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RESEARCH ARTICLE

IN SILICO STUDIES ON *Tithonia Diversifolia* DERIVED PHYTOCHEMICALS AS POTENT INHIBITOR OF CYCLOOXYGENASE

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ABSTRACT

Objectives: Prostaglandins released by the activity of the bifunctional enzyme, cyclooxygenase (COX) are involved in physiological functions such as protection of the stomach mucosa, aggregation of platelets and regulation of kidney function. Conversely, prostaglandins have been implicated in inflammation, thereby representing an important target for non-steroidal anti-inflammatory drugs.**Material and methods:** In this study, natural compounds from Tithonia diversifolia (a known anti-inflammatory plant) have been evaluated for their interaction with COX in comparison with ibuprofen using computational methods. T. diversifolia phytocompounds were docked into COX active site using Autodock/Vina Plugin in PyMol.**Result:** All phytocompounds from T. diversifolia show better interactions than ibuprofen with myricetin and quercetin having the highest binding energy of -9.1 Kcal/mol (ibuprofen -6.2 Kcal/mol).**Conclusion**: These phytocompounds can be considered as potential COX inhibitor thereby leading credence to the use of T. diversifolia as an anti-inflammatory plant.

Keywords: Cyclooxygenase, saponin, quercetin, myricetin, apigenin, gingerol,caffeic acid, kaurenoic acid, Ibuprofen.

INTRODUCTION

Inflammation is an important physiological reaction occurring in response to a wide variety of injurious agents in order to limit tissue damage and promotes tissue repair [1][2]. Prostaglandins (PGs) are an important mediator of inflammation and are released by almost any type of chemical or mechanical stimulus [3]. The key enzyme in their synthesis is prostaglandin endoperoxide synthase (PGHS) or cyclooxygenase (COX) which possesses two catalytic sites. The first, a cyclooxygenase active site, converts arachidonic acid to the endoperoxide PGG2. The second, a peroxidase active site, then converts the PGG2 to another endoperoxide, PGH2. PGH2 is further processed by specific synthases to form PGs, prostacyclin and thromboxane A2. Of the PGs, GE2 and prostacyclin are the main inflammatory mediators [3]. Plants are a good source of biologically active compounds. The decoctions of various parts of *Tithonia diversifolia* are used for the treatment of malaria, diabetes mellitus, sore throat, menstrual pains and inflammation [4][5]. Modern pharmacological investigations revealed that it has extensive bioactivities including antimalarial [6], anticancer [7] antidiabetic and anti-inflammatory [8]. In recent years, plant products play a dominant role in the discovery of phytodrugs for treating human diseases. There is a worldwide interest in searching for a safer and newer anti-inflammatory drugs especially from medicinal plants due to their inherent phytocompounds which have been documented to ascribe pharmacological potency to medicinal plants [9][10]. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) widely used to relieve pain and inflammation in many disorders via inhibition of cyclooxygenases [2]. Vane in 1995 showed that the pharmacological actions of ibuprofen and similar drugs were due to the inhibition of cyclooxygenase. Thus, ibuprofen-like drugs exert their anti-inflammatory, antipyretic and analgesic effects by inhibition of cyclooxygenase [11]. Pythocompounds from *Tithonia diversifolia* such as; saponin, chlorogenic acid, apigenin, quercetin, myricetin, and quercetin have been documented to have the ability to inhibit COX both *in vivo* and *in-vitro* experimental models [12]. However, this claim has not been validated by *in silico* approaches. Using computational methods, the present study is aimed to determine the molecular interaction of ibuprofen and six phytocompounds from *Tithonia* with COX, a target protein for an anti-inflammatory drug.

MATERIALS AND METHODS

Protein preparation and generation of 3d structure through homology modeling

The starting structure (PDB ID: 1CX2) required for docking was retrieved from the protein data

bank repository (HTTP: //www.rcsb.org). Prior to docking, water and ligand coordinates were

deleted. Human cyclooxygenase (COX) “Fasta” file downloaded from www.pubmed.org was use to model the starting structure of COX protein. Homology modeling was done on Swiss Model Server (http://swissmodel.expasy.org). This requires one sequence of known 3D structure with significant similarity with the target sequence. The coordinate file of the template from protein data bank (PDB ID: 1CX2) was used to model the 3D structure of COX [13].

Ligand preparation for docking

The 2D structure of compounds viz; Ibuprofen ID: 3672, saponin ID: 92825, apigenin ID:5280443, chlorogenic acid ID:794427, quercetin ID:5280343 and myricetin ID:5281672, were retrieved from PubChem database. The ligands were optimized into 3D for docking using Marvinsketch.

MOLECULAR DOCKING

Molecular Docking

Algorithm for active site determination was performed using BSP-SLIM server and AutodockVina was used for molecular docking. Each of the ligands was loaded on Autodock/Vina [13] and all the water molecules were removed prior to docking. All calculations were computed using the facilities of Center for Bio-Computing and Drug Development, Adekunle Ajasin University, Akungba-Akoko. Nigeria.

DATA ANALYSIS

Bonding interaction and amino acid residue around the active site were estimated using Ligplot. The binding pose of the COX docked with the ligands was viewed and snapshots were taken using PyMOL

RESULTS AND DISCUSSION

The structure of protein viewed with PYMOL. The docking poses were ranked according to their docking scores, together with the ranked list of docked ligands and their corresponding binding poses [14]. Ten (10) docking runs were performed. Grid parameters were set spacing between grid points was 0.375A and the coordinates are X=231.39, y=97.19, z=48.04.

There is increasing evidence that the presence of bioactive compounds in natural plants is responsible for their pharmacological effect [12]. The current study showed the potential of the phytocompounds (saponin, chlorogenic acid, apigenin, quercetin and myricetin) from *Tithonia diversifolia* to interact with COX and also compare their interactions with ibuprofen. The Protein-Ligand interaction plays a meaningful role in structural based designing [13]. The phytocompounds from *T. diversifolia* bind at the active sites of COX with good docking score and glide energy (Figure 3-7) compared with ibuprofen, a COX inhibitor drugs (Figure 2). In the current study, quercetin (Figure 5), and myricetin (Figure 6) exhibited higher binding energy of interaction with COX (-9.1Kcal/mol) while the energy of binding for apigenin (Figure 4), saponin (Figure 3), chlorogenic acid (Figure 7) are (-8.9, -8.4, -7.9) Kcal/mol respectively. This study showed that all the phytocompounds docked with COX exhibited higher docking scores and good binding interaction at the active sites of COX compared to ibuprofen (-6.5 Kcal/mol).

The binding affinity alone is not a sufficient tool to describe the enzyme-ligand complex stability hence; molecular interactions such as hydrogen bonding and hygroscopic interaction of ligands [15] were studied using Ligplot. For each ligand molecule, a Ligplot figure shows a schematic depiction of the hydrogen bonds and non-bonded interactions between the ligand and the amino acid residues of the protein [15]. A higher binding affinity is a function of the ability of the ligand to form more hydrophobic interactions with the hydrophobic amino acids around the binding site of the ligand as well as the number of hydrogen bonds. In the current study, quercetin (Figure 5) and myricetin (Figure 6) formed eight(8) hydrophobic interactions with hydrophobic amino acids within their binding site and three(3) hydrogen bonding. Quercetin, myricetin and apigenin formed three(3) hydrogen bonding with ASN381, THR205 and ALA198 contributing to the high docking score observed in this study. The green dotted line represents the H-bonding interaction of the ligand with the amino acid of the protein depicted in green color. The red arc with spikes indicates the hydrophobic interaction with the amino acids represented in black. It can be said that TYR384, HIS387, HIS385, PHE209 form a hydrophobic interaction with all the phytocompounds studied thus contributing to the binding affinity of the Phytocompounds to COX. Saponin and chlorogenic acid formed two H-bonds (THR211 and ASN381), (THR211 and HIS206) respectively while ibuprofen formed a single H-bond (THR211) accounting for the lowest docking scored observed in this study.

This study concludes that saponin, chlorogenic acid, apigenin, myricetin and quercetin will be of great interest to the pharmaceutical industry as a source of inflammatory agent most especially quercetin and myricetin with binding energy -9.1Kcal/mol.

CONCLUSION

The present study showed that saponin, chlorogenic acid, apigenin, quercetin, and myricetin, could be the potential inhibitor of COX protein. The phytocompounds of *Tithonia diversifolia* showed a better molecular interaction than the existing COX inhibitor (ibuprofen). Animal model studies to determine their level of safety are suggested.

Table1: Binding Affinity (Kcal/mol) of phytocompounds from *T. diversifolia* and Ibuprofen with cyclooxygenase.

	Ibuprofen	Quercetin	Myricetin	Apigenin	Chlorogenic	Saponin
Mode	Afinity	Afinity	Afinity	Afinity	Afinity	Afinity
	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol
1	-6.5	-9.1	-9.1	-8.9	-7.9	-8.4
2	-6.3	-8.9	-8.6	-8.3	-7.7	-8.4
3	-5.9	-8.5	-8.5	-8.3	-7.6	-8.4
4	-5.8	-8.1	8.4	-8.1	-7.5	-8.2
5	-5.8	-8	-8.2	-7.6	-7.4	-7.7
6	-5.7	-8	-8.1	-7.4	-7.2	-7.6
7	-5.6	-7.7	-7.9	-7.3	-7	-7.1
8	-5.6	7.7	-7.6	-7.3	-6.9	-7
9	-5.6	-7.6	-7.6	-7.2	-6.6	-7
10	-5.4	-7.6	-7.6	-7.2	-6.6	-6.8

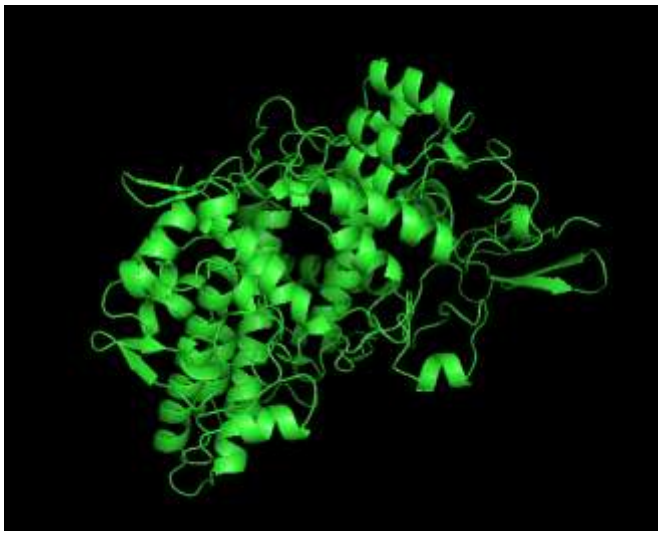

Fig.1: 3D structure of Human Cyclooxygenase.

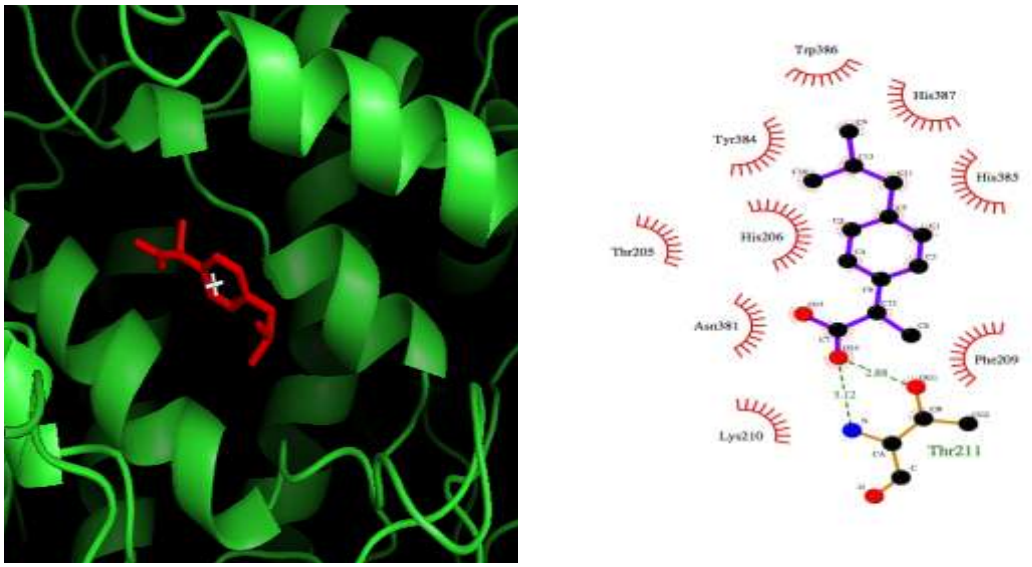

Fig.2: Protein-ligand interaction of COX with Ibuprofen using Ligplot diagram

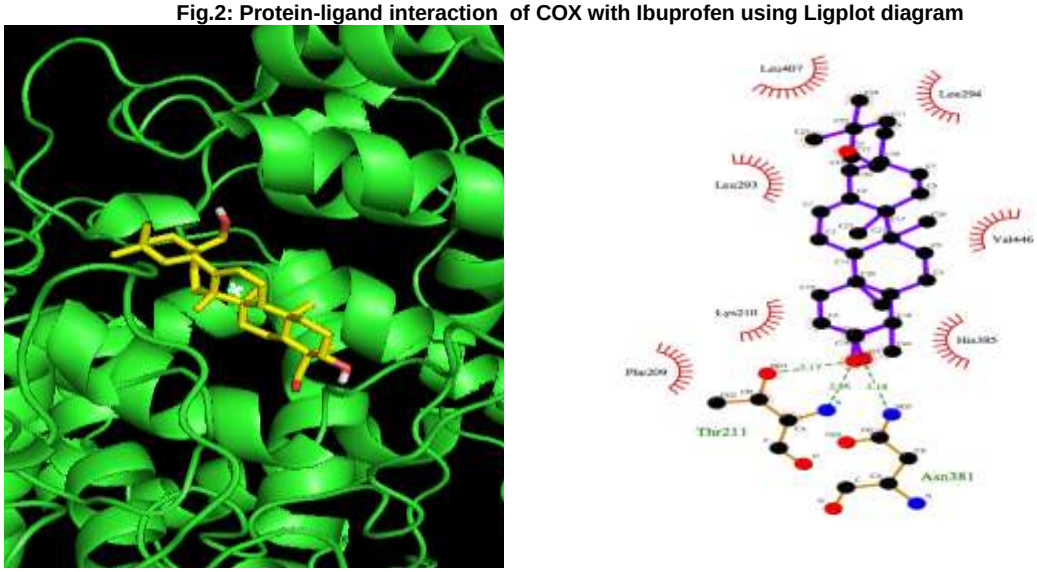

Fig.3: Protein-ligand interaction of COX with saponin using Ligplot diagram

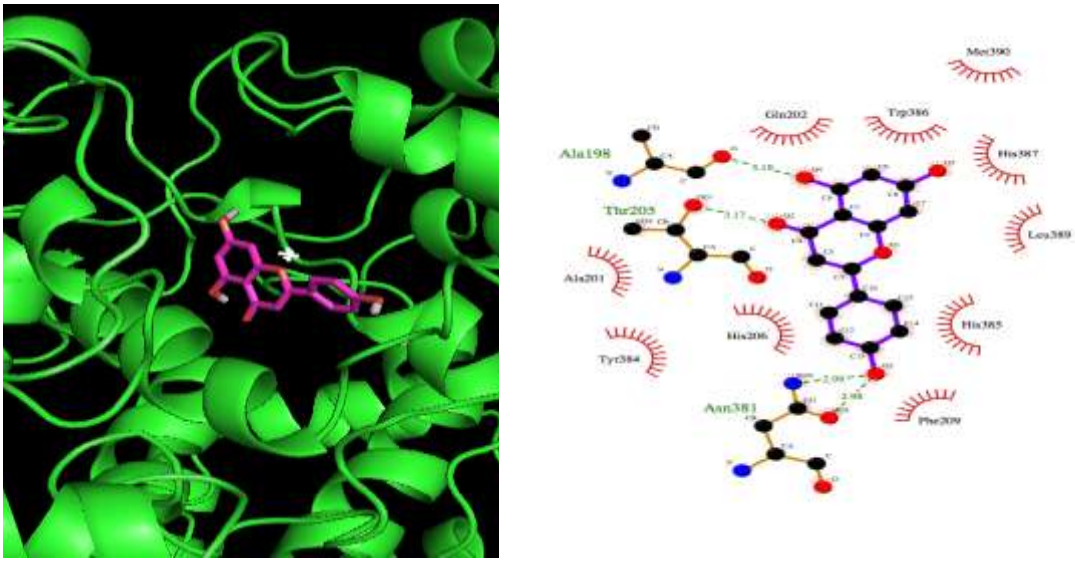

Fig.4: Protein-ligand interaction of COX with Apigenin using Ligplot diagram

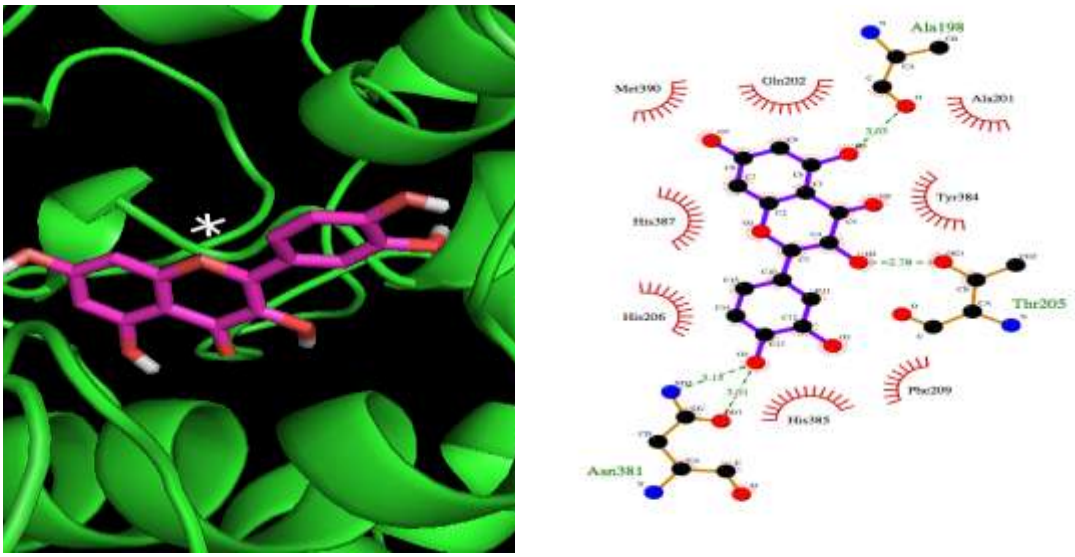

Fig.5: Protein-ligand interaction of COX with Quercetin using Ligplot diagram

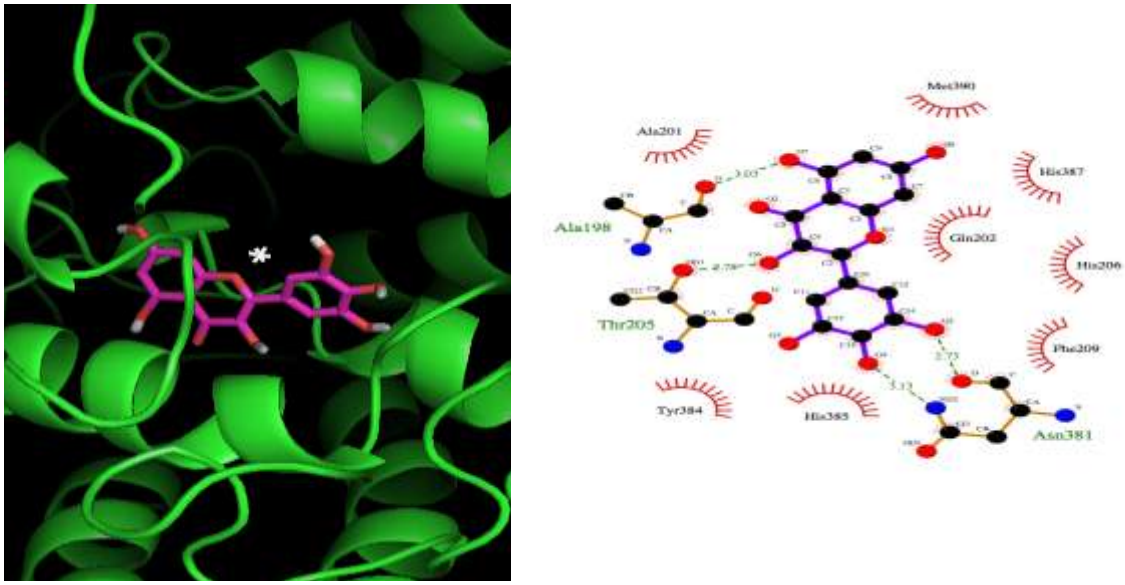

Fig.6: Protein-ligand interaction of COX with Myricetin using Ligplot diagram.

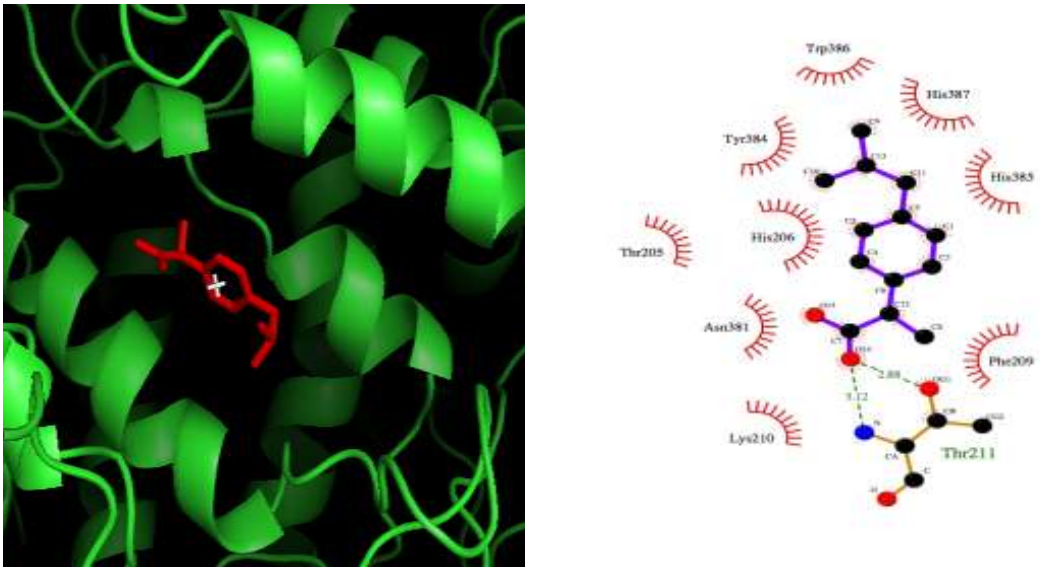

Fig.7: Protein-ligand interaction of COX with Chlorogenic acid using Ligplot diagram

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