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In-Silico Anti-Inflammatory Activity Evaluation of Phytochemical Through Molecular Docking Approach

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ABSTRACT
Inflammation can be detected when a wound swells up, gets red, and aches. Inflammation is the body's immune system's reaction to an irritant in broad terms. The irritant that may be possible that the irritant is a microbe, or it might also be a foreign body. This suggests that inflammation does not begin solely when a wound has been infected with germs, gushing pus, or if it is not healing properly. It begins when the body tries to protect itself against toxic irritation. Microorganisms, physical agents, chemicals, incorrect immune responses, and tissue loss, are the factors that may cause inflammation. Infectious agents, such as viruses and bacteria, are among the most prevalent inflammatory triggers. Inflammation is caused by viruses infiltrating and killing bodily cells, while bacteria release chemicals called endotoxins that can cause inflammation. This study aims to find suitable and effective bioactive compounds by screening numerous phytochemical molecules using a bioinformatic approach to develop an effective treatment for inflammation.
Methods: In this study, the proteins that play a crucial role in inflammation were used. The phytochemicals with known anti-inflammatory properties were scrutinized based on pathophysiological relevance, pharmacokinetic characteristics, and drug-like properties using the SwissADME web server. These phytochemicals were filtered through the process of docking. Further, shortlisted molecules' bioavailability and toxicity profiles were assessed using SwissADME and ProTox-II - Prediction of Toxicity of Chemicals web server, respectively.
Result: The two target receptors such as COX1 and iNOS were identified using a bibliographic study. Further, the screening of 59 phytochemicals resulted in 10 molecules that satisfied the Lipinski rule for both COX1 and iNOS. Owing to the binding affinity scores of 10 molecules, these 10 molecules with each receptor were chosen, resulting in 10 drug candidates. Out of these ten, four were safe and non-toxic, whereas the other 6 were partially toxic but as they have good binding affinity hence these molecules can also be used as drug candidates by modifying the LD50 value or by modification of the drug molecule to get the desired effect.
Conclusion: Screening numerous phytochemicals against target proteins led to promising therapeutic candidates. We believe these findings will aid in developing traditional medicine-based therapy methods and identifying viable hits for future discoveries in inflammation medication development.
Keywords: *Insilco, Molecular simulation, anti-inflammatory, COX-2, iNOS, toxicity*

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I. INTRODUCTION

Inflammation is an immune response to invading foreign objects to defend their host and protect it from any external invasion. It causes redness, swelling, pain, itching, burning, and increased temperature as a defense mechanism which can also be addressed as a sign of averting superficial threats [Roma Pahwa; Amandeep Goyal; Ishwarlal Jialal, 2000]. This immune response results in the release of antibodies and also directs increased blood flow to the affected area. In the course of the inflammation process, various inflammatory mediators, including pro and anti-inflammatory mediators, are synthesized and secreted from inflammatory cells and generated many cellular effects [G. Villarreal, J. Zagorski, S.M. Wahl, 2000]. The inflammation Is branched into further two types i.e., acute and chronic [Roma Pahwa; Amandeep Goyal; Ishwarlal Jialal, 2000]. Drugs that are in use in the treatment of pain and inflammatory conditions are either steroidal or NSAIDs. In the following research, we considered iNOS and COX-1. COX1, in particular, involves inflammation, COX-1 is required to treat the inflammation [Rahul Kumar Vishwakarma, Ayesha Negi, Devendra s. Negi, 2022]. These have symptoms such as Body pain, arthralgia, myalgia, Chronic fatigue and insomnia, Depression, anxiety, mood disorders, Weight gain or weight loss, Frequent infections, etc. [Roma Pahwa; Amandeep Goyal; Ishwarlal Jialal, 2000]

In inflammation cyclooxygenases-1 (COX-1) is a stimulus enzyme, so the activity of inhibition will be targeted for inflammation treatment. To treat inflammatory disease non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used. Commonly and widely used drugs mostly fail to inhibit COX-1

INOS, is another inducible enzyme, significantly, overproduction of nitric oxide (NO) implicating several pathophysiological states, like various inflammation, septic shock, and vascular dysfunction in diabetes and cancer patients.

Nitric oxide (NO) is a biological messenger molecule and neurotransmitter, which is synthesized by NO synthase (NOS) in multiple cells. NOS, specifically neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS), produce NO through the catalytic reaction of L-arginine and dioxygen. Distinct from nNOS and eNOS, iNOS is expressed just after being induced by extracellular stimuli such as stimulation with lipopolysaccharide (LPS)

Phenolic compounds and flavonoids manifest anti-inflammatory activities controlled by levels of inflammatory mediators including IL-1, IL-6, IL-10, TNF-α, NF-KB, NO, INOS and COX-2.[4]

The main aim of this study is to find suitable and effective bioactive compounds by screening numerous phytochemical molecules using a bioinformatic approach for the development of an effective treatment for breast cancer. In addition, marketed anti-cancer standard drugs were used for comparative evaluation.

II. MATERIALS AND METHODS USED:

1) **Protein Retrieval:** The three-dimensional structure of the proteins involved in Inflammation such as COX-1 (PDB ID-6Y3C) and iNOS (PDB ID-3HR4) were obtained from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/).

Table 1, *Target proteins and their respective functions.*

Proteins	Protein Description	Protein Function	PDB ID
COX-1	Cyclooxygenase-1	These lipid mediators play important roles in inflammation and pain and in normal physiological functions.	6Y3C
iNOS	Inducible nitric oxide synthase	iNOS functions as a nodal point for the development of inflammatory spinal	3HR4

2) **Ligand Preparation**: The list of phytochemicals with proven anti-inflammatory properties was retrieved from Dr. Duke's Phytochemical and Ethnobotanical Database (https://phytochem.nal.usda.gov/phytochem/search). Further, the canonical smiles and three-dimensional structure of phytochemicals were obtained from the PubChem library (https://pubchem.ncbi.nlm.nih.gov/).

3) **Pharmacological Properties of Compounds:** For initial screening, SwissADME (http://www.swissadme.ch/), an online web-based platform, was used, which evaluates the pharmaceutical fidelity of the drug candidates. Various attributes such as molecular weight, lipophilicity, number of hydrogen-bond acceptors, and donors were analyzed using this tool. As these attributes form the foundation for the Lipinski rule of five, any molecule deviating from the threshold values was eliminated from further analysis.

4) **Molecular Docking**: Before molecular docking analysis, proteins were pre-processed using Discovery Studio 2021. This step includes the removal of any hetero-groups and the addition of Hydrogen atoms and different charges. Further, the preparation of ligands and receptors in the PDBQT file format was carried out in the AutoDock tool. The molecular docking was carried out using Autodock Vina to understand the interaction between receptors and ligands. A rigid-flexible docking was performed after setting a grid box surrounding the binding sites of the receptors.

5) **Bioavailability Radar & Toxicity:** Drug-likelihood was comprehensively evaluated for candidates, considering six physicochemical properties such as solubility, molecular size, polarity, lipophilicity, saturation, and flexibility, and a bioavailability radar was obtained using the SwissADME tool (http://www.swissadme.ch/). At the same time, the ProTox-II (https://tox-new.charite.de/protox_II/index.php?site=compound_input) was used to predict the toxicity of the ligand

6) **Instrument used for software:** Asus Tuf F17 Gaming laptop, Intel(R) Core (TM) i5-10300H CPU @ 2.50GHz.

III. RESULT:

Despite the advancement in medical research and the evolution of drugs inflammation remain a serious threat if not treated properly. Our study mainly focused on finding the potential inhibitors of inflammatory agents using various computational tools. An attempt was made to explore various phytochemicals against two proteins involved in the progression in inflammatory activities and considered it as a potential drug target.

1) **Ligand Selection**: Molecules investigated in this study were selected from a diverse range of classes of phytochemicals such as Bavachinin, Bassic-acid, Berberastine, etc. Some of the molecules have already been reported in different literature as inhibitors of various enzymes involved in inflammation. In the current study, we examined 150 phytochemicals that have already been identified as anti-inflammatory compounds. Initially, pharmacokinetic properties were evaluated and Lipinski's rule of five was considered for the assessment of the drug-likeness of molecules. Out of all the selected phytochemicals, 10 molecules satisfied Lipinski's rule and were considered for further analysis.

2) **Molecular Docking**: Molecular docking is one of the most applied virtual screening methods that is used to predict the interaction between receptors and ligands. This method could predict both the binding affinity and the structure of the protein-ligand complex, which is valuable information for lead optimization. In the current study, the selected receptors were docked against the screened molecules to examine the binding affinity. The detailed information about the interactions between the amino acid residues of selected receptors and ligands is present in the supplementary file. The docking score of the ligands against the receptors used in the study is mentioned in the following study.

3) **Screening of Drug candidates for COX-1**: Amongst the 10 phytochemical compounds, the top five ligands viz Bavachinin, Berberastine, Calophyllolide, Alkannin, Artecanin, Bassic-acid exhibited the highest binding affinity and were considered as the best one (figure no 3-8). The binding energy of these following compounds ranged between -9.0 kcal/mol to -8.1 kcal/mol. The graphical representation of binding energy (-kcal/mol) of these phytochemicals are shown in the figure no 1.

4) **Screening of Drug Candidates for iNOS**: The top seven phytochemicals viz Chlorogenin, Acetylshikonin, Artemetin, Atractylochromene, Bavachinin, Bassic-acid, Berberastine (figure no 9-13) had the highest binding affinity -10.2 kcal/mol, -8.6 kcal/mol, -8.5 kcal/mol, -8.5 kcal/mol, -8.3 kcal/mol, -8.3 kcal/mol and -8.1 kcal/mol, respectively. The binding energy of the standard drugs was in the range of -8.0 kcal/mol to -10.0 kcal/mol. The graphical representation of binding energy (-kcal/mol) of these phytochemicals are shown in the figure no 2.

Table 2, *The binding affinity of COX and iNOS with selected ligands*

COX-1	
MOLECULE	Binding Energy n(ΔG) (kcal/mol)
BAVACHININ	-9.0
BERBERASTINE	-8.8
CALOPHYLLOLIDE	-8.8
ALKANNIN	-8.5
ARTECANIN	-8.1
BASSIC-ACID	-8.1

iNOS	
MOLECULE	Binding Energy n(ΔG) (kcal/mol)
CHLOROGENIN	-10.2
ACETYLSHIKONIN	-8.6
ARTEMETIN	-8.5
ATRACTYLOCHROMENE	-8.5
BAVACHININ	-8.3
BASSIC-ACID	-8.1
BERBERASTINE	-8.1

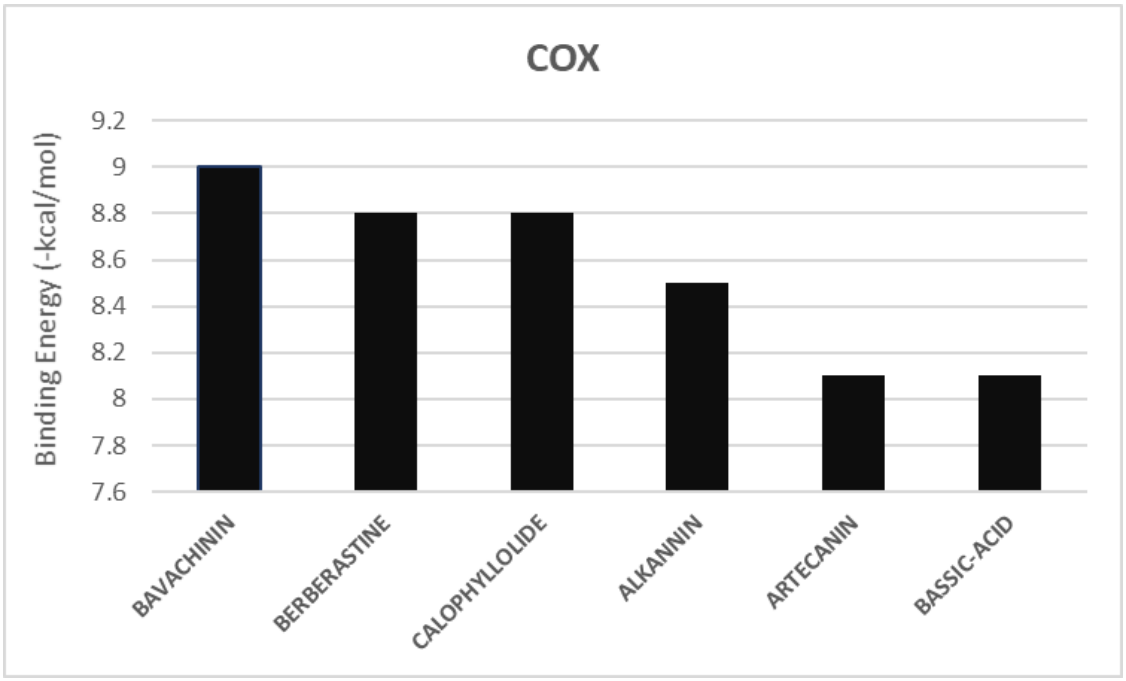


Figure 1, Graphical representation of the binding energy (-kcal/mol) of top 6 phytochemicals with cox.

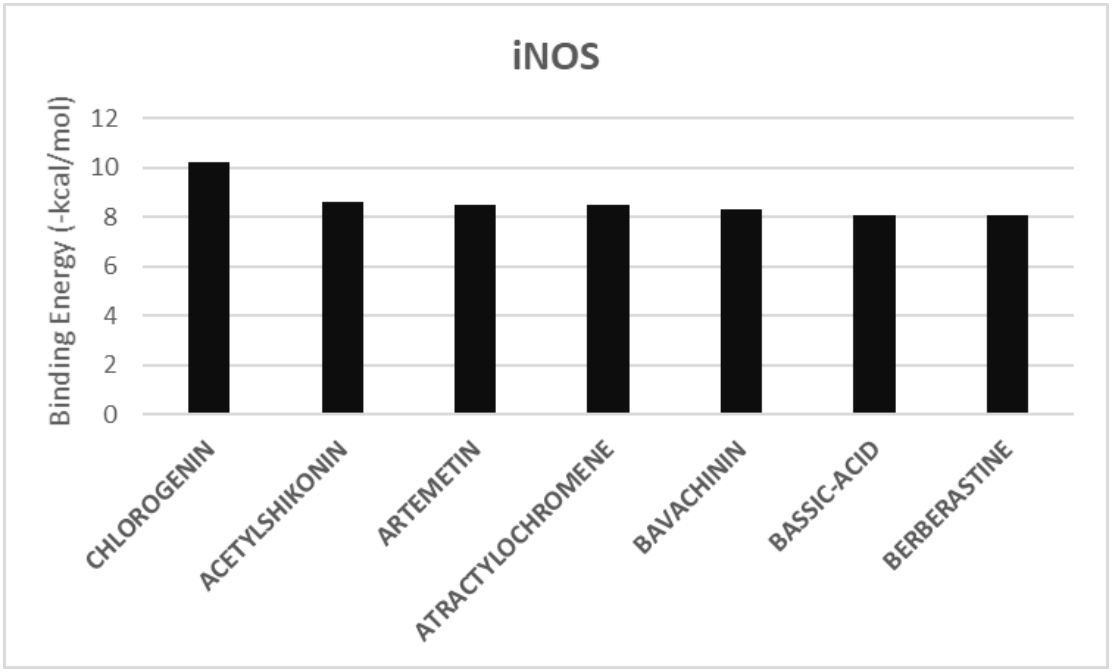


Figure 2, Graphical representation of the binding energy (-kcal/mol) of top 7 phytochemicals with iNOS.

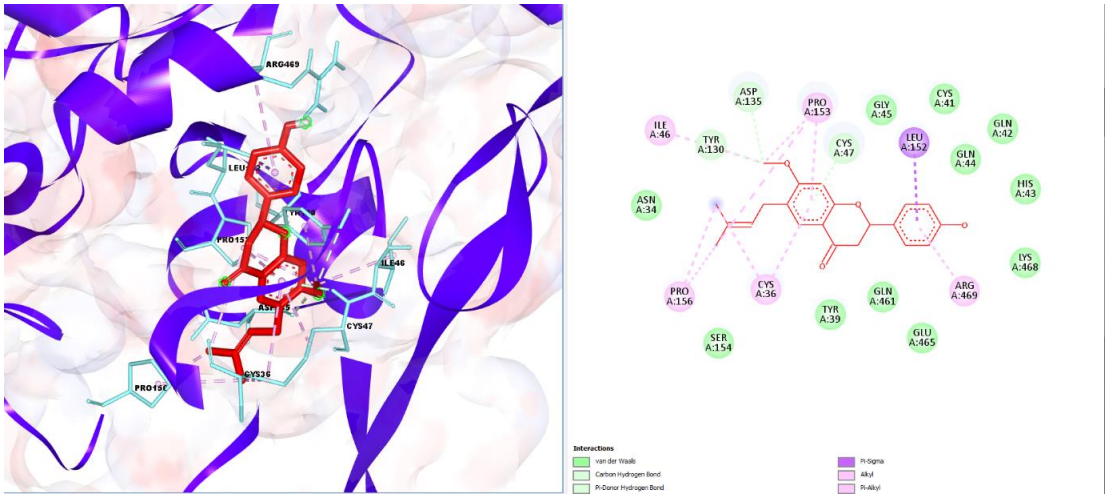


Figure 3, Docking pose of Bavachinin with the entity of COX (left), 2d interaction plot of COX-Bavachinin complex visualized(right).

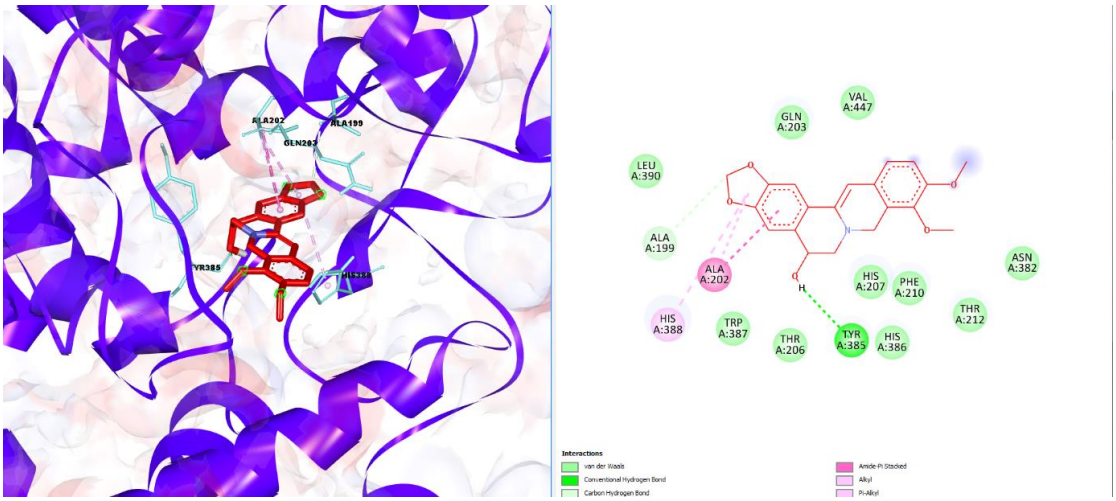


Figure 4, Docking pose of Berberastine with the entity of COX (left), 2d interaction plot of COX-Berberastine complex visualized(right).

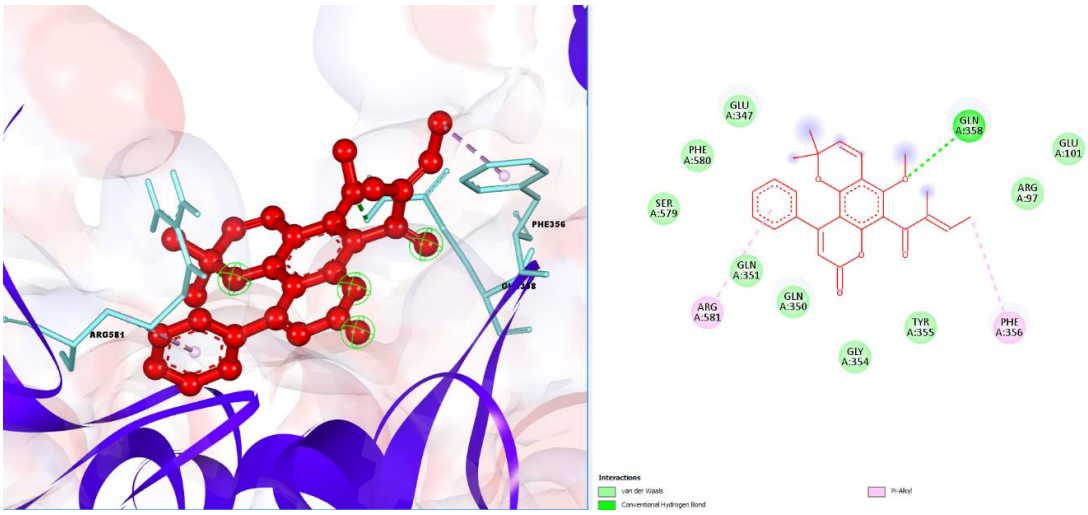


Figure 5, Docking pose of Calophyllolide with the entity of COX (left), 2d interaction plot of COX-Calophyllolide complex visualized(right).

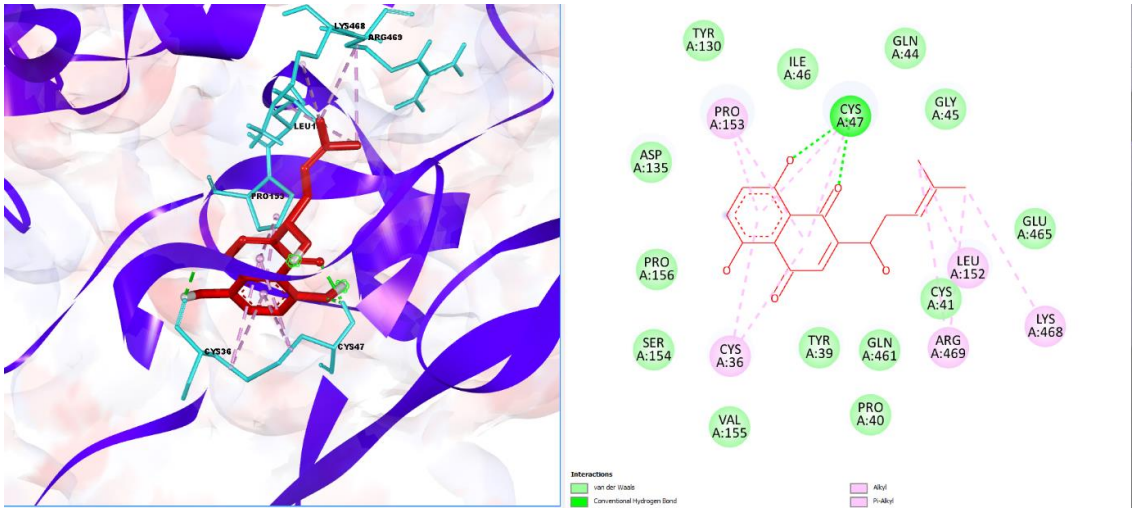


Figure 6, Docking pose of Alkannin with the entity of COX (left), 2d interaction plot of COX-Alkannin complex visualized(right).

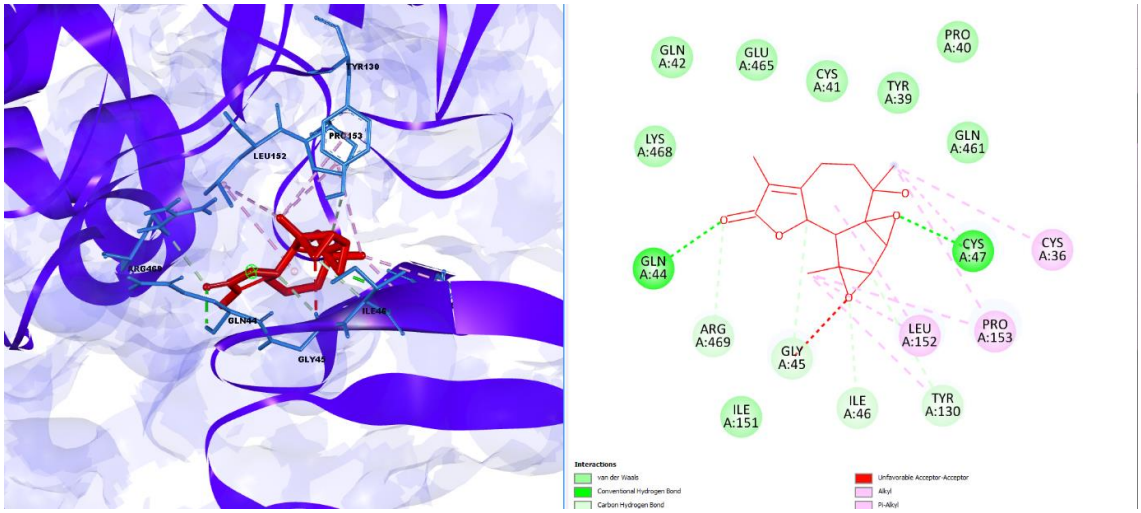


Figure 7, Docking pose of Artecanin with the entity of COX (left), 2d interaction plot of COX-Artecanin complex visualized(right).

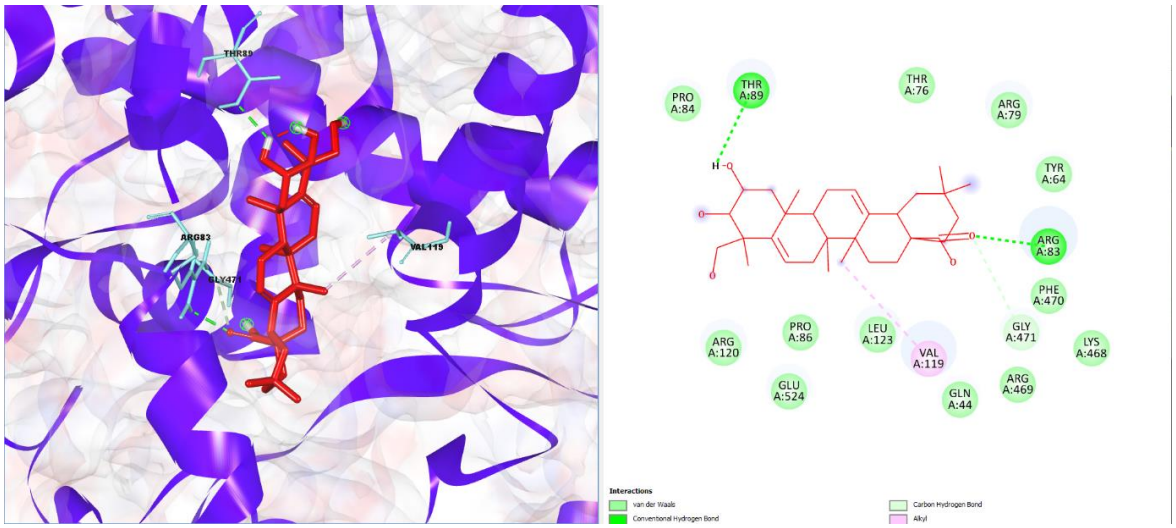


Figure 8, Docking pose of Bassic-acid with the entity of COX (left), 2d interaction plot of COX-Bassic-acid complex visualized(right).

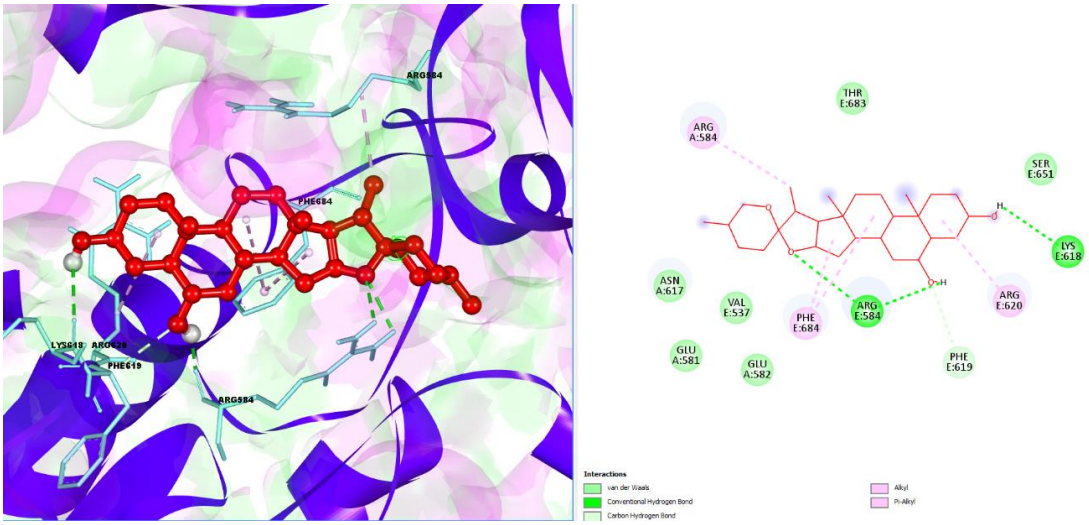


Figure 9, Docking pose of Chlorogenin with the entity of iNOS (left), 2d interaction plot of iNOS-Chlorogenin complex visualized(right).

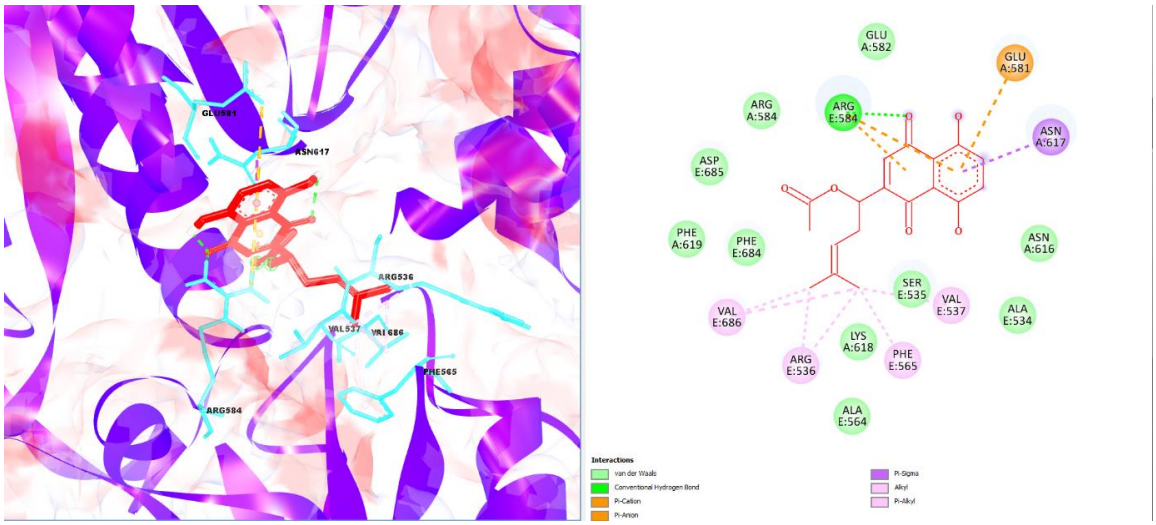


Figure 10, Docking pose of Acetylshikonin with the entity of iNOS (left), 2d interaction plot of iNOS-Acetylshikonin complex visualized(right).

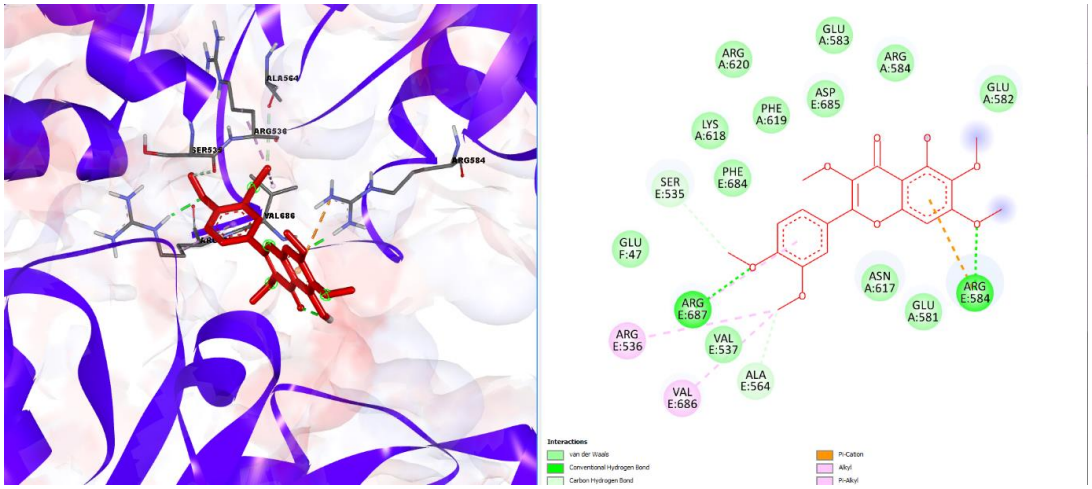


Figure 11, Docking pose of Artemetin with the entity of iNOS (left), 2d interaction plot of iNOS-Artemetin complex visualized(right).

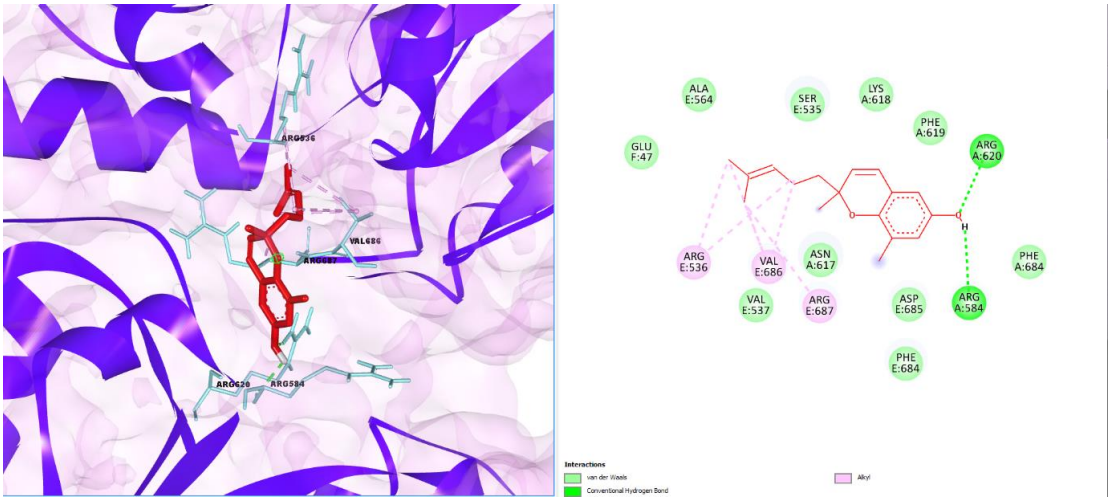


Figure 12, Docking pose of Atractylochromene with the entity of iNOS (left), 2d interaction plot of iNOS-Atractylochromene complex visualized(right).

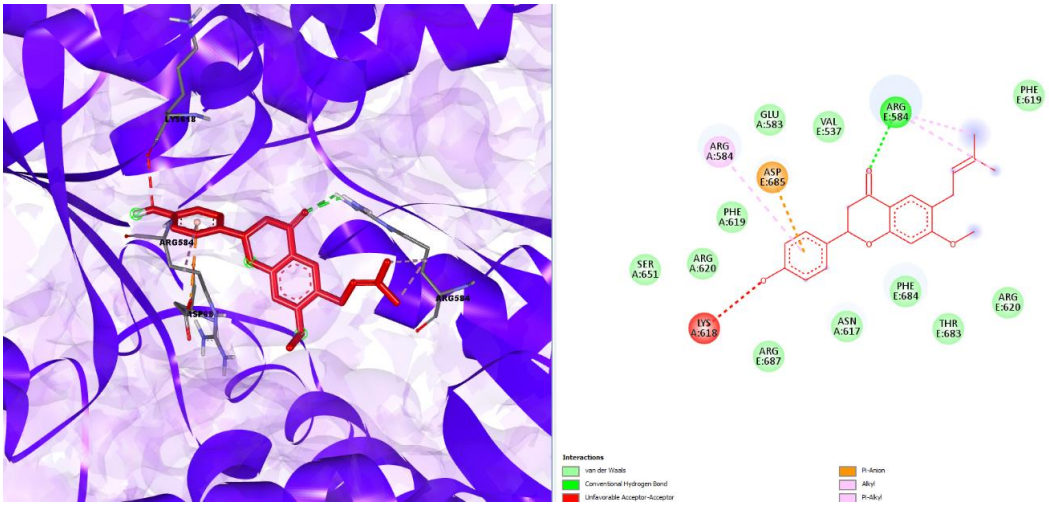


Figure 13, Docking pose of Bavachinin with the entity of iNOS (left), 2d interaction plot of iNOS-Bavachinin complex visualized(right).

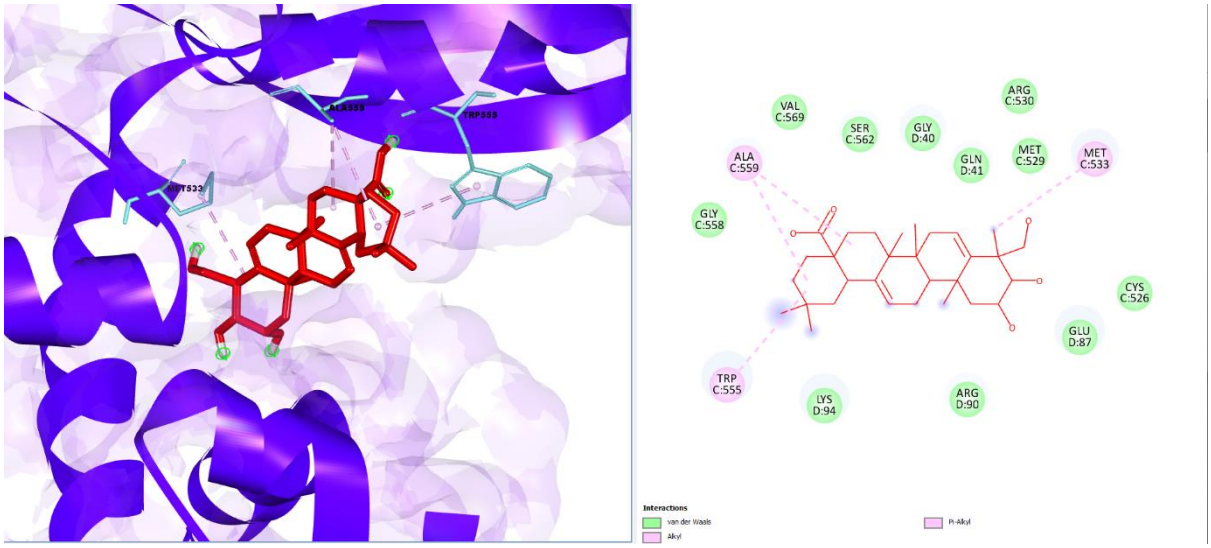


Figure 14, Docking pose of Bassic-acid with the entity of iNOS (left), 2d interaction plot of iNOS-Bassic-acid complex visualized(right).

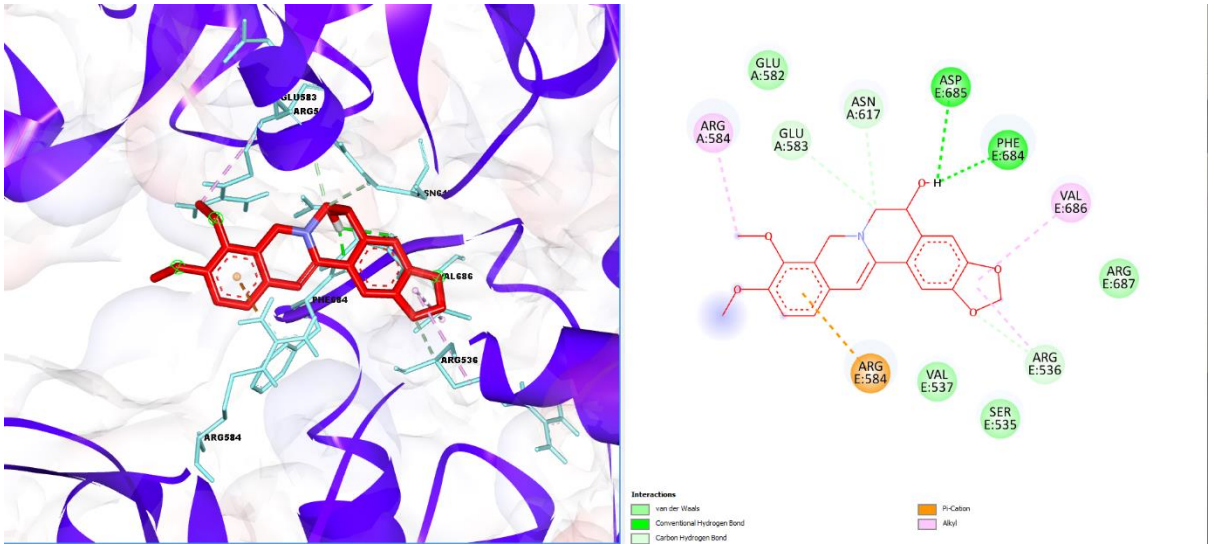
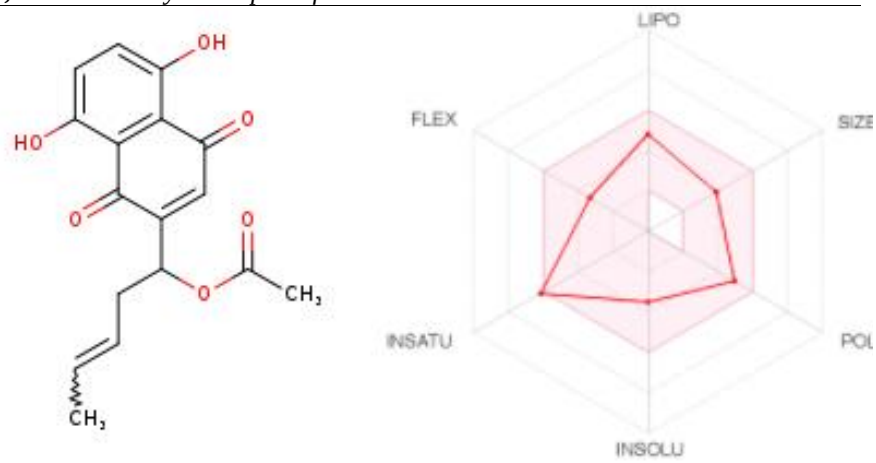


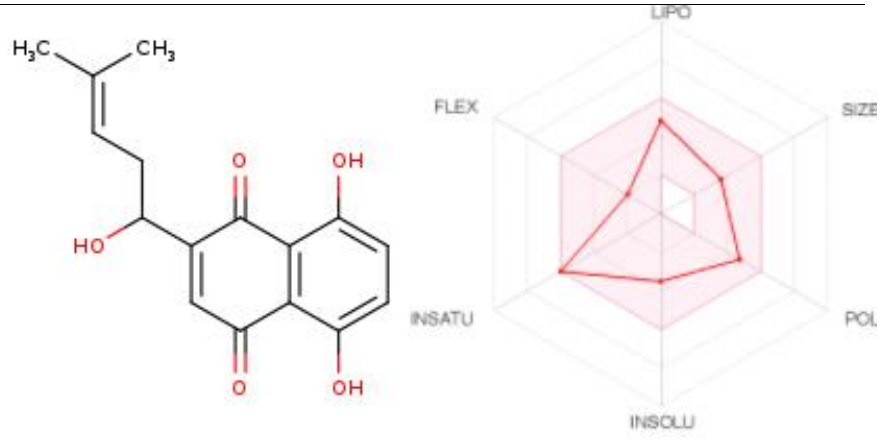
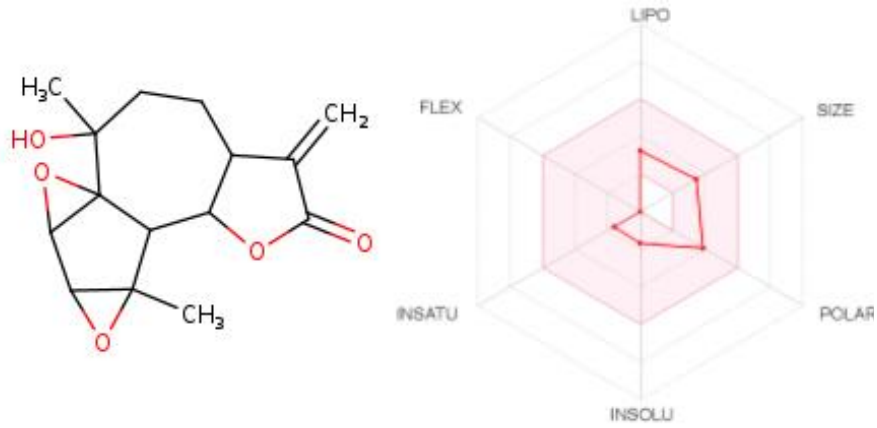
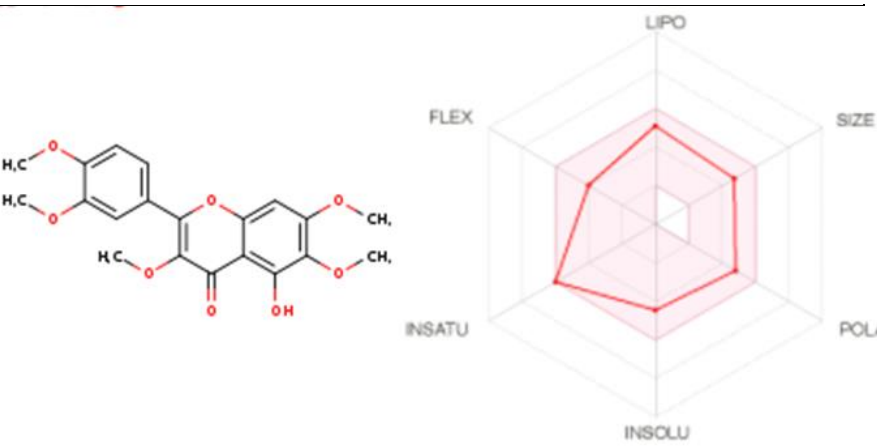
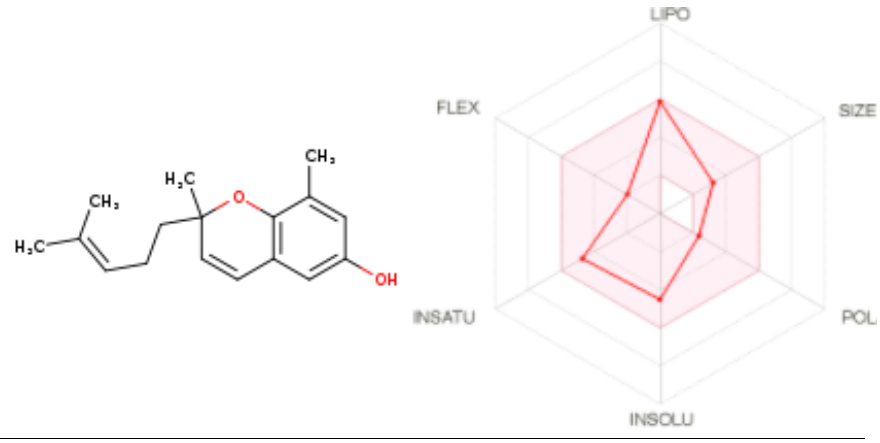
Figure 15, Docking pose of Berberastine with the entity of iNOS (left), 2d interaction plot of iNOS-Berberastine complex visualized(right).

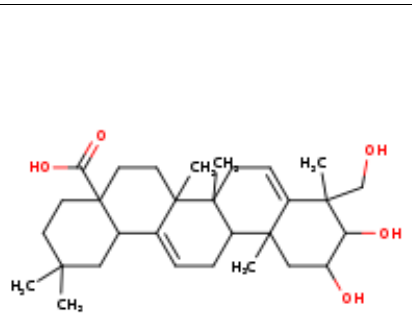
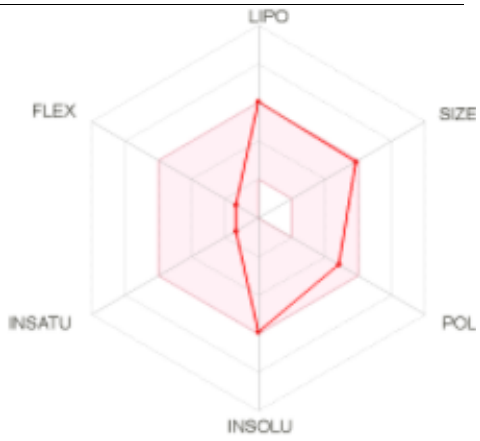
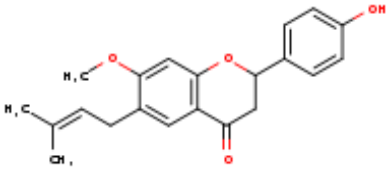
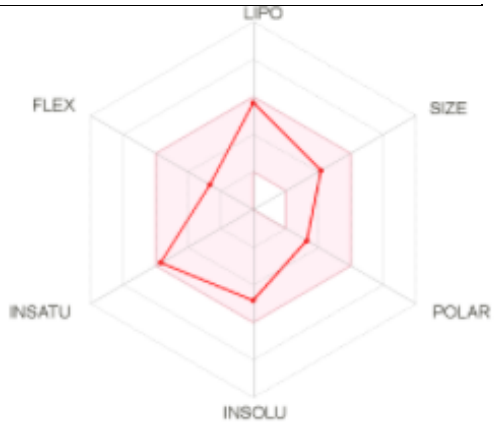
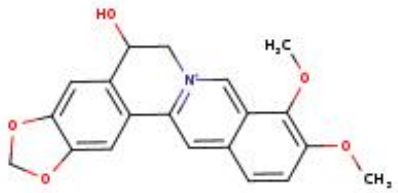
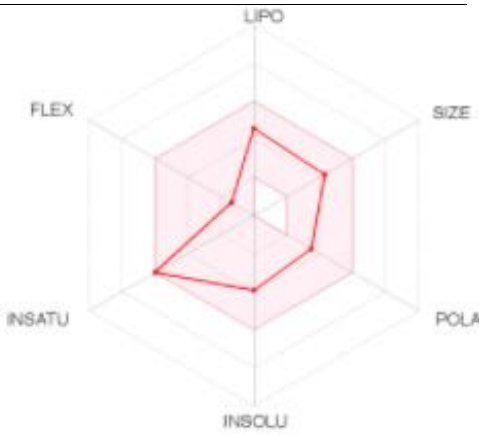
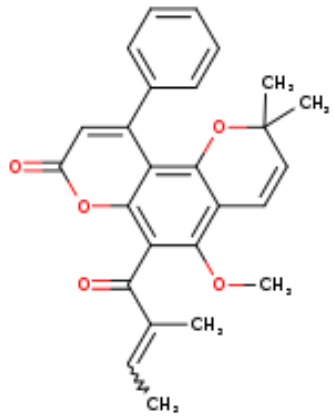
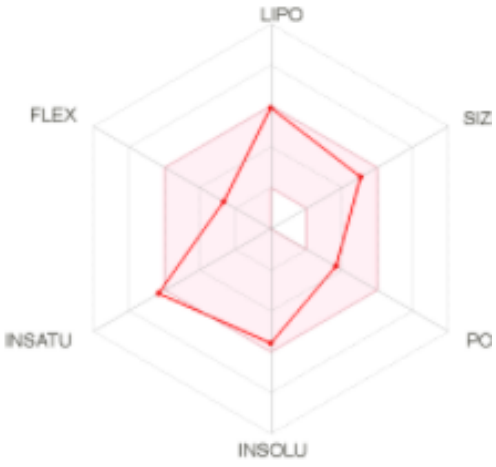
IV. DISCUSSION:

Inflammatory disease is the leading cause of death worldwide. The World Health Organisation (WHO) claims that chronic inflammation poses a serious threat to human health. Stroke, chronic respiratory diseases, heart issues, cancer, obesity, and diabetes kill three out of every five people worldwide. The need for an effective treatment for inflammation is urgent due to the long history of its causes. In our study, pharmacokinetic criteria were used to screen 150 phytochemicals, and 50 lead molecules were found to address this issue. When these 50 molecules interact with COX and iNOS, the top 10 potential binders are created. After being tested for bioavailability and toxicity on these top 10 molecules, the phytochemicals Bavachinin, Berberastine, Calophyllolide, Alkannin, Artecanin, Bassic-acid, Chlorogenin, Acetylshikonin, Artemetin, and Atractylochromene were also found to be secure and non-toxic. Thus, it can be said that these molecules are effective at neutralising the targeted proteins. We believe the results will aid in the development of conventional medicine-based therapy methods in addition to identifying promising hits for potential lead optimization in the development of anti-inflammatory medications.

Table 3, *Bioavailability radar plot of 10 shortlisted molecules.*

ACETYLSHIKONIN	

ALKANNIN	
ARTECANIN	
ARTEMETIN	
ATRACTYLOCHROMENE	

BASSIC-ACID	 
BAVACHININ	 
BERBERASTINE	 
CALOPHYLLOLIDE	 

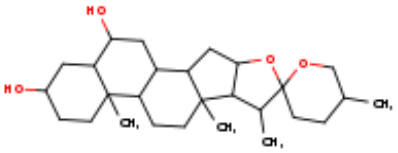
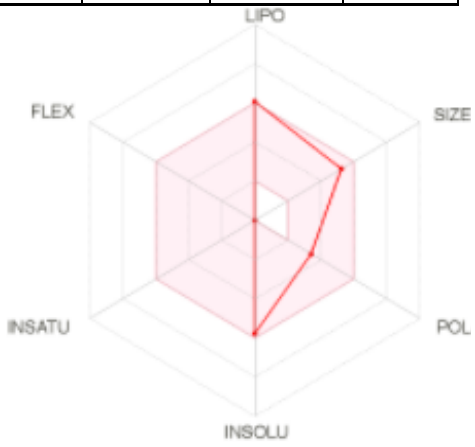
Ligands	Class	LD 50 (mg/kg)	Hepato-toxicity	Carcino-genicity	Immuno -toxicity	Mutagen -icity	Cyto-toxicity
ACETYLSHIKONIN	4	1000mg/kg	Inactive	Inactive	Active	Inactive	Active
ALKANNIN	4	1000mg/kg	Inactive	Inactive	Active	Inactive	Active
ARTECANIN	6	39800mg/kg	Inactive	Inactive	Active	Inactive	Inactive
ARTEMETIN	5	5000mg/kg	Inactive	Inactive	Active	Inactive	Inactive
ATRACTYLOCHROMENE	4	750mg/kg	Inactive	Inactive	Inactive	Inactive	Inactive
BASSIC-ACID	4	2000mg/kg	Inactive	Inactive	Inactive	Inactive	Inactive
BAVACHININ	4	2000mg/kg	Inactive	Inactive	Active	Inactive	Inactive
BERBERASTINE	3	200mg/kg	Inactive	Active	Active	Active	Active
CALOPHYLLOLIDE	5	2500mg/kg	Inactive	Inactive	Active	Inactive	Inactive
CHLOROGENIN	5	2600mg/kg	Inactive	Inactive	Active	Inactive	Inactive
CHLOROGENIN							

Table no 4, Toxicity profile of the shortlisted molecule

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REFERENCE

[1]. https://www.ncbi.nlm.nih.gov/books/NBK493173/

[2]. G. Villarreal, J. Zagorski, S.M. Wahl Inflammation, (2000). Acute Ethno pharmaco., 72

[3]. Rahul Kumar Vishwakarma, Ayesha Negi, Devendra s. Negi, (2022).: Abortitristoside A and Desrhamnosylverbanscoside The Potential COX-2 Inhibitor from the Leaves of Nyctanthes Arbor-tristis as Anti-inflammatory Agents Based on the in-vitro Assay, Molecular Docking and ADMET Prediction). https://doi.org/10.21203/rs.3.rs-1345800/v

[4]. Sounok Sengupta, Ratul Bhowmik, Satarupa Acharjee, and Suchandra Sen (2021). In-Silico Modelling of 1- 3- [3-(Substituted Phenyl) Prop-2-Enoyl) Phenyl Thiourea Against Anti-Inflammatory Drug Targets

[5]. Thamaraiselvi L, Selvankumar T, Wesely E, et al, (2021), In-silico molecular docking analysis of some plant-derived molecules for anti-inflammatory inhibitory activity

[6]. Utami W, Aziz H, Fitriani I, Zikri A, Mayasri A, and Nasrudin D, (2020). In silico anti-inflammatory activity evaluation of some bioactive compound from ficus religiosa through molecular docking approach.

[7]. Syed Shams ul Hassan, Wei-Dong Zhang, Hui-Zi Jin, Syed Hussain Basha &S. V. S. Sasi Priya, (2020) In-silico anti-inflammatory potential of guaiane dimers from Xylopia vielana targeting COX-2.

[8]. Bahuguna, Ashutosh Ramalingam, Srinivasan Arumugam, Aruna Natarajan, Devarajan Kim, Myunghee, (2020). Molecular and in silico evidence explain the anti-inflammatory effect of Trachyspermum Ammi essential oil in lipopolysaccharide-induced macrophages.

[9]. Kumar A, Fausto M. Robbins, (2007) Basic Pathology. 8th Ed. Saunders: Elsevier :31-41.

[10]. Furman, D. et al, (2017). Expression of specific inflammasome gene modules stratifies older individuals into two extreme clinical and immunological states. Nat. Med. 23, 174–184

[11]. Netea, M. G. et al, (2017) A guiding map for inflammation. Nat. Immunol. 18, 826–831.

[12]. Slavich, G. M. (2015). Understanding inflammation, its regulation, and relevance for health: a top scientific and public priority. Brain Behav. Immun. 45, 13–14.

[13]. Bennett, J. M., Reeves, G., Billman, G. E. & Sturmberg, J. P. (2018). Inflammation–nature's way to efficiently respond to all types of challenges: implications for understanding and managing "the epidemic" of chronic diseases. Front. Med.5, 316

[14]. http://www.medicinenet.com/nonsteroidal_antiinflammatory_drugs/article.htm. Nonsteroidal Anti-inflammatory Drugs (NSAIDs), Inc. 2012.

[15]. Chetty M, Sivaji K and Rao KT. (2008). Flowering plants of Chittoor district Andhra Pradhesh, 1 edition, Published by student offset printer, Tirupati,; 193. 3.

[16]. Rathee JS, Shyam, Hassarajani and Subrata C. (2007). Antioxidant activity of Nyctanthes arbortristis leaf extract. Food Chem.; 103:1350-1357.

[17]. Omkar A, Jeeja T and Chhaya G. (2006). Evaluation of anti-inflammatory activity of Nyctanthes arbortristis and Onosma echiodes. Phrmacog. mag.

[18]. Chronic inflammation …… citation left (for reference)

[19]. Mital N. Manvar…(shodhganga) …. (For reference)

[20]. Derle DV, Gujar KN, Sagar SH, (2006) Adverse effects associated with the use of nonsteroidal anti-inflammatory drugs: An overview. Indian Journal of Pharmaceutical Sciences. http://www.ijpsonline.com/text.asp?2006/68/4/409/27809

[21]. Tabas Ira, Christopher G. (2013) Anti-Inflammatory Therapy in Chronic Disease: Challenges and Opportunities. Science (80-).; 339(6116): 166–72.

[22]. Orlando BJ, Malkowski MG. (2016) Substrate-selective inhibition of cyclooxygenase-2 by fenamic acid derivatives is dependent on peroxide tone. J Biol Chem [Internet]. 2016 http://dx.doi.org/10.1074/jbc.M116.725713

[23]. Dannhardt, G., & Kiefer, W. (2001). Cyclooxygenase inhibitors-current status and future prospects. European Journalofmedicinalchemistry,36(2),109–126. https://doi.org/10.1016/s0223-5234(01)01197-7

[24]. Mamidisetti, Y. D., Yammada, N., Kumar, H., Siddamsetty, V. B., & Boggula, N (2018) Phytochemical and analgesic, anti-inflammatory screening of methanolic extract of Ficus religiosa fruits: An in vivo design The Pharma Innovation 7 6 69-74

[25]. Remadevi V, Mohan Lathika L, Sasikumar Sujatha A, Sreeharshan S (2019) Ficus extract-A promising agent for anti-mammary tumorigenesis: A review on the current status and future possibilities Phytotherapy research 33 6 1597-1603

[26]. Bhalerao SA, Sharma AS (2014) Ethnomedicinal, the phytochemical and pharmacological profile of Ficus religiosa Roxb. Int J Curr Microbiol App Sci 3 11 528-538

[27]. Kumar A, Sandeep D, Tomer V, Gat Y, Kumar V (2018) Ficus religiosa: A wholesome medicinal tree Journal of Pharmacognosy and Phytochemistry 7 4 32-37

Singh S, Jaiswal S (2014) Therapeutic properties of Ficus religiosa Int J Eng Res Gen Sci 2 5 149-158

[28]. Singh S, Ahmad MP, Sarraf DP, Mishra C, Singh PK (2018) Anticonvulsant effect of aqueous extract of the aerial root of Ficus religiosa in animal models Journal of Drug Delivery and Therapeutics 8 1 13-16

[29]. Anjum, K., Abbas, S. Q., Akhter, N., Shagufta, B. I., Shah, S. A. A., & Hassan, S. S. U. (2017). Emerging biopharmaceuticals from bioactive peptides derived from marine organisms. Chemical Biology & Drug Design, 90(1), 12–30. https://doi.org/10.1111/cbdd.12925

[30]. Anjum, K., Abbas, S. Q., Shah, S. A. A., Akhter, N., Batool, S., & Hassan, S. S. U. (2016). Marine sponges as a drug treasure. Biomolecules & Therapeutics, 24(4), 347–362. https://doi.org/10.4062/biomolther.2016.067

[31]. Hassan, S. S., Ul, Anjum, K., Abbas, S. Q., Akhter, N., Shagufta, B. I., Shah, S. A. A., & Tasneem, U. (2017). Emerging biopharmaceuticals from marine actinobacteria. Environmental Toxicology and Pharmacology, 49, 34–47. https://doi.org/10.1016/j.etap.2016.11.015

[32]. Hassan, S. S u., & Shaikh, A. L. (2017). Marine actinobacteria as a drug treasure house. Biomedicine & Pharmacotherapy & Biomedecine & Pharmacotherapies, 87, 46–57. https://doi.org/10.1016/j.biopha.2016.12.086

[33]. Khan, S. A., Imam, S. M., Ahmad, A., Basha, S. H., & Husain, A. (2018). Synthesis, molecular docking with COX 1& II enzyme, ADMET screening, and in vivo anti-inflammatory activity of oxadiazole, thiadiazole and triazole analogs of felbinac. Journal of Saudi Chemical Society, 22(4), 469–484. https://doi.org/10.1016/j.jscs.2017.05.006

[34]. Xiao, Y., Zhu, S., Wu, G., Ul Hassan, S. S., Xie, Y., Ishaq, M., Sun, Y., Yan, S., Kai, Qian, X., Ping., & Jin, H. z. (2020). Chemical Constituents of Vernonia parishii. Chemistry of Natural Compounds, 56(1), 134–136. https://doi.org/10.1007/s10600-020-02963-x

[35]. Guo, Y. G., Xie, Y. G., Wu, G. J., Cheng, T. F., Zhu, S. L., Yan, S. K., Jin,

[36]. H. Z., & Zhang, W. D. (2019). Xylopidimers A-E, five new guaiane dimers with various carbon skeletons from the roots of xylopia vielana. ACS Omega, 4(1), 2047–2052. https://doi.org/10.1021/acsomega.8b00828

[37]. Xie, Y. G., Zhao, X. C., ul Hassan, S. S., Zhen, X. Y., Muhammad, I., Yan, S., Kai, Yuan, X., Li, H., Liang., & Jin, H. z. (2019). One new sesquiterpene and one new iridoid derivative from Valeriana amurensis. Phytochemistry Letters, 32, 6–9. https://doi.org/10.1016/j.phytol.2019.04.020

[38]. T. Kaur, R.K. Bijarnia, S.K. Singla, C. Tandon, (2009). In vivo efficacy of Trachyspermum ammi anticalcifying protein in urolithiatic rat model, J. Ethnopharmacol. 126 (3) 459–462. doi:10.1016/jjep2009.09.015

[39]. R. Bairwa, R.S. Sodha, B.S. Rajawat, (2012). Trachyspermum ammi, Pharmacogn. Rev. 611: 56–60.

[40]. C. Thangam, R. Dhananjayan, (2003). Anti-inflammatory potential of the seeds of Carum copticum Linn, Indian J. Pharmacol. 35 388–391.

[41]. Zhang QL, Zhang J, Xia PF, Peng XJ, Li HL, Jin H, Li Y, Yang J, Zhao L (2019) Anti-inflammatory activities of gentiopicroside against iNOS and COX-2 targets Chinese Herbal Medicines 11 1 108-12

[42]. Yatam S, Gundla R, Jadav SS, reddy Pedavenkatagari N, Chimakurthy J, Kedam T (2018) Focused library design and synthesis of 2-mercapto benzothiazole linked 1, 2, 4-oxadiazoles as COX2/5-LOX inhibitors Journal of Molecular Structure 1159 193-204

[43]. O. Trott, A. J. Olson, (2010). # # AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31 455-461