Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Molecular Docking Analysis of Phytic Acid and 4-hydroxyisoleucine as Cyclooxygenase-2, Microsomal Prostaglandin E Synthase-2, Tyrosinase, Human Neutrophil Elastase, Matrix Metalloproteinase-2 and -9, Xanthine Oxidase, Squalene Synthase, Nitric Oxide Synthase, Human Aldose Reductase, and Lipoxygenase Inhibitors

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Submitted: 12-04-2016 Revised: 11-07-2016 Published: 11-10-2017

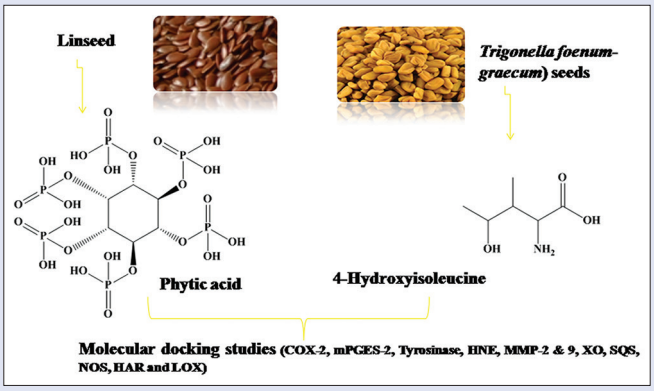
ABSTRACT

Background: The phytoconstituents phytic acid and 4-hydroxyisoleucine have been reported to posses various biological properties. **Objective:** This prompted us to carry out the docking study on these two ligands (phytic acid & 4-hydroxyisoleucine) against eleven targeted enzymes. **Materials and Methods:** Phytic acid & 4-hydroxyisoleucine were evaluated on the docking behaviour of cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-2 (mPGES-2), tyrosinase, human neutrophil elastase (HNE), matrix metalloproteinase (MMP 2 and 9), xanthine oxidase (XO), squalene synthase (SQS), nitric oxide synthase (NOS), human aldose reductase (HAR) and lipoxygenase (LOX) using Discovery Studio Version 3.1 (except for LOX, where Autodock 4.2 tool was used). **Results:** Docking and binding free energy analysis revealed that phytic acid exhibited the maximum binding energy for four target enzymes such as COX-2, mPGES-2, tyrosinase and HNE. Interestingly, we found that 4-hydroxyisoleucine has the potential to dock and bind with all of the eleven targeted enzymes. **Conclusion:** This present study has paved a new insight in understanding 4-hydroxyisoleucine as potential inhibitor against COX-2, mPGES-2, tyrosinase, HNE, MMP 2, MMP 9, XO, SQS, NOS, HAR and LOX.

Key words: 4-hydroxyisoleucine, cyclooxygenase-2, microsomal prostaglandin E synthase-2, molecular docking, phytic acid, tyrosinase

SUMMARY

- 4-hydroxyisoleucine has the potential to dock and bind with all 11targeted enzymes such as (cyclooxygenase-2 [COX-2], microsomal prostaglandin E synthase-2 [mPGES-2], tyrosinase, human neutrophil elastase [HNE], matrix metalloproteinase [MMP-2 and -9], xanthine oxidase, squalene synthase, nitric oxide synthase, human aldose reductase, and lipoxygenase)
- Moreover, docking studies and binding free energy calculations revealed that phytic acid exhibited the maximum binding energy for four target enzymes such as COX-2, mPGES-2, tyrosinase, and HNE; however, for other six target enzymes, it fails to dock.



Abbreviations used: COX-2: Cyclooxygenase-2, mPGES-2: Microsomal prostaglandin E synthase-2, HNE: Human neutrophil elastase, MMP-2 and -9: Matrix metalloproteinase-2 and -9, XO: Xanthine oxidase, SQS: Squalene synthase, NOS: Nitric oxide synthase, HAR: Human aldose reductase, LOX: Lipoxygenase, ADME: Absorption, distribution, metabolism, and excretion, TOPKAT: Toxicity Prediction by Computer-assisted Technology.

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DOI: 10.4103/pm.pm_195_16

Access this article online
Website: www.phcog.com
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INTRODUCTION

Phytic acid is abundant in nature (especially in plants). It accounts for 1%–5% weight of edible cereals, legumes, nuts, oil seeds, and tubers. Although it is well known as antinutrient agent, in recent years, phytic acid has been reported to possess a number of biological activities such as antibacterial, antidiabetic, anti-inflammatory, anticarcinogenic, antioxidant, antiangiogenic, antiulcer, antiviral, hypoallergenic, hypolipidemic, immunomodulation, and neuroprotection.[1,2]

Various reports have revealed that fenugreek (*Trigonella foenum-graecum*) seeds' extract exhibits anti-inflammatory

Cite this article as: Narayanaswamy R, Wai LK, Esa NM. Molecular docking analysis of phytic acid and 4-hydroxyisoleucine as cyclooxygenase-2, microsomal prostaglandin E synthase-2, tyrosinase, human neutrophil elastase, matrix metalloproteinase-2 and -9, xanthine oxidase, squalene synthase, nitric oxide synthase, human aldose reductase, and lipoxygenase inhibitors. Phcog Mag 2017;13:S512-8.

activity, as well as lower blood glucose and cholesterol levels in humans and experimental animals.[3-5] 4-hydroxyisoleucine is a unique amino acid isolated and identified from fenugreek (*T. foenum-graecum*) seeds. It accounts for about 80% of the total amino acid within the seeds. Further two diastereoisomers of 4-hydroxyisoleucine have been reported from fenugreek seeds, the major one is (2S, 3R, 4S) configuration and another (minor) one is (2R, 3R, 4S) configuration, respectively. (2S, 3R, 4S) configuration of 4-hydroxyisoleucine has been reported as an antidiabetic agent.[6] 4-hydroxyisoleucine has been reported as hepatoprotective agent[7] and also reported to inhibit palmitate-induced reactive oxygen species generation.[8] Therefore, these two phytoconstituents, namely, phytic acid and 4-hydroxyisoleucine were selected to be evaluated in this study on the docking behavior of cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-2 (mPGES-2), tyrosinase, human neutrophil elastase (HNE), matrix metalloproteinase (MMP-2 and -9), xanthine oxidase (XO), squalene synthase (SQS), nitric oxide synthase (NOS), human aldose reductase (HAR), and lipoxygenase (LOX) with investigation on the enzymes putative binding sites using Discovery Studio version 3.1 (except for LOX, where Autodock 4.2 [Scripps Research Institute, San Diego, USA] tool was used).

MATERIALS AND METHODS

In this section, ligand preparation, target protein identification and preparation, molecular descriptors calculation, absorption, distribution, metabolism, and excretion (ADME), and Toxicity Prediction by Komputer-assisted Technology (TOPKAT) analysis were carried out according to the previously reported method[9] as briefly stated below.

Ligand preparation

Chemical structures of the ligands, i.e., (i) phytic acid (ID 16735966) and (ii) 4-hydroxyisoleucine (CID2773624) were downloaded from ChemSpider (www.chemspider.com) and PubMed (www.pubmed.com) databases. Both the ligands were drawn in ChemBioDraw Ultra 12.0 (www.cambridgesoft.com), and subsequently, molecular mechanics 2 minimization of ligands was carried out using ChemBio3D Ultra 12.0 (PerkinElmer, Waltham, USA) (molecular mechanics (MM2)). Thus, these energy-minimized ligands (structures) were employed for Autodock 4.2, whereas in the case of CDOCKER inbuild ligand preparation protocol (Accelrys, San Diego, USA) was adopted.

Target protein identification and preparation

The three-dimensional protein structures of the COX-2 (PDB ID: 3 LN1 with resolution of 2.40 Å), mPGES-2 (PDB ID: 1Z9H with resolution of 2.60 Å), tyrosinase (PDB ID: 2Y9W with resolution of 2.30 Å), HNE (PDB ID: 1H1B with resolution of 2.00 Å), MMP-2 (PDB ID: 1QIB with resolution of 2.80 Å), MMP-9 (PDB ID: 4H1Q with resolution of 1.59 Å), XO (PDB ID: 3NRZ with resolution of 1.80 Å), SQS (PDB ID: 3ASX with resolution of 2.00 Å), NOS (PDB ID: 4NOS with resolution of 2.30 Å), HAR (PDB ID: 1US0 with resolution of 0.66 Å), and LOX (PDB ID: 1JNQ with resolution of 2.10 Å) were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) (Anonymous, www.rcsb.org). A chain of all proteins (except for XO and COX-2, where C chain; mPGES-2, where A, B, C, and D chains; and tyrosinase, where A and B chains) was preprocessed separately by deleting other chains (B, C, and D), ligand, as well as the crystallographically observed water molecules (water without hydrogen bonds). All the proteins above mentioned were prepared using UCSF Chimera software (www.cgi.ucsf.edu/chimera) for Autodock 4.2, whereas in the case of CDOCKER inbuild protein preparation protocol (Accelrys, San Diego, USA) was adopted.

Molecular descriptors calculation

Molinspiration online database was used for the two selected ligands to calculate thirteen descriptors (www.molinspiration.com) which are logP, polar surface area, molecular weight (MW), number of atoms, number of O or N, number of OH or NH, number of rotatable bonds, volume, drug-likeness including G-protein-coupled receptors ligand, ion channel modulator, kinase inhibitor, and nuclear receptor ligand, and the number of violations to Lipinski's rule.

Absorption, distribution, metabolism, and excretion and Toxicity Prediction by Komputer-assisted Technology analysis

Both ADME and TOPKAT analyses were performed using Discovery Studio® 3.1 (Accelrys, San Diego, USA). ADME analysis was performed using six descriptors such as human intestinal absorption, aqueous solubility, blood–brain barrier, cytochrome P450 2D6, plasma protein binding, and hepatotoxicity. As for the TOPKAT analysis, five descriptors were used which includes aerobic biodegradability (AB), Ames mutagenicity, ocular irritancy, skin irritancy, and skin sensitization.

Docking studies

Docking studies were performed on the protein crystal structures of COX-2, mPGES-2, tyrosinase, HNE, MMP-2, MMP-9, XO, SQS, NOS, and HAR obtained from PDB using the CDOCKER protocol under the protein–ligand interaction section in Discovery Studio® 3.1 (Accelrys, San Diego, USA). In general, CDOCKER is a grid-based molecular docking method that employs CHARMM force fields. Protein was first held rigid while the ligands were allowed to flex during the refinement. Two hundred random ligand conformations were then generated from the initial ligand structure through high-temperature molecular dynamics followed by random rotations, refinement by grid-based (GRID I) simulated annealing, and a final grid-based or full force field minimization.[10] In this experiment, the ligand was heated to a temperature of 700 K in 2000 steps, and the cooling steps were set in 5000 steps to 300 K with the grid extension set to 10 Å. Hydrogen atoms were added to the structures, and all ionizable residues were set at their default protonation state at a neutral pH. For each ligand, top ten ligand binding poses were ranked according to their CDOCKER energies, and the predicted binding interactions were then analyzed, from which the best among the ten ligand binding poses were chosen and carried out *in situ* ligand minimization using a standard protocol.

Docking was performed using Autodock 4.2 version, in which combined energy evaluation through precalculated grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein (LOX). All rotatable bonds in the ligands were kept free to allow flexible docking. Grid size was set to 60 × 60 × 60 grid points (x, y, and z), with spacing between grid points kept at 0.375 Å. The Lamarckian genetic algorithm was chosen to search for the best conformers. Standard docking protocol was applied. One hundred independent docking runs for each ligand were generated using genetic algorithm search.[11]

RESULTS AND DISCUSSION

Phytic acid and 4-hydroxyisoleucine were the two ligands selected for the present study; it could be beneficial to know the physicochemical and drug-likeness properties of these ligands before carry out docking studies. Lipinski's rule of five was applied to know the above said properties and further helps to determine whether a lead compound having a certain pharmacological or biological activity could be made into an orally active drug for human.[12] Violation of the Lipinski's rule of five is when logP >5,

MW >500, number of N, O (hydrogen bond acceptor) >10, number of OH and NH (hydrogen bond donor) >5, and number of rotatable bond (rotb) >15. In the present study, 4-hydroxyisoleucine showed no violation with respect to thumb rule of five (Lipinski's rule of five). On the other hand, phytic acid showed three violations as given in Table 1. With regard to drug-likeness score, if the score is >0 is active, −5.0–0.0 is moderate active, and <−5.0 is inactive.[13] Phytic acid showed active bioactivity score (>0) toward all descriptors, whereas 4-hydroxyisoleucine has shown active bioactivity score toward two descriptors as shown in Table 2. ADME prediction is also required before carry out docking studies and which is now commonly acceptable in early stage of drug discovery, drug screening, and drug design, owing to its unique characteristic nature.[14] Table 3 shows the ADME profile of the two selected ligands, wherein phytic acid was predicted to have very poor intestinal absorption and hepatotoxic effect. On the other hand, 4-hydroxyisoleucine was predicted to have good intestinal absorption. The toxicity profile of the two ligands as depicted in Table 4 shows that phytic acid was nondegradable toward AB nature, and both the ligands were predicted to have ocular/eye irritancy effect in humans.

COX is the key enzyme which catalyzes the conversion of arachidonic acid (AA) to prostaglandins. In human, COX exists in two isoforms; COX-1 is a constitutive enzyme, whereas COX-2 is an inducible enzyme. Cytokines and growth factors increase the expression of COX-2 mainly at inflammatory sites.[15] The docking studies and binding free energy reported in Table 5 show that phytic acid had the highest interaction energy (−42.20 kcal/mol) with COX-2 and both ligands exhibited interaction with Glu539 amino acid residue of COX-2 as shown in Table 5. Phytic acid has been reported to suppress the COX-2 expression in azoxymethane -induced colon cancer cells.[16] Similarly, in the present study, phytic acid exhibited interaction with Glu350, Trp531, and Asn546 amino acid residues of COX-2; this finding was in good agreement with Khokra *et al.* report.[17] In the case of 4-hydroxyisoleucine, there is no available reported investigation for their COX-2 inhibitory activity.

mPGES-2 has exhibited board substrate specificity and also expressed constitutively in a variety of human tissues. Interestingly, it is not induced by pro-inflammatory signals like that of mPGES-1.[18] Recently, mPGES-2 has been reported to play a protective role against different types of liver injury.[19] As for the docking studies and binding free energy calculations with mPGES-2, phytic acid exhibited the highest interaction energy (−56.21 kcal/mol), and both ligands showed interaction with Ser295 amino acid residue of mPGES-2 as shown in Table 6. Interestingly, phytic acid interacts with all the four chains (A, B, C, and D) of mPGES-2. In the present study, both ligands had potential to dock with mPGES-2; however, until now, there is no report available with regard to their docking studies.

Table 1: Molecular physicochemical descriptors analysis on two ligands using Molinspiration online software tool

Ligand	Log A[a]	TPSA[b]	N atoms[c]	MW[d]	noN[e]	nOH NH[f]	N violations[g]	N rotb[h]	Volume[i]
Phytic acid	−hyti	400.6	36	660.0	24	12	3	12	423
4-hydroxyisoleucine	−-hyd	83.55	10	147.2	4	4	0	3	142.6

[a]Octanol-water partition coefficient; [b]Topological polar surface area; [c]Number of nonhydrogen atoms; [d]Molecular weight; [e]Number of hydrogen bond acceptors (O and N atoms); [f]Number of hydrogen bond donors (OH and NH groups); [g]Number of rule of five violations; [h]Number of rotatable bonds; [i]Molecular volume

Table 2: Bioactivity score of two ligands using Molinspiration online software tool

Ligand	GPCRs ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Phytic acid	0.38	0.44	0.37	0.24	0.31	0.48
4-hydroxyisoleucine	−0.49	0.03	−1.36	−0.72	−0.13	0.15

GPCRs: G-protein-coupled receptors

Table 3: Absorption, distribution, metabolism, and excretion analysis of phytic acid and 4-hydroxyisoleucine

Ligand	HIA			AS		BBB		Prediction		
	PSA	ALogP8	Level*	Log (SW)	Level**	Log BB	Level***	PPB	CYP2D6	HT
Phytic acid	407.2	−2.38	3	−14.92	0	0	4	False	False	True
4-hydroxyisoleucine	85.5	−0.68	0	0.602	5	−1.71	3	False	False	False

*0 - good, 1 - moderate, 2 - poor and 3 - very poor; **0 - extremely low, 1 - very low, 2 - low, 3 - good, 4 - optimal, 5 - too soluble, and 6 - warning; ***0 - very high penetrate, 1 - high, 2 - medium, 3 – low, and 4 - undefined. HIA: Human intestinal absorption; AS: Aqueous solubility; BBB: Blood–brain barrier; PPB: Plasma protein binding; CYP2D6: Cytochrome P450 2D6; HT: Hepatotoxicity; PSA: Polar surface area

Table 4: Toxicity prediction analysis of phytic acid and 4-hydroxyisoleucine

Ligand	AB	AM	OI	SI	SS
Phytic acid	Nondegradable	Nonmutagen	Irritant	Irritant	Sensitizer
4-hydroxyisoleucine	Degradable	Nonmutagen	Irritant	Nonirritant	Sensitizer

AB: Aerobic biodegradability; AM: Ames mutagenicity; OI: Ocular irritancy; SI: Skin irritancy; SS: Skin sensitization

Table 5: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with cyclooxygenase-2 using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	42.20	Glu350	0.57 and 2.2
		Trp531	1.2 and 2.3
		Glu539	1.8 and 2.2
		Asn546	2.1
4-hydroxyisoleucine	21.14	Glu539	1.7 and 2.1

Tyrosinase is the key regulatory enzyme in melanin biosynthesis pathway that too particularly in the first two steps such as (i) tyrosine hydroxylation to 3, 4-dihydroxyphenylalanine (DOPA) and (ii) the oxidation of DOPA to dopaquinone.[20] The maximum interaction energy in the docking studies and binding free energy calculations with that of tyrosinase was exhibited by phytic acid (−33.26 kcal/mol), and 4-hydroxyisoleucine showed interaction with Pro14 amino acid residue of tyrosinase as shown in Table 7. Graf *et al.* have reported that phytic acid as inhibitor of mushroom tyrosinase,[21] this was good in agreement with the present finding. Previously, we reported L-cysteine as tyrosinase inhibitor using molecular docking;[22] similarly, in the present study, we again revealed another amino acid (4-hydroxyisoleucine) as tyrosinase inhibitor.

HNE is key enzyme which plays a major role in degenerative and inflammatory diseases, through proteolysis and extracellular matrix (ECM) components.[23] HNE is another target protein/enzyme which its docking studies and binding free energy calculations showed phytic acid having the maximum interaction energy (−47.59 kcal/mol). Interestingly, both ligands exhibited interaction with Phe192 and Ser195 amino acid residues of elastase as shown in Table 8. In the present study, both ligands showed interaction with Ser195 amino acid residue of HNE; this was good in agreement with previous reports.[23,24]

MMPs are a group of zinc-dependent endopeptidase which is capable of degrading ECM components, and among the MMPs family, MMP-2 and -9 were reported to be elevated in the pathological conditions such as inflammation, wound healing, cancer, and aging.[24] The docking studies and binding free energy reported in Table 9 show that 4-hydroxyisoleucine had the highest interaction energy (−43.47 kcal/mol) with that of MMP-2, but phytic acid fails to dock with the MMP-2. 4-hydroxyisoleucine showed interaction with Ala165, His201, and Glu202 amino acid residues of MMP-2 as shown in Table 9. As for the docking studies and binding free energy calculations with MMP-9, 4-hydroxyisoleucine exhibited the highest interaction energy (−38.60 kcal/mol), but phytic acid fails to dock with the same. 4-hydroxyisoleucine exhibited interaction with Ala189 and Gln227 amino acid residues of MMP-9 as shown in Table 10. In the present study, 4-hydroxyisoleucine exhibited interaction with Ala165, His201, and Glu202 amino acid residues of MMP-2 and with Ala189 and Gln227 amino acid residues of MMP-9, respectively, this finding was in good agreement with previous reports.[9,23] However, phytic acid fails to dock with the MMP-2 and -9 might due to the general poor binding phenomenon as reported by Akdogan *et al.*[25]

XO is the key enzyme which catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid.[9] XO is another target protein/enzyme which its docking studies and binding free energy calculations showed 4-hydroxyisoleucine having the maximum interaction energy (−29.23 kcal/mol), but phytic acid fails to dock with the XO. Interestingly, 4-hydroxyisoleucine showed interaction with

Table 6: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with microsomal prostaglandin E synthase-2 using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	56.21	GlnA198	1.1 and 1.6
		ArgB292	2.5
		SerB295	1.7, 1.7 and 2.1
		SerD295	2.4 and 2.4
		ArgB296	1.9
		ArgB298	2.2 and 2.5
		ArgD298	2.5
4-hydroxyisoleucine	24.13	SerB295	2.1
		SerD295	2.4

Table 7: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with tyrosinase using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	33.26	Lys129	1.1, 1.7, 2.2 and 2.3
4-hydroxyisoleucine	14.73	Pro14	1.8 and 2.5

Table 8: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with human neutrophil elastase using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	47.59	Arg147	2.3, 2.6, and 3.1
		Cys191	1.5 and 2.8
		Phe192	2.7 and 3.0
		Gly193	2.7
		Asp194	3.0
		Ser195	1.9, 2.0, 2.6, 2.7, and 3.1
4-hydroxyisoleucine	23.51	Phe192	2.8
		Ser195	1.6, 1.6, and 1.7
		Ser214	2.2

Table 9: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with matrix metalloproteinase-2 using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	F	-	-
4-hydroxyisoleucine	43.47	Ala165	1.5
		His201	1.7
		Glu202	1.1 and 1.2

F: Fails to dock

molybdenum-oxygen-sulfur (MOS) complex which is the key component in XO as shown in Table 11. In the present study, 4-hydroxyisoleucine showed interaction with Arg880, Thr1010, and MOS1328 amino acid residues of XO; this was in good agreement with the previous report.[9] However, phytic acid fails to dock with the XO might due to the general poor binding phenomenon as reported by Akdogan *et al.*[25]

SQS is the key enzyme involves in cholesterol biosynthesis pathway, and it is inhibition leads to direct decrease in cholesterol biosynthesis resulted in reduction of plasma cholesterol level.[9] The maximum interaction energy in the docking studies and binding free energy calculations with that of SQS was exhibited by 4-hydroxyisoleucine (−22.59 kcal/mol), but phytic acid fails to dock with the same. 4-hydroxyisoleucine exhibited interaction with Val175 and Gln212 amino acid residues of SQS as shown in Table 12. In the present study, 4-hydroxyisoleucine exhibited interaction with Val175 and Gln212 amino acid residues of SQS; this finding was in good agreement with the previous report.[9] However, phytic acid fails to dock with the SQS might due to the general poor binding phenomenon as reported by Akdogan *et al.*[25]

NOS is a family of enzymes that catalyze the production of nitric oxide from L-arginine. Nitric oxide is a key cellular signaling molecule which plays a vital role in various cellular processes.[9] The docking studies and binding free energy reported in Table 13 show that 4-hydroxyisoleucine had the highest interaction energy (−24.66 kcal/mol) with that of NOS, but phytic acid fails to dock with the NOS. Interestingly, 4-hydroxyisoleucine does not interact with any amino acid residue of NOS. In the present study, phytic acid fails to dock with the NOS might due to the general poor binding phenomenon as reported by Akdogan *et al.*[25]

HAR is the key enzyme which plays a major role in the development of secondary diabetic complications through polyol pathway, which follows the entry of excess glucose into the body.[26] HAR is another target protein/enzyme which its docking studies and binding free energy calculations showed 4-hydroxyisoleucine had the maximum interaction energy (−34.50 kcal/mol), but phytic acid fails to dock with the same. Interestingly, 4-hydroxyisoleucine showed interaction with six amino acid residues (Thr19, Trp20, Lys21, Asp43, Ser210, and Ile260) of HAR as shown in Table 14. Thus, the present finding was in good agreement with Umamaheswari *et al.* report.[27] However, phytic acid fails to dock with the HAR might due to the general poor binding phenomenon as reported by Akdogan *et al.*[25]

LOXs are the class of oxidative enzymes, which catalyze the formation of hydroperoxy eicosatetraenoic acids (HPETEs) from AA. Moreover, these HPETEs are further reduced and transformed to form so-called eicosanoids.[28] The docking studies and binding free energy reported in Table 15 show that 4-hydroxyisoleucine with the lowest binding energy (−5.15 kcal/mol) using Autodock 4.2, and interestingly, 4-hydroxyisoleucine exhibited interaction with three amino acid residues (Ser510, His513, and Gln716) of LOX. The present finding was in good agreement with the previous report.[29] Phytic acid showed very least binding energy (+42.57 kcal/mol) with LOX, which might due to unfavorable interactions phenomenon as reported by Castro *et al.*[30]

CONCLUSION

In the present study, it was found that 4-hydroxyisoleucine has the potential to dock and bind with all of the 11 targeted enzymes, whereas phytic acid failed to dock and bind with six enzymes except COX-2, mPGES-2, tyrosinase, HNE, and LOX. Hence, it is strongly suggested that the results of this study have paved better understanding of 4-hydroxyisoleucine as potential COX-2, mPGES-2, tyrosinase, HNE, MMP-2, MMP-9, XO, SQS, NOS, HAR, and LOX inhibitor in

Table 10: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with matrix metalloproteinase-9 using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	F	-	-
4-hydroxyisoleucine	38.60	Ala189	1.7
		Gln227	0.88, 1.2 and 2.7

F: Fails to dock

Table 11: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with xanthine oxidase using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	F	-	-
4-hydroxyisoleucine	29.23	Arg880	2.7 and 3.2
		Thr1010	1.7 and 2.7
		Glu1261	1.8
		MOS1328	2.5

F: Fails to dock; MOS: Molybdenum-oxygen-sulfur

Table 12: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with squalene synthase using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	F	-	-
4-hydroxyisoleucine	22.59	Val175	1.6
		Gln212	1.3

F: Fails to dock

Table 13: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with nitric oxide synthase using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	F	-	-
4-hydroxyisoleucine	24.66	No interaction	-

F: Fails to dock

Table 14: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with human aldose reductase using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	F	-	-
4-hydroxyisoleucine	34.50	Thr19	2.3
		Trp20	2.4
		Lys21	2.1
		Asp43	2.3
		Ser210	2.3
		Ile260	1.8

F: Fails to dock

Table 15: The interaction energy analysis of phytic acid and 4-hydroxyisoleucine with lipoxygenase using Autodock 4.2

Ligand name	Lowest binding energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Phytic acid	42.57[+]	NA	NA
4-hydroxyisoleucine	−5.15	Ser510	2.1 and 3.0
		His513	3.0
		Gln716	1.9 and 2.0

[+]Positive binding energy, which might due to unfavorable interactions phenomenon as reported by Castro *et al.* and NA. NA: Not analyzed

relation to the prevention of associated disorders of inflammation, hyperpigmentation, wound healing, hyperuricemia, hyperlipidemia, and hyperglycemia.

Acknowledgement

The author (Radhakrishnan Narayanaswamy) would like to thank the Research Management Center of Universiti Putra Malaysia, for the postdoctoral financial support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Oatway L, Vasanthan T, Helm JH. Phytic acid. Food Rev Int 2001;17:419-31.
2. Pathak N, Rai AK, Kumari R, Bhat KV. Value addition in sesame: A perspective on bioactive components for enhancing utility and profitability. Pharmacogn Rev 2014;8:147-55.
3. Timotius KH, Sari IN, Santoso AW. Major bioactive compounds of pilis plant materials: A GC-MS analysis. Pharmacognosy Commun 2015;5:190-6.
4. Bera TK, Ali KM, Jana K, Ghosh A, Ghosh D. Protective effect of aqueous extract of seed of *Psoralea corylifolia* (Somraji) and seed of *Trigonella foenum-graecum* L. (Methi) in streptozotocin-induced diabetic rat: A comparative evaluation. Pharmacognosy Res 2013;5:277-85.
5. Perera PK, Li Y. Functional herbal food ingredients used in type 2 diabetes mellitus. Pharmacogn Rev 2012;6:37-45.
6. Broca C, Manteghetti M, Gross R, Baissac Y, Jacob M, Petit P, *et al.* 4-Hydroxyisoleucine: Effects of synthetic and natural analogues on insulin secretion. Eur J Pharmacol 2000;390:339-45.
7. Haeri MR, Izaddoost M, Ardekani MR, Nobar MR, White KN. The effect of fenugreek 4-hydroxyisoleucine on liver function biomarkers and glucose in diabetic and fructose-fed rats. Phytother Res 2009;23:61-4.
8. Maurya CK, Singh R, Jaiswal N, Venkateswarlu K, Narender T, Tamrakar AK. 4-Hydroxyisoleucine ameliorates fatty acid-induced insulin resistance and inflammatory response in skeletal muscle cells. Mol Cell Endocrinol 2014;395:51-60.
9. Narayanaswamy R, Isha A, Wai LK, Ismail IS. Molecular docking analysis of selected *Clinacanthus nutans* constituents as xanthine oxidase, nitric oxide synthase, human neutrophil elastase, matrix metalloproteinase 2, matrix metalloproteinase 9 and squalene synthase inhibitors. Pharmacogn Mag 2016;12 Suppl 1:S21-6.
10. Wu G, Robertson DH, Brooks CL 3rd, Vieth M. Detailed analysis of grid-based molecular docking: A case study of CDOCKER-A CHARMm-based MD docking algorithm. J Comput Chem 2003;24:1549-62.
11. Swamy RN, Gnanamani A, Shanmugasamy S, Gopal RK, Mandal AB. Bioinformatics in crosslinking chemistry of collagen with selective cross linkers. BMC Res Notes 2011;4:399.
12. 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;46:3-26.
13. Singh S, Gupta AK, Verma A. Molecular properties and bioactivity score of the *Aloe vera* antioxidant compounds – In order to lead finding. Res J Pharm Biol Chem Sci 2013;4:876-81.
14. Wang J, Urban L. The impact of early ADME profiling on drug discovery and development strategy. Drug Discov World 2004;5:73-86.
15. Crofford LJ. COX-1 and COX-2 tissue expression: Implications and predictions. J Rheumatol Suppl 1997;49:15-9.
16. Saad N, Esa NM, Ithnin H. Suppression of ß-catenin and cyclooxygenase-2 expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA). Asian Pac J Cancer Prev 2013;14:3093-9.
17. Khokra SL, Jyoti, Chetan, Kaushik P, Alam MM, Zaman MS, *et al.* Quinoline based furanones and their nitrogen analogues: Docking, synthesis and biological evaluation. Saudi Pharm J 2016;24:705-17.
18. Chang HH, Meuillet EJ. Identification and development of mPGES-1 inhibitors: Where we are at? Future Med Chem 2011;3:1909-34.
19. Sun Y, Jia Z, Yang G, Kakizoe Y, Liu M, Yang KT, *et al.* mPGES-2 deletion remarkably enhances liver injury in streptozotocin-treated mice via induction of GLUT2. J Hepatol 2014;61:1328-36.
20. Radhakrishnan N, Ashok S, Kavitha V, Rameshkumar G, Gnanamani A. Molecular docking studies of embelin (simple natural benzoquinone) and its derivatives as a potent tyrosinase inhibitor. J Chem Pharm Res 2013;5:320-6.
21. Graf E, Empson KL, Eaton JW. Phytic acid: A natural antioxidant. J Biol Chem 1987;262:11647-50.
22. Radhakrishnan N, Ashok S, Kavitha V, Rameshkumar G, Gnanamani A. Molecular docking studies of L-cysteine (a non-essential sulfur containing amino acid) as a potent tyrosinase inhibitor. J Appl Cosmetol 2013;31:183-9.
23. Narayanaswamy R, Wai LK, Ismail IS. *In silico* analysis of selected honey constituents as human neutrophil elastase (HNE) and matrix metalloproteinases (MMP 2 and 9) inhibitors. Int J Food Properties 2015;18:2155-64.
24. Narayanaswamy R, Wai LK, Ismail IS. Molecular docking analysis of natural compounds as human neutrophil elastase (HNE) inhibitors. J Chem Pharm Res 2013;5:337-41.
25. Akdogan ED, Erman B, Yelekci K. *In silico* design of novel and highly selective lysine-specific histone demethylase inhibitors. Turk J Chem 2011;35:523-42.
26. Maanvizhi S, Narayanaswamy R, Wai LK, Gnanamani A. Molecular docking analysis of embelin and its metal complexes as human aldose reductase (HAR) inhibitor. Pharm Lett 2014;6:165-8.
27. Umamaheswari M, Aji CS, Kuppusamy A, Thirumalaisamy S, Varadharajan S, Puliyath J. *In*

silico docking studies of aldose reductase inhibitory activity of selected flavonoids. Int J Drug Dev Res 2012;4:328-34.

28. Wisastra R, Dekker FJ. Inflammation, cancer and oxidative lipoxygenase activity are intimately linked. Cancers (Basel) 2014;6:1500-21.
29. Ng CH, Rullah K, Aluwi MF, Abas F, Lam KW, Ismail IS, *et al.* Synthesis and docking studies of 2,4,6-trihydroxy-3-geranylacetophenone analogs as potential lipoxygenase inhibitor. Molecules 2014;19:11645-59.
30. Castro JS, Trzaskowski B, Deymier PA, Bucay J, Adamowicz L, Hoying JB. Binding affinity of fluorochromes and fluorescent proteins to Taxol™ crystals. Mater Sci Eng C 2009;29:1609-15.