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IN SILICO ANALYSIS AND MOLECULAR DOCKING STUDIES OF C-GLYCOSYL FLAVONOIDS OF MIMOSA PUDICAFOR NEUROPATHIC PAIN

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ABSTRACT

The purpose of the study was to evaluate the activity of C-glycosyl flavonoids for neuropathic pain by computational docking studies. For this, natural metabolite C-glycosyl flavonoids from Mimosa pudica were used as ligands for molecular interaction. The crystallographic structure of molecular target was obtained from PDB database. Pregabalin, a well-known antiepileptic was taken as the standard for comparative analysis. Computational docking analysis was performed using PyRx, AutoDock Vina option based on scoring functions. These results showed binding affinity ranging between -5.9 to -7.1 kcal/mol when compared with that of the standard (-4.3 & -4.1kcal/mol). These results indicated that these C-glycosyl flavonoids could be one of the potential ligands to treat neuropathic pain.

INTRODUCTION

Neuropathic pain is a common problem that presents a major challenge to health-care providers owing to its complex natural history, uncertain aetiology and poor response towards therapy. It is a chronic pain condition that arises from a disease or injury to the central nervous system (CNS) or the peripheral nervous system (PNS) leading to its damage or abnormal function. Common symptoms of neuropathic pain include sensory abnormalities such as burning sensations, hyperalgesia, allodynia, hyperesthesia and dysesthesia. The current analgesics are unable to treat cancer chemotherapy-induced neuropathic pain which is severe enough for patients to discontinue their cancer chemotherapy treatment and worsens the quality of their life.Thus, the search for new chemical entities that can act as promising molecules to treat chemotherapy-induced neuropathic pain has emerged. Medicinal plants are potential sources of commercial drugs and lead compounds in drug development forming important sources of new chemical substances with potential therapeutic effects.[1] Mimosa pudica from latin "pudica" means shy, shrinking is also called a sensitive plant and touch me not is a creeping annual and perennial herb. The species is native to South America and Central America. Mimosa belongs to the taxonomic group Magnoliopsida and belonging to family Mimosaseae. It folds itself when touched and spreads its leaves

once again after a while. Thigmonastic movements in the sensitive plant Mimosa pudica L., associated with fast responses to environmental stimuli, appear to be regulated through electrical and chemical signal transductions. These are plants used in traditional medicine in Cameroon to treat insomnia, epilepsy, anxiety, agitation, leprosy, dysentery, depression, vaginal, uterine complaints, inflammations, burning sensation, asthma, leucoderma, fatigue and blood diseases. The major components said to be responsible for activities are C-glycosyl flavones namely, isorientin, orientin, isovitexin and vitexin.[2]

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. They belong to a class of low-molecular-weight phenolic compounds that are widely distributed in the plantkingdom.[3] Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid aglycone consists of a benzene ring (A) condensed with a six membered ring (C), which in the 2-position carries a phenyl ring(B) as a substituent.[4] The dietary flavonoids, especially their glycosides, are the most vital phytochemicals in diets and are of great general interest due to their diverse bioactivity. Almost all natural flavonoids exist as their O-glycoside or C-glycoside forms in plants. Among the flavonoid C-glycosides,, especially vitexin, isoorientin, orientin, isovitexin (figure 1-4) and their multiglycosides are more frequently mentioned than others.[5] Although, current drugs available for the effective management of neuropathic pain such as tricyclic antidepressants, antiepileptic drugs, cannabinoid receptor agonists and sodium channel blockers are effective, but, their usage is associated with many side effects.[6] The side effects of the currently used drug made us to explore an alternative and traditional approach to finding out new drug compound from the natural flavonoid, compounds which are having activity and also not having any side effects to human normal cell. These plant compounds which are also having high binding affinity for neuropathic pain receptors could lead to its treatment by docking method and to determine the drug likeness of these new molecules by estimation of the Lipinski's Rule of Five.[7]

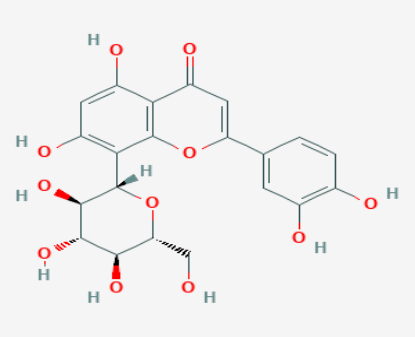


Fig1: 2D structure of orientin

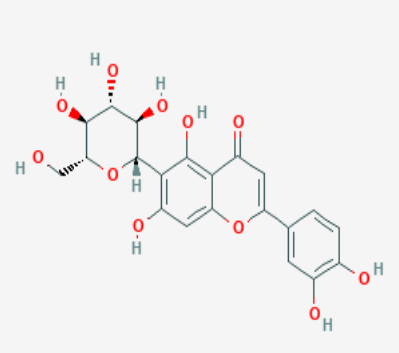


Fig 2: 2D structure of isoorientin

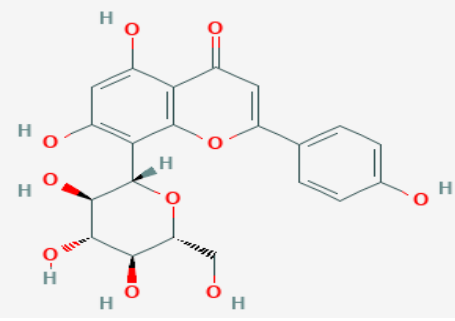


Fig 3: 2D structure of vitexin

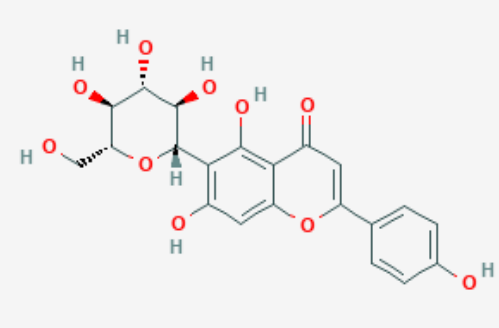


Fig 4: 2D structure of isovitexin

MATERIALS AND METHODS

Bioinformatics online databases such as PubMed, PubChem and PDB were used. PubMed database is designed to provide access to citations from biomedical journals. PubChem database provides information on the biological activities of small molecules. PubChem also provides a fast chemical structure similarity search tool. The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids.[8]

Protein preparation

The three dimensional crystal structure of human GABA (PDB ID: 4COF) and P2X4 receptors (PDB ID: 4DWI) was downloaded from the RCSB Protein Data Bank.[9]

Ligand preparation

The chemical structure of the ligands was obtained from PubChem compound database. It was prepared by ChemBioDraw and MOL SDF format of this ligand was converted to PDBQT file using PyRx tool to generate atomic coordinates.

ADMET, molecular and bioavailability scores

The ADME (absorption, distribution, metabolism and excretion) properties were calculated by using smile notation in Swiss ADME web based tool.[10]This website allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, drug like nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery. The toxicity was calculated by using PROTOX web based tool.[11]The molecular properties including Log p, PSA, rule of five parameters and drug likeness of the compounds were predicted using Molinspirationchemiinformation software.

Docking studies

The active site of the protein was first identified and it is defined as the binding site. A computational ligand-target docking approach was used to analyze structural complexes of the target with ligand in order to understand the structural basis of this protein target specificity. Finally, docking was carried out by PyRx, AutoDock Vina option based on scoring functions.

Drug-likeness prediction

Drug-likeness is the concept used in drug design, to estimate the molecular structure before the molecular structure is synthesized and tested. A drug-like molecule has properties such as solubility (LogP), potency, lipophilic efficiency, number of hydrogen bond donors vs alkyl sidechains, molecular weight and mutagenic and carcinogenic properties. Drug-likeness is predicted by online molsoft software. The drug likeness model score of the compounds will be obtained. For a molecule to consider as drug-like, the score should be between 0-1.

RESULTS

Molecular Docking: Docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure based drug design process. AutoDock Vina is a open-source program

for drug discovery, molecular docking and virtual screening, offering multicore capability, high performance and enhanced accuracy and ease of use. The parameters chosen for the docking can be judged by the docking tool's ability to reproduce the binding mode of a ligand to protein, when the structure of the ligand–protein complex is known. The docking interactions are shown in Figure 5-14 and compiled results are shown in Table 1.

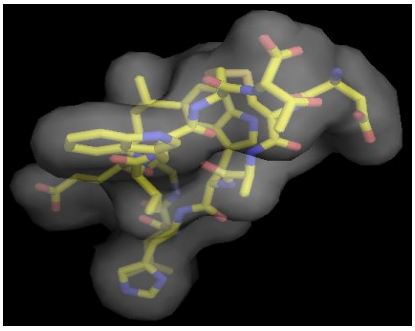


Fig 5: docked transparency image oforientin with GABA

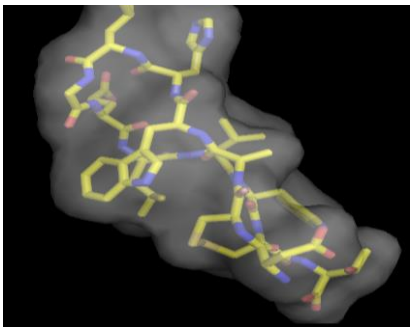


Fig 6: docked transparency image of orientinwith P2X4

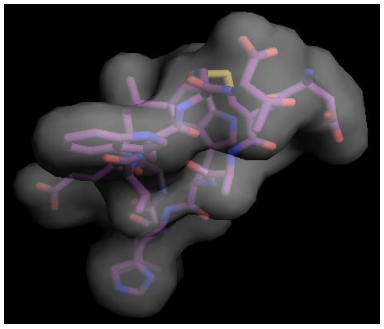


Fig 7: docked transparency image ofisoorientin with GABA

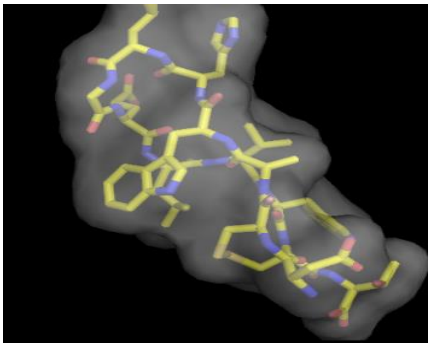


Fig 8: docked transparency image ofisoorientin with P2X4

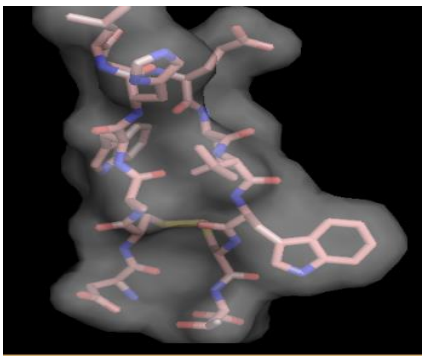


Fig 9: docked transparency image ofvitexin with GABA

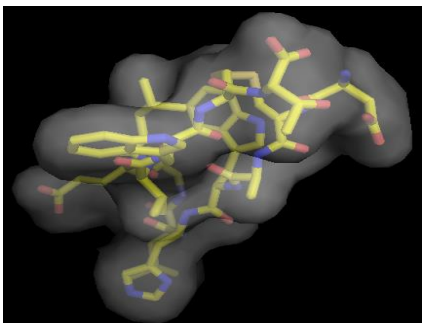


Fig 10: docked transparency image of vitexin with P2X4

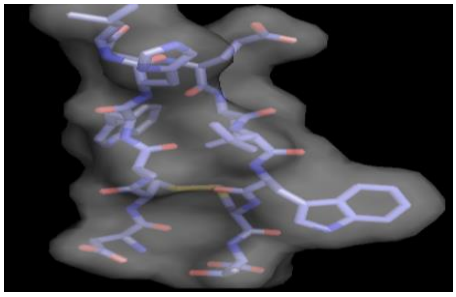


Fig 11: docked transparency image ofisovitexin with GABA

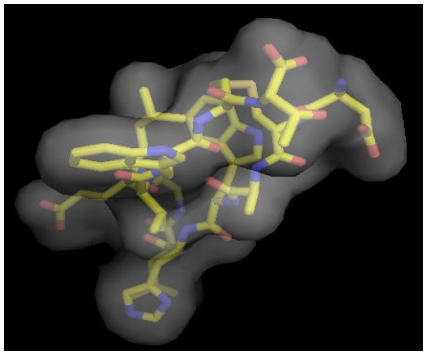


Fig 12: docked transparency image ofisovitexinwith P2X4

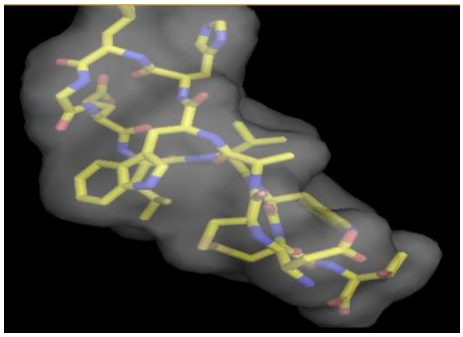


Fig 13. Docked transparency image of pregbalin with GABA

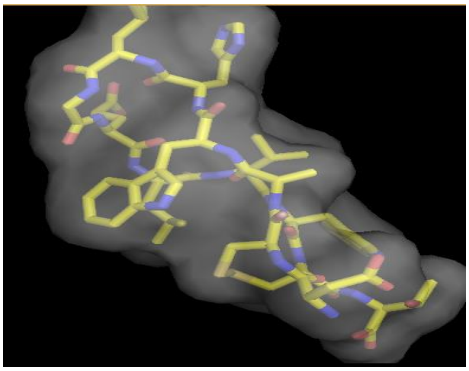
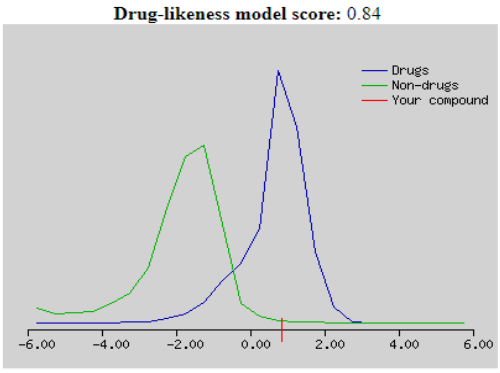


Fig 14: docked transparency image of pregabalin with P2X4

Table 1: Docking results of compounds

Compounds	Binding affinity (kcal/mol)	
	GABA	P2X4
Isoorientin	-7.1	-6.1
Isovitexin	-7.1	-5.9
Vitexin	-6.7	-6.3
Orientin	-6.5	-6.3
Pregabalin	-4.3	-4.1



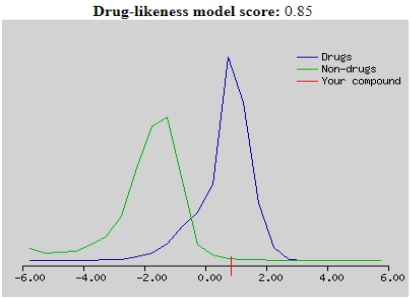
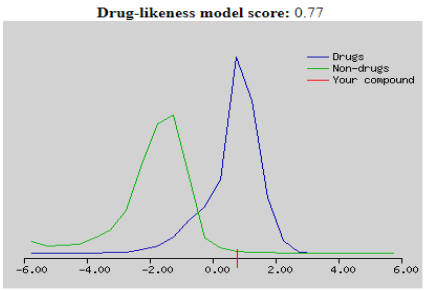


Fig 15: drug likeness model score isoorientin

Fig 16: drug likeness model scoreof isovitexin



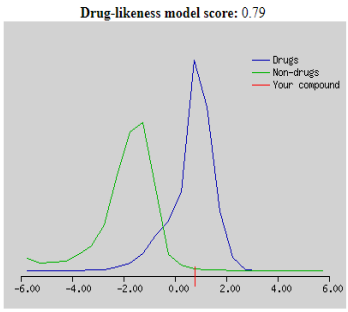


Fig 17: drug likeness model scoreof orientin & Fig18: drug likeness model scoreof vitexin

Table 2: Lipophilicity of C-glycosyl flavonoids of *Mimosa pudica*

Compounds	Isovitexin	Isoorientin	Orientin	Vitexin	Pregabalin
LogPo/w (iLOGP)	1.94	2.12	1.27	1.38	1.06
Log Po/w (XLOGP3)	0.21	-0.15	-0.15	0.21	-1.55
Log Po/w (WLOGP)	-0.23	-0.53	-0.53	-0.23	1.08
Log Po/w (MLOGP)	-2.02	-2.51	-2.51	-2.02	1.08
Log Po/w (SILICOS-IT)	0.33	-0.14	-0.14	0.33	0.67
Consensus Log Po/w	0.05	-0.24	-0.41	-0.07	0.47

Compounds	Isovitexin	Isoorientin	Orientin	Vitexin	Pregabalin
Log *S* (ESOL)	-2.84	-2.70	-2.70	-2.84	0.48
Solubility	6.29e-01mg/ml; 1.46e-03mol/l	9.00e-01mg/ml; 2.01e-03 mol/l	9.00e-01mg/ml; 2.01e-03 mol/l	6.29e-01mg/ml; 1.46e-03mol/l	4.80e+02 mg/ml; 3.02e+00mol/l
Class	Soluble	Soluble	Soluble	Soluble	Highly soluble
Log *S* (Ali)	-3.57	-3.62	-3.62	-3.57	0.73
Solubility	1.16e-01mg/ml;2.68e-04mol/l	1.07e-01mg/ml; 2.39e-04 mol/l	1.07e-01mg/ml; 2.39e-04 mol/l	1.16e-01mg/ml;2.68e-04mol/l	8.50e+02 mg/ml; 5.34e+00mol/l
Class	Soluble	Soluble	Soluble	Soluble	Highly soluble
Log *S* (SILICOS-IT)	-2.38	-1.79	-1.79	-2.38	-0.97
Solubility	1.81e+00mg/ml; 4.20e-03mol/l	7.32 e+00mg/ml; 1.63e-02 mol/l	7.32 e+00mg/ml; 1.63e-02 mol/l	1.81e+00mg/ml; 4.20e-03mol/l	1.71e+01mg/ml; 1.07e-01 mol/l
Class	Soluble	Soluble	Soluble	Soluble	solube

Table 3: Water Solubility of C-glycosyl flavonoids of *Mimosa pudi*

Table 4: Pharmacokinetics of C-glycosyl flavonoids of *Mimosa pudica*

Compounds	**Isovitexin**	**Isoorientin**	**Orientin**	**Vitexin**	**Pregabalin**
GI absorption	Low	Low	Low	Low	High
BBB permeant	No	No	No	No	Yes
P-gp substrate	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Lop K_P (skin permeation)	-8.79cm/s	-9.14cm/s	-9.14cm/s	-8.79cm/s	-8.37 cm/s

Table 5: Drug likeness of C-glycosyl flavonoids of *Mimosa pudica*

Compounds	**Isovitexin**	**Isoorientin**	**Orientin**	**Vitexin**	**Pregabalin**
Lipinski	**Yes; 1 violation: NHorOH>5**	**No;2 violations: NorO>10,NHOROH>5**	**No;2 violations: NorO>10,NHOROH>5**	**Yes; 1 violation: NHorOH>5**	**Yes; 0 violation**
Ghose	Yes	No; 1 violation: WLOGP<-0.4	No; 1 violation: WLOGP<-0.4	Yes	No; 1 violation:MW <160
Veber	No; 1 violation: TPSA>140	No; 1 violation: TPSA>140	No; 1 violation: TPSA>140	No; 1 violation: TPSA>140	Yes
Egan	No; 1 vioaltion: TPSA >131.6	No; 1 vioaltion: TPSA >131.6	No; 1 vioaltion: TPSA >131.6	No; 1 vioaltion: TPSA >131.6	Yes
Muegge	No; 2 violations: TPSA >150, H-don>5	No;3 violations: TPSA>150, H-acc>10,H-don>5	No;3 violations: TPSA>150, H-acc>10,H-don>5	No; 2 violations: TPSA >150, H-don>5	No; 1 violation:MW <200
Bioavailability score	0.55	0.17	0.17	0.55	0.55

Compounds	Isovitexin	Isoorientin	Orientin	Vitexin	Pregabalin
PAINS	0 alert	1 alert: catechol- A	1 alert: catechol- A	0 alert	0 alert
Brenk	0 alert	1 alert:catechol	1 alert:catechol	0 alert	0 alert
Leadlikeness	No; 1 violation: MW>350	No; 1 violation: MW>350	No; 1 violation: MW>350	No; 1 violation: MW>350	No; 1 violation: MW<250
Synthetic accessibility	4.99	5.04	5.17	5.12	1.87

Table 6: Medicinal chemistryof C-glycosyl flavonoids of *Mimosa pudica*

Compounds	Isovitexin	Isoorientin	Orientin	Vitexin	Pregabalin
miLogP	0.52	0.03	0.03	0.52	0.66
TPSA	181.04	201.27	201.27	181.04	63.32
Natoms	31	32	32	31	11
MW	432.38	448.38	448.38	432.38	159.23
nON	10	11	11	10	3
nOHNH	7	8	8	7	3
nviolations	1	2	2	1	0
Nrotb	3	3	3	3	5
volume	355.20	363.22	363.22	355.20	168.11

Table 7: Molecular properties of C-glycosyl flavonoids of *Mimosa pudica*

Table 8: Bioavailability scores of C-glycosyl flavonoids of *Mimosa pudica*

Scores	Isovitexin	Isoorientin	Orientin	Vitexin	Pregabalin
GPCR ligand	0.12	0.11	0.12	0.13	-0.24
Ion channel modulator	0.02	0.01	-0.14	-0.14	0.23
Kinase inhibitor	0.15	0.16	0.20	0.19	-0.81
Nuclear receptor ligand	0.23	0.20	0.20	0.23	-0.79
Protease inhibitor	0.04	0.01	0.01	0.03	-0.02
Enzyme inhibitor	0.47	0.46	0.45	0.46	0.02

Table 9: Oral toxicity prediction results of C-glycosyl flavonoids of *Mimosa pudica*

Compounds	Isovitexin	Isoorientin	Orientin	Vitexin	Pregabalin
Predicted LD50	159mg/kg	159mg/kg	1213mgkg	1213mg/kg	9000mg/kg
Predicted toxicity class	3	3	4	4	6
Average similarity	63.84%	64.87%	58.25%	58.285%	85.26%
Prediction accuracy	68.07%	68.07%	67.38%	67.38%	70.97%

ADMET, molecular and bioavailability score

The ADMET properties for the compounds were determined using Swiss ADME: a free web tool. The toxicity was calculated using PROTOX web based tool. Toxic doses are often given as LD50 values in mg/kg body weight. The ADME properties, the molecular properties, the bioavailability scores and toxicity analysis of C-glycosyl flavonoids of Mimosa pudica are given in Tables 2-9.

Drug Likeness Model Scores Of C-Glycosyl Flavonoids Of Mimosa Pudica

The drug likeness model scores of compounds were obtained from online molsoft software. . The drug likeness scores of C-glycosyl flavonoids of Mimosa pudica were given in figure 15-18

DISCUSSION

A large number of medicinal plants are proven to possess beneficial therapeutic potentials and act a rich source of secondary metabolites with diverse array of biological activities. These secondary metabolites may act as lead molecules. Molecular docking continues to hold great promise in the field of computer based drug design, as a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. In recent times number of reports citing successful application of in silico studies in drug developing program in different therapeutic areas is expanding rapidly. Understanding the interactions between proteins and ligands is crucial for the pharmaceutical and functional food industries. The emergence of bioinformatics has offered a platform to explore diseases at molecular level using computational tools. The Protein-Ligand interaction plays a significant role in structure based drug designing. The experimental structures of these protein/ligand complexes are usually obtained, by time-consuming techniques such as X-ray crystallography or NMR. These screening methods are routinely and extensively used to reduce cost and time of drug discovery. The development of novel compounds with biological activity is an urgent need for developing effective drugs.The ability of these C-glycosyl flavonoids to bind with the targets is given in terms of binding affinity (kcal/mol). The ligand possessing the highest binding affinity shows a strong affinity towards its target. The ‘drug likeness properties’ of the phytoconstituents was evaluated according to the ‘The Lipinski rule of five’ and to develop them as potential lead compound for neuropathic pain. The rules, based on the 90-percentile values of the drug’s property distributions, apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are actively transported through cell membranes by transporter proteins are exceptions to the rule. The Lipinski criteria are widely used by medicinal chemists to predict not only the absorption of compounds, as Lipinski originally intended, but also overall drug-likeness. Further studies can be performed to evaluate the in-vitro and in-vivo activity of the selected medicinal plants and to discover pharmacokinetic properties of the phytoconstituents to know the absorption, distribution, metabolism and excretion of the phytoconstituents. Docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure based drug design process. AutoDock Vina is a open-source program for drug discovery, molecular docking and virtual screening, offering multicore capability, high performance and enhanced accuracy and ease of use. The parameters chosen for the docking can be judged by the docking tool’s ability to reproduce the binding mode of a ligand to protein, when the structure of the ligand–protein complex

is known. The ADMET properties for the compounds were determined using SwissADME: a free web tool. The estimated number of hydrogen bonds that would be donated are in the range of 3-8. Estimated number of hydrogen bonds that would be accepted are in the range of 3-11. Number of violations of Lipinski's rule of five is 0-2. The compounds have good logP value, thus lipophilic in nature and are permeant to blood brain barrier.The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments. ADME stands for "Absorption, Distribution, Metabolism and Excretion" and describes the disposition of a pharmaceutical compound within an organism. The four criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug. The toxicity was calculated using PROTOX web based tool. Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound. Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS). The drug likeness model scores of compounds were obtained from online molsoft software. For a compound to be drug like, the drug likeness model score should be between 0-1. Here all the four compounds are in the range, and can be considered as drug-like.

CONCLUSION

Among those compounds, isoorientin and isovitexin were found to have the best binding energy of 7.1kcal/mol. On the whole, we conclude that these two flavonoids can be used as potent drug for neuropathic pain which may be worth for further investigations to enrich the activity of the natural flavonoid compounds especially in experimental studies with clinical trials to determine the dosage of safety levels.

ACKNOWLEDGEMENTS

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