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**Original Research Article**

# Mechanistic Insight the Anti-Inflammatory Potential of *Ziziphus jujuba* Fruit Bioactive: *In-Silico* Molecular Docking

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**Abstract:** ***Background*:** Inflammation denotes the body's standard physiological response to tissue damage. The injury may stem from physical or mechanical damage, trauma, autoimmune response, microbial invasion, or burns. Inflammation may be classified as either acute or chronic. Ziziphus jujube (ZJ), also known as Chinese date or Indian ber, is a widely researched medicinal plant due to its abundant phytochemical content, which may promote a healthy diet. It is a medium-sized indigenous plant belonging to the Rhamnaceae family. Ziziphus jujube (Z. jujube) is traditionally employed by locals to address dandruff, arthritis, chronic constipation, acne, antibacterial concerns, and cardiac ailments. Jujube comprises several constituents, such as flavonoids, triterpenic acids, amino acids, cerebrosides, mineral elements, phenolic acids, and polysaccharides. ***Aim*:** This study seeks to examine the effectiveness of *Ziziphus jujuba* flavonoid and terpenoid against COX-2 & 12- LOX to clarify their anti-inflammatory potential. ***Method*:** COX-2 & 12- LOX was chosen as the target proteins in the current investigation. The bond was found using the Auto Dock software using a grid-based docking method. Compounds' 2D structures were generated, converted to 3D, and subsequently energetically lowered up to an arms gradient of 0.01 using the Merck Molecular Force Field (MMFF). ***Result*:** Flavonoids (isoquercetrin) and terpenoid (zizyberanolic acid) of *Z.jujuba* effectively binds to be target protein *COX-2 & 12-LOX* respectively with binding energy -5.46 & -5.95 kcalmol$^{-1}$ respectively. ***Conclusion*:** The finding of the *in-silico* molecular docking showed that both lead compound is effective binds & inhibitory action on target protein. The molecular docking outcome revealed that isoquercetrin binds COX2 whereas zizyberanolic acid binds 12-LOX, thereby suppressed the synthesis of PGs and ILs.

**Keywords:** *Z.Jujuba*, Molecular Docking, *COX-2*, 12- LOX, Isoquercetrin & Zizyberanolic Acid.



## INTRODUCTION

Inflammation denotes the body's standard physiological response to tissue damage. The injury may come from physical or mechanical damage, trauma, autoimmune response, microbial invasion, or burns. Inflammation may be classified as either acute or chronic [1-5]. During an acute inflammatory response, lipoxins, leukotrienes, bradykinin, platelet-activating factor, and lymphokines are involved [6]. Histamine enhances tissue permeability, induces smooth muscle contraction, and results in bronchoconstriction [7]. Chronic inflammatory conditions, such as arthritis and hemorrhoids, persist in disrupting cellular and molecular processes that mitigate potential harm or infection. This mitigation process aids in the restoration of tissue homeostasis and the resolution of acute inflammation. Unregulated acute inflammation may, however, progress to a chronic state, contributing to many chronic inflammatory disorders [8]. Inflammation and the immune system are intricately linked. Mediators of inflammation (autacoids) encompass serotonin, histamine, and prostaglandins, highlighting humanity's reliance on nature's medicinal plant resources. Numerous botanicals utilized in traditional medicine for the treatment of inflammation remain scientifically unassessed [9]. The existing models for studying anti-inflammatory activity possess significant limitations and problems. The fruits of Ziziphus jujuba, referred to as jujube, red date, or Chinese date, are consumed fresh or dried and utilized in traditional medicine globally due to its significant

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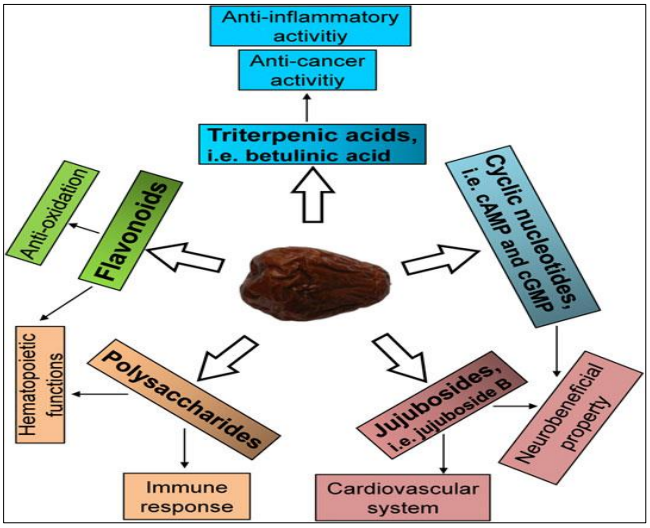
nutritional and physiological benefits. In China, jujube is traditionally regarded as a medicinal fruit utilized for the treatment of blood insufficiency [10].



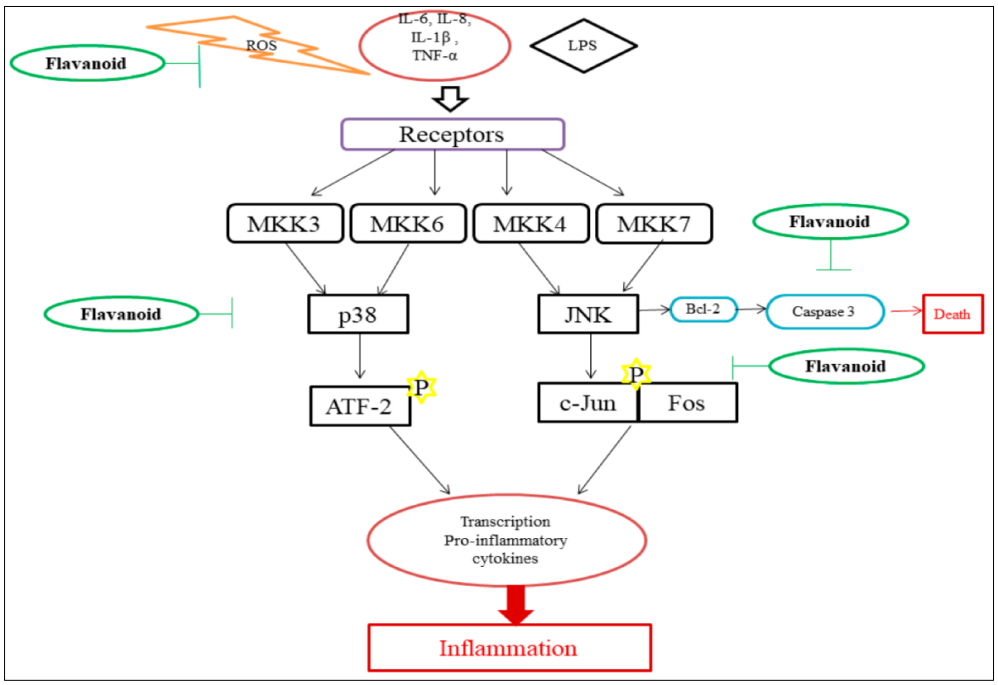

**Pharmacological potential of Ziziphus**

**Experimental Works**

***In-Silico* Molecular Docking**

**Selection of Lead Molecules**

As per literature survey Zhengzheng Wang *et al*., 2023 isolated and purified flavonoids from aqueous fruit extract. Five flavonoids, including epicatechin, quercetin, rutin, isoquercitrin, and hyperin were identified [11]. Studies show that flavonoids activate antioxidant pathways that render an anti-inflammatory effect. They inhibit the secretions of enzymes such as lysozymes and β-glucuronidase and inhibit the secretion of arachidonic acid, which reduces inflammatory reactions. Flavonoids such as quercetin, genistein, apigenin, kaempferol, and epigallocatechin 3-gallate modulate the expression and activation of a cytokine such as interleukin-1beta (IL-1β), Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8); regulate the gene expression of many pro-inflammatory molecules such s nuclear factor kappa-light chain enhancer of activated B cells (NF-κB), activator protein-1 (AP-1), intercellular adhesion molecule-1 (ICAM), vascular cell adhesion molecule-1 (VCAM), and E-selectins; and also inhibits inducible nitric oxide (NO) synthase, cyclooxygenase-2, and lipoxygenase, which are pro-inflammatory enzymes [12].

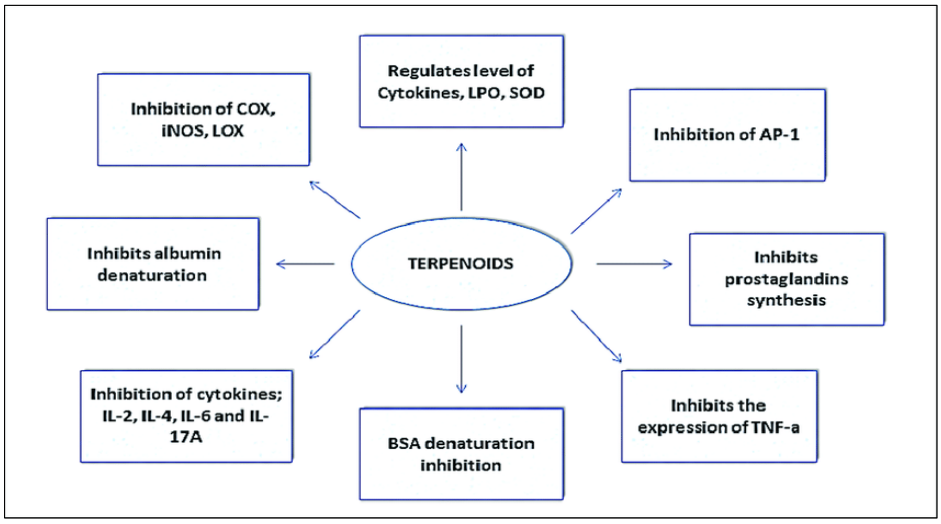


**Role of flavonoid in Inflammation**

As per previous studied epicatechin, quercetin and rutin are well known for anti-inflammatory activity. So, in current investigation isoquercitrin is selected as lead molecule for molecular docking study.

Z.jujuba (ZJ) is rich in a variety of active ingredients, with triterpenoids being a unique active ingredient, which are present in the fruit, leaves, branches, and roots. More than 120 triterpenoids have been identified in ZJ and has various biological activities. For example, betulinic and ursolic acids have anticancer, antioxidant, antibacterial and antiviral activities. ceanothic, alphitolic, and zizyberanalic acids possess anti-inflammatory activities [13].

Established terpenes are a broad class of secondary metabolites that are rich in monoterpenes, diterpenes, triterpenes, tetraterpenes, ceramides, and sesquiterpenes and have various therapeutic applications, such as antitumor, antibacterial, antimicrobial, analgesic and anti-inflammatory activities. Numerous studies have shown that terpenes can decrease inflammation-associated symptoms by reducing the release of proinflammatory cytokines, including nuclear transcription factor-kappa B, interleukins, tumor necrosis factor-alpha, and other inflammatory mediators [14].




**Role of Terpenoid in Inflammation**

So, due to versatility of terpenoids zizyberanalic acid was taken as another lead molecule for molecular docking study.

**Selection of Target Receptor**

***COX-1*** is thought to mediate 'housekeeping' functions, and thus is responsible for the production of prostaglandins that are required for normal physiological activities, inducible ***COX-2*** expressed in immune cells is a key player in initiating the inflammatory response by converting arachidonic acid (AA, C20:4), an ω-6 polyunsaturated fatty acid (PUFA), into proinflammatory prostaglandins (mainly PGE 2) and triggering production of other proinflammatory chemokines and cytokines [15].

***12-LOXs:***

Many studies have demonstrated that 12-LOXs and their eicosanoid metabolite 12-hydroxyeicosatetraenoate (12-HETE), have significant pathological implications in inflammatory diseases. Increased level of 12-LOX activity promotes stress (both oxidative and endoplasmic reticulum)-mediated inflammation, leading to damage in these tissues. 12-LOXs are also associated with enhanced cellular migration of immune cells—a characteristic of several metabolic and autoimmune disorders. Genetic depletion or pharmacological inhibition of the enzyme in animal models of various diseases has shown to be protective against disease development and/or progression in animal models in the setting of diabetes, pulmonary, cardiovascular, and metabolic disease, suggesting a translational potential of targeting the enzyme for the treatment of several disorders [16].

*So, Cox-2 and 12 LOX were taken as target receptor for current investigation.*

**Molecular Docking Studies**

***Ligand Preparation***

2D Structure of isoquercetin and zizyberanolic acid was drawn using ChemSketch [17], the two-dimensional structure of the prepared ligand was converted into their 3-D structures optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligand were given below:

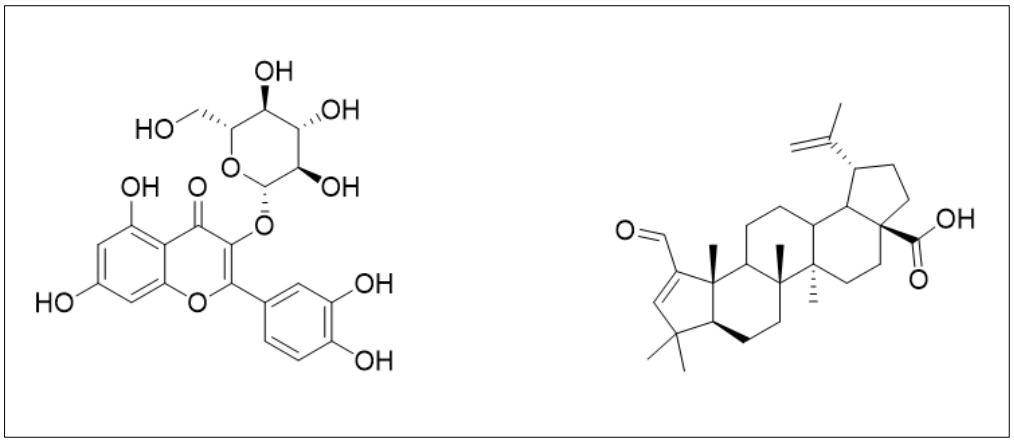



**Figure 1: 2D structure of isoquercetin and zizyberanolic acid**

***Preparation of the Grid File***

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points for the considered receptor in the current study are given in table 1 [18].

**Table 1: Grid parameters used in current docking analysis of COX 2 and 12 LOX receptor**

| S. No. | Receptor | x-axis | y-axis | z-axis | Spacing | x center | y center | z center |
|---|---|---|---|---|---|---|---|---|
| 1 | COX2 | 46 | 44 | 46 | 0.375 | 38.042 | 2.132 | 61.28 |
| 2 | 12LOX | 50 | 50 | 50 | 0.414 | 127.366 | 138.894 | 138.224 |

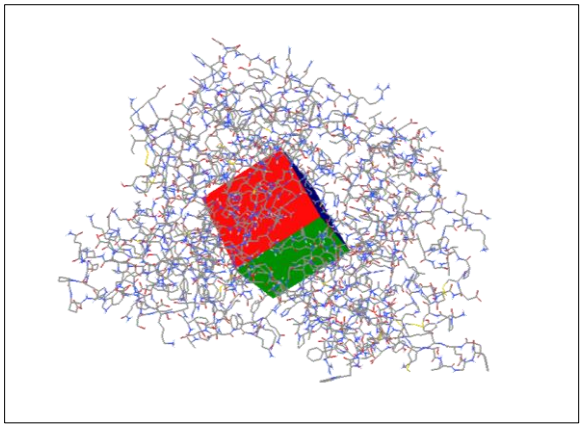


**Figure 2: Grid box covering all active sites in COX2 receptor**

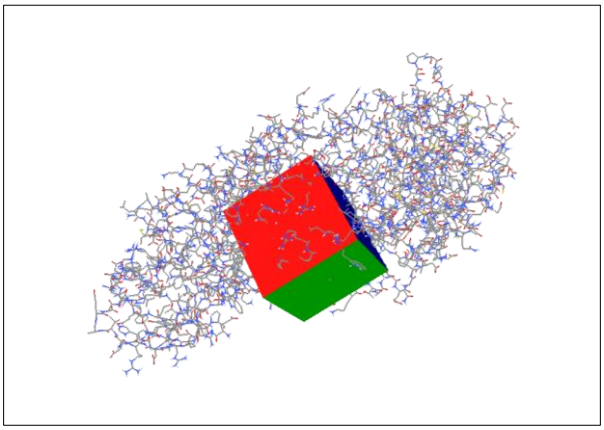


**Figure 3: Grid box covering all active sites in 12LOX receptor**

***Preparation of the Docking File***

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [19, 20].

**Docking Study**

***Crystal Structure***

The crystal structure of the protein consisting of COX2 and 12LOX receptor is downloaded from the Protein Data Bank portal. All the primary information regarding all the receptor's structure was registered in the Protein data bank [21-22]. The complex ligand was separated by using Chimera software for all the target receptors.

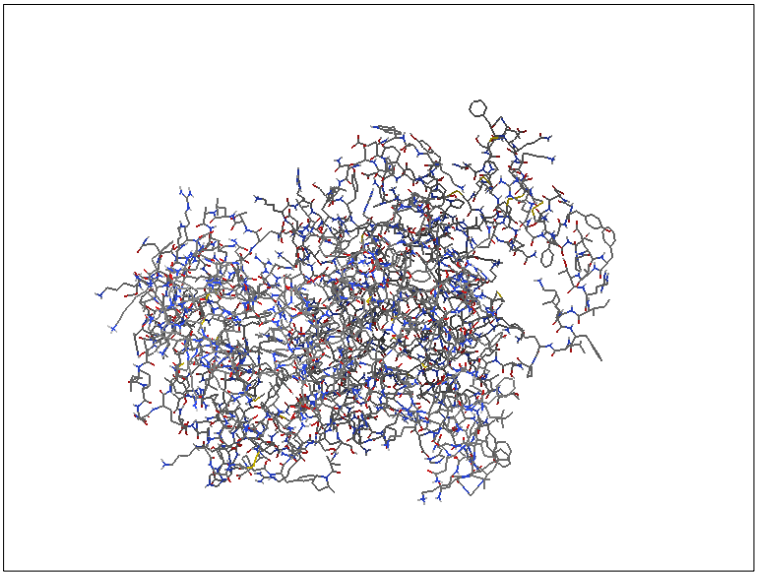


**Figure 4: Crystal structure of COX2 receptor (PDB ID-5ikr)**

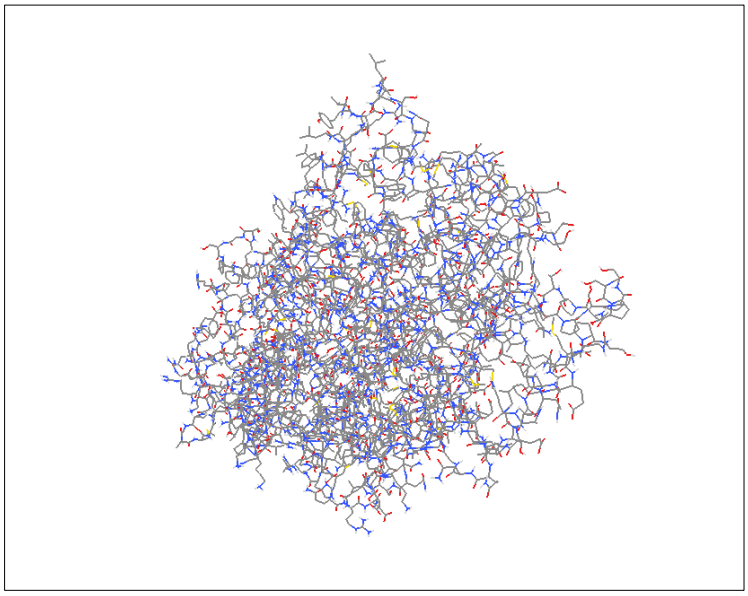


**Figure 5: Crystal structure of 12LOX receptor (PDB ID-8ghb)**

***Processing of Protein***

All the downloaded receptor proteins are having only one chains, i.e. chain A, which has been selected for experimental purpose and complex ligand was removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [23, 24].

**Molecular Docking Simulation Studies**

Docking of ligand isoquercetin and zizyberanolic acid against COX2 and 12LOX receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [25-27].

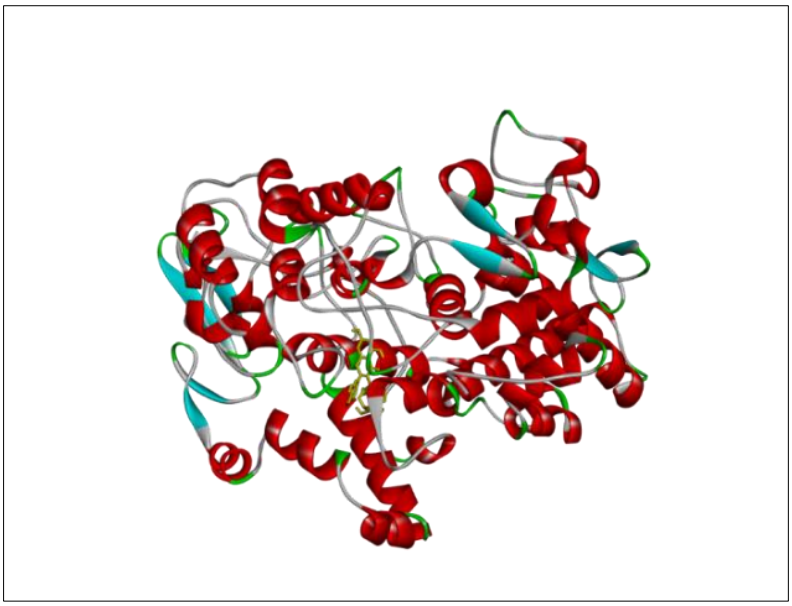


**Figure 6: Binding mode of isoquercetin within the active site of COX2 receptor**

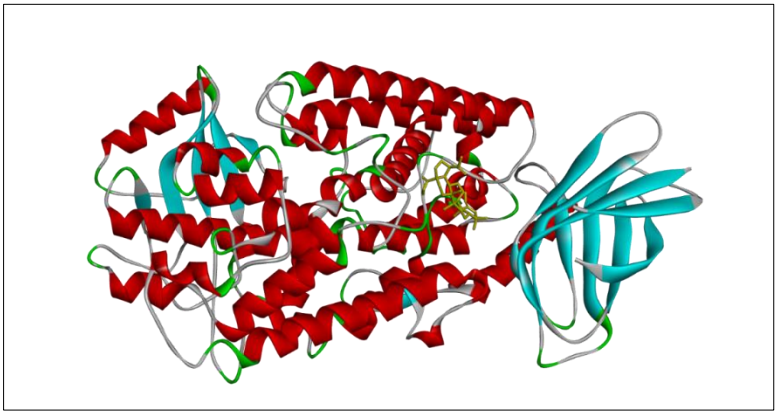


**Figure 7: Binding mode of zizyberanolic acid within the active site of 12LOX receptor.**

**Toxicity & ADME-T Studies**

The ligand molecules viz. isoquercetin and zizyberanolic acid was studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [28].

## RESULT AND DISCUSSION

Inflammation often arises when infectious microorganisms, including bacteria, viruses, or fungi, infiltrate the body, inhabit specific tissues, and/or disseminate through the bloodstream. Inflammation may also occur in reaction to events such as tissue injury, cellular necrosis, malignancy, ischemia, and degeneration. A variety of inflammatory mediators are synthesized and released during various types of inflammatory responses. Inflammatory chemicals are typically classified into two primary categories: pro-inflammatory and anti-inflammatory mediators.

Medicinal plants possess therapeutic qualities owing to the presence of different compounds. Contemporary herbal goods represent safety, in contrast to synthetics, which are deemed hazardous to both individuals and the environment. Ziziphus jujuba is a member of the Rhamnaceae family. It is a medium-sized, rapidly growing, deciduous spiky tree with a dense, spreading crown. Leaves exhibit variability, accompanied by greenish-yellow flowers. Fruits are green and turn pale yellow upon ripening. The plant Ziziphus jujuba is utilized therapeutically for many disorders. The seeds of jujuba have been utilized in traditional medicine for alleviating anxiety and insomnia, as well as serving as an appetite stimulant and digestive aid. The mucilaginous fruit is highly soothing to the throat, and jujuba decoctions have frequently been utilized in pharmacy to alleviate sore throats.

The scientific validation of the anti-inflammatory activity of flavonoids and terpenoids found in Z. jujuba fruit was conducted using molecular docking against the target proteins COX-2 and 12-LOX. Elevated 12-LOX activity exacerbates stress-induced inflammation, both oxidative and endoplasmic reticulum-related, resulting in tissue damage. 12-LOXs

are linked to increased cellular migration of immune cells, a feature of certain metabolic and autoimmune diseases. COX-2, the inducible isoform, is expressed in reaction to inflammatory and various physiological stimuli as well as growth factors, and it plays a role in the synthesis of prostaglandins that facilitate pain and promote the inflammatory response.

The results of molecular docking of the lead molecule against various selected receptors indicated that Isoquercetin targeting with COX-2 revealed binding energy 5.46 kcal $mol^{-1}$ having IC50 0.113 and drug score 0.43(table 2) whereas zizyberanolic acid have strong affinity for 12 LOX, with binding energies of -5.95 kcal $mol^{-1}$ (Table 3) having IC50 0.105 with drug score 0.1. The 2D and 3D binding interactions of the lead bioactive against the designated receptor are illustrated in Figures 8-11. The binding interaction of the selected molecule with the molecular target is demonstrated as follows:

| Lead molecule | Vander waal's | Hydrogen | C-H | donar-donar | π-σ | π-Alkyl | π-sulfur | π- π |
|---|---|---|---|---|---|---|---|---|
| Isoquercetin | Leu359<br>Val 116<br>Pro526<br>Leu31<br>Thr 94<br>Leu 384<br>Try 387<br>Phe381<br>Tyr385 | Arg 120<br>Ser 353 | Val523<br>Val 349 | His 90<br>Ser 530 | Tyr 355<br>Leu 352 | Ala 527 | Met 522 | Gly526<br>Phen 518 |
| Zizyberanolic acid | His 609<br>His 610<br>Glu612<br>Arg 394<br>Glu169<br>Asp 603<br>Pro 602 | Lys 611<br>Arg 600<br>Met 604 | Val 605 | ---- | ----- | ----- | ----- | Pro 606 |

The pharmacokinetic profiling of the Isoquercetin and zizyberanolic acid ligand had revealed that it is having good pharmacokinetic profile associated without the presence of major toxic effects like mutagenic, reproductive effects, irritant effect, and tumorogenic properties. The pharmacokinetic and toxicity profiling results of Isoquercetin and zizyberanolic acid were shown in figure 12-13 & table 4-6. Theoretically, all the ligand molecules have shown encouraging docking score. All compound followed Lipinski rule and showed all most similar drug likeness score.

**Table 2: Result of docking of Lead molecule with COX-2**

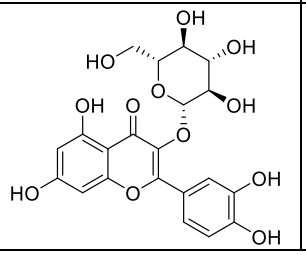
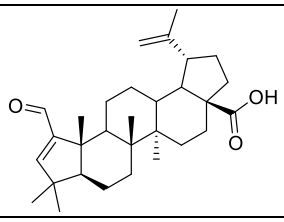
| S. No. | Molecule | Structure | B.E | KI | IC50 |
|---|---|---|---|---|---|
| **1.** | **Isoquercetin** |  | -5.46 | 9.286 | 0.113 |

**Table 3: Result of docking of Lead molecule with 12-LOX**

| S. No. | Molecule | Structure | B.E | KI | IC50 |
|---|---|---|---|---|---|
| **1.** | zizyberanolic acid |  | -5.95 | 10.042 | 0.105 |

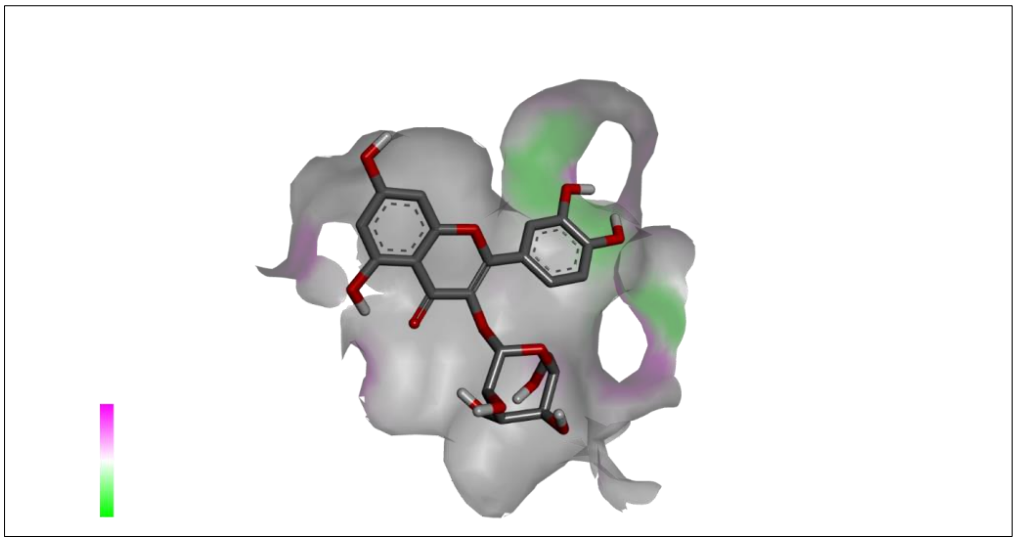


**Figure 8: Three-dimensional binding mode of isoquercetin within the active site of COX2 receptor**

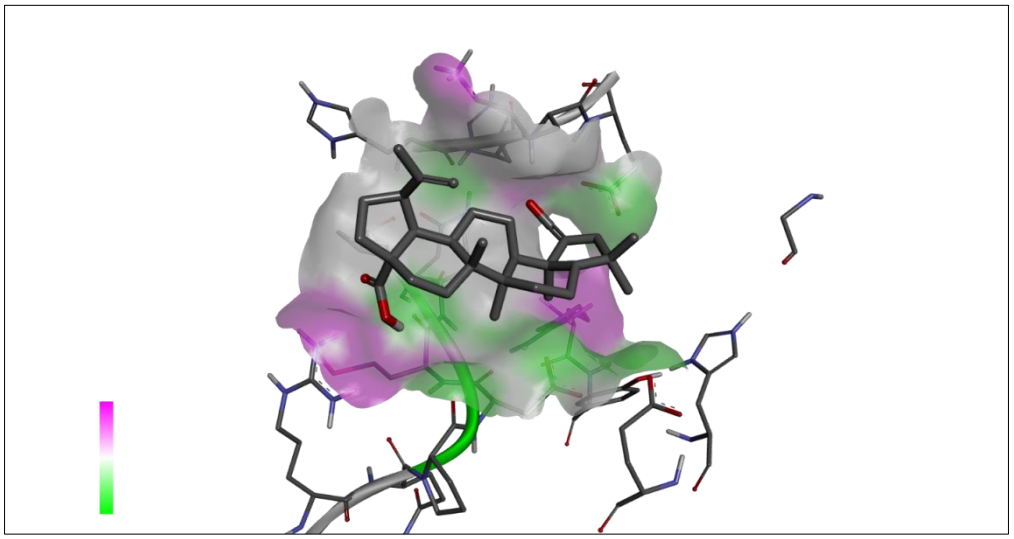


**Figure 9: Three-dimensional binding mode of zizyberanolic acid within the active site of 12LOX receptor**

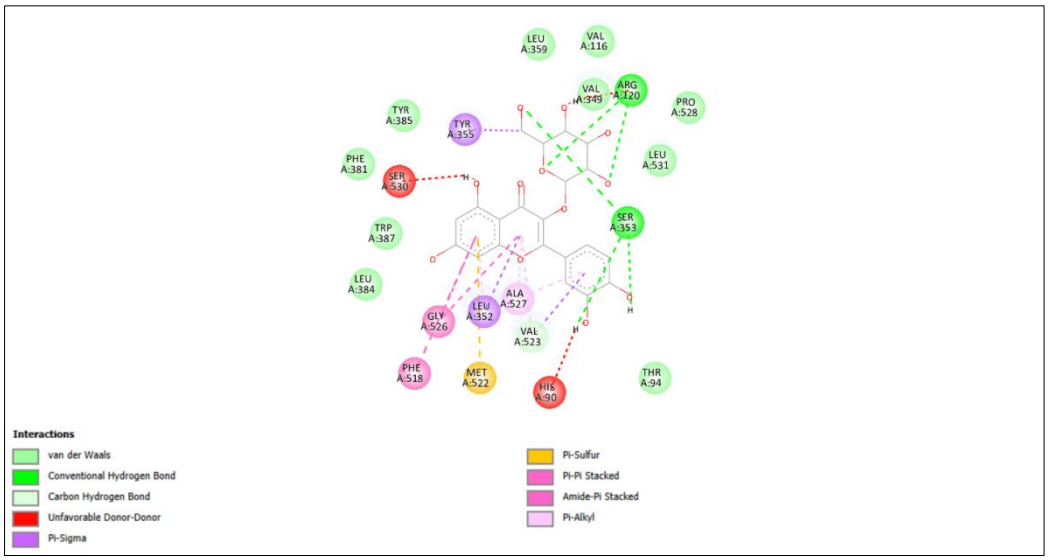



**Figure 10: Two-dimensional binding mode of isoquercetin within the active site of COX2 receptor.**

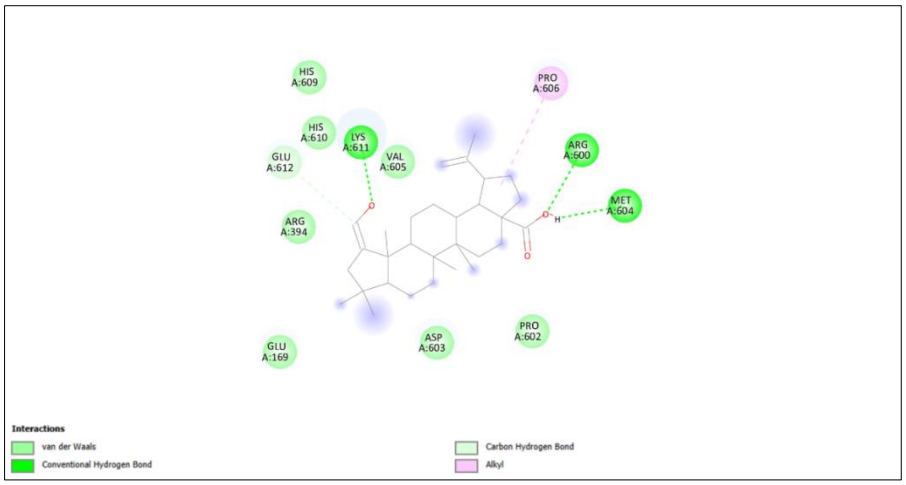



**Figure 11: Two-dimensional binding mode of zizyberanolic acid within the active site of 12LOX receptor**

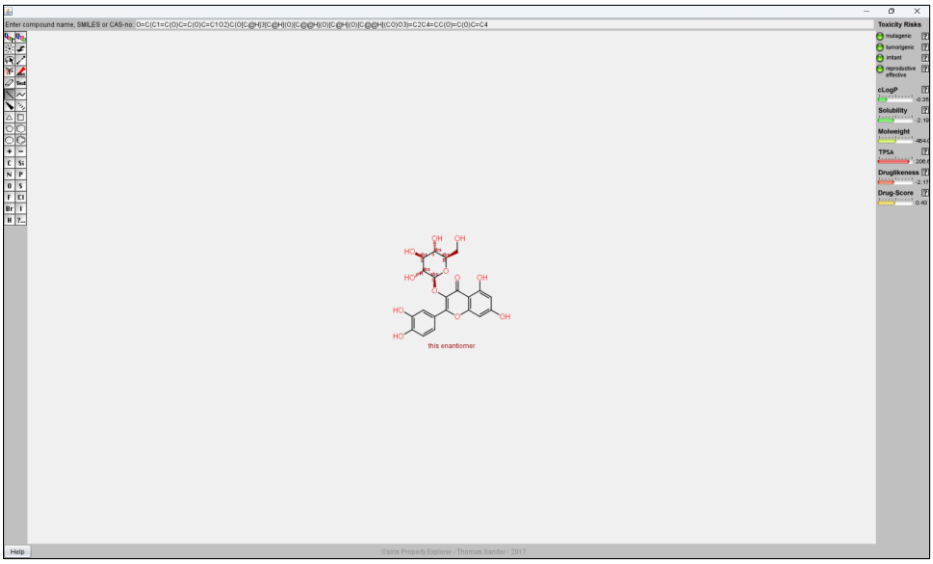


**Figure 12: Pharmacokinetic and toxicity profiling of isoquercetin**

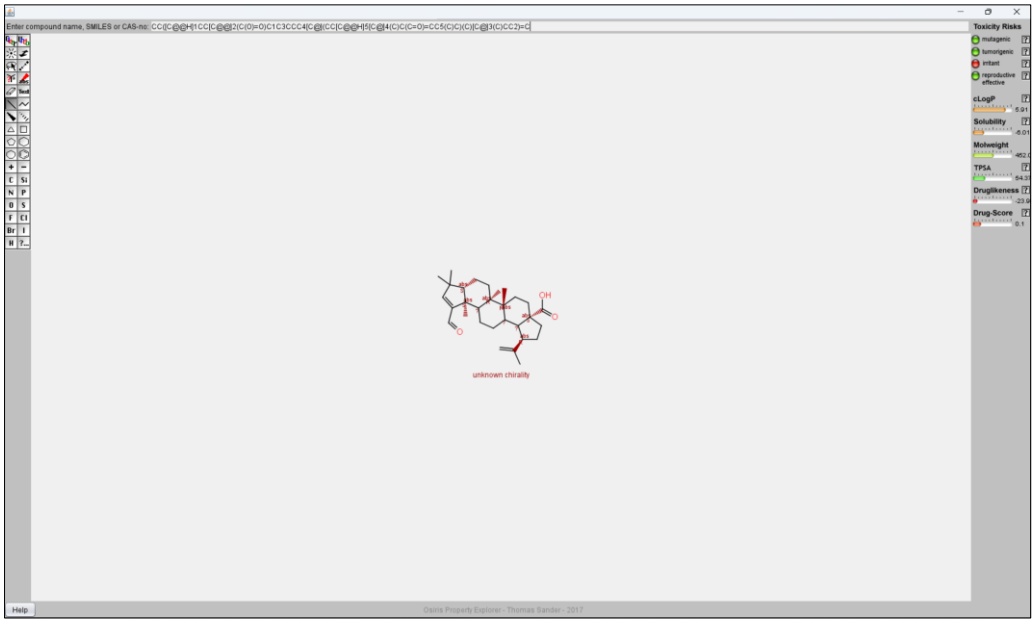


**Figure 13: Pharmacokinetic and toxicity profiling of zizyberanolic acid**

**Table 4: Pharmacokinetic Profiling of lead molecules**

| Compound | ADMET | | | |
|---|---|---|---|---|
| | Mutagenic | Tumorigenic | Irritant | Reproductive effectivity |
| Isoquercetin | NO | NO | Yes | NO |
| Zizyberanolic acid | NO | NO | NO | No |

**Table 5: Lipinski Properties of lead molecules**

| Compound | *cLogP* | Solubility | Mol.wt. | TPSA | Drug likeness | Drug score |
|---|---|---|---|---|---|---|
| Isoquercetin | 0.56 | -2.1 | 464 | 20.6 | 2.12 | 0.43 |
| Zizyberanolic acid | 0.01 | -6.01 | 470 | 54.3 | 2.31 | 0.1 |

**Table 6: Drug likeness of lead molecules**

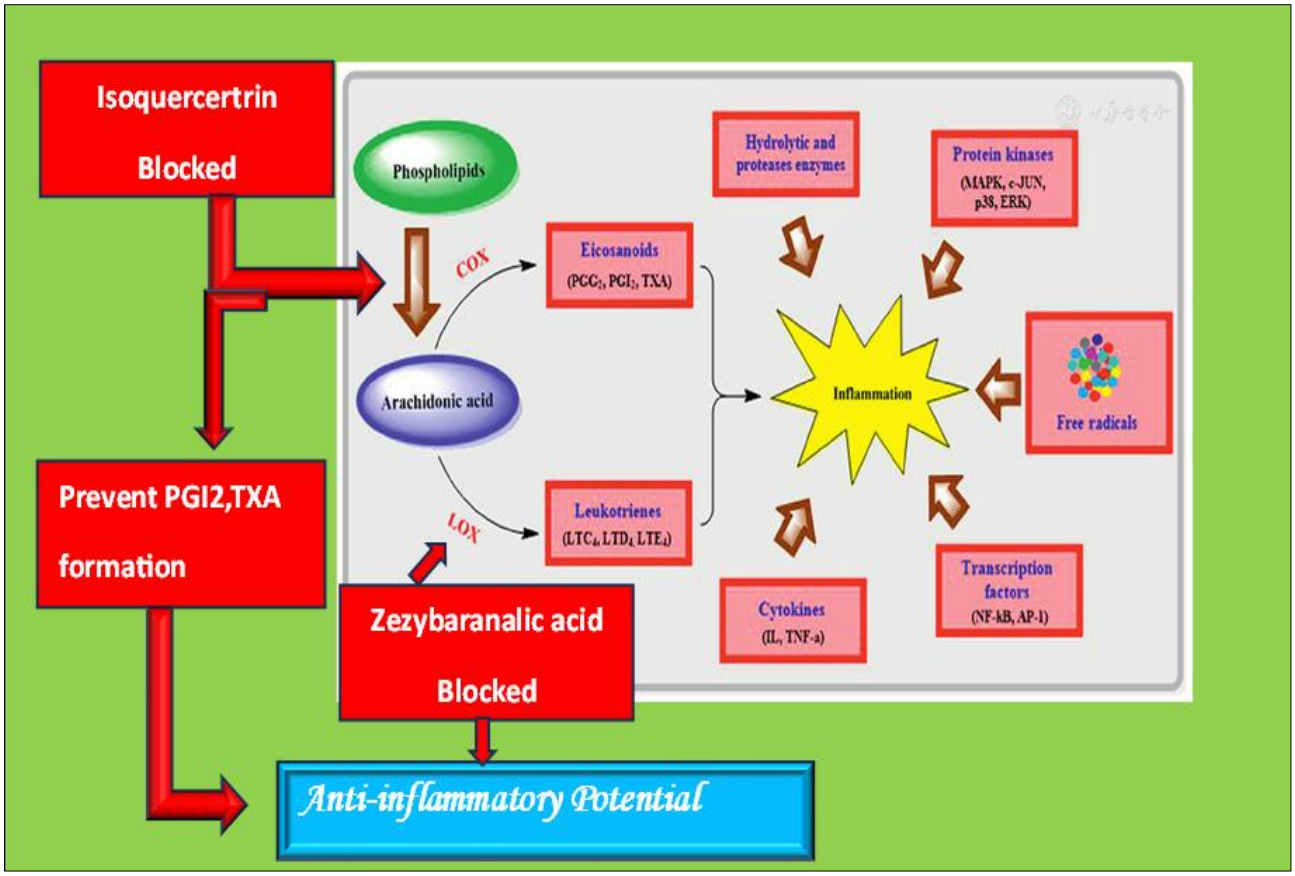
| Compound | Lipinski rule of five | H bond donar | H bond acceptor |
|---|---|---|---|
| Isoquercetin | Yes | 8 | 12 |
| Zizyberanolic acid | Yes | 2 | 4 |

## CONCLUSION

The scientific validation of anti-inflammatory action was conducted using in-silico molecular docking targeting COX-2 and 12 LOX as the proteins of interest. The literature survey indicates the presence of flavonoids and terpenoids in the fruit of Ziziphus jujuba. The chosen lead compound isoquercetrin exhibited inhibitory activity on the COX2 enzyme, thereby obstructing the conversion of arachidonic acid into proinflammatory prostaglandins, primarily PGE2, and initiating the creation of further proinflammatory chemokines and cytokines. Consequently, it demonstrated significant anti-inflammatory properties, while zizyberanalic acid, a new pentacyclic triterpenoid, successfully binds to and inhibits 12-LOX. This resulted in the suppression of the synthesis of leukotrienes, lipoxins, and cytokines (TNF-α, IL-1β). The synergistic activity of flavonoids and terpenoids in *Ziziphus jujuba* fruit renders it an anti-inflammatory agent.

### Divulgence of Investigation

The proposed mechanism of action of lead molecules against inflammation showed as:



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