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Research Article

Computational Studies of Vitexin isolated from *Vitex negundo* against Rheumatoid Arthritis Targets

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ABSTRACT

One chronic inflammatory autoimmune disease that needs attention to be treated is rheumatoid arthritis (RA). Especially, RA encounters more female cases that manifest progressive destruction of bone and cartilage. Expression of more inflammatory cytokines and matrix metalloproteins at the synovial tissue destruct collagen structure and worsen the condition. Generally, anti-inflammatory drugs and immune-modulatory agents are used to deduce the progression of the disease. Such chemical entities have proven many side effects and expensive. This study provides a glimpse of structural based drug designing, using bioactive pharmakon, from a folk chloric herb. *Vitex negundo* is a traditionally available plant that has been reported to possess pharmaco-beneficiary bioactive flavonoids, especially vitexin. Virtual screening involves docking of vitexin with three different targets responsible for triggering the collagenase enzyme achieved using AutoDock. The binding energy of vitexin and TNF α receptor1 (TNFR1) was found to be slightly higher when compared to docking results of TNF α converting enzyme (TACE) and human inhibitory kinase β (hIKβ) proteins. The molecular interactions were visualized using BIOVIA discovery studio visualizer. This study may become evident that vitexin has enhanced pharmacological benefits that need to be ensured through *in vitro* and *in vivo* assays.

Introduction

Rheumatoid arthritis (RA) is a progressive chronic inflammatory autoimmune disease that leads to the destruction of cartilage and bone. More than 15% of the world's populations are affected by encountering higher female cases. Since the exact cause is not known, disease-modifying anti-inflammatory drugs (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are administered even in the advanced stages of RA.[1] DMARDs and NSAIDs are better immuno-modulatory agents reported adverse side effects after prolonged use.[2] Natural molecular entities may replace chemical leads in healing diseases. Biological compounds are cost-effective, ease of production, safety, and efficacy levels also high.[3] *V. negundo* is an annual herb available throughout India, commonly called as "*nirgundi*" by *Ayurvedic* pharmacopoeia of India reported excellent anti-inflammatory property, which may occur due to the presence of bioactive pharmakon, vitexin.[4] As an alternate, a biological pharmakon isolated from *V. negundo* may satisfy the above need. Vitexin is a flavone glycoside considered an important flavonoid of interest that possesses ample pharmacological benefits.[5] It is reported that vitexin serves as an excellent anti-inflammatory pharmakon, which reduces the level of cytokine expression at the site of inflammation.[6] TNF α is an important cytokine that plays a crucial role in the immune system's signaling

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pathways and inflammation.[7] TACE is responsible for the activation of TNF α to bind its receptor TNFR.[8] A human inhibitory kinase (Ikβ) upon activation facilitates the upregulation of translocated NFkβ at the nuclear level and enhances matrix-metallo protein expression.[9] Matrix metallo proteins (MMPs) abundant in synovial tissue, once activated may significantly destruct extracellular matrix, and erode bone and cartilage.[10] Attenuation of TACE, TNFR1, and human IKβ may reduce the sequestering of more inflammatory cytokines and MMPs.

Structure-based drug discovery promotes a better understanding of three-dimensional protein targets that actively performs in a disease. Computer-aided drug discovery may highlight and scientifically validate the traditional folkloric applications of certain herbs and their biologically active compounds.[11] The efficacy of vitexin to repress the above targets may examine by computational molecular docking analysis. The pharmacokinetic profile of the vitexin has been evaluated to check the pharmacological potency. Virtual screening may help to assure drug-likeness of vitexin and may pay the way for further *in vitro* and *in vivo* studies in the treatment of rheumatoid arthritis.

Materials and Methods

Protein Preparation

The three-dimensional structure file of drug targets, such as, TACE, TNFR1, and hIKβ was retrieved from the Research Collaboratory for Structural Bioinformatics-Protein data Bank (RCSB-PDB) database. All water molecules were removed, and Gasteiger charges were assigned to the protein structure.[12]

Ligand Preparation

SMILES structure of vitexin retrieved from PubChem was submitted in SwissADME, an online tool that predicts the selected compound's efficiency by checking various physicochemical properties.[13] *In silico* toxicity predictor tool predicts the lethal dose 50 (LD_{50}) value in mg/kg body weight. Protox II, an online tool, predicts and assesses the toxicity of vitexin (Table 1).[14] 2D structure of vitexin was drawn using ChemSketch from the SMILES data available in the PubChem database. The 3D structure of vitexin was generated, optimized, and saved in a .mol file. Further conversions were made using the open babel molecular converter program and saved in PDB format.[12]

Protein-Ligand Interaction Prediction

AutoDock is one of the best suites of automated docking tools. The software is used for modeling flexible small molecules, such as, drug molecule binding to target proteins of known structure. Genetic algorithms are used in this suite for checking the conformational search. AutoDock is a user-friendly tool to perform blind docking, where the location of the binding site is not known. Molecular docking was performed using the AutoDock Tools 4.2 graphical user interface, which generates ten conformations (poses) of the protein-ligand complex customized in the order from lowest to highest binding free energy (ΔG).[15]

BIOVIA Discovery Studio Visualizer

BIOVIA visualization tool helps to visualize the docking interactions between vitexin and different protein targets of interest in both 2D and 3D. Emphasized images of different interactions are easily visualized in the Discovery Studio tool.[16]

Result and Discussion

Vitexin Structure and its Properties

The 2D (Fig. 1) and 3D structure (Fig. 2) of vitexin were retrieved and saved in PDB format. Membrane permeability and bioavailability of vitexin are always assessed with some basic molecular properties, such as, log p (partition coefficient), molecular weight (MW), or counts of hydrogen bond acceptors and donors in a molecule.[17] These molecular properties were used in formulating the "rule of five."[18] The rule states that most molecules with good membrane permeability have molecular weight ≤ 500, calculated octanol-water partition coefficient, log P ≤ 5, hydrogen bond donors ≤ 5, and acceptors ≤ 10.[19]

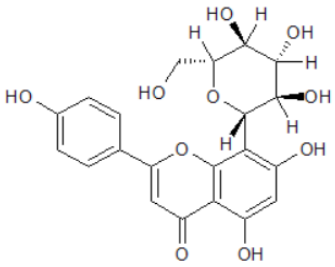


Fig. 1: 2D structure of vitexin

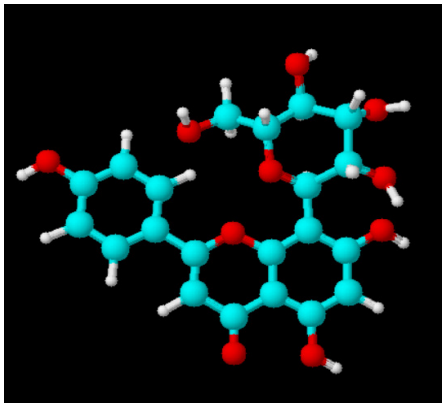


Fig. 2: 3D structure of vitexin

Table 1: LD_{50} and toxicity class for vitexin

Ligand name	*LD_{50} mg/kg*	*Toxicity class*
Vitexin	1,213	4

Therefore, Lipinski's rule of five was used to test the bioavailability characteristics, such as, absorption, distribution, metabolism, elimination (ADME) of the lead compounds, which was predicted by molinspiration[20] and SwissADME.

Physicochemical properties depicting oral bioavailability was predicted using SwissADME (Table 2), in which lipophilicity was calculated in terms of XLOGP3 as 0.21. Insolubility log value depicted as -2.84 indicates solubility of vitexin, and unsaturation as per Csp3 was found to be 0.29 (Fig. 3).[21] Properties, such as, G-protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor, were predicted with Molinspiration, and bioactive scores were tabulated (Table 3).[22]

Molecular Docking of Vitexin against Drug Targets

AutoDock 4.2 suite was used to perform molecular docking analysis of vitexin against TACE, TNFR1, and hIKβb. Hydrogen atoms were added, water molecules removed, and Gasteiger charges were added to the ligand and protein. The rotatable bonds of the ligand were altered using the "choose torsions" option. Further flexible bonds of the ligand were checked to dock with rigid protein targets. Blind docking was performed using grid point value (X, Y, and Z) of 126 Å and spacing between the grid points was 0.375 Å. The Lamarckian genetic algorithm was selected for ligand conformational searching,[23] and default docking parameters were used. A total of 10 docking configurations were determined in each docking calculation.

A preferable docking configuration was chosen based on the lowest empirical binding free energy and the most frequent cluster. AutoDock results are ranked according to the highest negative binding free energies and corresponding root mean square deviation (RMSD) values from the experimentally determined binding site. AutoDock shares functional commonalities, including the global optimization of the scoring function, pre-calculation of grid maps, and the pre-calculation of distant dependent pair-wise energetics between each atom type. However, they employ a different scoring function and algorithms to obtain binding free energies and should be considered as different programs.[24] The docked protein-ligand complex (Table 4) visualized using BIOVIA Discovery Studio visualizer, and the distances of interactions are calculated (Table 5).

Table 2: SWISSADME-predicted Lipinski rule values, including TPSA, bioavailability, and water solubility for vitexin

Properties	*Predicted value*
Mi Logp	0.52
TPSA	181.05
Number of atoms	31
Mw	432.38
H bond acceptor	10
H bond donor	7
Number of violations	1
Number of rotational bonds	3
Volume	355.2
Bioavailability	0.55
Water solubility	Soluble

Table 3: Bioactivity score for vitexin

Bioactivity	*Probability score*
GPCR ligand	0.13
Ion channel modulator	0.14
Kinase inhibitor	0.19
Nuclear receptor ligand	0.23
Protease inhibitor	0.03
Enzyme inhibitor	0.46

Visualization of Protein-Vitexin Complex Interactions

The best-docked ligand models were selected according to the lowest binding energy. Two and three-dimensional conformational structures of the ligand-protein complexes were visualized using BIOVIA Discovery Studio Visualizer v.4.5[25] to investigate the binding modes. The ligand binds at the target protein by non-covalent interactions, such as, H-bonding, alkyl, alkyl-π, π-π, π-σ, and van der Waals interactions. Simplified visualization is illustrated in 2D, which displays the H-bonding, van der Waals forces, carbon-oxygen dipole-dipole interaction, alkyl-pi interactions, T-shaped pi-pi stacking, and pi-pi stacking. Images

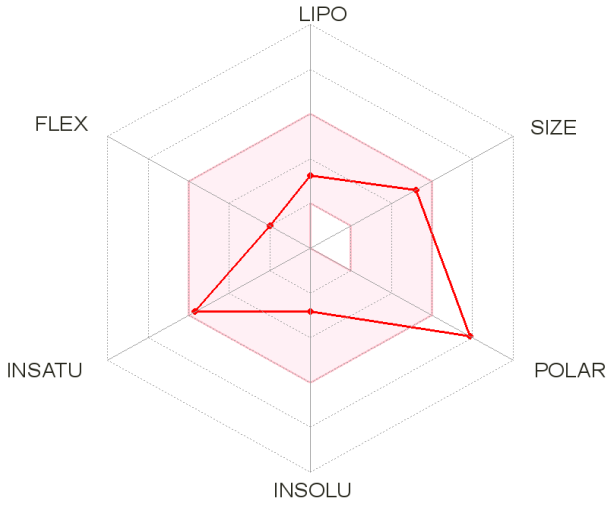


Fig. 3: Bioavailability radar (pink zone depicts suitable physicochemical space for oral bioavailability) for vitexin (LIPO indicates lipophilicity in terms of XLOGP3, SIZE indicates in terms of molecular weight, POLAR indicates polarity in terms of topological polar surface area, INSOLU depicts insolubility in water in terms of log S scale, INSATU refers to unsaturation as per fraction of carbons in the sp3 hybridization, and finally FLEX indicates flexibility as per rotatable bonds)

Table 4: Docking score predictions of protein-vitexin complex

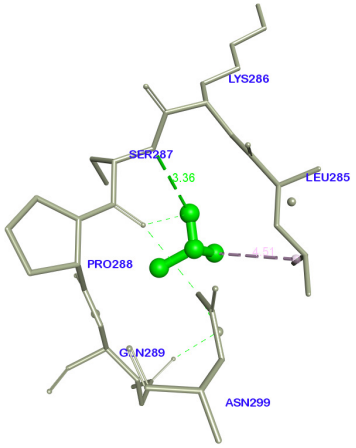
S. No.	*Conformation parameters*	*TACE*	*TNFR1*	*hIKβb*
1	Binding energy	-5.71	-6.1	-4.7
2	Inhibitory constant (μm)	65.22	33.94	360.35
3	van der Waal interaction	-8.39	-8.64	-7.32
4	Electrostatic energy	-0.3	-0.44	-0.36
5	Torsional energy	2.98	2.98	2.98
6	Unbound energy	-7	-6.66	-7.54
7	Amino acids	SER287, LEU285, TYR238, GLU463, PRO218	CYS158, CYS127, GLN131, ASN130	GLN548, ARG549, ARG452, GLN548, GLU149, CYS99

Table 5: Predicted distance and interacted amino acid residues of protein targets with vitexin

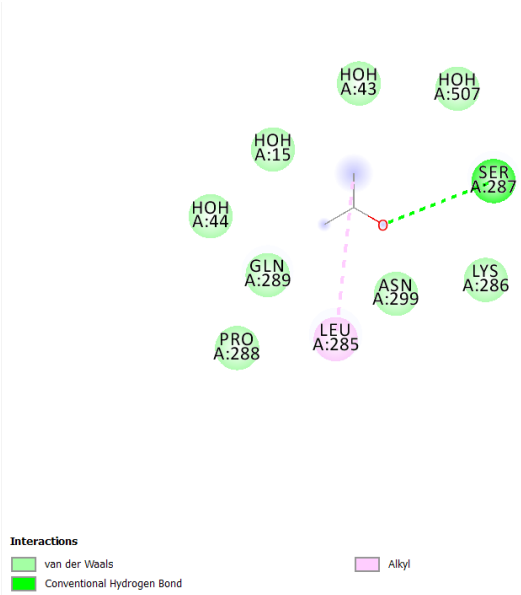
Vitexin (ligand)	*AA-residue*	*Distance (Å)*	*Docking energy (kcal/mol)*
TACE (protein)	SER287	3.36	-5.71
	LEU285	2.94	
	TYR238	2.186	
	GLU463	2.086	
	PRO218	2.149	
	PRO218	1.983	
TNFR1 (protein)	CYS158	2.07	-6.1
	CYS127	2.017	
	CYS127	2.48	
	CYS127	1.82	
	GLN131	1.822	
	ASN130	3.3	
hIKβb (protein)	GLU149	2.71	-4.7
	GLU97	2.96	
	CYS99	2.93	
	GLN548	1.956	
	ARG549	2.042	
	ARG452	1.817	
	GLN548	2.199	

were generated using Discovery Studio Visualizer 4.5.[26] Interactions between TNFR1 and vitexin (Fig. 4) showed the lowest binding energy when compared to TACE-vitexin interactions (Fig. 5) and vitexin-hIKβb kinase protein binding interactions (Fig. 6).

Computational analysis is less laborious, easy to perform, and yield quick results than conventional drug designing techniques. Cost-effective computational approaches may also seem to screen docking hits to produce small novel herbal-based drugs. AutoDock Vina docking enrolls better-performing speed simultaneously, elucidate better result accuracy.[27] The resulting docking complexes are expected to produce an excellent pharmacological effect in the treatment of RA. This investigation may serve as a tool for the evaluation of drug-like properties of pharmakon from folkloric herb

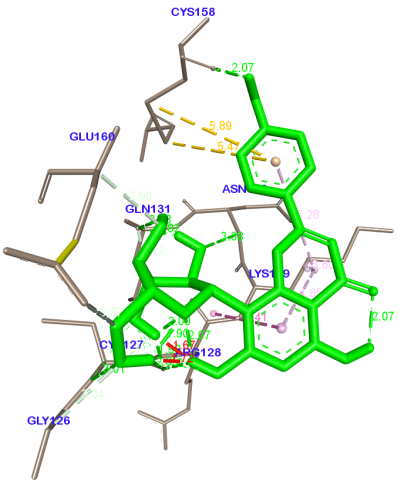


a) 3D interactions



b) 2D interactions

Fig. 4: 3D and 2D representations of vitexin-TACE protein interactions visualized by BIOVIA Discovery Studio visualizer; **a)** 3D interactions: TACE (protein)-line model; vitexin (ligand)-ball and stick model; green dotted lines-hydrogen bond interactions; **b)** 2D diagram: Ligand line model and protein interactions are colored depending on their type: conventional hydrogen bonds are colored in green, van der Waals interactions are colored in light green, and π-alkyl interactions are colored in light pink, respectively; the blue halo surrounding the interacting residues represents solvent-accessible surface that is proportional to its diameter



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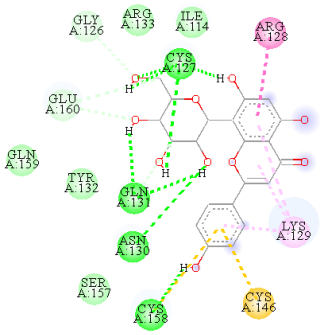
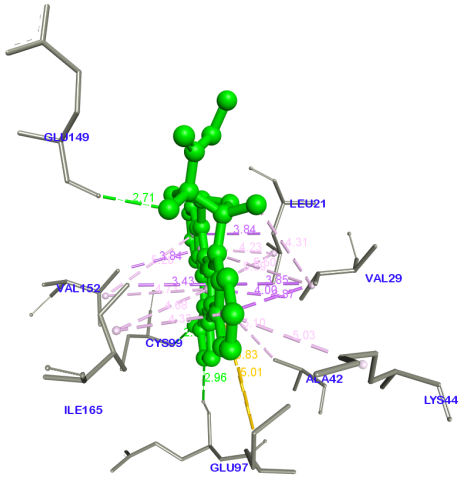


b) 2D interactions

Fig. 5: 3D and 2D representations of vitexin-TNRF1 receptor protein interactions visualized by BIOVIA Discovery Studio visualizer;
a) 3D interactions: TNFR1 (protein)-line model; vitexin (ligand)-ball and stick model; green dotted lines-hydrogen bond interactions;
b) 2D diagram: Ligand line model and Protein interactions are colored depending on their type; conventional hydrogen bonds are colored in green, van der Waals interactions are colored in light green, π-sulfur interactions are colored in yellow, amide-π stacked, and π-alkyl interactions are colored dark pink and light pink, respectively; the blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter



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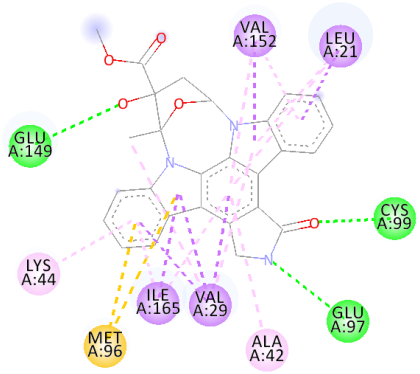

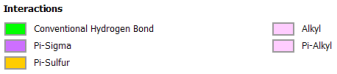

b) 2D interactions

Fig. 6: 3D and 2D representations of vitexin- hIKβb kinase protein interactions visualized by BIOVIA Discovery Studio visualizer;
a) 3D interactions: hIKβb (protein)-line model; vitexin (ligand)-ball and stick model; green dotted lines-hydrogen bond interactions;
b) 2D diagram: Ligand line model and protein interactions are colored depending on their type; conventional hydrogen bonds are colored in green, π-sulfur interactions are colored in yellow, π-sigma interactions are colored purple, alkyl and π-alkyl interactions are colored light pink, respectively; the blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter

V. negundo. It can be further utilized for the beneficiary of mankind after *in vivo* and clinical trial studies.

Conclusion

The present computational studies, concludes that vitexin might serve as better potent herbal pharmakon in repressing the activated inflammatory protein targets of RA such as, TACE, TNFR1, and hIKβb. Vitexin from *V. negundo* might serve as an efficient pharmaco-beneficiary lead in replacement of commercial drugs due to its lesser side effects and cost effectiveness, thereby the folk-chloric bioactive compound vitexin might act as a promising lead in therapeutics of Rheumatoid arthritis.

References

1. Verma MK, Sobha K. Understanding the major risk factors in the beginning and the progression of rheumatoid arthritis: current scenario and future prospects. J Inflamm Res. 2015;64:647-659.
2. Benjamin O, Bansal P, Goyal A, Lappin SL. Disease Modifying Anti-Rheumatic Drugs (DMARD). Star Pearls Publishing. 2020.

3. Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res. 2012;3:200-201
4. Roy, Somendu. Analysis of flavonoids and iridoids in *Vitex negundo* Linn by HPLC-PDA and method validation. Natural product communications. 2013.
5. Yahaya M, Zolkiffly S , Moklas M , Abdul Hamid H,Stanslas J ,Zainol M, *et al*. Possible Epigenetic Role of Vitexin in Regulating Neuro inflammation in Alzheimer's Disease. J Immunology Research. 2020;7.
6. Nikfarjam BA, Hajiali F, Adineh M, Nassiri-Asl M. Anti-inflammatory Effects of Quercetin and Vitexin on Activated Human Peripheral Blood Neutrophils:-The effects of quercetin and vitexin on human neutrophils. J Pharmacopuncture. 2017;20(2):127-131.
7. Awaluddin R, Muhtadi WK, Chabib L, Ikawati Z, Martien R, Ismail H. Molecular docking and ADME-toxicity studies of potential compounds of medicinal plants grown in Indonesia as an anti-rheumatoid arthritis. InAIP Conference Proceedings 2017 Mar 17 (Vol. 1823, No. 1, p. 020033). AIP Publishing LLC.
8. Wong E, Cohen T, Romi E, Levin M, Peleg Y, Arad U, Yaron A, Milla ME, Sagi I. Harnessing the natural inhibitory domain to control TNFα converting enzyme (TACE) activity in vivo. Scientific reports. 2016 Dec 16;6:35598.
9. Matthew P, Vincenti, constance E Brinckerhoff. Transcriptional regulation of collagenase (MMP-1, mmP-13) genes in arthritis: integration of complex signalling pathways for the recruitment of gene-specific transcription factors. http://arthritis-research.com/content/4/3/157. 2001
10. Zhang LM, Zhou JJ, Luo CL. CYLD suppression enhances the pro-inflammatory effects and hyperproliferation of rheumatoid arthritis fibroblast-like synoviocytes by enhancing NF-κB activation. Arthritis Res Ther. 2018; 20(1):219.
11. Lionta E, Spyrou G, Vassilatis DK, Cournia Z. Structure-based virtual screening for drug discovery: principles, applications and recent advances. Curr Top Med Chem. 2014;14(16):1923-1938.
12. Syed Mohd, Shazi Shakil, Mohd. Haneef. A simple click by click protocol to perform docking:Autodock 4.2 made easy for non-bioinformaticians. EXCLI Journal. 2013
13. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717.
14. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46:W257-263
15. Shah N, Rao H, Verma SK. Structure-based drug designing methodology to elucidate the binding affinities of phyto-ligands of Annona muricata against oncogenic PirH2. MOJ Proteomics Bioinform. 2018;7(2):113-120.
16. Dhananjayan K. Molecular docking study characterization of rare flavonoids at the Nac-binding site of the first bromodomain of BRD4 (BRD4 BD1). Journal of Cancer Research. 2015;2015.
17. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem. 2000; 43 (20):3714-3717.
18. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001; 46(1-3):3-26.
19. Muegge I. Selection criteria for drug-like compounds. Med Res Rev. 2003; 23(3):302-321.
20. Molinspiration.molinspiration cheminformatics. https://molinspiration.com/cgi-bin/properties. 2020
21. Karmakar B, Talukdar P, Talapatra SN. An in silico study for two anti-inflammatory flavonoids of Nerium oleander on proinflammatory receptors. Research Journal of Life Sciences, Bioinformatics, Pharmaceuticals and Chemical Sciences. 2019;5(1):582-596.
22. Mahesh kumar MVS, Rajagopal SV. Antioxidant, antimicrobial, antiproliferative activities and *in silico* drug likeness prediction of purified MHH compound, isolated from *millingtonia hortensis* linn. Int j pharm sci. 2015;7(9):251-257.
23. Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. Docking studies: In silico phosphodiesterase inhibitory activity of commercially available flavonoids. ||| Bangladesh Journal of Pharmacology|||. 2012 May 29;7(1):70-75.
24. Trott O, Olson A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 2010;31: 455-461.
25. Systèmes D. BIOVIA Discovery Studio[Internet]. Dassault Syst mes BIOVIA, Discovery Studio Modeling Environment, Release 2017. San Diego: Dassault Syst mes; 2016. Available from: http://accelrys.com/products/collaborative-science/biovia-discovery-studio/
26. Qasaymeh RM, Rotondo D, Oosthuizen CB, Lall N, Seidel V. Predictive Binding Affinity of Plant-Derived Natural Products Towards the Protein Kinase G Enzyme of *Mycobacterium tuberculosis* (*Mt*PknG). Plants (Basel). 2019; 8(11):477.
27. Meng XY, Zhang HX, Mezei M, Cui M. molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011; 7(2):146-57.

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