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Computational study of the inhibitory potential of *Gongronema latifolium* (benth) leave on farnesyl pyrophosphate synthase, a target enzyme in the treatment of osteoporosis. A molecular modelling approach

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ABSTRACT

Background & Aim: Osteoporosis is an increasing medical threat which is referred to as a systemic skeletal disorder that is characterized mainly by low bone mass and microarchitectural wear of bone tissue and strength, which eventually results in an increase in the fragility of bone and makes bone to be susceptible to fracture. Osteoporosis is known globally as a severe health problem affecting approximately 200 million people worldwide. Therefore, a pharmacological solution is urgently needed. Studies have shown that farnesyl pyrophosphate synthase is a crucial enzyme in the mevalonate pathway that causes bone resorption, thus serving as a key pharmacological target.

Experimental: *Gongronema latifolium*'s (Benth) phytoconstituents were screened against the mevalonate pathway enzyme farnesyl pyrophosphate synthase computationally using molecular docking, pharmacokinetics screening and Molecular Mechanics/Generalized Born Surface Area approach to identify compounds with the better inhibitorypotentials against this target in this study.

Results: The study resulted that five compounds; hyperoside, rutin, epigallocatechin-3-gallate, kaempferol-3-arabinoside, and isoquercetin show a better inhibitory potential by binding to the active site of farnesyl pyrophosphate synthase compared with a co-crystalized ligand. These hit compounds were further subjected to pharmacokinetics studies to predict their drug-likeness and toxicity characteristics which show that all hit compounds except Rutin are drug-like leaving Kaempferol-3-Arabinoside as the most drug-like hit compound compared to the co-crystallized ligand.

Recommended applications/industries: This study suggests that *G. latifolium* leaf could be a good plant source for a drug-like compound that may treat osteoporosis by inhibiting the farnesyl pyrophosphate synthase, in the mevalonate pathway, thereby stopping bone resorption.

1. Introduction

Osteoporosis is an increasing medical and socioeconomic threat which is referred to as a systemic skeletal disorder that is characterized mainly by low bone mass and microarchitectural wear of bone tissue and strength, which eventually results in an increase in the fragility of bone and makes bone to be susceptible to fracture (Cooper, 1999; Prevention, 2001).

The clinical significance of osteoporosis is observed and seen in the fractures that occur. These include

vertebral fractures, hip fractures, and Colles' fracture of the distal forearm, but the risk of fracture that occurs at many other sites also rises when bone density is reduced (Seeley *et al.*, 1991). There is a substantially higher risk of osteoporosis in females than among men and there are significantly different risks for both sexes from country to country. In the United States and Northern Europe, women above the age of 50 years are at risk of hip fracture of the same magnitude as the risk of other major osteoporosis fractures. The number of patients affected ranges from 11% to 18% and may last a lifetime (Kanis *et al.*, 1994). Advanced age is the best predictor of osteoporosis, but also other factors like; early menopause, a fracture after 40 years of age, maternal history of hip fracture, low body weight, or specific diseases and treatments increase susceptibility to fractures. All fractures, either wrist, ribs, vertebrae, or hip fracture are associated with considerable morbidity, a decline in quality of life, and increased mortality (JAMA, 2001).

Osteoporosis is known globally as a severe health problem affecting approximately 200 million people worldwide (Vijayakumar & Büsselberg, 2016). The incidence of osteoporosis in postmenopausal women increases steadily with increasingly aging populations, according to Reginter and co-worker (Reginster & Burlet, 2006). Approximately 10 million people, including men and women in the United States, have been affected by osteoporosis (Wright *et al.*, 2014). About 1.5 million fractures are attributable to osteoporosis annually in the United States (Riggs & Melton, 1995). In the year 2000, in the European Union, the estimated number of osteoporotic fractures was at 3.79 million, and 0.89 million were hip fractures (Kanis & Johnell, 2005). The prevalence of osteoporosis is predicted to rise in the United States from approximately 10 million people to more than 14 million people by 2020 (Burge *et al.*, 2007). Thus, a pharmacological solution is necessary.

Studies have shown that farnesyl pyrophosphate synthase (FPPS), is a target enzyme in the mevalonate pathway which condenses the diphosphates of C5 alcohols (isopentenyl and dimethylallyl) to form C10 and C15 diphosphates (geranyl and farnesyl) and thereby causes bone resorption (Gabelli *et al.*, 2006) or otherwise called osteoporosis.

Gongronema latifolium, otherwise known as Benth or Asclepiadaceae, is a climber with woody hollow stems, glabrous stems below and always covered with greenish-yellow flowers (Edim *et al.*, 2012). The plant has also been widely used in folk medicine for maintaining healthy blood glucose levels. The plant leaves are found to be effective as an anti-diarrhea and anti-tussive. Furthermore, the leaves are also used to prepare food for nursing mothers, where it is believed to reduce post-partum contraction, enhance the return of the menstrual cycle and work as a stimulant for appetite (Imo & Uhegbu, 2015).

In this study, a molecular docking, binding energy calculations and ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) screening approach was utilized to identify potential FPPS inhibitors from *G. latifolium* characterized phytochemicals.

2. Materials and Methods

2.1. Virtual screening and docking platform

In silico screening was done on Schrodinger Suite software using Maestro 11.1 (Schrödinger, 2017). A total of 65 compounds that have been characterized with *G. latifolium*was mined and docked to the active site of FPPS retrieved from an online database to predict compounds with the best inhibitory potential to inhibit the action of FPPS in the genesis of osteoporosis. The molecular docking was done following standard guidelines

2.2. Ligand library generation and preparation

Two-dimensional (2D) structures of secondary metabolites from *G. latifolium* leaves that have been characterized (Imo & Uhegbu, 2015) was mined from Pubchem online database (Kim *et al.*, 2016) in Standard data format (SDF). The mined structures were prepared into a three-dimensional (3D) structure using ligprep tool (Release Schrödinger, 2017), by adding hydrogen atoms, ionizing at pH (7.2 ± 0.2) and removing salt using Epik (Shelley *et al.*, 2007; Schrodinger, 2021). Optimised Potentials for Liquid Simulation 3 (OPLS3) force field (Harder *et al.*, 2016) was used for ionization and generation of tautomeric states. 32 stereoisomers were set to be generated per ligand which leads to the generation of 232 structures from 65 compounds. The generated structures were used for the virtual screening as obtained.

2.3. Target preparation

X-ray crystallographic structure of human FPPS complexed with an inhibitor (PDB ID: 2F8C) (Asthana *et al.*, 2014) was retrieved from Protein Data Bank. The structure was visualized using the molecular graphics program PyMol (DeLano, 2002) proposed for the structural visualization of proteins. Protein was prepared using the Protein preparation wizard tool of Maestro, Schrodinger Suite. During the protein preparation, bond orders were assigned for atoms in the molecule to have high connection and rotatability (Dehof *et al.*, 2011), hydrogens were added to atoms that lack hydrogen during protein crystalization, zero-order bonds to metals were created to substitutes covalent bonds with zero-order (dashed) bonds, adjusting the formal charges of the metal and ligating atoms correspondingly (Schrodinger.com), disulfide bonds were created to enhance the protein stability, water molecules were deleted as the precision of docking and enrichment results can be influenced by water molecules (Madhavi Sastry *et al.*, 2013) and het states were generated using Epik at pH 7.0± 0.2. The protein was refined by optimizing the H-bond assignment and was finally minimized using the OPLS3e force field.

2.4. Receptor grid generation

The receptor grid shows the area of interaction between the ligand and the protein. The prepared protein grid was generated on the binding site through the Receptor Grid Generation tool (Glide Grid). The binding site was located by selecting the co-crystalized ligand at the active site of 2F8C. The ligand on the crystal structure of the protein provided information about the active site. A cubic grid box was automatically generated which encompasses all the amino acid residues at the active site. The three-dimensional coordinates X, Y and Z of the generated grid was size 51.67A^{o}, 63.91A^{o}, and 35.04 A^{o}respectively

2.5. Docking

The Docking was performed using Glide tool (Friesner *et al.*, 2004) on maestro 11.1 (Schrödinger Release, 2017). The prepared crystal structure of FPPS (2F8C) was used to virtually screen the prepared compounds to identify compounds with the lowest docking score using Standard Precision (SP) and Extra Precision (XP) docking algorithms. The docking experiment was done treating the protein as a rigid body**,** while the ligand's rotatable bonds were set to be free.

2F8C co-crystallized ligand was docked using the same procedure to serve as a standard andwas compared with the hit compounds.

*2.6. ADME/Tox screening***:**

Hit compound from docking was further subjected to Absorption, Distribution, Metabolism, Excretion, and Toxicity using the swissADME (http://www.swissadme.ch) and Pro-Tox II online servers(https://tox-new.charite.de/protox_II) to determine the pharmacokinetic profile, drug-likeness and toxicity of the hit compounds.

2.7. Bindng energy calculation

The docked protein-ligand complex binding free energy was calculated using the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) continuum solvent model.

To accomplish this work, rotamer search algorithms from prime were employed using the OPLS3 force field, and VSGB solvent model was used.

3. Results and discussion

This study features a computational approach to reveal the molecular interaction, inhibitory potentials, and binding pose of compounds from *G. latifolium* leaves against FPPS, The co-crystallized ligand with FPPS was also docked to serve as a standard and compared with the hit compound. Our result shows that five compounds; Hyperoside, Rutin, Epigallocatechin-3-gallate, Kaempferol-3-Arabinoside and Isoquercetin show a better inhibitory potential by having a lower docking score when compared to the co-crystalized ligand.

Differences in the docking scores and MM/GBSA screening result of the hit compounds compared with the co-crystalized ligand is pictorially shown in Figure 1. Figure 2 represents the 2D structures of the lead compounds.

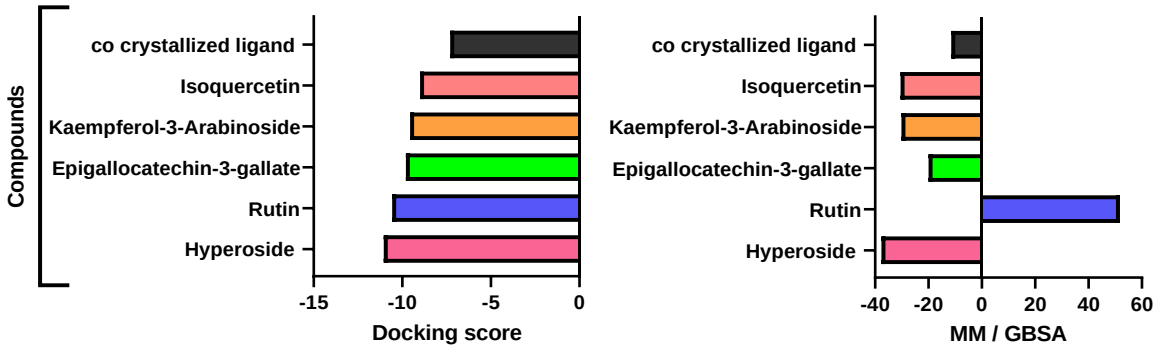

Figure 1. Bar chart showing the differences in the docking scores of hit compounds and the co-crystalized ligand.

The molecular docking scores result shows that five compounds: hyperoside, rutin, epigallocatechin-3-gallate, kaempferol-3-arabinoside and isoquercetinhas a better inhibitory effect on FPPS among the other compounds screened by having a better docking score and interaction with the amino acid residues at the active site of the protein. The result of the docking show that hyperoside, rutin, epigallocatechin-3-gallate, kaempferol-3-arabinoside, isoquercetin and the co-crystalized ligand have docking scores of -11.048 Kcal/mol, -10.567 Kcal/mol, -9.795 Kcal/mol, -9.553 Kcal/mol, -8.996 Kcal/mol and -7.298 Kcal/mol, respectively.

The differences in the docking scores were pictorially represented in Figure 1.

MM-GBSA is one of the enticing techniques used to improve the results of virtual screening. The MM-GBSA approach is a better way to estimate the free binding energies (dG) of protein-ligand complexes with a much greater level of precision. (Bandyopadhyay *et al.*, 2021)

A negative dG value indicates that the complexes formed were stable in the binding pocket of the target (Bathula *et al.*, 2021). All hit compounds show a negative dG value except Rutin which shows a positive dG value as shown in Figure 1.

The binding free energy of docked complexes were -37.651, 51.757, -19.938, -30.114, -30.482 and -11.478 for hyperoside, rutin, epigallocatechin-3-gallate, kaempferol-3-arabinoside, isoquercetin and co-crystallized ligand, respectively.

This indicates that all the hit compounds are more stable in the binding pocket of the target than the co-crystallized ligand except Rutin.

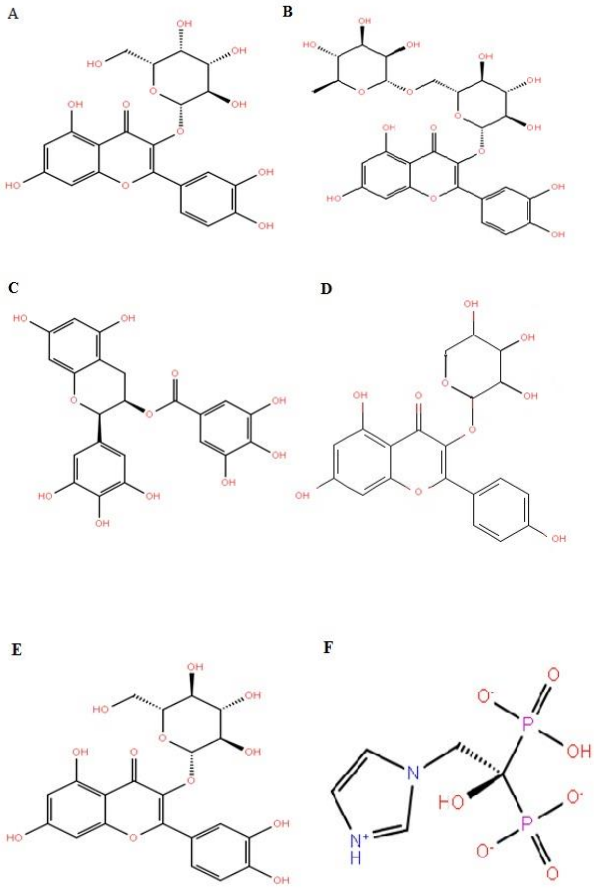

Figure 2. 2D structures of hit compounds and co-crystallized ligand. **A** – 2D structure of Hyperoside. **B**- 2D structure of Rutin, **C**- 2D structure of Epigallocatechin-3-gallate. **D**- 2D structure of Kaempferol-3-Arabinoside, **E**- 2D structure of Isoquercetin, **F**- 2D structure of Co-crystalized ligand.

Post-docking analysis of the docking experiment which includes the analysis of the binding pose and the interaction of hit compounds with the amino acid residues at the active site of 2F8C and the amino acid residues involved in the interaction are shown in 2D format in Figure 3.

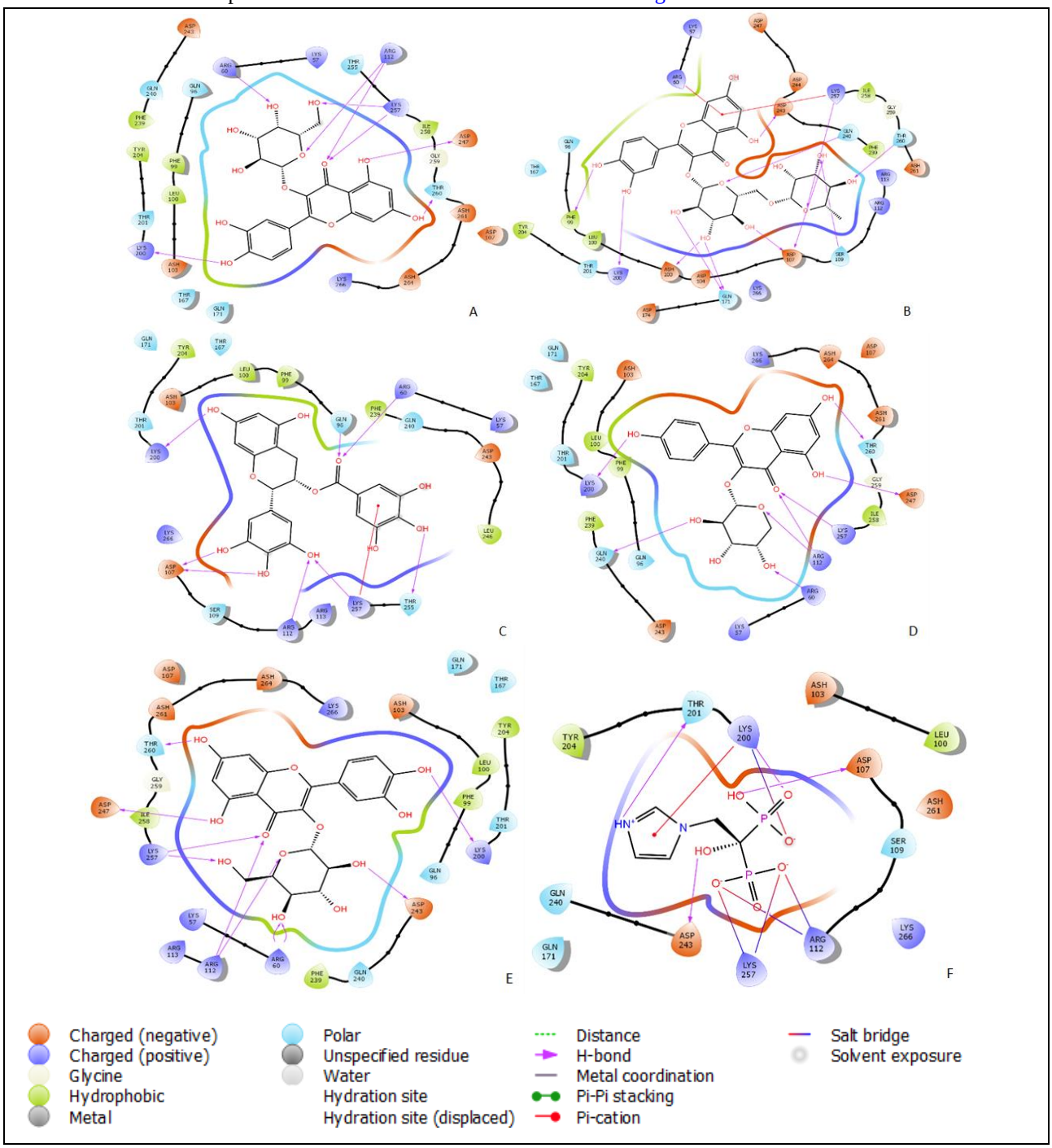

Figure 3. 2D interaction of hit compounds and the co-crystalized ligand with the amino acid residues at the active site of 2F8C expanded at 4 Å radius and amino acid residues involved in the interaction. **A-** 2D interaction of hyperoside with FPPS., **B-** 2D interaction of Rutin with FPPS, **C-** 2D interaction of epigallocatechin-3-gallate with FPPS, **D-** 2D interaction of kaempferol-3-arabinoside with FPPS, **E-** 2D interaction of isoquercetin with FPPS, **F-** 2D interaction of co-crystallized ligand with FPPS.

Protein-ligand interaction is critical in structural-based drug design. (Inyang *et al.*, 2017) Significant inhibition is largely determined by the combination of several amino acid residues placed within 4 hydrogen bonding distances in the enzyme's active region in a research project that involves FPPS antagonism (Olubode *et al.*, 2022). These amino acid residues play an important role in the synthesis of Protein-ligand complexes involving FPPS and the hit compounds (Olubode *et al.*, 2021).

The interactions which greatly contribute towards the inhibitory potentials of the hit compounds compared to the co-crystallized ligand are shown in Figures 3 and 4.

The 2d and 3d interaction of this work shows that: Hyperoside has hydrogen bond with ARG 60, ARG 112, LYS 257, ASP 247, THR 260 and LYS 200.

Rutin has hydrogen bond with ASP 243, LYS 257, GLN 240, THR 260, SER 109, ASP 107, GLN 171, ASH 103, LYS 200 and PHE 99, and also Pi- Cation with ARG 60 and LYS 25.

Epigallocatechin-3-gallate has hydrogen bond withLYS 200, GLN 96, ARG 60, ASP 107, ARG 112, LYS 257 and THR 255 and also Pi- Cation with LYS 257.

Kaempferol-3-arabinoside has hydrogen bond with LYS 200, THR 260, ASP 247, LYS 257, ARG 112, ARG 60, and GLN 240. Isoquercetin has hydrogen bond with THR 260, LYS 200, ASP 243, ARG 112, LYS 257, ASP 247 and ARG 60.

Co-crystallized ligand has hydrogen bond with THR 201, LYS 200, ASP 107 and ASP 243, and also Pi-Cation with LYS 200 and Salt Bridges with LYS 200, ARG 112and LYS 257.

ADME/Tox screening was employed to predict the lead compounds pharmacokinetic profile, drug-likeness and toxicity as shown in Table 1, 2 and 3.

Table 1. Physicochemical properties, bioavailability and drug-likeness.

Compound	mol MW	Consensus Log P	ESOL Log S	ESOL class	Bioavailability score	Rule of five
Hyperoside	464.382	-0.38	-3.04	Soluble	0.17	2
Rutin	610.524	-1.51	-3.3	Soluble	0.17	3
Epigallocatechin-3-gallate	458.378	0.95	-3.56	Soluble	0.17	2
Kaempferol-3-Arabinoside	418.356	0.35	-3.12	Soluble	0.55	1
Isoquercetin	464.382	-0.48	-3.04	Soluble	0.17	2
2F8C co crystallized ligand	272.091	-2.72	1.23	Highly soluble	0.11	0

Table 2. SwissADME pharmacokinetic profiles of the test compounds.

Compound	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Hyperoside	Low	No	No	No	No	No	No	No
Rutin	Low	No	Yes	No	No	No	No	No
Epigallocatechin-3-gallate	Low	No	No	No	No	No	No	No
Kaempferol-3-Arabinoside	Low	No	No	No	No	No	No	No
Isoquercetin	Low	No	No	No	No	No	No	No
2F8C co crystallized ligand	Low	No	No	No	No	No	No	No

Table 3. Pro-Tox II toxicity profile of test compounds.

compound	LD50	Toxicity class	Carcinogenicity	Hepatotoxicity
Hyperoside	5000 mg/kg	5	-	-
Rutin	5000 mg/kg	5	-	-
Epigallocatechin-3-gallate	1000 mg/kg	4	-	-
Kaempferol-3-arabinoside	5000 mg/kg	5	-	-
Isoquercetin	5000 mg/kg	5	-	-
2F8C co crystallized ligand	3500 mg/kg	5	-	-

- Inacctive + Active

Computer-aided ADME/Tox predictions have become extremely important in modern-day in silico drug design due to its extremely fast approach and very little cost attached to it (Akinnusi *et al.*, 2022). ADME/Tox screening technically predicts the absorption, distribution, metabolism, excretion and toxicity of compounds with small molecular weight (Kenakin, 2016). Lipophilicity and gastrointestinal absorption are integral physicochemical properties that contribute to the absorption of these compounds (Verma *et al.*, 2004). A drug candidate must exhibit a significant level of lipophilicity to enable it to cross the lumen of the small intestine (Adebesin, 2022). Water solubility aids the distribution of molecules to cells. In addition to lipophilicity, a drug candidate must also possess a good level of water solubility to enable it to move in the systemic circulation. The ADME screening guided by swissADME (Table 1) showed that epigallocatechin-3-gallate had the highest level of lipophilicity. Although, the predicted gastrointestinal (GI) absorption was low, nevertheless, it tends to cross the lumen of the intestine better than the other compounds. Similarly, all the hit compounds had better-predicted lipophilicity than the co-crystallized ligand. An inverse relationship was observed between the lipophilicity and water solubility of the test compounds.

The ESOL model of water solubility showed that the co-crystallized ligand which had the least level of lipophilicity exhibited the most robust water solubility. This implies that the movement of thecompound in systemic circulation could be slightly more progressive than the other compounds. However, water solubility alone does not confirm this assertion. Other bases like the rate of binding to plasma proteins must be taken into consideration.

The bioavailability score and drug-likeness of the compounds based on the Lipinski rule (Table 1) showed that only kaempferol-3-arabinoside had a positive bioavailability score (0.55). A bioavailability score of 0.55 shows that the compound passes (not completely) the Lipinski rule-based filter of drug-likeness and is considered drug-like. The co-crystallized ligand passes all the rules completely but turned out a negative bioavailability score. This makes kaempferol-3-Arabinoside the most drug-like out of all the tested compounds.

The swissAMDE pharmacokinetic predictions also showed that none of the compounds can permeate the Blood-Brain barrier. Furthermore, only rutin is predicted to be a substrate of P-glycoprotein. P-glycoprotein is a member of ATP binding cassette (ABC) proteins which actively participate in the efflux of molecules from the cell. This implies that rutin, being a substrate of P-glycoprotein would be prevented from bioaccumulating in cells and eventually rendered ineffective. The further analyses showed that none of the compounds could inhibit the selected Cytochrome P450 (CYP) isoforms and therefore, would not induce a drug-drug interaction. (CYP) is a family of enzymes that catalyze phase 1 metabolism of xenobiotics at large.

The toxicity predictions guided by Pro-tox II online server showed that none of the test compounds were carcinogenic and would not have a toxic effect on the liver.

4. Conclusion

Our study subjected phytoconstituents from *G. latifolium*leaf to *In silico* study and predicted five compounds; hyperoside, rutin, epigallocatechin-3-gallate, kaempferol-3-arabinoside, and isoquercetinbind robustly to FPPS compared with the co-crystalized ligand.The subjection of these hit compounds to ADME/Tox screening shows that the hit compounds except rutin are partially drug-like leaving kaempferol-3-arabinoside as the most drug-like compound compared to the co-crystallized compound. Thissuggests that these compounds might be better drug-like molecule and *G. latifolium*leafcould be a good plant source for a drug-like compound that may treat osteoporosis by inhibiting a target enzyme FPPS, in the mevalonate pathway, thereby stopping bone resorption. However, lead compound optimization and further *in vitro* and *in vitro* analysis are recommended to validate this experiment.

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