Original Research Article

Elucidating the Anti-inflammatory Properties of *Garcinia kola* and *Vernonia amygdalina* through *In Silico* Molecular Biology Techniques

Abstract

Introduction

- Inflammation is implicated in many disorders, including communicable and noncommunicable diseases. Cyclooxygenase-2 (Cox-2) is a key enzyme involved in the production of prostaglandins implicated in inflammatory disorders. *Garcinia kola* and *Vernonia amygdalina* are medicinal plants being used for treating various ailments in many parts of the world and extensive *in vitro* and *in vivo* studies have been conducted on them. Five phytochemicals were selected from the two plants; aspirin and celecoxib were used as reference drugs. This study investigated the interactions of the seven ligands with the Cox-2 enzyme, using *in silico* molecular biology techniques.

Materials and Method

- The 3-D structures of the seven ligands were retrieved from the PubChem database in their Structure Data Format (SDF). Cox-2 was retrieved in its Protein Data Bank (PDB) format. The ligands and the protein were converted to their pdbqt formats through the open babel software. The cox-2 was docked with the ligands using the Auto-Dock Vina software. The binding energies and the root mean square deviation values were noted. Pharmacophore modeling were visualized by using the Biovia Discovery Studio Visualizer. One of the ligands (luteolin) was further subjected to molecular dynamics simulation using the desmond maestro software.

Results

- While celecoxib had the best binding property with Cox-2 (-10.8 kcal/mol, 3 H bonds), the five ligands from the two plants had better binding properties than aspirin (which had -6.5kcal/mol, 1 H bond). Kolaviron, from *G. kola* (-9.1 kcal/mol, 3 H bonds) and luteolin, from *V. amygdalina* (-8.5kcal/mol, 2 H bonds) demonstrated the best binding properties among the five phytochemicals. Additional interactions of H bonds and hydrophobic bonds were noticed post molecular dynamics simulation of luteolin with Cox-2, indicating dynamic forces fluctuations. MD simulations showed that Ser530 and Tyr385 are the best amino acid side chains that interacted with luteolin for the stabilization of the protein-ligand complex.

Conclusion

- The energy values and protein-ligand interactions indicate affinity and stability of complex. This can be taken as a promising drug target and subjected to ADMET

(absorption, distribution, metabolism, excretion, toxicity) properties analysis and clinical trials. This is especially important in view of the various side effects of using aspirin and the selective Cox-2 inhibitors, including celecoxib.

Keywords: In silico, Vernonia amygdalina, Garcinia kola, Kolaviron, Luteolin, Docking, Molecular Dynamics, Simulations

INTRODUCTION

Medicinal plants have been used for centuries to treat various diseases (Liew & Yong, 2016; Morebise, 2015). Research reports have also backed the folk use of many of these plants for disease treatment or management (Morebise & Fafunso, 1998; Morebise et.al, 2001, 2002, 2006; Olajide et.al, 2000). Moreover, notable drugs discovered from medicinal plants are currently in use for disease treatment or management (Keglevich et.al, 2012). Among the medicinal plants popularly used for various ailments in different communities are *Garcinia kola* (Bitter kola) and *Vernonia amygdalina* (Bitter leaf).

Garcinia kola Heckel (Guttiferae) is a flowering plant that is found in the forest areas of West and Central Africa. Its seeds, known as bitter kolas, are popular in Africa for traditional religious and medicinal uses (Olaleye et.al, 2000; Onasanwo & Rotu, 2016). Reports indicate that Garcinia kola seeds are used for the ethnomedical treatments of various disorders, including diabetes mellitus, ulcers, cancers and hypertension (Dogara et.al, 2022; Onasanwo & Rotu, 2016). Laboratory studies have also confirmed the anti-inflammatory, antinociceptive, antidiabetic, anticancer, antihypertensive, antihyperlipidemic, antioxidant and antimicrobial properties of the seeds of this plant (Dogara et.al, 2022; Olaleye et.al, 2000; Onasanwo & Rotu, 2016).

Vernonia amygdalina Del (Asteraceae) is a small shrub that has been domesticated in many parts of West Africa where it is commonly known as bitter leaf because of the bitter taste of concoctions or decoctions made from its leaves (Igile et.al, 1994; Oyeyemi et.al, 2018). The leaves are also used for soups where they add bitter-sweet flavours (Igile et.al, 1994; Farombi & Owoeye, 2011). There have been reports of ethnomedical use of leaves, bark and roots of this plant to treat various diseases, including diabetes mellitus, cancers, ulcers, infectious disorders, stomach pains, among others (Igile et.al, 1994; Farombi and Owoeye, 2011). Laboratory studies also conformed the antidiabetic, anticancer, anti-inflammatory, antinoniceptive, antioxidant and antimicrobial properties of extracts of Vernonia amygdalina leaves (Igile et.al, 1994; Farombi & Owoeye, 2011; Opeyemi et.al, 2017; Joseph et.al, 2021).

Inflammation has been the bedrock of many health disorders, including communicable and noncommunicable diseases (Furman et.al, 2019). Among the mediators of inflammation, the prostaglandins play chief roles. The prostaglandins that are implicated in inflammation are produced by the cyclooxygenase-2 (Cox-2) enzyme (Park & Christman, 2006). Over the years, aspirin and other nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) have been used to treat inflammatory disorders. However, this comes with a price: synthesis of prostaglandins that are responsible for house-keeping jobs, like those responsible for maintaining the stomach

integrity, is inhibited, leading to side effects such as gastric erosion and ulcers (Kwok & Loke, 2010). Along the line selective Cox-2 inhibitors were produced as drugs for the purpose of targeting Cox-2 enzyme thereby diminishing the side effects of aspirins and the other nonselective NSAIDs. However, this also comes with more serious side effects, including cardiovascular disorders and deaths (Attiq et.al, 2018). This has made attention to be shifted to natural products that can cure or manage inflammatory disorders without the resulting serious side effects; hence the studies on drug discovery from natural products (Attiq et.al, 2018; Deng et.al, 2022).

Computer-aided drug discovery studies have gained ground and have been found very useful in supporting and complementing studies from wet labs (Vignani et.al, 2019). In silico molecular biology techniques have made drug discovery and design more rapid by revealing hidden information about structural and functional knowledge of nucleic acids, proteins and potential drug targets (Bayat, 2002; Behera et.al, 2021a, b; Schneider et.al, 2014).

This study employed computer-aided drug discovery techniques to investigate the inhibitory effects of selected ligands from *Garcinia kola* and *Vernonia amygdalina* on the Cox-2 enzyme.

MATERIALS AND METHOD

Kolaviron, beta-amyrin and elaidic acid, from *G. kola* and luteolin and isoflavone glycoside, from V. amygdalina were used for this study, using standard in silico molecular biology techniques. Aspirin (a nonselective NSAID) and celecoxib (a selective Cox-2 NSAID) were used as reference ligands. The 3-D structures of the seven ligands were retrieved from the National Center for Biotechnology Information (NCBI) PubChem database in their Structure Data Format (SDF) (https://pubchem.ncbi.nlm.nih.gov/). Cox-2 was retrieved in its Protein Data Bank (PDB) format from the NCBI GenBank (https://www.ncbi.nlm.nih.gov/). Cox-2 was cleaned by removal of all nonstandard amino acids and water through the discovery studio (BIOVIA, San Diego, CA, USA). The ligands and protein were converted to their pdbqt formats through the Open Babel software (https://github.com/openbabel/openbabel). The Cox-2 was docked with the ligands using the Auto-Dock Vina software (https://vina.scripps.edu/). The binding energies and the root mean square deviation values were noted. Pharmacophore modeling were visualized by using the Biovia Discovery Studio Visualizer ((BIOVIA, San Diego, CA, USA). Molecular dynamics simulations were conducted using the desmond maestro software (https://www.schrodinger.com/products/desmond). Simulations were run for both 50ns and 100ns.

RESULTS AND DISCUSSIONS

Molecular Docking and Discovery Studio Visualization

Table 1 shows the seven ligands with their most negative docked binding energies and least root mean square root deviation (RMSD).

Table 1. ***Ligands with their PubChem CIDs, Binding Energies and RMSD***

S/N	LIGAND	PUBCHEM CID	BINDING ENERGY (KCAL/MOL)	RMSD (Å)
1	Luteolin	5280445	-8.5	0.000
2	Isoflavone glycoside	121596018	-7.9	0.000
3	Kolaviron	155169	-9.1	0.000
4	Beta-Amyrin	73145	-8.4	0.000
5	Elaidic acid	637517	-6.8	0.000
6	Aspirin	2244	-6.5	0.000
7	Celecoxib	2662	-10.8	0.000

From Table 1 above, it can be seen that the five ligands from the two plants exhibited higher negative binding energies than the reference drug, aspirin though the other reference drug, celecoxib, exhibited the best binding energy of all. The higher the negative values of binding energy and the lower the RMSD, the better is the docking (Behera et.al, 2021a).

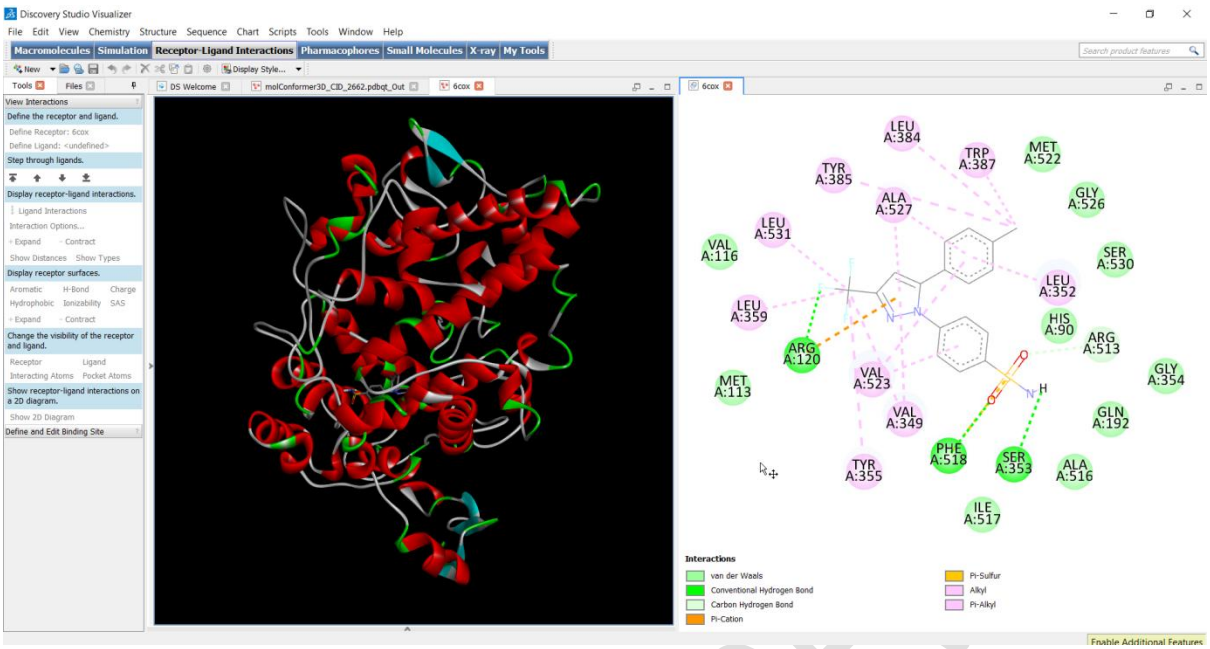
Table 2 gives the summary of the interactions between the ligands and amino acid residues of Cox-2, as obtained from the discovery studio visualization after docking.

Table 2. ***Docking Interactions of Cox-2 Amino Acid Residues with Ligands***

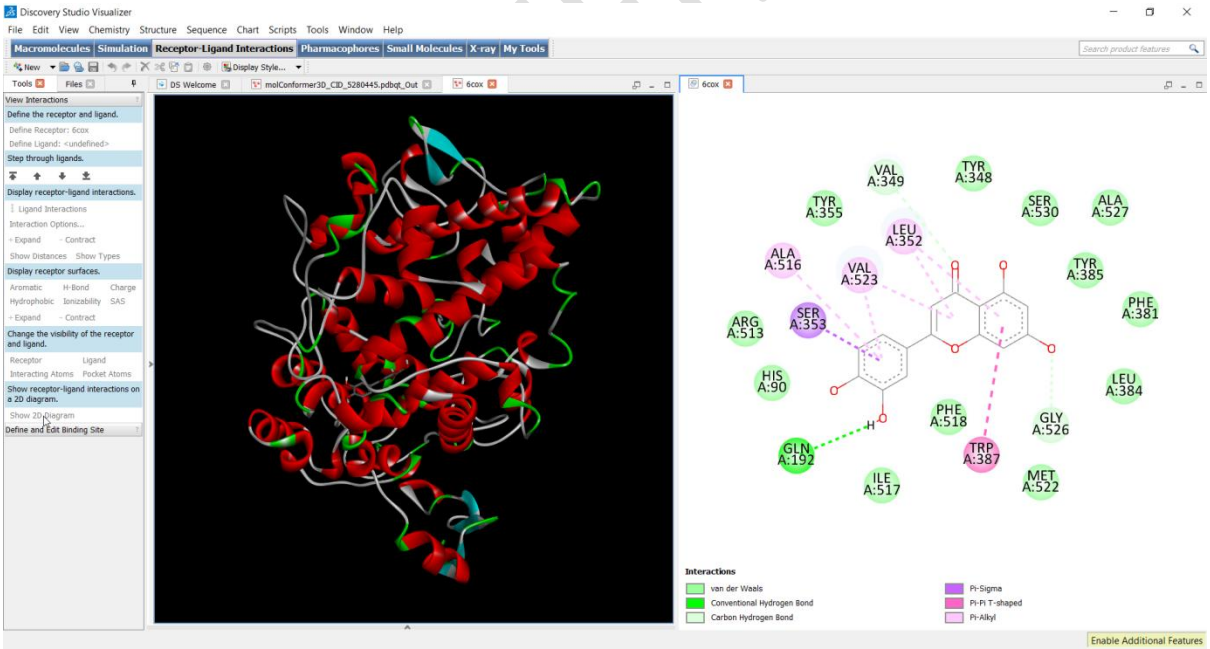
S/N	**LIGAND**	**CID NUMBER**	**DOCKING INTERACTIONS WITH COX-2 AMINO ACID RESIDUES**
1	Luteolin	5280445	H bond with Gln A192, H bond with Ser A530, Pi-Sigma with Ser 353, Pi-Sigma with Val A523, Pi-Sigma with Leu A352 Pi-Pi T shaped with Trp A387 Pi-Alkyl with Ala516

2	Isoflavone glycoside	121596018	H bonds with Asn A581, Covalent bond with Val A582
3	Kolaviron	155169	H bonds with Val A291, His 386, Thr A212
4	Beta-Amyrin	73145	Van der Waals with various amino acids
5	Elaidic acid	637517	H bond with Gln A192 Alkyl/Pi-Alkyl bonds with Val A349, Leu A531, Ala A527, Tyr A385, Tyr A387, Phe A518, Leu A352, Val A523, Tyr A355
6	Aspirin	2244	H bonds with Ser A530 Pi-Sgma with Leu A352 Pi-Alkl with Val A523 & Val A349
7	Celecoxib	2662	H bonds with His A90, Ser A353 & Arg A120 Pi Cation with Arg A120 Alkyl/Pi-Alkyl with Tyr A355, Val A349, Leu A531, Leu A359, Leu A352, Ala A527, Tyr A385, Leu A384 &Tyr A387. Pi-Sulfur with Phe A518.

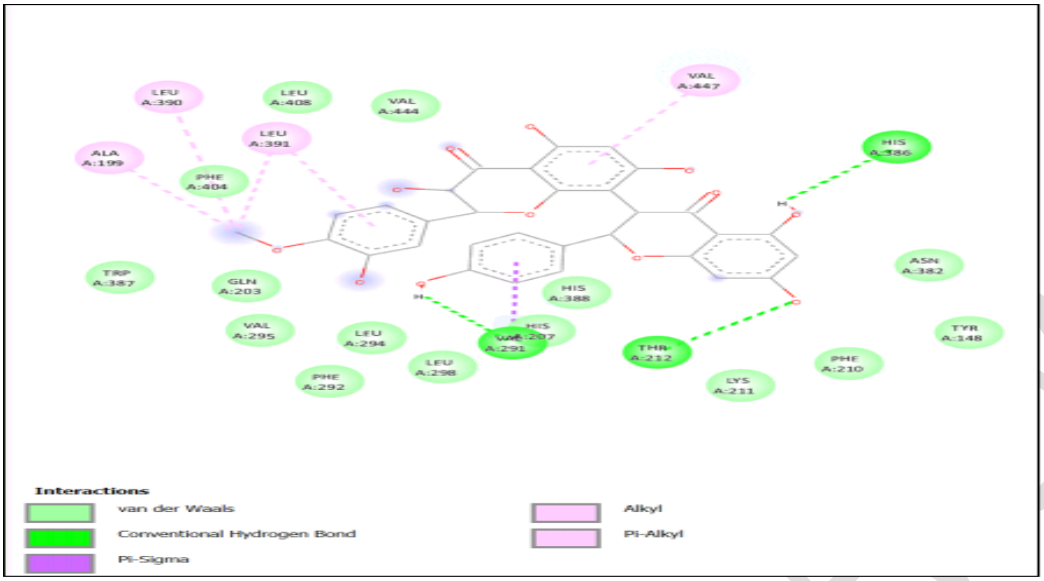
Celecoxib (reference ligand), luteolin (from bitterleaf) and kolaviron (from bitter kola) exhibited good docking properties (Tables 1 and 2). Figure 1 below shows the pharmacophore modeling for celecoxib, luteolin and kolaviron when docked with the Cox-2 enzyme.



CID2662 Celecoxib Docked with Cox-2



CID 5280445 Luteolin Docked with Cox-2



CID155169 Kolaviron, Docked with Cox-2

Figure 1: Pharmacophore Modeling of Cox-2 Docked with Celecoxib, Luteolin and Kolaviron, respectively

In this study, luteolin was further subjected to the molecular dynamics simulation.

Figure 2 shows the primary structure of the protein (Cox-2) prior to simulation.

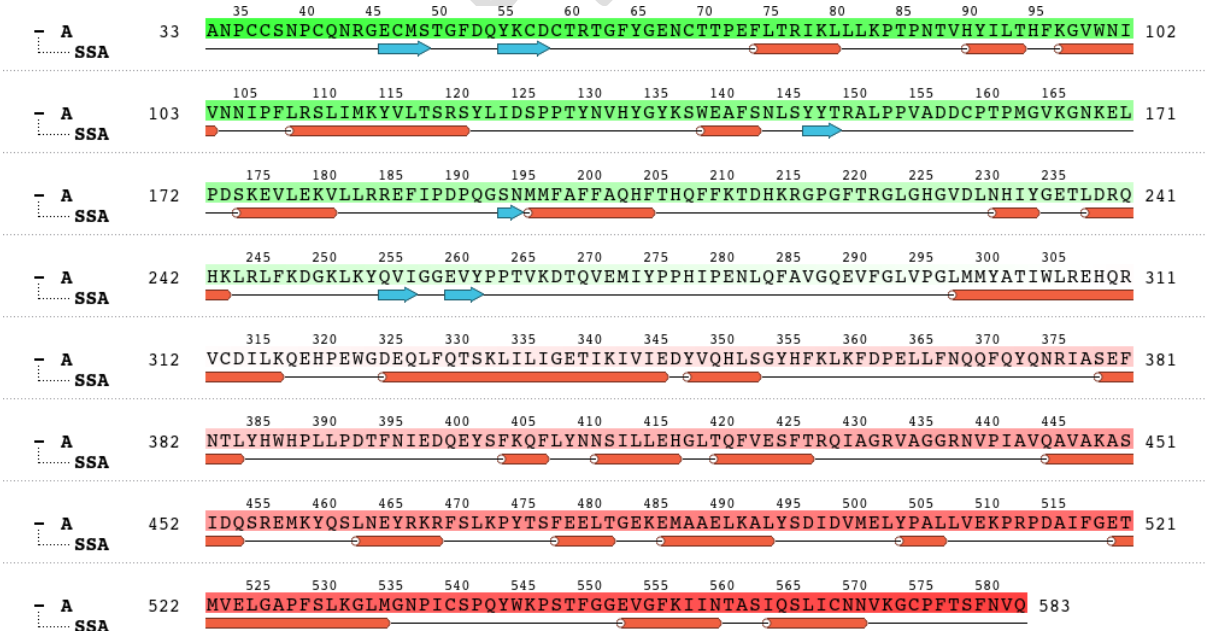


Figure 2: Primary Structure of Cox-2 Prior to MD Simulation

Figure 3 shows the protein-ligand contact post MD simulation while Figure 4 shows the MD simulation result at 50ns.

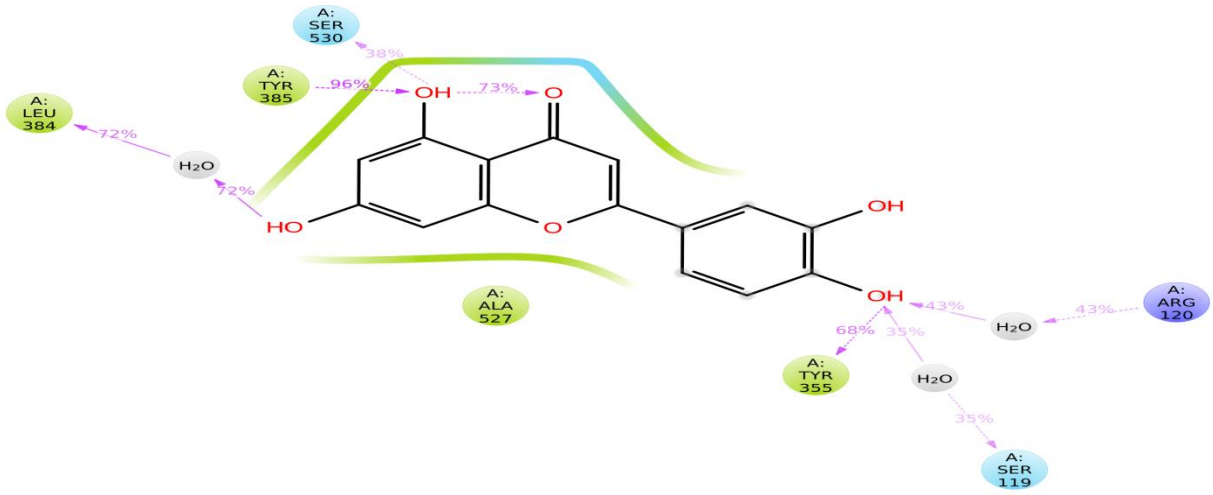


Figure 3: Cox-2 -Luteolin Contact Post MD Simulation

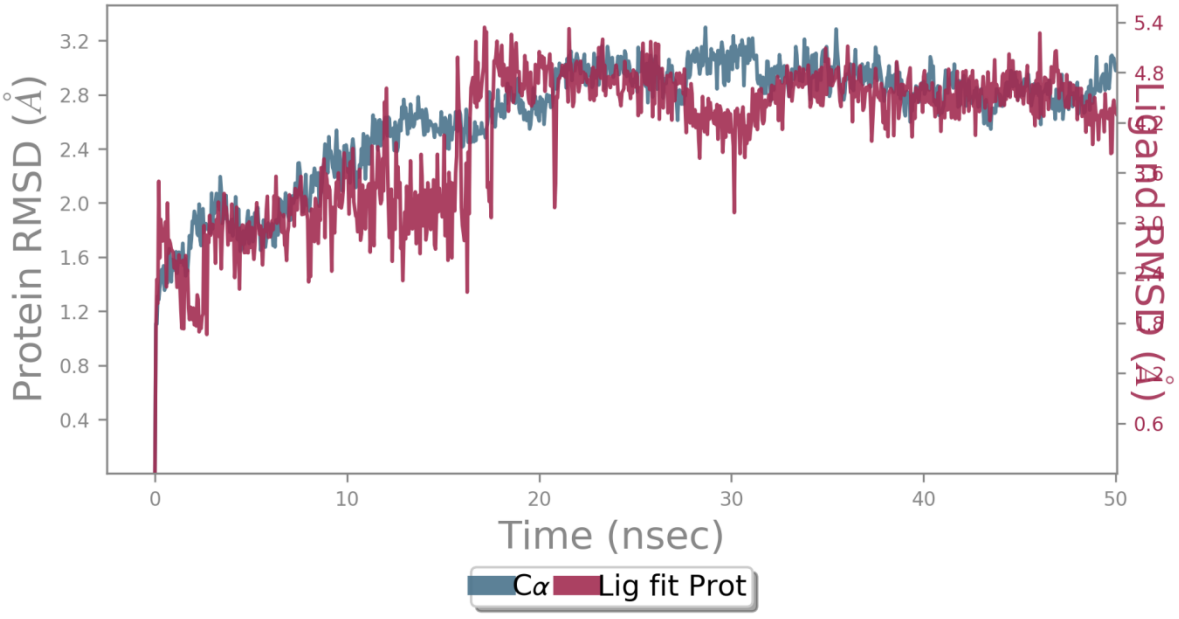


Figure 4: MD Simulation Result at 50 ns

Protein-Ligand RMSD Fluctuations

As indicated in the Figure above, when the MD simulation was studied for 50 ns, intactness of protein was conserved, with maximum fluctuations from 1.4 Å to 2.4 Å for 0 to 10 ns, 2.4 Å to 2.8 Å for 10-20ns, 3.2 Å for 20-30ns, 3.2 Å -3.0 Å for 30-40ns, and 3.0 Å-3.2 Å for 40-50ns. Likewise, the ligand exhibited a similar mode of conformational changes: it is 3.6 Å at 0 ns, it is 1.8 Å at 3ns, and from 3ns to 10 ns it is 2.4 Å to 3.6 Å. From 10ns to 20ns it is 3 Å. It reached 5 Å from 20 to 30ns and dropped till it reached 4 Å from 30 to 40ns. From 40-50ns it was in the range of 4.2 Å to 4.8 Å, with maximum reaching of 5 Å at 45 ns (Figure 4).

Figure 5 shows the MD simulation result when the simulation was run for 100 ns.

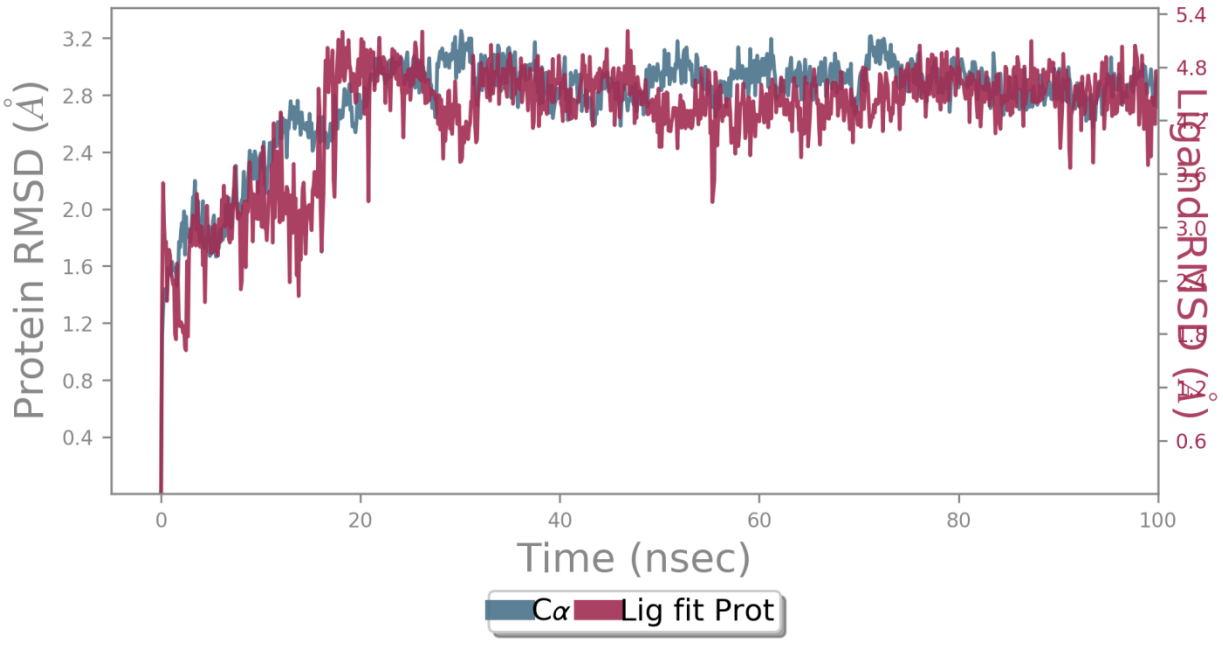


Figure 5: MD Simulation at 100 ns

Figure 5 above indicates that when the simulation was run for 100 ns, the protein (Cox-2) exhibited RMSD of 2.2-2.4 Å at 0-10 ns. However, from 10-20ns, it exhibited 2.4-2.8 Å. At 20-30ns, it exhibited 3.2 Å; at 30-40ns, it exhibited 3.2-3.0 Å. At 40-50ns, it exhibited 3.0-3.2 Å. From 50-100ns it exhibited 3.0-3.2 Å. Likewise, the ligand (luteolin) also showed RMSD fluctuations: it is 3.6 Å at 0-10ns; from 3ns to 10ns it is 2.4-3.6 Å. From 10-20ns it is 3.6-2.4 Å. From 18-20ns it reached 5 Å and from 20-30ns it dropped till it reached 4 Å. From 30-40ns, and from 40-50ns, it is in the range of 4.2 to 4.8 Å. From 50ns to 100ns, it is in the range of 3.6-4.8 Å (Figure 5).

The ligand is nicely associated with protein throughout with exceptional diffusion at 13-18nsn, 27-31 ns and 45-55 ns. At the end, the association between protein and ligand was great (Figure 5).

Figure 6 illustrates the protein-ligand contact after the MD simulation for a period of 100 ns.

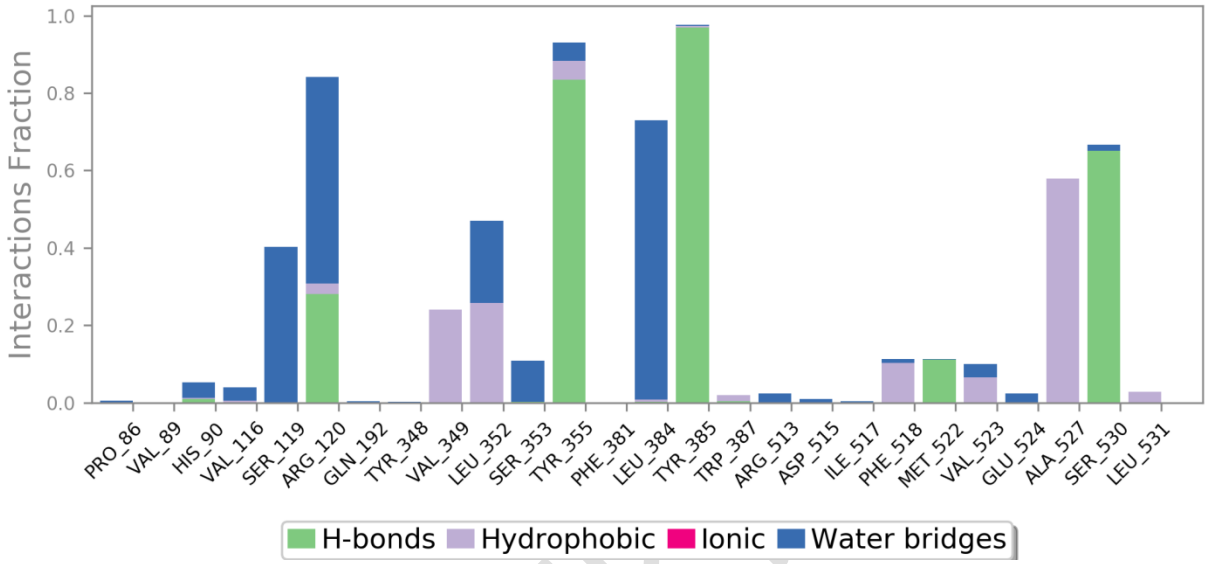


Figure 6: Protein-Ligand Contacts at Simulation (100 ns)

As indicated in Figure 6 above, Arg120, Tyr355, Trp387, Met522 and Ser530 residues of Cox-2 protein exhibited hydrogen bonds with luteolin after molecular dynamics simulations at 100 ns. Likewise, Arg120, Val349, Leu352, Tyr355, Leu354, Trp387, Phe128, Val523, Ala527 and Leu531 of the Cox-2 enzyme exhibited hydrophobic interactions with luteolin post-simulation. There were water bridges interactions with Pro86, Val116, Arg120, Gln192, Tyr348, Leu352, Ser353, Tyr355, Leu384, Arg513, Glu524 and Ser530 residues of the protein with the ligand (Figure 6).

The overall protein, ligand and complex dynamicity and conformational stability suggest that the interaction of the ligand (luteolin) with the Cox-2 binding site region is highly preferable for the desired activity.

The anti-inflammatory, analgesic, anticancer, antioxidant and neuroprotective properties of luteolin have been reported by several authors (Gupta et.al, 2018; Lin et.al, 2008; Ntalouka & Tsirivakou, 2023). Inhibition of the cyclooxygenase-2 enzyme could play a significant role in eliciting these properties.

Conclusion

In view of the serious side effects associated with both the nonselective NSAIDs and the selective Cox-2 inhibitors, there is the need to shift attention to alternative anti-inflammatory

agents with minimal or no side effects. This study has validated the medicinal uses of *G. kola* and *V. amydalina* in different communities. Consumption of these plants should therefore be encouraged. Ligands from the two medicinal plants also exhibited good docking properties with the Cox-2 enzyme and could be responsible for the anti-inflammatory properties of these plants. Our team is in the process of conducting molecular dynamics simulation for the binding of Cox-2 with kolaviron.

Luteolin exhibited excellent docking and molecular dynamics simulation properties and is hereby recommended for ADMET: (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties analysis and clinical trials. A positive outcome of the ADMET studies would make luteolin a potential drug which could serve as a better alternative to the current anti-inflammatory drugs that pose attendant serious side effects.

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