The right panel shows the 2D interaction identifying conventional hydrogen bonds (green dashed lines) and Pi-Alkyl interactions (purple) with key residues such as VAL96, TYR95, GLU94, LYS45, and LEU146. The interactions suggest stronger binding affinity and stability for Naringenin compared to Metformin.

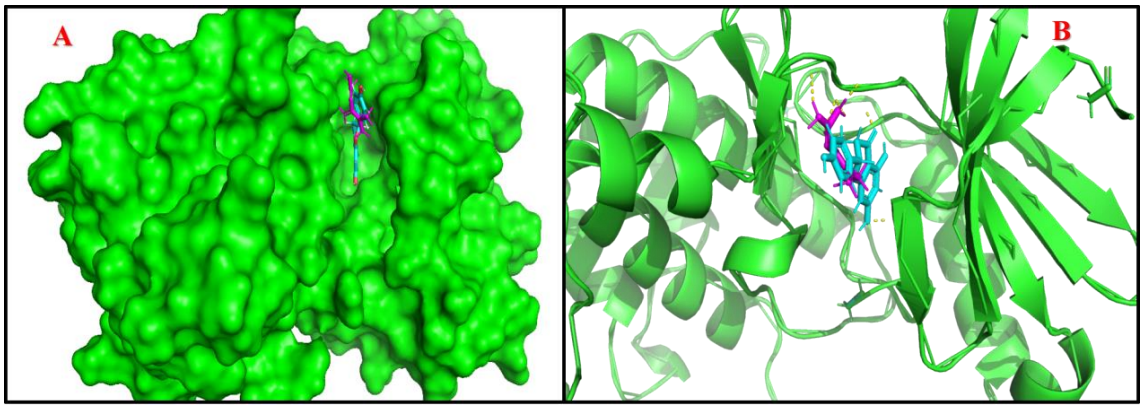

Figure 6. *Surface and Ribbon Representations of AMPK Binding with Metformin and Naringenin.*

Left Panel (A) is the surface view of AMPK showing the binding of Metformin (magenta) and Naringenin (cyan) at the same active site, indicating a similar inhibitory pattern on the enzyme.

Right Panel (B) is the ribbon representation of AMPK, showing the binding interactions of the two ligands within the active site. The structural overlap suggests that both compounds may exert comparable effects on AMPK inhibition.

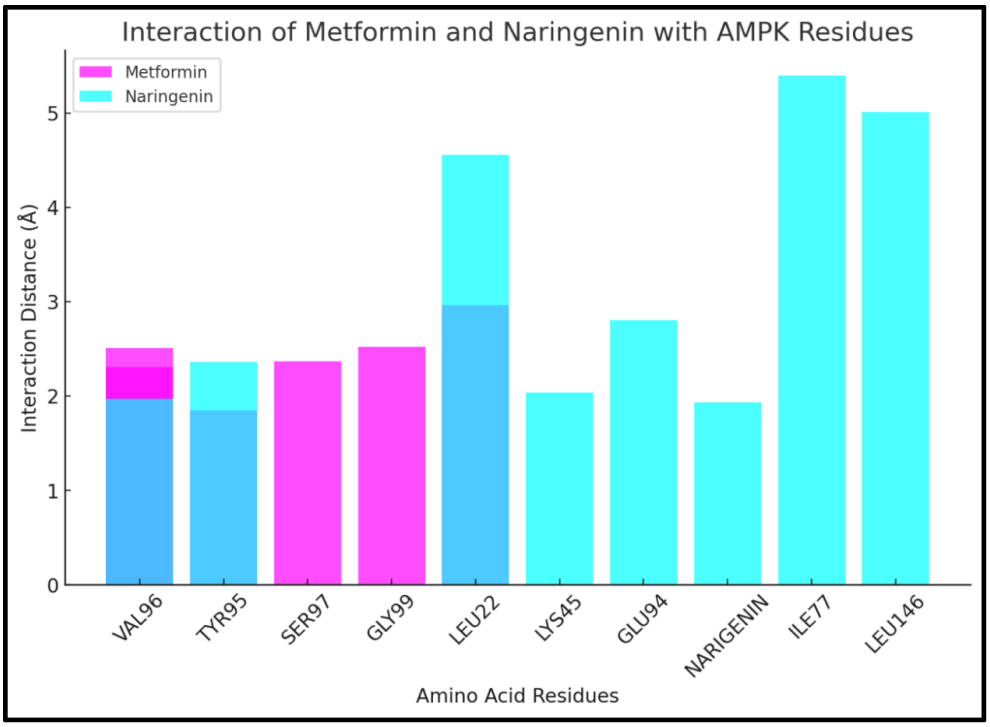

Figure 7. *Interaction Distances of Metformin and Naringenin with AMPK Residues.*

Figure 7 is a comparison of the interaction distances (Å) of Metformin (magenta) and Naringenin (cyan) with AMPK residues. The shorter distances indicate stronger interactions, with Naringenin showing greater interaction stability with multiple residues, particularly LEU22, ILE77, and LEU146.

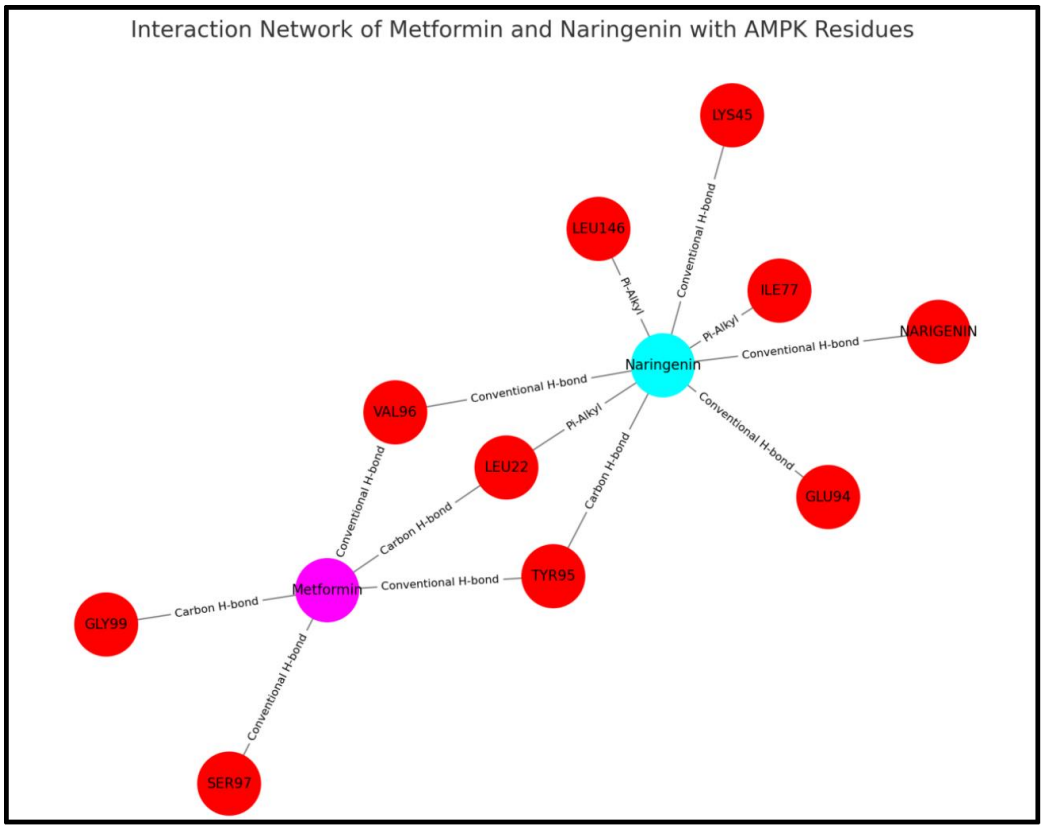

Figure 8. *Interaction Network of Metformin and Naringenin with AMPK Residues.*

Figure 8 shows the binding interactions of Metformin (magenta) and Naringenin (cyan) with AMPK residues (red nodes) at the active site. The edges represent interaction types, such as conventional hydrogen bonds and Pi-Alkyl interactions, demonstrating how each compound stabilizes within the active site.

Table 3. *Molecular Interaction Parameters of Hesperidin and Nitroglycerin with eNOS (PDB:8UFU).*

Compound Names (CID)	Binding Affinity (kcal/mol)	Hydrogen Bond Score	Binding Free Energy (kcal/mol)
Endothelial Nitric Oxide Synthase (8UFU)			
NITROGLYCERIN (4510)	-2.299	-0.83	-17.26
HESPERIDIN (10621)	-13.876	-6.342	-30.83

Table 3 shows the binding characteristics of Hesperidin (PubChem CID: 10621) and Nitroglycerin (CID: 4510) with endothelial nitric oxide synthase (eNOS, PDB ID: 8UFU). The interaction parameters include binding affinity (kcal/mol), hydrogen bond score, and binding free energy (kcal/mol), which indicate the strength and stability of their molecular interactions with eNOS. Notably, Hesperidin exhibits a significantly stronger binding affinity and higher hydrogen bond score than Nitroglycerin, suggesting a more stable and favourable interaction.

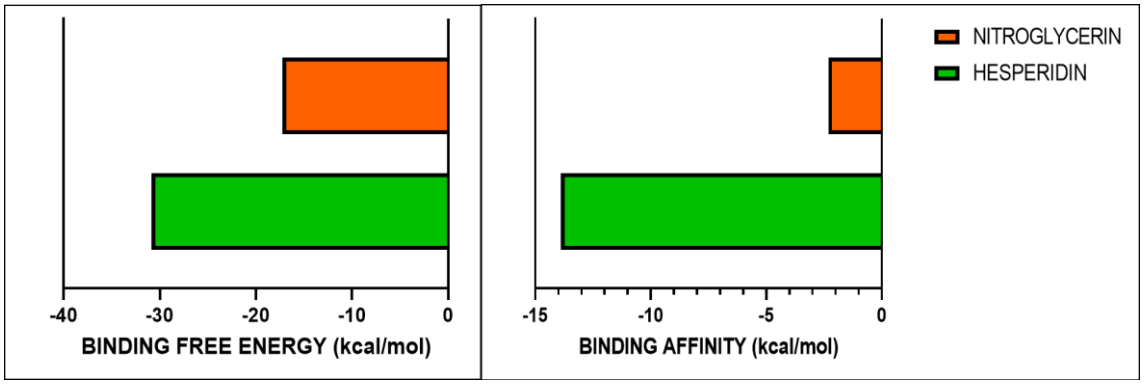

Figure 9. *Comparative Binding Affinity and Binding Free Energy of Hesperidin and Nitroglycerin with Enos.*

Figure 9 shows the binding affinity (kcal/mol) and binding free energy (kcal/mol) of Hesperidin (green) and Nitroglycerin (orange) with endothelial nitric oxide synthase (eNOS, PDB: 8UFU), as summarized in Table 3. The negative values indicate the binding strength, with lower values signifying stronger interactions. Hesperidin exhibits a significantly more favourable binding affinity and binding free energy than Nitroglycerin, suggesting a more stable and energetically favourable interaction with eNOS.

Figure 10 shows the 3D (left) and 2D (right) molecular interaction profile of Hesperidin within the active site of endothelial nitric oxide synthase (eNOS, PDB: 8UFU). The 3D representation shows the structural conformation of Hesperidin (green) within the enzyme's binding pocket, interacting with key amino acid residues. The 2D interaction map shows the specific molecular interactions, including hydrogen bonds, pi-pi stacking, pi-alkyl, and van der Waals forces, contributing to Hesperidin's stability and binding affinity with eNOS.

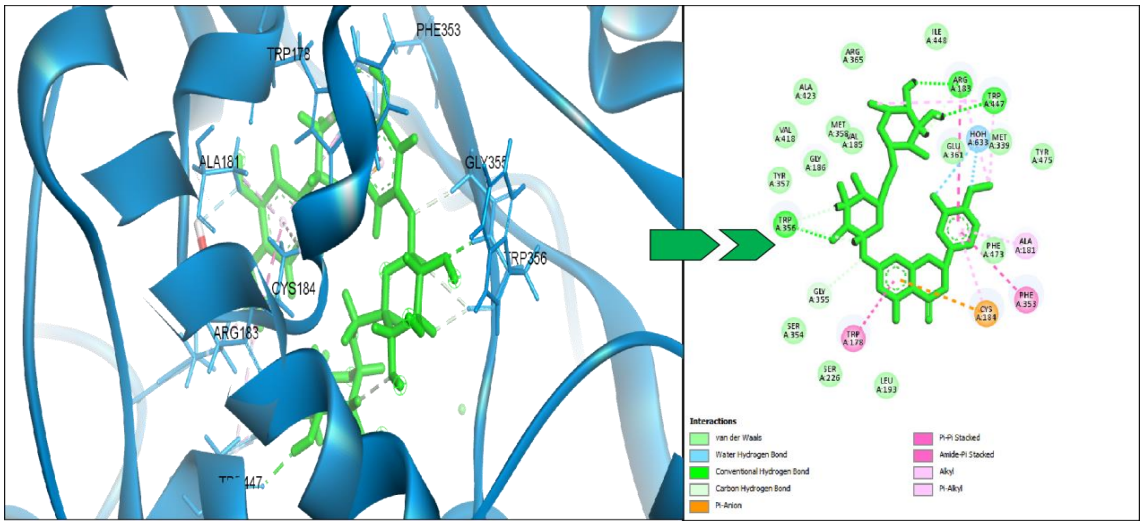

Figure 10. *3D and 2D Interaction Profile of Hesperidin with eNOS.*

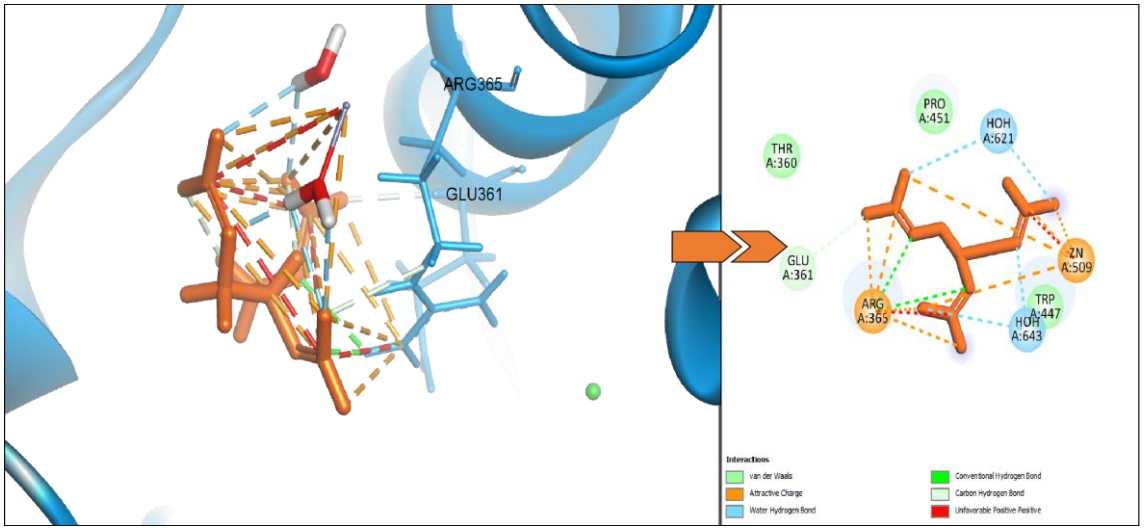

Figure 11. *3D and 2D Interaction Profile of Nitroglycerin with eNOS.*

Figure 11 shows the 3D (left) and 2D (right) molecular interaction profile of Nitroglycerin within the active site of endothelial nitric oxide synthase (eNOS, PDB: 8UFU). The 3D representation highlights Nitroglycerin (orange) interacting with key residues in the binding pocket, forming electrostatic and hydrogen bond interactions. The 2D interaction map shows the specific molecular interactions, including attractive charge, water hydrogen bonds, conventional hydrogen bonds, and hydrophobic interactions, which influence Nitroglycerin's binding affinity and stability within eNOS.

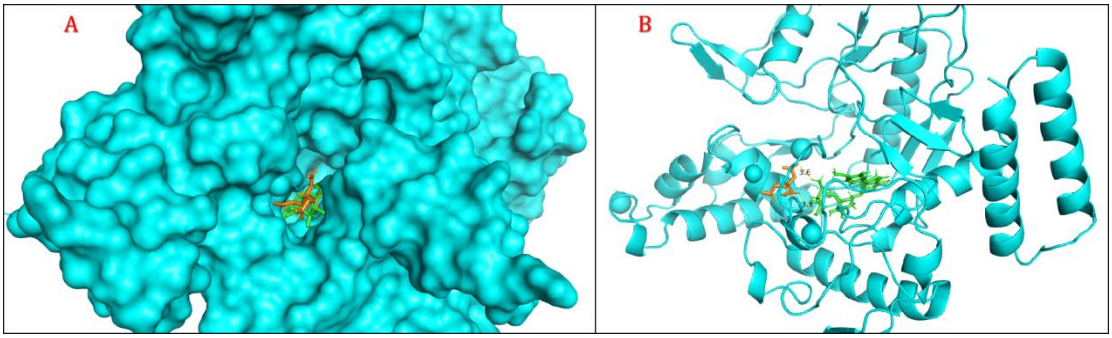

Figure 12. *Binding Orientation of Hesperidin and Nitroglycerin within the eNOS Active Site.*

Figure 12 shows the structural representation of endothelial nitric oxide synthase (eNOS, PDB: 8UFU) in surface (A) and ribbon (B) modes, highlighting the binding positions of Hesperidin (green) and Nitroglycerin (orange) within the active site. Panel A shows the overall molecular surface of eNOS, emphasizing the ligand-occupied binding cavity. In contrast, Panel B shows the binding orientations of both ligands within the active site, revealing their spatial positioning and potential molecular interactions with key residues. The difference in binding positioning observed in the ribbon representation suggests distinct interaction mechanisms for Hesperidin and Nitroglycerin, which may contribute to their respective binding affinities and functional effects on eNOS activity.

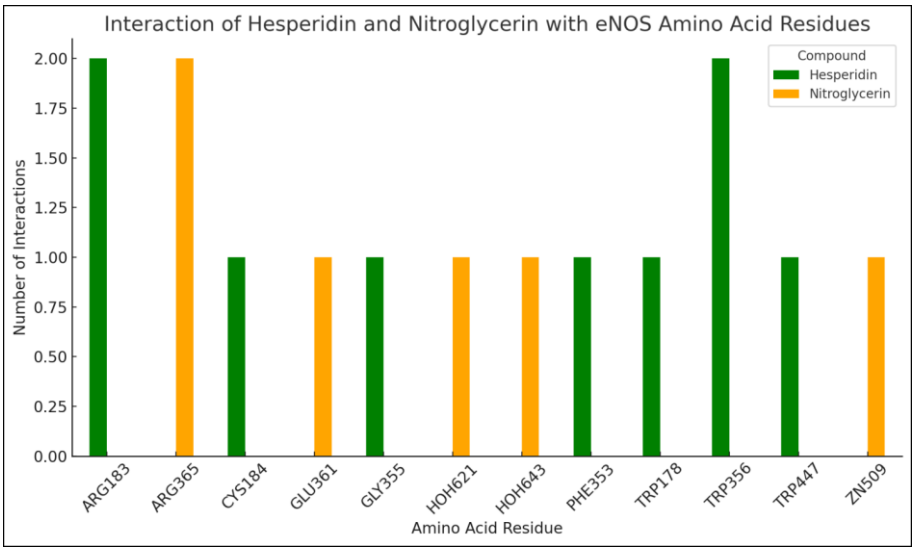

Figure 13. *Interaction of Hesperidin and Nitroglycerin with eNOS Amino Acid Residues.*

Figure 13 shows the number of interactions between Hesperidin (green) and Nitroglycerin (orange) with specific amino acid residues of endothelial nitric oxide synthase (eNOS, PDB: 8UFU). The differences in interaction frequency suggest that Hesperidin forms more stabilizing interactions with eNOS than Nitroglycerin, which may contribute to its stronger binding affinity.

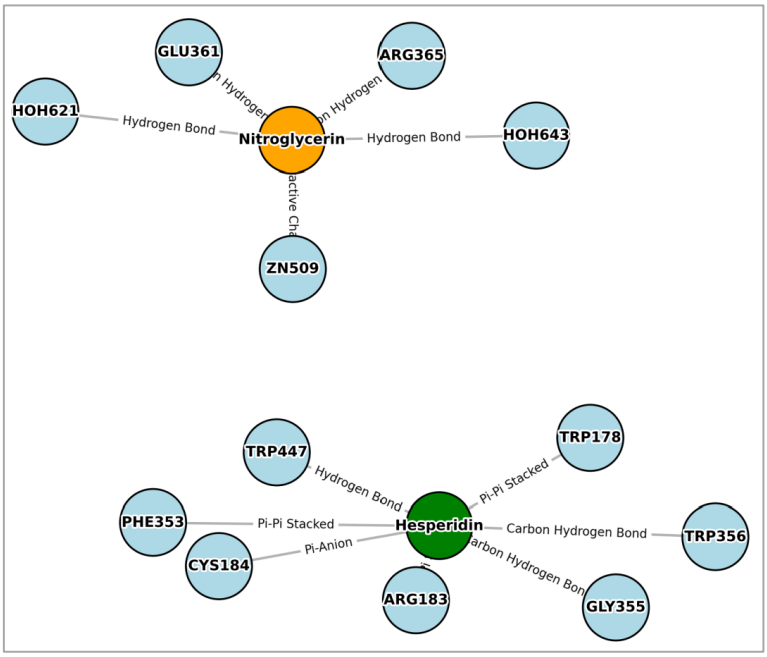

Figure 14. *Interaction Network of Hesperidin and Nitroglycerin with eNOS.*

Figure 14 shows the molecular interactions between Hesperidin (green), Nitroglycerin (orange), and key amino acid residues in the eNOS binding site. The connectivity between nodes represents different interaction types, such as hydrogen bonding, pi-pi stacking, carbon-hydrogen bonding, and attractive charge interactions, contributing to ligand binding stability. Hesperidin exhibits a more diverse interaction network, indicating a potentially stronger and more stable binding than Nitroglycerin.

The general classes of phytochemicals present in the fruit extract of T. tetraptera include the saponins (consisting of carbohydrate and either terpenoid or steroid aglycone moieties (glycosylated triterpenoids) present in the cell membrane of many plant species that have detergent properties and are noted for their multiple biological activities. Saponin's fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects have all been observed [39, 40], making them valuable in traditional medicine and modern drug discovery [41].

Phytosterols, including fatty acid esters, are biosynthetic precursors of many hormones, including vitamin D and steroids [42]. Phytosterols can help to safely lower cholesterol levels to avoid health risks such as heart attacks and stroke. They also neutralize the compensatory increase in intestinal cholesterol absorption induced by statins as a side effect [43]. Phytosterols are also suggested to offer protection against various chronic ailments, including cancer, obesity, and diabetes [44, 45].

Tannins, which include polyphenolic biomolecules, exert several pharmacological effects, including antioxidant and free radical scavenging activity, antimicrobial, anticancer, antinutritional, and cardio-protective properties [46]. Plant phenolic compounds exert protective effects against oxidative damage and inflammation caused by airborne particulate matter, in addition to a range of anti-inflammatory, anticancer, anti-ageing, antibacterial, antiviral and antidiabetic activities [47, 48]. Flavonoids possess several medicinal benefits, including anticancer, antioxidant, anti-inflammatory and antiviral properties. They also have neuroprotective and cardioprotective effects. These biological activities, however, depend on the type and nature of the flavonoid, its mode of action and its bioavailability [49].

Glycosides play numerous important roles in living organisms. Many plants store chemicals as inactive glycosides that can be activated by enzyme hydrolysis [50]. The steroid glycosides (cardiac glycosides) are an essential class of organic compounds that increase the output force of the heart. They help the heart muscles have stronger contractions and decrease their rate of contractions. Cardiac glycosides slow down the speed of heartbeats by inhibiting the cellular sodium-potassium ATPase pump. As a result, the "biological target" of the cardiac glycoside is Na + / K+ ATPase and is thus used in treating congestive heart failure [51].

Terpenoids derived from Isoprene show various pharmacological activities such as antiviral, antibacterial, antimalarial, anti-inflammatory, anticancer, and hypoglycemic [52].

Molecular docking was performed to identify potential drug candidates by predicting the binding affinity of selected bioactive phytocompounds naringenin and hesperidin to receptors of interest. The choice of the targets, 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS), is due to their respective roles in energy metabolism and influence on the NO (Nitric oxide) pathway and synthesis. AMP-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine kinase identified initially as the key player in maintaining cellular energy homeostasis. When activated, AMPK enhances insulin sensitivity and increases the phosphorylation of proteins, such as the glucose transporter type 4 (GLUT4), which is involved in glucose transport, facilitating glucose uptake into cells. It also modulates insulin secretion by pancreatic β-cells [53]. In the liver, AMPK inhibits gluconeogenesis by inhibiting transcription factors, including hepatocyte nuclear factor 4 (HNF4) and cyclic AMP-responsive element-binding protein (CREB) regulated transcription coactivator 2 (CRTC2), that promote the expression of gluconeogenetic enzymes, including phosphoenolpyruvate carboxy kinase and glucose-6-phosphatase [54-56]. AMPK also inhibit gluconeogenesis by phosphorylating and inducing the nuclear exclusion of class IIa histone deacetylates, which normally deacetylate and activate transcription factor FOXO in the nucleus, which results in the expression of gluconeogenic enzymes during fasting [54, 57]. Thus, AMPK regulates diverse metabolic and physiological processes and is dysregulated in major chronic diseases, such as obesity, inflammation, cancer and diabetes. Based on these critical roles in physiology and pathology, AMPK is emerging as one of the most promising targets for preventing and treating these diseases. Endothelial NOS (eNOS), also known as nitric oxide synthase 3 (NOS3), catalyzes the synthesis of nitric oxide (NO) from L-arginine and oxygen and yields citrulline as a byproduct. NO is an important regulator and mediator of numerous nervous, immune and cardiovascular processes. These include vascular smooth muscle relaxation, arterial vasodilation, and increased blood flow [58]. Nitric oxide, when produced by eNOS, dilates blood vessels, raising blood supply and lowering blood pressure. As a result, humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways [59].

This study shows that naringenin exhibits a stronger binding affinity and lower binding free energy than metformin, suggesting potentially higher interaction stability with AMPK (Table 2). Notably, hesperidin exhibits a significantly stronger binding affinity and higher hydrogen bond score than nitroglycerine, suggesting a more stable and favourable interaction (Table 3).

4. Conclusion

The GC-MS results of the methanol fruit extract of T. tetraptera have revealed some of the chemical components of the fruits of the plant. Most of these chemical components have considerable therapeutic value. This study also investigates the therapeutic effects of T. tetraptera against diabetes and hypertension-associated target proteins using in silico experiments. Based on their critical roles in physiology and pathology, AMPK and eNOS are emerging as promising targets for preventing and treating diseases such as diabetes and hypertension, respectively. The results show that T. tetraptera may be a key bio-resource for developing affordable antidiabetic and antihypertensive drugs. These investigations provide supporting evidence for using the fruits of T. tetraptera in ethnomedicine in Nigeria and Sub-Saharan Africa to treat diseases and infections.

Abbreviations

GC-MS Gas Chromatography-Mass Spectrometry

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Conflict of Interest

Authors have declared that no conflicts of interest exist.

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