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 was 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 were 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 the 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 associated with both nonselective and selective Cox-2 inhibitors, including aspirin and 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 (1, 2). Research reports have also backed the folk use of many of these plants for disease treatment and management (3-7). Moreover, notable drugs discovered from medicinal plants are currently in use for disease treatment or management (8). 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 (9, 10). Reports indicate that Garcinia kola seeds are used for the ethnomedical treatment of various disorders, including diabetes mellitus, ulcers, cancers, and hypertension (10, 11). Laboratory studies have also confirmed the anti-inflammatory, antinociceptive, antidiabetic, anticancer, antihypertensive, antihyperlipidemic, antioxidant and antimicrobial properties of the seeds of this plant (9-11).

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 (12, 13). The leaves are also used for soups where they add bitter-sweet flavours (12, 14). 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 (12, 14)). Laboratory studies also confirmed the antidiabetic, anticancer, anti-inflammatory, antinoniceptive, antioxidant and antimicrobial properties of extracts of *Vernonia amygdalina* leaves (12, 14, 15).

Inflammation has been the bedrock of many health disorders, including communicable and noncommunicable diseases (16). 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 (17). 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 (18). 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 (19). 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 (19, 20).

Computer-aided drug discovery studies have gained ground and have been found very useful in supporting and complementing studies from wet labs (21). *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 (22-25).

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 (23).

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.

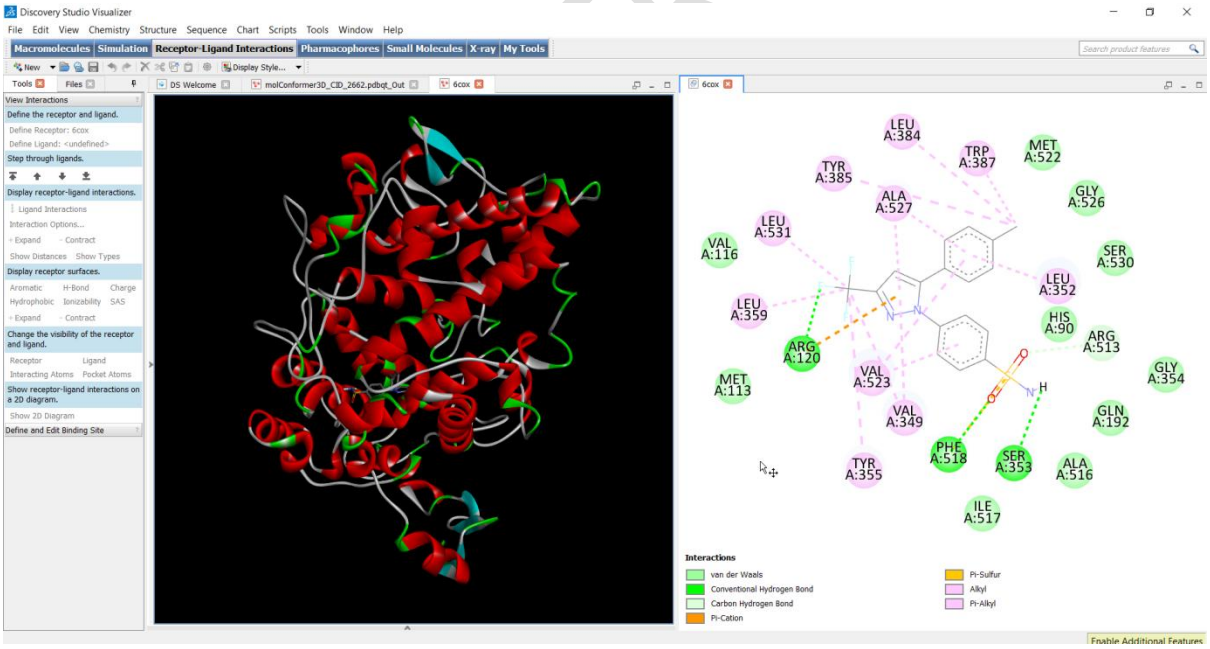
Table 2. ***Docking Interactions of Cox-2 Amino Acid Residues with Ligands***

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

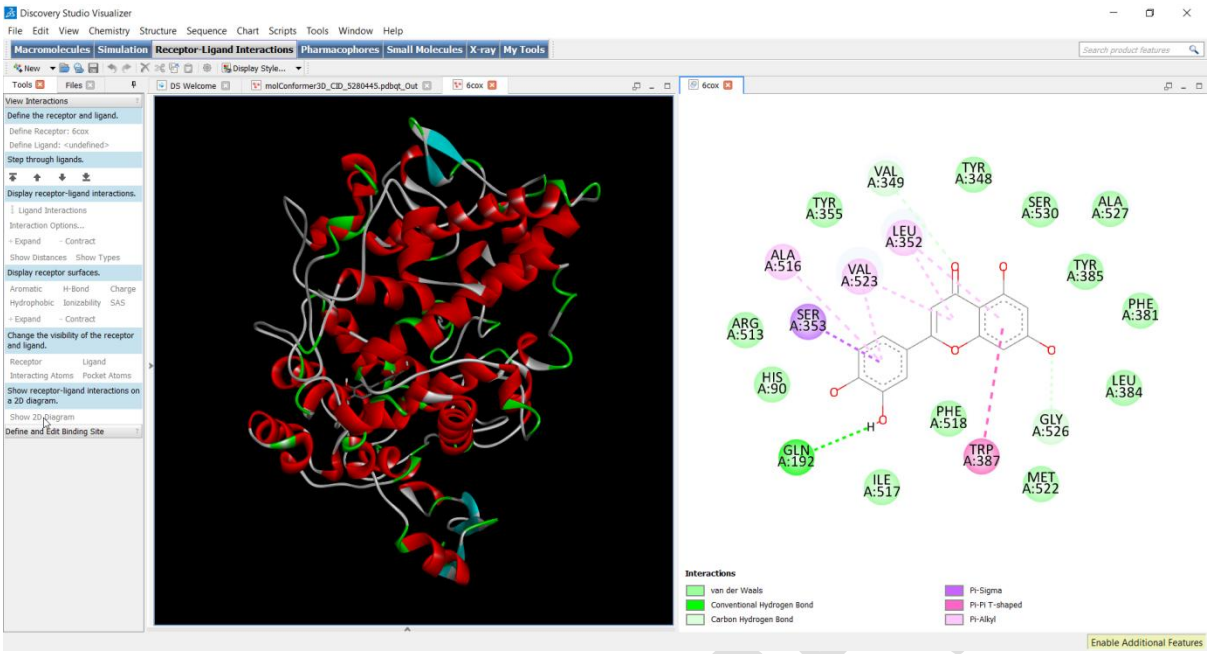
			Pi-Sgma with Leu A352 Pi-Alkl with Val A523 & Val A349
7	Celecoxib	2662	H bonds with His A90, Ser A353 & Arg A120 Pi Cation with Arg A120 Alkyl/Pi-Alkyl with Tyr A355, Val A349, Leu A531, Leu A359, Leu A352, Ala A527, Tyr A385, Leu A384 &Tyr A387. Pi-Sulfur with Phe A518.

Celecoxib (reference ligand), luteolin (from bitterleaf) and kolaviron (from bitter kola) exhibited good docking properties (Tables 1 and 2). This is an indication of anti-inflammatory property of kolaviron and luteolin, as molecular docking of herbal compounds against COX-2 has been used to detect their anti-inflammatory property (26).

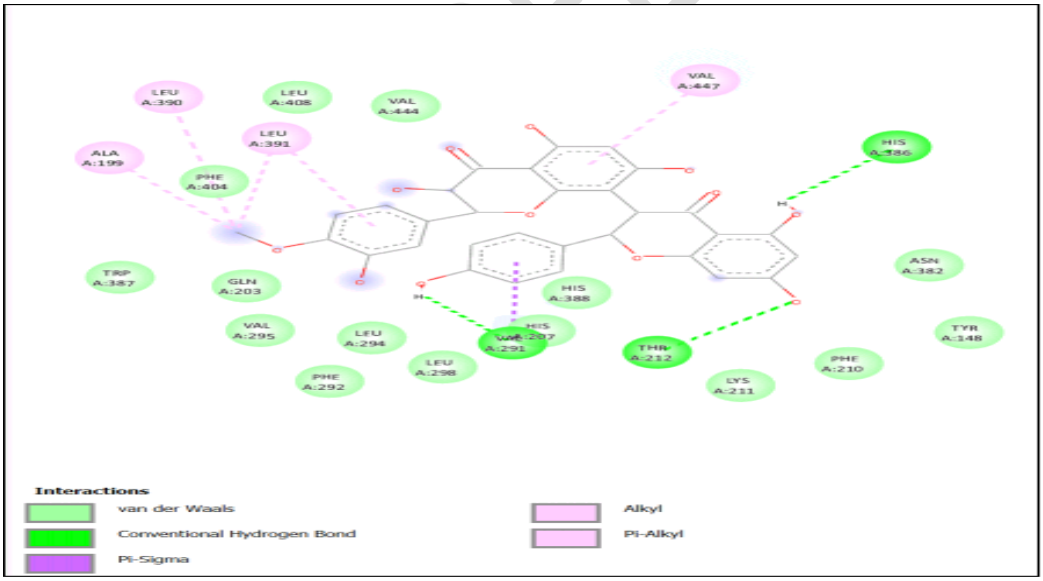
Figure 1 below shows the pharmacophore modeling for celecoxib, luteolin and kolaviron when docked with the COX-2 enzyme.



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

Molecular Dynamics Simulation of Luteolin with COX-2

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.

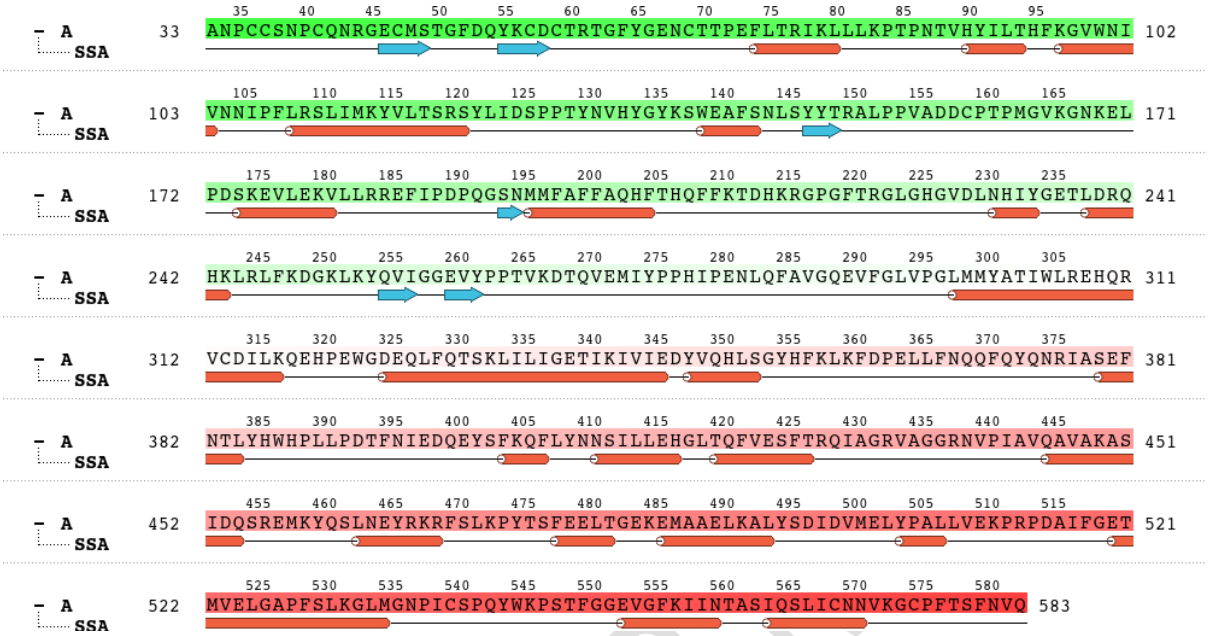


Figure 2: Primary Structure of COX-2 Prior to MD Simulation

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

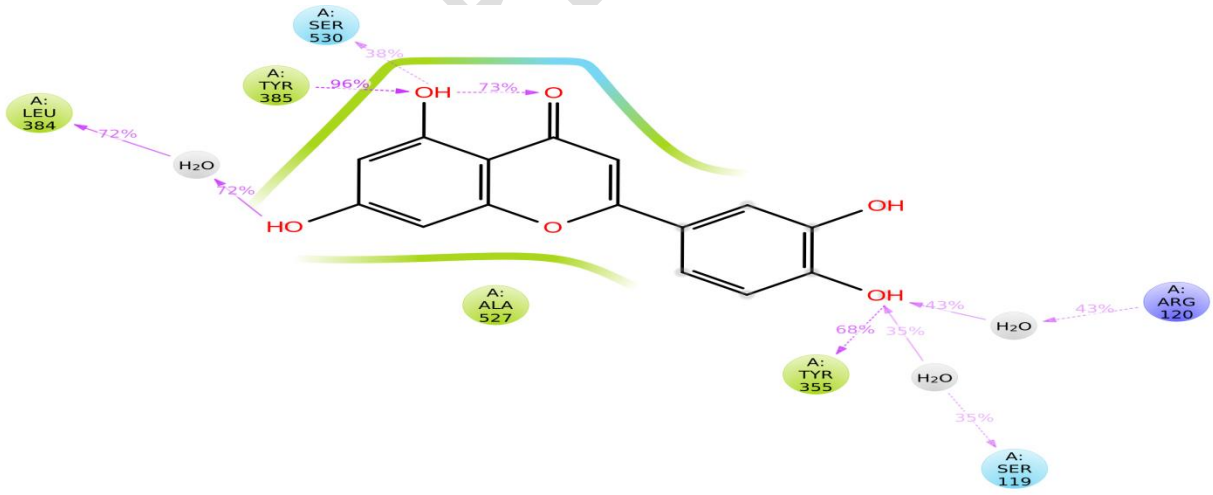


Figure 3: Cox-2 -Luteolin Contact Post MD Simulation

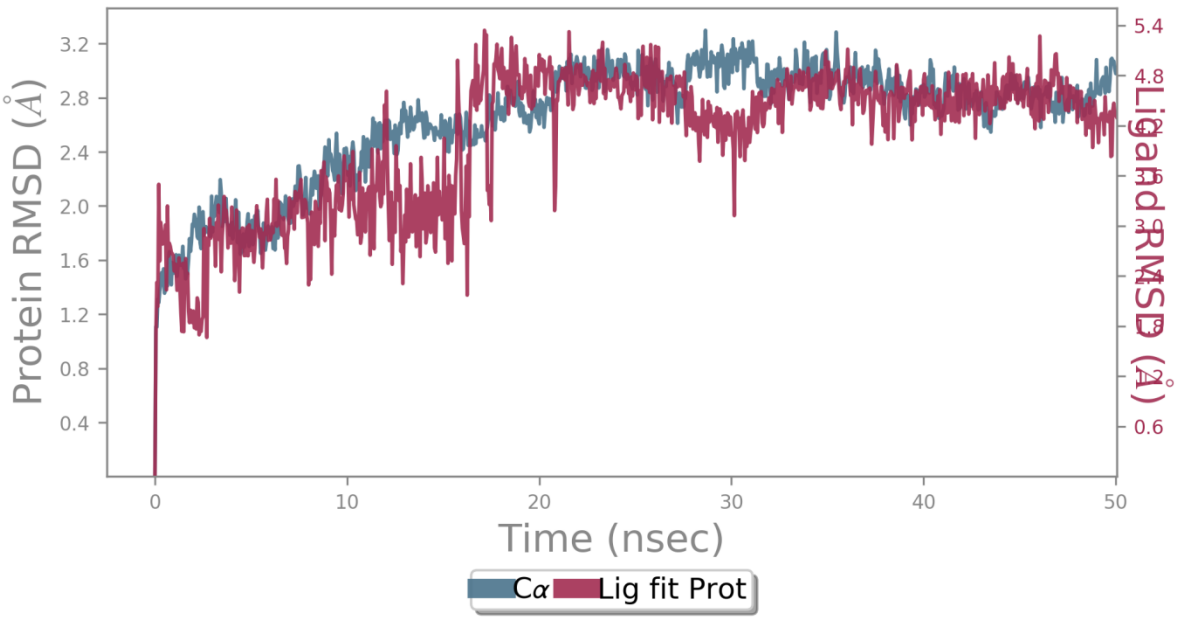


Figure 4: MD Simulation Result at 50 ns

Protein-Ligand RMSD Fluctuations re Simulation

As indicated in Figure 4 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 0ns to 10 ns, 2.4 Å to 2.8 Å for 10ns to 20ns, 3.2 Å for 20-30ns, 3.2 Å to 3.0 Å for 30-40ns, and 3.0 Å to 3.2 Å for 40ns-50ns. Likewise, the ligand exhibited a similar mode of conformational changes: it was 3.6 Å at 0 ns; it was 1.8 Å at 3ns, and from 3ns to10 ns it was 2.4 Å to 3.6 Å. From 10ns to 20ns it was 3 Å. It reached 5 Å from 20ns to 30ns and dropped till it reached 4 Å from 30 to 40ns. From 40ns to 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.

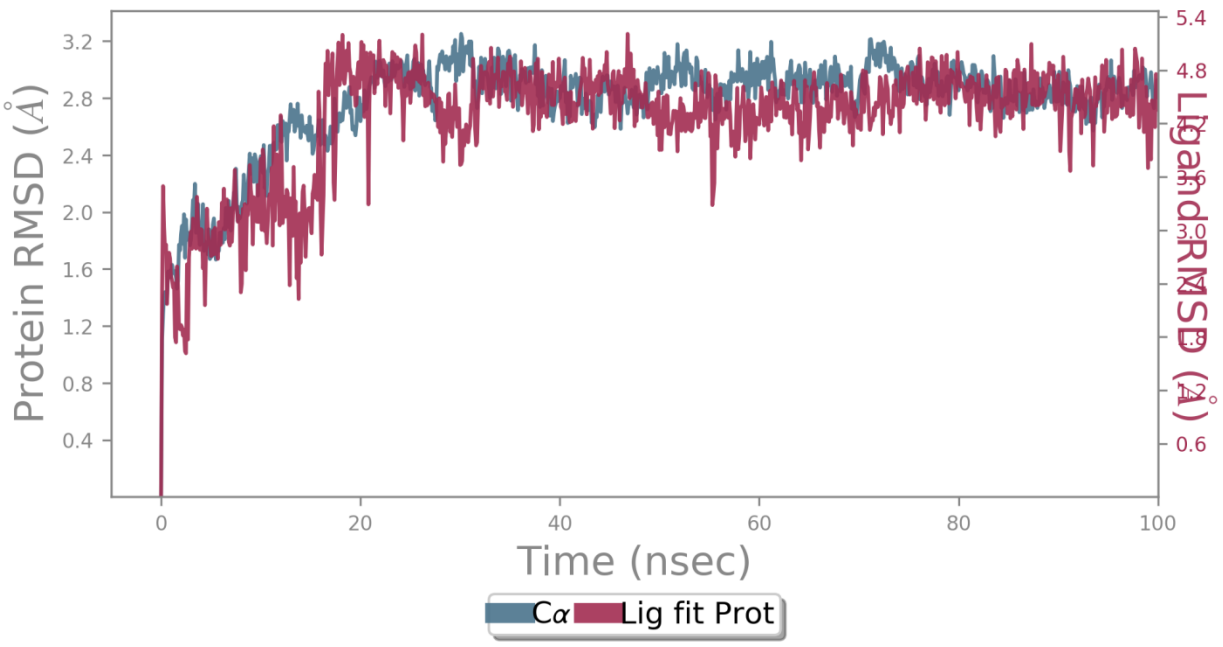


Figure 5: MD Simulation at 100 ns

Figure 5 above indicates that when the simulation was run for 100 ns, the protein (Cox-2) exhibited RMSD of 2.2 to 2.4 Å at 0 to10 ns. However, from 10-20ns, it exhibited 2.4 Å to 2.8 Å. At 20ns to 30ns, it exhibited 3.2 Å; at 30ns to 40ns, it exhibited 3.2 Å to 3.0 Å. At 40ns to 50ns, it exhibited 3.0 Å to 3.2 Å. From 50ns to 100ns, it exhibited 3.0 Å to 3.2 Å. Likewise, the ligand (luteolin) also showed RMSD fluctuations: it was 3.6 Å at 0s to10ns; 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 30ns to 40ns, and from 40-50ns, it was in the range of 4.2 Å to 4.8 Å. From 50ns to 100ns, it was in the range of 3.6 Å to 4.8 Å (Figure 5).

The ligand was nicely associated with protein throughout with exceptional diffusion at 13ns to 18ns, 27ns to 31 ns, and 45ns to 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.

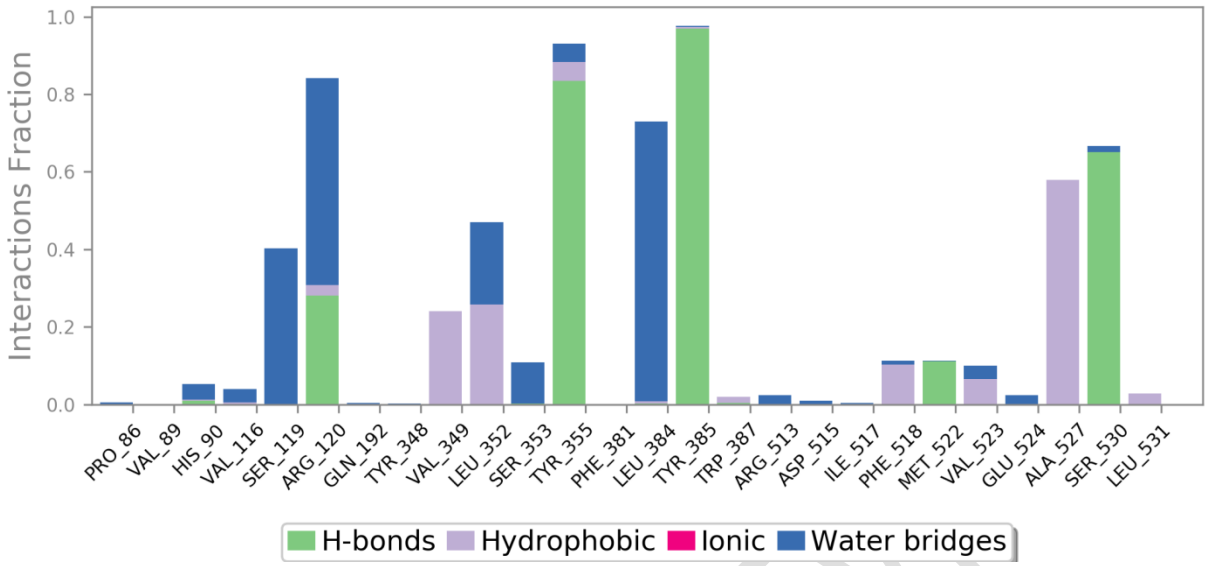


Figure 6: Protein-Ligand Contacts at Simulation (100 ns)

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.

Kolaviron and Luteolin as Potential Anti-inflammatory Drugs

Several studies on the anti-inflammatory, analgesic, and anti-nociceptive actions of kolaviron have been reported and several mechanisms of action have been proposed. Ayepola *et.al* (27) suggested that the anti-inflammatory effects of kolaviron could be due to its suppression of macrophage infiltration and/ or by inhibiting NF-κB activation. Onasanwo and Rotu (10) suggested actions through the opioidergic and adrenergic receptor pathways.

Farombi et.al (28) had earlier reported the inhibitory effect of kolaviron on COX-2, through their wet lab studies. Our present *in silico* study has supported this earlier report and has provided details of molecular interactions between kolaviron and the cyclooxygenase-2 enzyme. In their study which involved using molecular docking to determine the neuroprotective effects of kolaviron against the Alzheimer's disease, Adewole *et.al* (29) reported that kolaviron destabilized the assembled Aβ fibrils, making kolaviron a potential anti-amyloidogenic drug. Thus, kolaviron could exercise its many beneficial actions (anti-inflammatory, antioxidative,

anti- amyloidogenic, anticancer properties) through a combination of molecular interactions in the human body. Our present study appears to be the first docking study on the interaction between kolaviron and COX-2.

The anti-inflammatory, analgesic, anticancer, antioxidant and neuroprotective properties of luteolin have also been reported by several authors (30-32). Our docking results compared well with those of D'Mello *et.al* (33) who obtained the binding energy of -8.7504 kcal/mol for luteolin against the COX-2 enzyme (our study indicated -8.50 kcal/mol). Several mechanisms have been proposed for the anticancer effects of luteolin; one of such is the inhibition of COX-2, an enzyme implicated in carcinogenesis (33, 34). The docking and simulation results of this present study indicate that luteolin inhibits COX-2. Inhibition of the cyclooxygenase-2 enzyme could therefore be a significant way by which luteolin exerts its anti-inflammatory and anticancer properties.

A major limitation of molecular docking is the inability to say with 100% certainty that the binding energies and molecular interactions are accurate; this is mostly due to the intermolecular interactions which may not be predicted accurately (35). That is why the best binding energies (most negative) and least root mean square deviations (rmsd) for each protein-ligand dock are chosen in docking studies, and this was done in our study. Besides, molecular dynamics simulations are usually performed to confirm dock complex stability, and this was done for luteolin in our study. Another limitation of docking is that the interaction of ligands with off-target proteins are not displayed through docking, and these could only be detected in the wet labs with human or animal trials (35). Finally, molecular docking results need to complement wet lab results to gain more confidence about the authenticity of results (21, 35). Nonetheless, molecular docking has proved to be very effective and advantageous in drug discovery process (23, 24, 35).

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 (luteolin and kolaviron) 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.

References

1. Liew, P.M., & Yong, Y.K. (2016). Stachytarpheta jamaicensis (L.) Vahl: From Traditional Usage to Pharmacological Evidence. *Evidence-Based Complementary and Alternative Medicine, 2016*, 1-7. http://dx.doi.org/10.1155/2016/7842340
2. Morebise, O. (2015). A review on *Gongronema latifolium*, an extremely useful plantwith great prospects. *European Journal of Medicinal Plants*.10 (1): 1-9.
3. Morebise, O. & Fafunso, M.A. (1998): Antimicrobial and Phytotoxic Activities of theSaponin Extracts from Two Edible Medicinal Plants. *Biokemistri* 8 (2): 69-77.
4. Morebise, O., Awe, E.O., Makinde, J.M., & Olajide, O. A. (2001): Evaluation of the Anti- inflammatory and Analgesic Properties of *Chasmanthera dependens* Leaf Methanol Extract.*Fitoterapia* 72: 497-502.
5. Morebise, O., Fafunso, J.M. Makinde, O.A. Olajide and E.O. Awe (2002): Anti-inflammatory Property of the Leaves of *Gongronema latifolium*. *Phytotherapy Research* 16:75-77.
6. Morebise, O., Fafunso, M.A., Makinde, J.M., & Olajide, O.A. (2006): Evaluation of theBioactivity of *Gongronema latifolium* Leaf Extract in Rodents. Science Focus 11 (1): 27-30.
7. Olajide, O.A., Awe, S.O., Makinde, J.M., Ekhelar, A.I., Olusola, A., Morebise, O., & Okpako, D.T. (2000). Studies on the Anti-inflammatory, Antipyretic and Analgesic Properties of*Alstonia boonei* Stem Bark. *Journal of Ethnopharmacology* 71: 179-186.
8. Keglevich P, Hazai L, Kalaus G, Szantay C. Modifications on the basic skeletons of vinblastine and vincristine. Molecules. 2012;17:5893-5914.
9. Olaleye S.B1, Farombi E. O, Adewoye E. A, Owoyele B. V, Onasanwo S. A. & Elegbe R.A(2000). Analgesic And Anti-Inflammatory Effects of Kolaviron (A Garcinia Kola Seed Extract). *African Journal of Biomedical Research, 3*,171 – 174
10. Onasanwo, S. & Rotu, R. (2016). Antinociceptive And Anti-Inflammatory Potentials of Kolaviron: Mechanisms of Action. *Journal of Basic and Clinical Physiology and Pharmacology, 27*(4), 363-370. https://Doi.Org/10.1515/Jbcpp- 2015-0075
11. Dogara, A. M., Hamad, S. W., Hama, H. A., Bradosty, S. W., Kayfi, S., Al-Rawi, S. S., & Lema, A. A. (2022). Biological Evaluation of *Garcinia kola* Heckel. *Advances in pharmacological and pharmaceutical sciences, 2022*, 3837965. https://doi.org/10.1155/2022/3837965
12. Igile, G.O., Oleszek, W., Jurzysta, M., Burda, S., Fafunso, M., & Fasanmade, A.A. (1994). Flavonoids from Vernonia amygdalina and their antioxidant activities. Journal of Agricultural and Food Chemistry, 42, 2445-2448. https://pubs.acs.org/doi/10.1021/jf00047a015
13. Oyeyemi, I.T., Akinlabi, A.A., Adewumi, A., Aleshinloye, A.O., & Oyeyemi, O.T. (2018). Vernonia amygdalina: A folkloric herb with anthelminthic properties. *Beni-Suef University Journal of Basic and Applied Sciences, 7*, 43-49. DOI:10.1016/J.BJBAS.2017.07.007

14. Farombi, E. O., & Owoeye, O. (2011). Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. *International journal of environmental research and public health*, *8*(6), 2533–2555. https://doi.org/10.3390/ijerph8062533
15. Joseph, J., Khor, K. Z., Moses, E. J., Lim, V., Aziz, M. Y., & Abdul Samad, N. (2021). In vitro Anticancer Effects of *Vernonia amygdalina* Leaf Extract and Green-Synthesised Silver Nanoparticles. *International journal of nanomedicine*, *16*, 3599–3612. https://doi.org/10.2147/IJN.S303921
16. Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0
17. Park, G. Y., & Christman, J. W. (2006). Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. *American journal of physiology. Lung cellular and molecular physiology*, *290*(5), L797–L805. https://doi.org/10.1152/ajplung.00513.2005
18. Kwok, C. S., & Loke, Y. K. (2010). Critical Overview on the Benefits and Harms of Aspirin. *Pharmaceuticals (Basel, Switzerland)*, *3*(5), 1491–1506. https://doi.org/10.3390/ph3051491
19. Attiq, A., Jalil, J., Husain, K., & Ahmad, W. (2018). Raging the war against inflammation with natural products. *Frontiers in Pharmacology*, *9*, 1-27. https://doi.org/10.3389/fphar.2018.00976
20. Deng, W., Du, H., Liu, D., & Ma, Z. (2022). Editorial: The Role of Natural Products in Chronic Inflammation. *Frontiers in pharmacology*, *13*, 901538. https://doi.org/10.3389/fphar.2022.901538
21. Vignani, R., Liò, P., & Scali, M. (2019). How to integrate wet lab and bioinformatics procedures for wine DNA admixture analysis and compositional profiling: Case studies and perspectives. *PloS one*, *14*(2), e0211962. https://doi.org/10.1371/journal.pone.0211962
22. Bayat A. (2002). Science, medicine, and the future: Bioinformatics. *BMJ (Clinical research ed.)*, *324*(7344), 1018–1022. https://doi.org/10.1136/bmj.324.7344.1018
23. Behera, S.K., N. Mahapatra, C.S. Tripathy and S. Pati. (2021a). Drug repurposing for identification of potential inhibitors against SARS-CoV-2 spike receptor-binding domain: An in silico approach. *Indian Journal of Medical Research, 153*(1 & 2):132-143. doi: 10.4103/ijmr.IJMR_1132_20.
24. Behera, S.K., N. Vhora, D. Contractor, A. Shard, D. Dinesh Kumar., K. Kalia and A. Jain (2021b). Computational drug repurposing study elucidating simultaneous inhibition of entry and replication of novel corona virus by Grazoprevir. *Scientific Reports*, *11*, 7303. https://doi.org/10.1038/s41598-021-86712-2
25. Schneider, B., Cerný, J., Svozil, D., Cech, P., Gelly, J. C., & de Brevern, A. G. (2014). Bioinformatic analysis of the protein/DNA interface. *Nucleic acids research*, *42*(5), 3381–3394. https://doi.org/10.1093/nar/gkt1273

26. Vyshnevska, L., Severina, H.I., Prokopenko, Y., & Shmalko, A. (2022). Molecular docking investigation of anti-inflammatory herbal compounds as potential LOX-5 and COX-2 inhibitors. Pharmacia, 69(3), 733-744. https://pharmacia.pensoft.net/article/89400/list/18/
27. Ayepola, O.R., Chegou, N.N., Brooks, N.L., & Oguntibeju, O.O. (2013). Kolaviron, a Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. BMC Complementary and Alternative Medicine, 13, 363-371. https://doi.org/10.1186/1472-6882-13-363
28. Farombi, E.O., Shrotriya, S., & Surh, Y. (2009). Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-κB and AP-1. Life Sciences, 84(5-6), 149-155. https://doi.org/10.1016/j.lfs.2008.11.012
29. Adewole, K.E., Gyebi, G.A., & Ibrahim, I.M. (2021). Amyloid β fibrils disruption by kolaviron: Molecular docking and extended molecular dynamics simulation studies. Computational Biology and Chemistry, 94, 107557, https://doi.org/10.1016/j.compbiolchem.2021.107557
30. Gupta, G., Tiwari, J., Dahiya, R., Kumar Sharma, R., Mishra, A., & Dua, K. (2018). Recent updates on neuropharmacological effects of luteolin. *EXCLI journal*, *17*, 211–214. https://doi.org/10.17179/excli2018-1041
31. Lin, Y., Shi, R., Wang, X., & Shen, H. M. (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. *Current cancer drug targets*, *8*(7), 634–646. https://doi.org/10.2174/156800908786241050
32. Ntalouka, F., & Tsirivakou, A. (2023). Luteolin: A promising natural agent in management of pain in chronic conditions. *Frontiers in Pain Research, 4,* 1-19. https://doi.org/10.3389/fpain.2023.1114428
33. D'Mello, P., Gadhwal, M.K., Joshi, U., & Shetgiri, P. (2011). Modeling of COX-2 inhibitory activity of flavonoids. International Journal of Pharmacy and Pharmaceutical Sciences, 3(4), 33-40.
34. Wang, D., & DuBois, R.N. (2004). Cyclooxygenase 2-derived prostaglandin E2 regulates the angiogenic switch. Proceedings of the National Academy of Sciences, 101(2), 415-416. https://www.pnas.org/doi/full/10.1073/pnas.0307640100
35. Sethi, A., Joshi, K., Sasikala, K., & Alvala, M. (2020). Molecular Docking in Modern Drug Discovery: Principles and Recent Applications. IntechOpen. doi: 10.5772/intechopen.85991