In-Silico Virtual Screening of Novel Antitoxic Agents from Talinum paniculatum as Inhibitors of Phospholipase A2 and Metalloproteinase Receptor for Antivenom Study

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In-Silico Virtual Screening of Novel Antitoxic Agents from *Talinum paniculatum* as Inhibitors of Phospholipase A2 and Metalloproteinase Receptor for Antivenom Study

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Abstract

Health agencies have reported deaths from the envenomation of snakes, scorpions, and spiders to be a global challenge. Phospholipase A2 (PLA2) and metalloproteinase have been investigated to be major venom enzymes and their inhibition is important for antivenom experimentation. Varespladib (and other drugs such as varespladib-methyl and darapladib) has been mostly used as an antivenin but its cytotoxicity and the drive to source for effective drugs from phytomedicine with little or no side effects and higher potency led to the screening of bioactive compounds of *Talinum paniculatum.* The bioactive components of the plant with high binding affinities to the protein targets used were identified and subjected to further analysis and studies. After the molecular screening of the compounds of *Talinum paniculatum*; rutin, kaempferol, quercetin, and Talinumoside1 were established to have high binding affinities to PLA2 and metalloproteinase in comparison to Varespladib (and other compounds such as varespladib-methyl, darapladib, marimastat and ilomastat) used for the study. Quercetin and kaempferol showed better results after the ADMET study and are better drug candidates than the other compounds studied. This research indicates that quercetin and kaempferol may inhibit PLA2, metalloproteinase, and potential antivenins. Further analysis is recommended to substantiate this study.

Keywords: Rutin, Quercetin, Kaempferol, Varespladib, Envenomation, ADMET

1.0 Background

Yearly, there are about 1.5 million reported cases of envenoming and there is a potential for an increment in this number due to exponential improvement of urbanization [1]. There is a variation in the degree of envenomation effect on the human body and this is dependent on some parameters,

but symptoms of it include hemorrhage, nausea, fever, dermatitis and hyperthermia which can degenerate into respiratory or heart failure, necrosis, hemorrhage, edema, inflammation, paralysis, coma, Hypotension and many more pathological outcomes [1,2]. The spread of venom is facilitated by enzymes such as phospholipases and metalloprotease through the disintegration of molecules within the matrix and the later brings about selective posttranslational processing of toxins caused by envenomation [3, 4]. SvPLA2 facilitates venom spread through hydrolysis of phospholipids, disruption of cell membranes, activation of inflammatory pathways, degradation of extracellular matrix and induction of necrosis. The early occurrence of death and other adverse effects encountered after untreated snakebite is also attributed to the action of PLA2 due to its negative impact on homeostatic mechanisms which are crucial for the circulation of oxygen and coagulation regulation [5-8]. The mode of action of PLA2 involves the hydrolyzation of glycerophospholipids at the position of SN-2 of phospholipids which constitutes the release of oleic acid, lysophospholipids and arachidonic acid [9, 10]. The effects of PLA2 include pre-synaptic neurotoxicity, coagulotoxicity, cardiotoxicity, and myotoxicity [11, 12]. Metalloproteinase is an important enzyme in envenomation research because it constitutes about 30% of the total protein content contained in the venom of numerous vipers and other snakes [13]. Metalloproteinase has been studied to cause skin hemorrhage, apoptotic, fibrinolytic, fibrinogenolytic, activation of prothrombin and Factor X and also the aggregation of platelet [14] which has established it has a significant target for antivenom study. Phospholipases and metalloproteinase are mediators of inflammation triggered by envenomation and their concentration can be adequately regulated by the presence and binding of inhibitors. The inhibition of PLA2 activity by Varespladib establishes it as a drug candidate for envenomation [15]. Methyl-varespladib and varespladib have shown the potency of inhibiting PLA2 obtained from six continents [16] and so are important treatment for envenomation. Darapladib is also an effective potent inhibitor of PLA2 and this has facilitated its usage for clinical conditions and envenomation treatment [17]. Marimastat and Ilomastat are svMP inhibitors and are being investigated as treatments to venom-induced pathogenesis. *Talinum paniculatum* also known as “major gomes” and “erva gorda” is a weed predominant in Brazilian Cerrado and it has been put to use in folk medicine and for food [18]. It has also been used for cardiovascular disorder treatment [19], gastrointestinal problems, skin infections, and wound healing [18]. Its numerous clinical importance and its richness in essential phytocompounds such as tannins, triterpenes, steroids,

saponins, and phytosterols [18] prompted the choice of this plant for this antivenom study. With the estimation of 15% of the whole animal biodiversity being poisonous and having toxins [20] and the limited supply of antivenins in so many areas, it is imperative to study a more effective and accessible antivenom with no side effect as compared to contemporary treatments which are still under-evaluated and under-investigated. An in-silico study {which includes molecular docking, molecular mechanics generalized Born surface area evaluation (MM/GBSA) and ADMET study} was done for the investigation of compounds (contained in *Talinum paniculatum*) with better inhibitory effects on PLA2 and metalloprotease and the pharmacokinetics of the lead compounds in comparison to the standard drugs (Varespladib, methyl-varespladib and darapladib).

1.1 Methodology

1.11 Ligands and protein target

For the identification and experimentation of the inhibitors of PLA2 and metalloproteinase which can ultimately serve as an essential antivenom constituent, the ligands (compounds of *Talinum paniculatum*) and standards utilized for this experiment were mined from PubChem in 2D SDF format. The crystal structures of the protein targets (PLA2 and Metalloproteinase) were obtained from protein data bank (http://www.rscb.org/). Their PDB ID are 1TK4 and 1KUG respectively.

1.12 Ligand preparation

The obtained compounds of *Talinum paniculatum* used for this study were prepared using LigPrep tool. This was made possible with the use of Schrodinger (release 2017).

1.13 Protein preparation and receptor grid generation

The protein targets used were prepared with protein preparation wizard (2020) in Schrodinger suite. This was done to ensure restrain minimization and optimization of structures. Correction of essential aspects such as bond orders, charges, hydrogen consistency and water removal were also done. The Ca^{2+} ion was introduced to the active site to make it optimal for docking (particularly, as Varespladib and varespladib methyl coordinate the Ca^{2+} ion). Thereafter, the receptor grid was generated for the docking protocol using the prepared structure.

1.14 Molecular docking

The grid generated was utilized as the site for docking the bioactive compounds prepared as well as the standards (Varespladib, Methyl-varespladib and Darapladib) to the protein targets. The

Standard Precision (SP) and a more rigid {Extra Precision (XP)} levels of docking were utilized for the obtainment of optimum results. Four compounds having the best pose and docking score were selected and subjected to further computational analyses such as binding energy and pharmacokinetic profiling for comparison with the standards. The interaction between the ligand-protein complexes formed were later visualized and analyzed.

1.15 MM/GBSA

The calculation for binding free energy in ligand-receptor (L-R) interactions can be expressed as:

$$(\Delta Gbind) = \Delta H - T\Delta S = \Delta E_{MM} + \Delta G_{sol} - T\Delta S \quad (1)$$

$$(\Delta E_{MM}) = \Delta E_{internal} + \Delta E_{electrostatic} + \Delta E_{vdw} \quad (2)$$

$$(\Delta G_{sol}) = \Delta G_{PB/GB} + \Delta G_{SA} \quad (3)$$

Upon binding, ΔE_{MM} = the MM energy

ΔG_{sol} = the solvation free energy

$-T\Delta S$ = the conformational entropy

These are representations upon changes of the gas phase.

Furthermore, ΔE_{MM} consists of $\Delta E_{internal}$ (including bond, angle, and dihedral energies), $\Delta E_{electrostatic}$, and ΔE_{vdw} (van der Waals) energies [21]. The summation of electrostatic solvation energy, non-electrostatic solvation component, $\Delta G_{PB/GB}$, and ΔG_{SA} brings about ΔG_{solv} [21].

1.16 Frontal Molecular Orbital Analysis

Frontier molecular orbital (FMO) theory is a concept in chemistry that helps explain and predict chemical reactivity based on the interaction between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of reacting molecules. The HOMO and LUMO, being the most likely orbitals to be involved in chemical reactions, are crucial in estimating various chemical reactivity descriptors, such as energy gap (Eg), ionization potential(I), electron affinity (A), electronegativity (χ), electrophilicity (δ) global hardness (η), softness (S), and dipole moment (ω). These descriptors are calculated using the following equations:

$$Eg = E_{LUMO} - E_{HOMO} \quad \ldots\ldots (1)$$

$$I = - E_{HOMO} \quad \ldots\ldots (2)$$

$$A = - E_{LUMO} \quad \ldots\ldots (3)$$

$$\chi = \frac{I+A}{2} \quad \text{..... (4)}$$

$$\eta = \frac{I-A}{2} \quad \text{..... (5)}$$

$$\delta = \frac{1}{\eta} \quad \text{..... (6)}$$

$$\omega = \frac{(E_{LUMO} + E_{HOMO})^2}{4(E_{LUMO} - E_{HOMO})} \quad \text{..... (7)}$$

1.17 Pharmacokinetic profiling

The study of Absorption, distribution, metabolism, excretion, and toxicity of potential drug candidates is very essential for drug discovery and experimentation. Therefore, SwissAdme (http://www.swissadme.ch) and Pro-tox II (http://tox.charite.de/protox_II) were utilized for such study. Descriptors such as, bioavailability score, ability to inhibit cytochrome P450 enzymes, P-glycoprotein substrate candidacy, topological surface area (TSA), blood brain barrier permeability, molecular weight of compounds, water solubility, lipophilicity, and absorption rate via the gastrointestinal tract were analyzed.

1.18 Results and Discussion

Docking entails having an output of scoring functions obtained through algorithms that calculate the level of binding affinity involved in protein–ligand interaction and this computation subsequently generates a scoring function for the ranking of the most energetical protein-bound conformation in line with the individual ligands [22]. About 40 compounds were obtained from *Talinum paniculatum* as well as five standards (Varespladib, Varespladib methyl, Darapladib, Marimastat and Ilomastat) were screened against two protein targets (Phospholipase A2 and Metalloproteinase) to investigate their inhibitory effect via the binding affinities. The four lead compounds (rutin, kaempferol, quercetin and talinumoside1) showed a good inhibitory effect in comparison to the standards. Quercetin had the highest binding affinity to PLA2 with docking score of -9.033 kcal/mol and Rutin was ranked highest for metalloproteinase with -12.953 kcal/mol. The three other lead compounds showed very impressive inhibitory potential to the targets having docking scores in the range of -5.768 and -8.622 kcal/mol for PLA2 (which were higher than the standards) and -6.974 and -9.145 kcal/mol for metalloproteinase as illustrated in Figure 1. After thorough investigation of the ligands' interaction with the targets, it was seen that with PLA2, rutin and quercetin interact with Phe5 through pi-pi stacking and hydrogen bond (H-bond) respectively. Kaempferol and quercetin interact with Hie48 via pi-pi stacking and H-bond.

Rutin binds to Ser23 and Gly30 via hydrogen bonding. While the binding of kaempferol to Ash49 was also through H-bond. The interaction between Quercetin and the target also involves pi-pi stacking with Ash49 and Leu2. Arg72 of PLA2 forms an H-bond and salt bridge with talinumoside1 while Leu3 and Lys69 interacts with the ligand via H-bond. These interactions are shown in Fig 2.

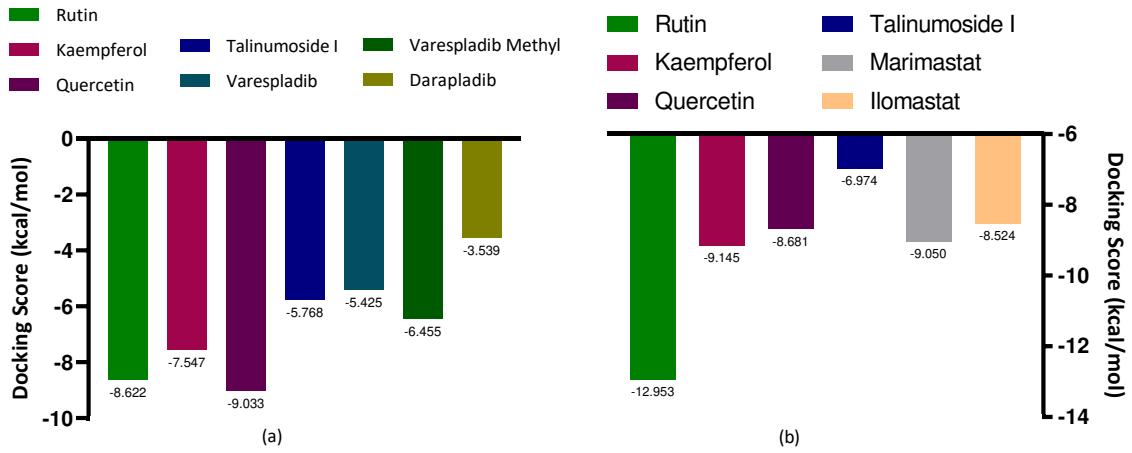
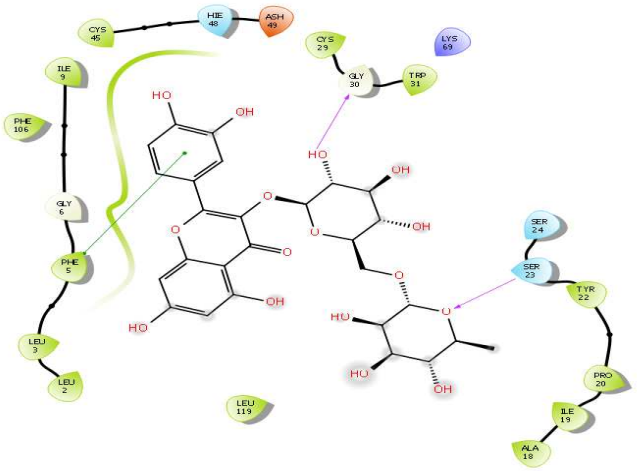
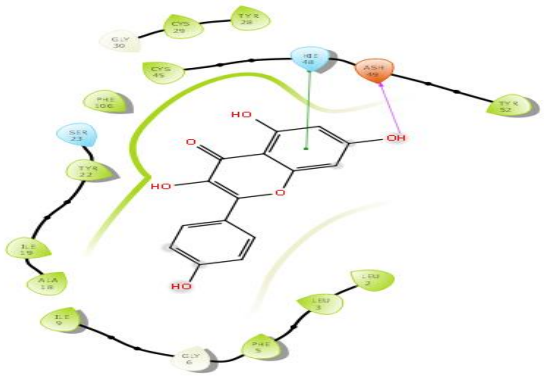


Figure 1: Diagrammatic Representation of Molecular Docking Score (kcal/mol) of Screened ligands from *Talinum paniculatum* and standards to PLA 2 and Metalloproteinase Respectively.

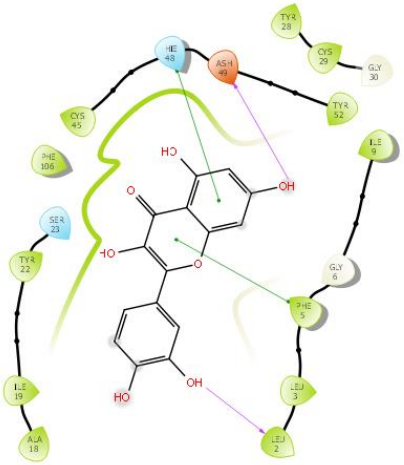
(a)



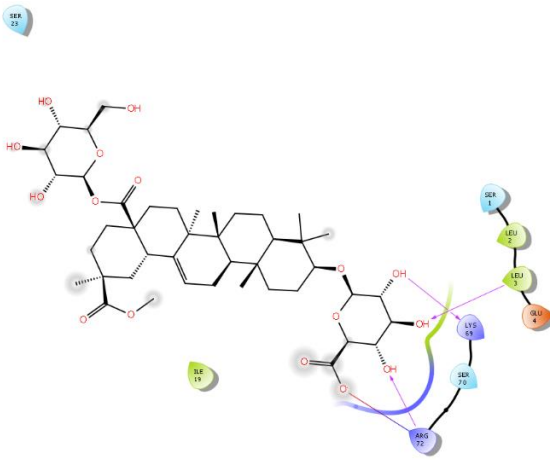
(b)



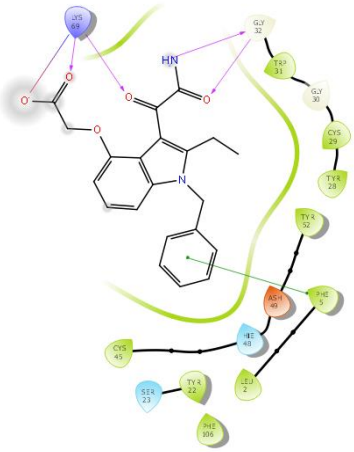
(c)



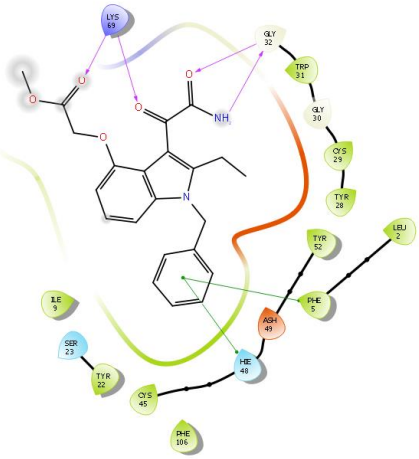
(d)

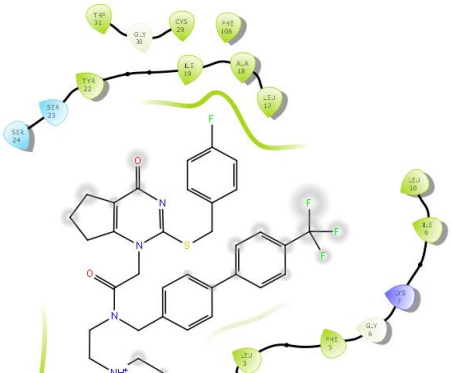


(e)



(f)

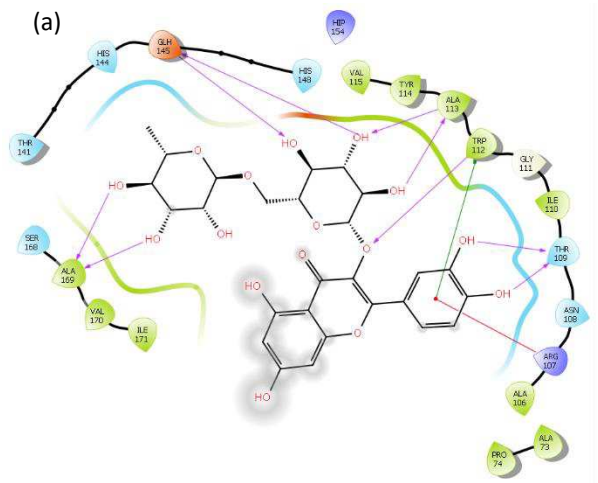


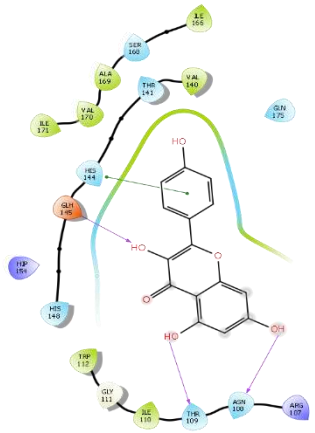


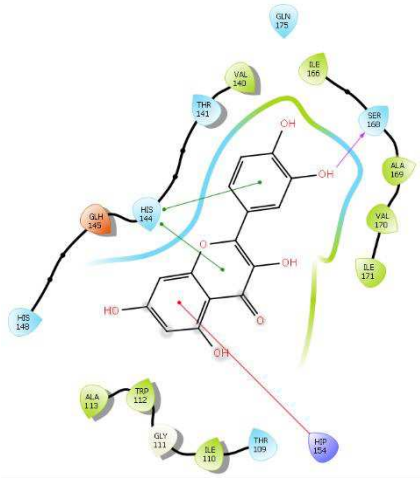
(g)

Figure 2: 2D Interactions of the Lead Compounds with Amino Acid Residues at the Active Sites of PLA 2 (a) Rutin, (b) Kaempferol, (c) Quercetin (d) Talinumoside1 (e) Varespladib (f) Varespladib Methyl (g) Darapladib.

The stability of rutin with metalloproteinase can be said to be due to the H-bond it forms with Glh145, Ala113 and Ala169 amongst other bonding. It is worth noting that Thr109 forms a hydrogen bond with rutin, kaempferol and talinumoside1 while Trp112 binds via pi-pi stacking to rutin and H-bond to it and talinumoside1. A pi-cation interaction occurred at the active pocket of metalloproteinase at Arg107 with rutin and the said amino acid also contacted talinumoside1 via H-bond. Asn108 established a hydrogen bond contact with Kaempferol and talinumoside1 while the later also formed a H-bond with Asp72. A pi-pi interaction of quercetin and kaempferol with metalloproteinase was established at His144. Quercetin also contacted the target via pi-cation and H-bond at Ser168 and Hip154. These interactions with metalloproteinase are illustrated in Fig 3.


(a)


(b)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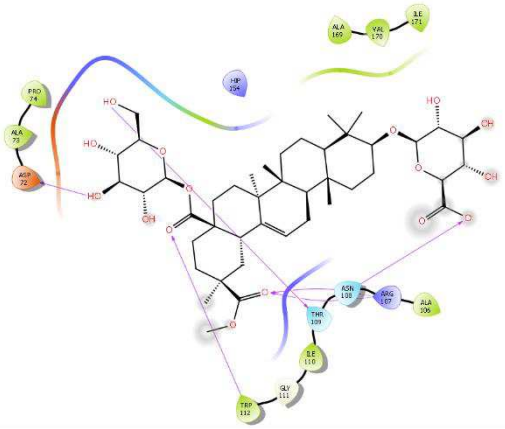
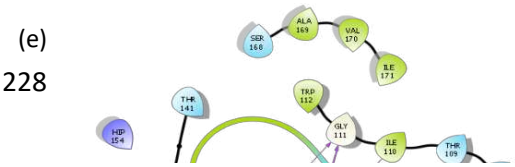
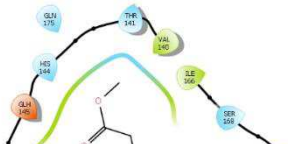

(d)


(e)


(f)

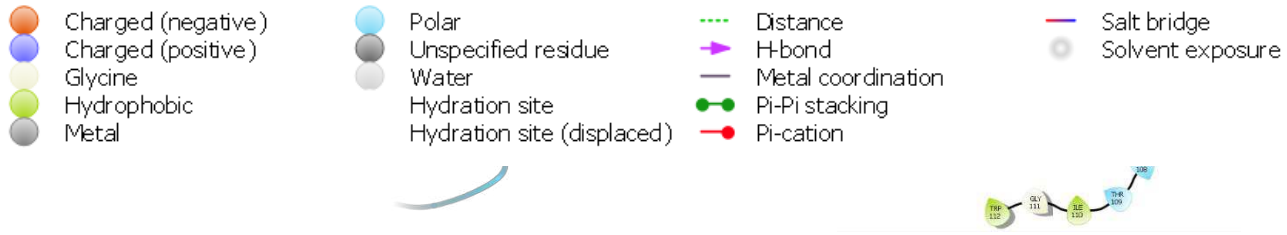

Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

Figure 3: 2D Interactions of the Lead Compounds with Amino Acid Residues at the Active Sites of Metalloproteinase (a) Rutin, (b) Kaempferol, (c) Quercetin (d) Talinumoside I (e)Marimastat (f)Ilomastat.

Salt bridges play a fundamental role for the stability of secondary-structural elements [23]. The connection of several subunits of proteins is also a function that salt bridges perform in interactions [24]. The H-bond is very essential in protein-ligand interactions because of its support in ligand binding affinity using the mechanism of displacement of protein-bound water molecules and also has a role in protein folding and catalysis [25]. Pi-stackings interact via non-covalent force for stabilization purpose. Protein aromatic residues are important for the substrate binding and also stability when interacting via pi-stacking [26]. The manner at which the interacting molecules orientate (geometrically) determines the strength of aromatic stacking interactions [27]. For molecular interactions, pi-cation also utilizes noncovalent forces [28] and it forms a bond between monopoles (cations) and a quadrupole (π system) while also promoting protein structure. Furthermore, the binding energy of each ligand to the protein targets were investigated to know the level of spontaneity of each compound in complex with the receptor. It is worth noting that the more negative the value of the binding energy (ΔGbind) of a compound to a target is, the higher its spontaneity. With a binding energy of -52.145 kcal/mol, rutin can be said to be the most spontaneous compound to PLA2 with respect to this study. Kaempferol and quercetin (-50.132 and -48.907 kcal/mol) had higher ΔGbind than the standards used, while talinumoside1 had a value of -41.995 kcal/mol which is an impressive value in comparison with the standard as shown grammatically in Fig 4a. A closely related trend was observed with the ΔGbind of the ligands' interaction with metalloproteinase. Although, Marimastat had the highest biding energy of -58.557 kcal/mol but it was closely followed by Rutin, quercetin and kaempferol which were ranked -57.712, -55.209 and -54.845 kcal/mol respectively while talinumoside1 having a value of -34.162 kcal/mol showed a fair level of spontaneity with respect to the standards. Molecular Mechanics/ Generalized Born Surface Area (MM/GBSA) method utilizes both molecular mechanics calculations and the continuum solvation models [21]. The scores of the MM/GBSA protocol with metalloproteinase are shown in Fig 4b.

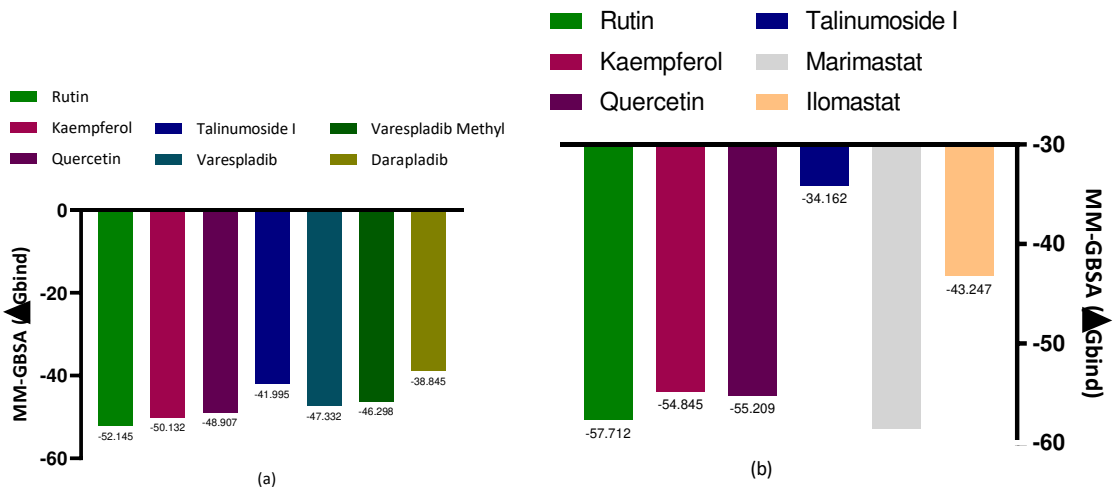
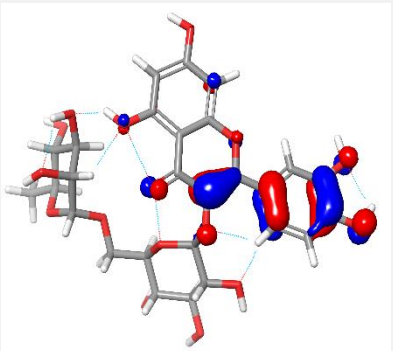
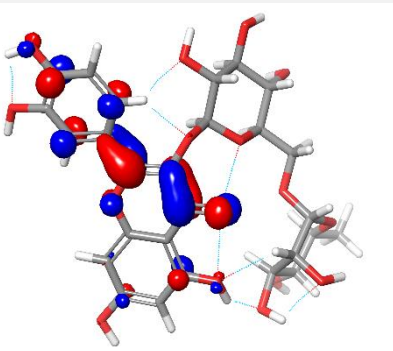
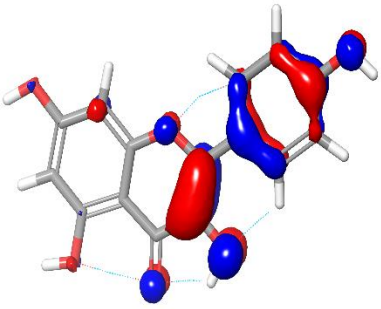
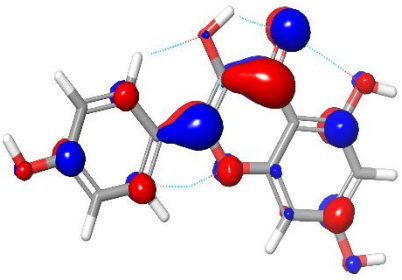
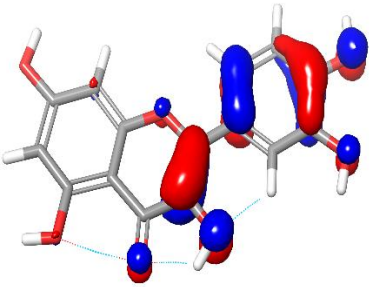
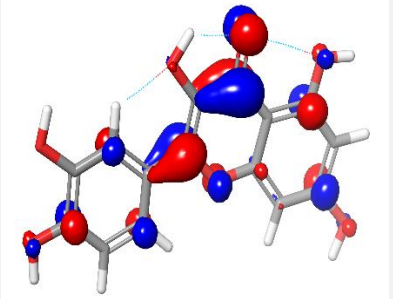
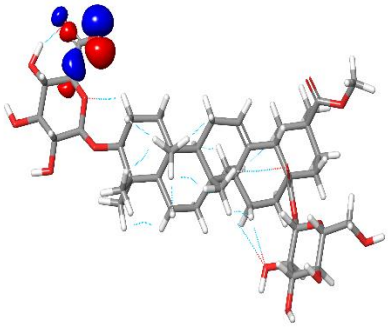
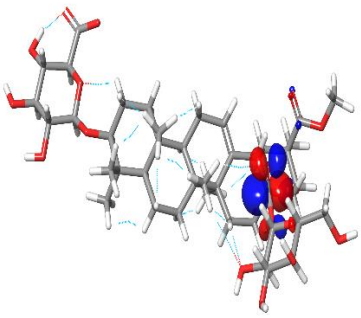


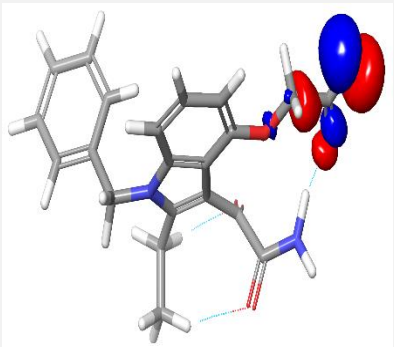
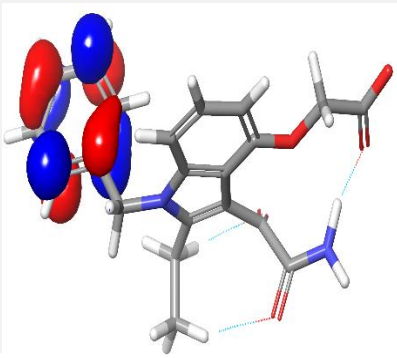
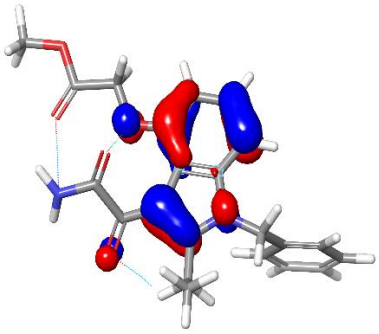
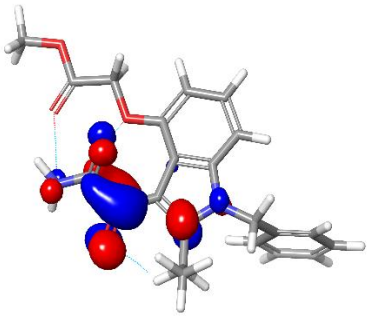
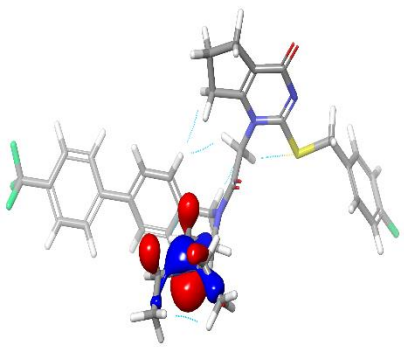
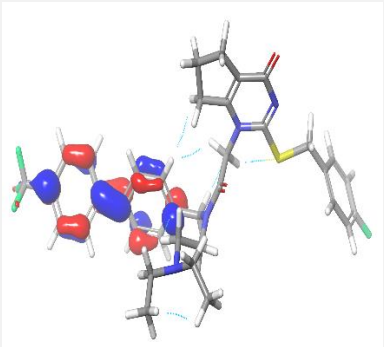
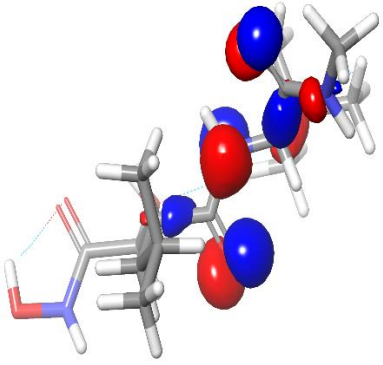
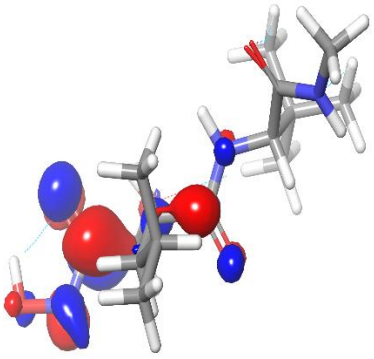
Figure 4: **Diagrammatic Representation of MM-GBSA of Screened Ligands from *Talinum paniculatum* and Standard to PLA 2 and Metalloproteinase Respectively.**

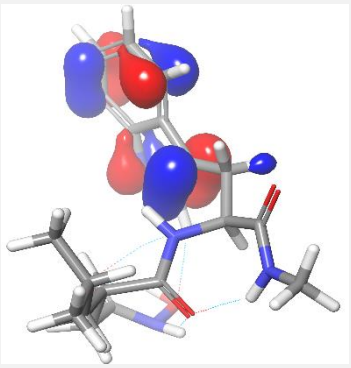
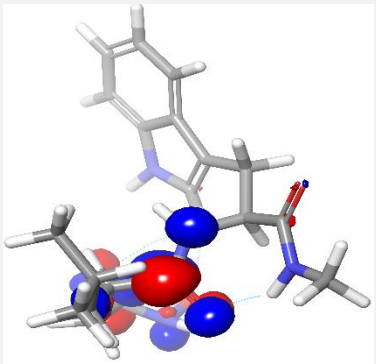
Table 1. Quantum Chemical Calculations of Lead Compound and the Standard Compound.

Compounds	Homo	Lumo	Eg	I	A	χ	η	δ	ω
Rutin	-0.19703	-0.04211	0.15492	0.19703	0.04211	0.11957	0.07746	12.9098	0.09228
Kaempferol	-0.19464	-0.05494	0.1397	0.19464	0.05494	0.12479	0.06985	14.3163	0.11147
Quercetin	-0.19392	-0.05648	0.13744	0.19392	0.05648	0.1252	0.06872	14.5518	0.11405
Talinumoside I	-0.06609	0.05151	0.1176	0.06609	-0.05151	0.00729	0.0588	17.0068	0.00045
Varespladib	-0.07068	0.05526	0.12594	0.07068	-0.05526	0.00771	0.06297	15.8805	0.00047
Varespladib Methyl	-0.19388	-0.04717	0.14671	0.19388	0.04717	0.12052	0.07335	13.6323	0.09901
Darapladib	-0.21105	-0.06087	0.15018	0.21105	0.06087	0.13596	0.07509	13.3173	0.12308
Marimastat	-0.24522	-0.00427	0.24095	0.24522	0.00427	0.12475	0.12048	8.30048	0.06458
Ilomastat	-0.19938	-0.01338	0.186	0.19938	0.01338	0.10638	0.093	10.75269	0.06084

Table 2. Illustration of the Molecular Orbitals of Lead Compounds and the Standard Compounds.

COMPOUNDS	HOMO	LUMO
Rutin		
Kaempferol		
Quercetin		
Talinumoside I		

Varespladib		
Varespladib Methyl		
Darapladib		
Marimastat		

Ilomastat		

1.19 Frontier Molecular Orbital

Many studies have proven that energy quantization in molecular orbitals is a key component in predicting spontaneity and stability of chemical reactions [29, 30]. The spatial distribution of electron in a molecule when two or more nuclei interact is known as molecular orbitals. During molecular interactions, electrons are distributed into orbitals according to specific energy level. The highest occupied molecular orbitals (HOMO) signifies the filled orbital which has the higher tendency of donating electrons while lowest unoccupied molecular orbital connotes the empty orbitals which accept electrons [31]. Frontier molecular orbital analysis is used to assess the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), ionization potential ($I = -E_{HOMO}$), electron affinity ($A = -E_{LUMO}$), electronegativity (χ), global hardness (η), softness (S), electrophilicity, and dipole moment (ω). These descriptors are essential for predicting the chemical reactivity of potential drug candidates. As depicted in Table 1, Talimunoside I has the highest HOMO energy, followed by Varespladib, Kaempferol, Varespladib Methyl, Quercetin, Rutin, and Darapladib, respectively. The higher the HOMO energy the lower the ionization energy, therefore these results indicate that Talimunoside I and Varespladib possess low ionization energies, suggesting they have a strong tendency to act as good electron donors to the target protein when compared to rutin. Furthermore, the low LUMO (Lowest Unoccupied Molecular Orbital) energies of Varespladib (0.055 eV) and Talimunoside I (0.051 eV) further support their potential as electron acceptors. Molecules with lower LUMO energies tend to be better electron acceptors. These findings align with the observed ionization potential and electron affinity values in Table 1. HOMO – LUMO energy gap is one of the critical indicators in predicting the interaction of drug candidate with target biomolecules. A smaller HOMO-LUMO gap generally indicates higher reactivity and lower stability [30]. When the gap is

narrow, it's easier for a molecule to lose an electron (oxidation in the HOMO) or gain an electron (reduction in the LUMO), making it more prone to reactions. The results from the studies shows the value of HOMO-LUMO energy gap in increasing order; Talinumoside I < Varespladib < Quercetin < Kaempferol < Varespladib Methyl < Darapladib < Rutin. This trend suggests a corresponding difference in their reactivity. Drug candidate with a smaller HOMO-LUMO gap (like Talinumoside I) are likely to be more reactive than rutin. The electronegativity value gives insight to the relative ability of molecules to attract electron towards itself during chemical bonding and its overall polarity [32]. As depicted in Table 1, the electronegativity values (χ) suggest that the compounds are moderately polar, with values ranging from 0.0072 to 0.135. Darapladib, with the highest electronegativity value of 0.135 eV compared to the other compounds, has the greatest potential to form strong bonds with target proteins. Energy gap determines the chemical hardness and softness of a molecule [33]. The larger the energy gap the harder the molecule and vice versa. As shown in Table 1, the drug candidate possess relatively moderate hardness and softness value hence are chemically moderately stable and reactive. Another key descriptor of reactivity is the dipole moment which measure the inhomogeneity of charge distribution and stability of drug candidate [34]. As shown in Table 1, darapladib exhibit high dipole moment of (0.123debye) compared to other ligands indicating it relative reactivity with the surrounding media. Furthermore, the calculated dipole moment reveal that all tested ligands are polar in nature, which can improve their solubility and absorption.

2.0 ADMET investigation

The ADMET property of a drug candidate is part of the determining factor of its success and acceptability [35]. Safety issues can be contained with the use of computational ADMET [36]. The physicochemical profiling of the lead compounds in this study are shown in Table 1. The molecular weight ranged between 286.24 and 610.52 g/mol with rutin and kaempferol having the highest and least values respectively.

Table 3: Physicochemical Properties of Top-Scoring Compounds

Compounds	Molecular weight(g/mol)	TPSA (Å^2)	Log P	Log S	nHA	nHD
Rutin	610.52	269.43	-1.29	-3.30	16	10
Kaempferol	286.24	111.13	1.58	-3.31	6	4
Quercetin	302.24	131.36	1.23	-3.16	7	5

Talinumoside I	838.97	259.20	1.80	-6.34	16	8
Varespladib	380.39	111.62	2.18	-3.80	5	2
Varespladib Methyl	394.42	100.62	2.56	-4.01	5	1
Darapladib	666.77	83.74	6.89	-7.75	8	0
Marimastat	331.41	127.76	1.23	-1.49	5	5
Ilomastat	388.46	123.32	1.80	-2.26	4	5

Table 4: Descriptors of Absorption and Distribution

Compounds	**GI absorption**	**P-gp substrate**	**BBB penetration**
Rutin	Low	Yes	No
Kaempferol	High	No	No
Quercetin	High	No	No
Talinumoside I	Low	Yes	No
Varespladib	High	No	No
Varespladib Methyl	High	No	No
Darapladib	Low	Yes	No
Marimastat	High	No	No
Ilomastat	High	Yes	No

Table 5: Descriptors for Pharmacokinetics

Compounds	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**
Rutin	No	No	No	No	No
Kaempferol	Yes	No	No	Yes	Yes
Quercetin	Yes	No	No	Yes	Yes
Talinumoside I	No	No	No	No	No
Varespladib	No	No	Yes	No	No
Varespladib Methyl	No	Yes	Yes	No	Yes
Darapladib	Yes	No	No	Yes	Yes
Marimastat	No	No	No	No	No
Ilomastat	No	No	No	No	No

Table 6: Excretion and Toxicity Parameters

Compounds	**LD50 (mg/kg)**	**Hepatotoxicity**	**Carcinogenicity**	**cytotoxicity**
Rutin	5000	No	No	No
Kaempferol	3919	No	No	No
Quercetin	159	No	Yes	No
Talinumoside I	3220	No	No	No
Varespladib	78	No	No	Yes
Varespladib Methyl	78	No	Yes	Yes
Darapladib	800	No	No	No
Marimastat	2000	No	No	No

Ilomastat	300	Yes	Yes	No

Table 7: Druglikeness and Bioavailability Score

Compounds	Lipinski rule Violations	Egan rule Violations	Veber rule Violations	Bioavailability score
Rutin	3	1	1	0.17
Kaempferol	0	0	0	0.55
Quercetin	0	0	0	0.55
Talinumoside I	3	1	1	0.11
Varespladib	0	0	0	0.56
Varespladib Methyl	0	0	0	0.55
Darapladib	2	1	1	0.17
Marimastat	0	0	1	0.55
Ilomastat	0	0	1	0.55

The topological polar surface area (TPSA) is essential for the prediction for absorption in intestine and brain. TPSA < 60 $Å^2$ shows that the drug can penetrate the blood brain barrier (BBB). Kaempferol and rutin having 111.13 and 269.43 $Å^2$ respectively as values of TPSA are the least and highest amongst the lead compounds. This confirms that all the four lead compounds do not penetrate the BBB and it is important so as not to induce an adverse drug reaction via the central nervous system. LogP is the term used to depict molecules' lipophilicity and the value is determined through the estimation of the partitioning between phases that is aqueous and lipophilic [37]. In drug research, logP is an essential parameter because it has an influence on absorption, distribution, drug clearance route and permeability [37]. The logP value should be <5 and the most ideal values are between 1.35 and 1.8. Kaempferol, quercetin and talinumoside1 have logP values of 1.58, 1.23, and 1.80 respectively which are suitable values for drug candidates. If the logP is very low, the drug will not be retained and when too high, there will be a deposition of the drugs in fatty tissues. The logS show water solubility level of individual molecule and this determines movement in hydrophilic state during distribution [38]. As shown in Table 1; Rutin, kaempferol and quercetin having logS values of -3.30, -3.31 and -3.16 respectively fall in the same range as varespladib and varespladib methyl which have their values as -3.80 and -4.01. Talinumoside1 with a value of -6.34 is significantly the same as Darapladib with a value of -7.75. These correlations show that the compounds have the potential of exhibiting similar hydrophilic property as the standards. P-glycoprotein (p-gp) is a transmembrane that expels numerous harmful

compounds inside the cell to the extracellular space, but also has the ability to efflux many drugs out of the cells which can adversely affect the activity of diverse drugs [37]. P-gp substrates reduce drug absorption and therefore, the knowledge of its status is critical for drug development. Kaempferol and quercetin are not substrates of P-gp and this depicts that the drug absorption level will be optimal. Rutin and talinumoside1 are P-gp substrate just as darapladib and as such the absorption rate will be minimal. Cytochrome P (CYP) enzymes are the most investigated phase 1 enzymes which plays the role of drug metabolism through the mediation of oxidation in numerous compounds [35]. Studies have shown that 75% of drugs in the market are metabolized by CYPs [39]. Rutin and talinumoside1 do not inhibit the activities of the CYP's analyzed (which include CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4) but just as darapladib, kaempferol and quercetin can be an inhibitor to CYP1A2, CYP2D6 and CYP3A4 which may elicit drug-drug interaction. For these compounds, provisions should be made to enable the improvement of drug metabolism that might have been impeded. The analysis of the toxicity of these compounds showed that they are all not an inducer of hepatoxicity, carcinogenicity and cytotoxicity asides quercetin which has a minimal potential of being carcinogenic. Further studies can be performed to investigate the degree and the dose that may likely prompt this. It is worth noting that varespladib and varespladib methyl both have the potential of being cytotoxic while the later can also be carcinogenic. The oral LD50 of the quercetin, talinumoside1, kaempferol and rutin are 159, 3220, 3919 and 5000 (mg/kg). These values are needed to be known for the indication of the acute toxicity of the test compounds. Abbott Bioavailability score is a representation of the dose fraction that gets into system circulation after oral administration or via the extravascular route. This is an important parameter for drug absorption. The optimal score for bioavailability is ≥ 0.55 and this is the case for kaempferol and quercetin with both having a score of 0.55. Therefore, Kaempferol and quercetin will have an ideal absorption rate. Scores of 0.11 and 0.17 possessed by talinumoside1 and rutin predicts poor oral bioavailability which is the same as darapladib. Kaempferol and quercetin obeyed all rules of Lipinski, Egan and Veber. Rutin and talinumoside1 violated 3 rules of Lipinski and 1 of the rules of Egan and Veber. For the lipinski violation, it is due to the molecular weight being >500Da and this can be solved through lead optimization according to [38]. Also, the hydrogen bond donors and hydrogen bond acceptors were greater than 5 and 10 respectively. For the Egan and Veber rule violation, it was due to the TPSA being greater than 131.6 and 140 (respectively) for the compounds. In conclusion, *Talinum paniculatum* has

been used for folk medicine and studied to treat several health challenges due to its phytoconstituents and this prompted the use of drug discovery tools to screen bioactive compounds of the plant against PLA2 and metalloproteinase which are major enzymes present in venom. The lead compounds analyzed showed a very good binding affinity and binding energy in comparison to the standards. Kaempferol and quercetin had a good ADMET result but rutin and talinumoside1 can undergo modifications (such as lead optimization) to make them suitable drug candidates. The compounds may be explored as antivenins individually or in combination for the treatment of envenomation.

Research Ethical Considerations and Approval

This study was conducted in accordance with ethical guidelines and regulations. The research procedures and methodology for handling of this experimental protocol for this study was approved by the Adekunle Ajasin University, Akungba Akoko Central Research Committee Council on Research Ethics prior to commencement of research. All procedures followed were in accordance with the ethical standards of Ministry of Health, Nigeria. All experimental procedures complied with the relevant institutional and international ethical standards.

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Authors' Contributions

This work was carried out in collaboration with all the authors from different Departments and Universities. Author O. S. Bakare did the experimental background proposal design of the study while author S.A. Praise, carried out the literature search of the plant materials as well as the docking process. Author O. S, Bakare and S. O. Olubode, also jointly collate the protocols for the experimental assays for the study while O.S. Bakare wrote the first draft of the manuscript. Authors A. T. Kolawole, M.M. Olusanya and C.E. Ikechukwu managed the analyses of the study and

managed the assays protocols and the molecular docking of the compounds and the final proofreading was done by author O.S. Bakare. All the authors read contributed to the write-up and approved the manuscript All the authors read contributed to the write-up and approved the manuscript

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This research was supported by the commitment of all the authors involved in the work, there was no funding agency and no funding available for the research work; neither was there any funding body role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Everything was solely done by the participation of all the authors as follows:

Consent for Publication

All authors have reviewed and approved the final version of this manuscript for publication. No identifiable personal data or images are included in this study that require additional consent for publication.

Conflicts of Interest: we declared that there are no conflicts of interest and there is no funding sponsor in executing this research work and in the publication of the results of the study.

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