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ORIGINAL ARTICLE

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

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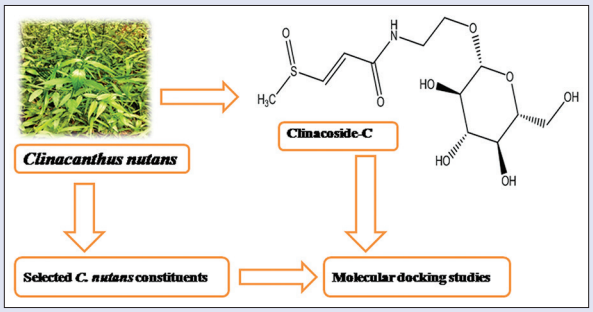
ABSTRACT

Background: *Clinacanthus nutans* (Burm. f.) Lindau has gained popularity among Malaysians as a traditional plant for anti-inflammatory activity. **Objective:** This prompted us to carry out the present study on a selected 11 constituents of *C. nutans* which are clinacoside A–C, cycloclinacoside A1, shaftoside, vitexin, orientin, isovitexin, isoorientin, lupeol and β-sitosterol. **Materials and Methods:** Selected 11 constituents of *C. nutans* were evaluated on the docking behavior of xanthine oxidase (XO), nitric oxide synthase (NOS), human neutrophil elastase (HNE), matrix metalloproteinase (MMP 2 and 9), and squalene synthase (SQS) using Discovery Studio Version 3.1. Also, molecular physicochemical, bioactivity, absorption, distribution, metabolism, excretion, and toxicity (ADMET), and toxicity prediction by computer assisted technology analyzes were also carried out. **Results:** The molecular physicochemical analysis revealed that four ligands, namely clinacoside A–C and cycloclinacoside A1 showed nil violations and complied with Lipinski's rule of five. As for the analysis of bioactivity, all the 11 selected constituents of *C. nutans* exhibited active score (>0) toward enzyme inhibitors descriptor. ADMET analysis showed that the ligands except orientin and isoorientin were predicted to have Cytochrome P_{450}2D6 inhibition effect. Docking studies and binding free energy calculations revealed that clinacoside B exhibited the least binding energy for the target enzymes except for XO and SQS. Isovitexin and isoorientin showed the potentials in the docking and binding with all of the six targeted enzymes, whereas vitexin and orientin docked and bound with only NOS and HNE. **Conclusion:** This present study has paved a new insight in understanding these 11 *C. nutans* ligands as potential inhibitors against XO, NOS, HNE, MMP 2, MMP 9, and SQS.

Key words: *Clinacanthus nutans*, clinacoside B, cycloclinacoside, isoorientin, isovitexin, shaftoside

SUMMARY

- Isovitexin and isoorientin (*Clinacanthus nutans* constituent) showed potentials in the docking and binding with all of the six targeted enzymes (xanthine oxidase [XO], nitric oxide synthase [NOS], human neutrophil elastase [HNE], matrix metalloproteinase [MMP 2 and 9], and squalene synthase [SQS])
- Moreover, clinacoside B (*C. nutans* constituent) exhibited the least binding energy for the target enzymes except for XO and SQS
- Interestingly, all of the selected ligands from *C. nutans* showed the potential to dock and bind with HNE.



Abbreviations used: *C. nutans: Clinacanthus nutans,* XO: Xanthine oxidase, NOS: Nitric oxide synthase, HNE: Human neutrophil elastase, MMP: Matrix metalloproteinase, SQS: Squalene synthase, ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity, TOPKAT: Toxicity prediction by the computer assisted technology.

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INTRODUCTION

Clinacanthus nutans (Burm. f.) Lindau is a small shrub, belongs to the family Acantaceae and native to tropical Asia. In Malaysia, it is commonly known as Sabah snake grass,[1] presently it is cultivated to meet the huge market demand.[2] It has been used traditionally to treat snake bites, dysentery, diabetes, fever, skin infections, and burns.[3,4] Recent studies have shown that *C. nutans* have the signifi ant biological activities such as antiviral, antioxidant, anti-inflammatory, and anti-bacterial activities.[1,5] However, only two enzymes which are myeloperoxidase (MPO) and elastase inhibition activity of *C. nutans* have been reported so far.

C. nutans has been chemically studied[2] wherein the important chemical constituents isolated from *C. nutans* are clinacoside A–C, cycloclinacoside A1, shaftoside, vitexin, orientin, isovitexin, isoorientin,[6] lupeol, and β-sitosterol.[7] Therefore, these 11 chemical constituents were selected to be evaluated in this study on the docking

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behavior of xanthine oxidase (XO), nitric oxide synthase (NOS), human neutrophil elastase (HNE), matrix metalloproteinase (MMP 2 and 9), and squalene synthase (SQS) with investigation on the enzymes putative binding sites using Discovery Studio Version 3.1. (Accelrys, USA).

MATERIALS AND METHODS

Ligand preparation

Chemical structures of the 11 selected ligands namely (i) shaftoside (CID442658); (ii) vitexin (C ID5280441); (iii) orientin (CID5281675); (iv) isovitexin (CID162350); (v) isoorientin (CID114776); (vi) lupeol (CID259846), and (vii) *β*-sitosterol (Chemspider ID192962) were retrieved from PubMed (www.pubmed.com) and Chemspider (www.chemspider.com) compound databases. Unavailable three dimensional structures of clinacoside A–C and cycloclinacoside A1 were generated using ACD (www.acdlabs.com/download/chemsk.html).

Target protein identification and preparation

The three-dimensional structures of the XO (Protein Data Bank (PDB) ID: 1FIQ with resolution of 2.50 Å), NOS (PDB ID: 4NOS 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), and SQS (PDB ID: 3ASX with resolution of 2.00 Å) were obtained from the Research Collaborator for Structural Bioinformatics PDB (Anonymous, www.rcsb.org). A chain of all of the proteins (except for XO, where C chain) was preprocessed separately by deleting other chains (B, C, and D), ligand, as well as the crystallographically observed water molecules (water without hydrogen bonds).

Molecular descriptors calculation

Molinspiration online database was used for all the selected twelve ligands to calculate thirteen descriptors (www.molinspiration.com) which are log P, polar surface area, molecular weight, 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, excretion, and toxicity, and toxicity prediction by computer assisted technology test

Both of absorption, distribution, metabolism, excretion and toxicity (ADMET) and toxicity prediction by computer assisted technology (TOPKAT) tests were performed using Discovery Studio® 3.1 (Accelrys, San Diego, USA). ADMET analysis was performed using human intestinal absorption, aqueous solubility, blood brain barrier, cytochrome P_{450}2D6 (CYP2D6), plasma protein binding, and hepatotoxicity descriptors. As for the TOPKAT analysis, aerobic biodegradability (AB), Ames mutagenicity, ocular irritancy, skin irritancy, skin sensitization, and oral toxicity in rat (LD_{50} in g/Kg of body weight) descriptors were used.

Docking studies

Docking studies were carried out on the crystal structures of XO, NOS, HNE, MMP 2, MMP 9, and SQS retrieved 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 fi lds. A protein was fi stly held rigid while the ligands were allowed to flex during the refi ement. Two hundred random ligand conformations were then generated from the initial ligand structure through the high temperature molecular dynamics followed by random rotations, refi ement by grid-based (GRID I) simulated annealing, and a fi al grid-based or full force fi ld minimization.[8] 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 10 ligand binding poses were ranked according to their CDOCKER energies, and the predicted binding interactions were then analyzed from which the best among the 10 ligand binding poses was chosen and carried out *in situ* ligand minimization using standard protocol.

RESULTS AND DISCUSSION

Lipinski's rule of five is a computational tool used to predict the solubility and permeability of chemical compounds based on their molecular properties.[9] According to Lipinski's rule, poor absorption or permeation is more likely when log *P* value >5; molecular weight >500; more than 5 hydrogen bond donors; more than 10 hydrogen bond acceptors, and greater than 15 rotatable bonds.[10] In the present study, clinacoside A–C and cycloclinacoside A1 showed no violation. On other hand, orientin and isoorientin showed two violations, while shaftoside showed three violations as given in Table 1.

Bioactivity score is another computational approach used to determine whether or not a particular molecule is similar to the known drugs in their molecular properties and structure features.[11] According to the bioactivity score, if >0 is active; if (−5.0 to − 0.0) is moderately active and if <−5.0 is inactive. In the present study, all of the 11 *C. nutans* constituents showed

Table 1: Molecular physicochemical descriptors analysis on 11 ligands using Molinspiration online software tool

Ligand	Log A[a]	TPSA[b]	Natoms[c]	MW[d]	noN[e]	nOH NH[f]	Nviolations[g]	Nrotb[h]	Volume[i]
Clinacoside A	−2.45	133.5	19	298.3	8	4	0	5	244.9
Clinacoside B	−2.85	116.5	18	282.3	7	4	0	5	239.2
Clinacoside C	−3.75	145.5	22	339.4	9	5	0	7	287.4
CycloclinacosideA1	−2.16	122.5	19	241.3	8	3	0	3	241.3
Shaftoside	−1.67	250.9	40	564.5	14	10	3	4	461.5
Vitexin	0.52	181.0	31	432.4	10	7	1	3	355.2
Orientin	0.03	201.3	32	448.4	11	8	2	3	363.2
Isovitexin	0.52	181.0	31	432.4	10	7	1	3	355.2
Isoorientin	0.03	201.3	32	448.4	11	8	2	3	363.2
Lupeol	8.29	20.2	31	426.7	1	1	1	1	461.6
β-sitosterol	8.62	20.2	30	414.7	1	1	1	6	456.5

[a]Octanol-water partition coeffici t; [b]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 5 violations; [h]Number of rotatable bonds; [i]Molecular volume

active score (>0) toward enzyme inhibitors descriptor. However, for other descriptors, these compounds exhibited active to moderate active scores with none showing inactive score (<−5.0), as shown in Table 2.

Optimizing desirable ADMET characteristics is now recognized as an important approach for drug discovery[12] as many drug candidates often fail to comply with the ADMET profiles.[13] Many pharmaceutical companies opt for using ADMET profiling with in some cases used it as an alternative for the wet screening method.[14] Table 3 shows the ADMET profile of the 11 ligands wherein shaftoside, vitexin, orientin, isovitexin, and isoorientin are predicated to have hepatotoxic effect compared to all other ligands. Most of the ligands except orientin and isoorientin were predicted to have CYP2D6 inhibition effect. The toxicity profile of the 11 ligands as depicted in Table 4 shows that cycloclinacoside A1 is nondegradable toward AB nature and all of the ligands were predicted to have ocular/eye irritancy effect in humans.

Table 2: Bioactivity score of 11 ligands using Molinspiration online software tool

Ligand	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Clinacoside A	0.01	−0.03	−0.25	−0.32	0.32	0.49
Clinacoside B	0.11	0.07	−0.33	−0.22	0.10	0.75
Clinacoside C	0.26	−0.07	−0.21	−0.15	0.38	0.57
Cycloclinacoside A1	−0.01	−0.24	−0.23	−0.45	0.17	0.36
Shaftoside	0.10	−0.33	0.00	0.02	−0.00	0.33
Vitexin	0.13	−0.14	0.19	0.23	0.03	0.46
Orientin	0.12	−0.14	0.19	0.20	0.01	0.45
Isovitexin	0.12	0.02	0.15	0.23	0.03	0.47
Isoorientin	0.11	0.01	0.16	0.20	0.01	0.46
Lupeol	0.27	0.11	−0.42	0.85	0.15	0.52
β-sitosterol	0.14	0.05	−0.51	0.73	0.07	0.51

GPCR: G protein coupled receptors

XO is the key regulatory enzyme in purine metabolism which catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid.[15] Hyperuricemia is a metabolic disorder characterized by elevated level of serum uric acid. Allopurinol and febuxostat are the two XO inhibitors widely used for the treatment of hyperuricemia. However, these drugs are associated with adverse effects such as gastrointestinal, hepatic, renal, and allergic reactions.[16] In this study, 11 selected constituents of *C. nutans* were evaluated on the docking behavior of XO. The docking studies and binding free energy calculations as in Table 5 show isoorientin to be having the highest interaction energy (−49.80 kcal/mol) with that of XO. In contrast, cycloclinacoside A1 showed the least interaction energy (−25.60 kcal/mol) compared to other ligands. However, clinacoside B, shaftoside, vitexin, orientin, lupeol and *β*-sitosterol were the six ligands which could not dock with XO due to the general poor binding phenomenon.[17] Interestingly, isoorientin showed interaction with Molybdenum-Oxygen-Sulfur (MOS) complex which is the key component in XO.[18] Lin *et al.*[19] reported isovitexin as a competitive inhibitor of XO and suggested that the bulky sugar moiety of isovitexin has been responsible for the interaction with XO. Isovitexin and isoorientin isolated from *Biophytum umbraculum*, an African medicinal plant were recently found to be the XO inhibitors.[20]

NOS are a family of enzymes that catalyze the production of NO from L-arginine. There are three isoforms of NOS in mammals, of which, two are constitutive and one is an inducible type. NO is an important

Table 3: ADMET analysis of 11 ligands

Ligand	HIA			AS		BBB		Predication		
	PSA	ALogP (8)	Level*	Log (SW)	Level**	Log BB	Level***	PPB	CYP2D6	HT
Clinacoside A	135.72	−2.57	3	1.41	5	0	4	False	False	False
Clinacoside B	118.42	−2.67	3	1.96	5	0	4	False	False	False
Clinacoside C	148.53	−3.40	3	1.99	5	0	4	False	False	False
Cycloclinacoside A1	123.84	−2.25	3	0.67	5	0	4	False	False	False
Shaftoside	252.25	−1.86	3	−4.49	2	0	4	False	False	True
Vitexin	180.87	0.02	3	−2.75	3	0	4	False	False	True
Orientin	201.68	−0.22	3	−3.32	3	0	4	False	True	True
Isovitexin	180.87	0.02	3	−2.67	3	0	4	False	False	True
Isoorientin	201.68	−0.22	3	−3.22	3	0	4	False	True	True
Lupeol	20.82	7.40	3	−8.76	0	0	4	True	False	False
β-sitosterol	20.82	8.08	3	−8.26	0	0	4	True	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: Undefi ed. HIA: Human intestinal absorption; AS: Aqueous solubility; BBB: Blood brain barrier; PPB: Plasma protein binding; CYP2D6: Cytochrome P_{450}2D6; HT: Hepatotoxicity; ADMET: Absorption, distribution, metabolism, excretion and toxicity; PSA: Polar surface area

Table 4: Toxicity predication analysis of 11 ligands

Ligand	AB*	AM**	OI#	SI##	SS♦	Oral toxicity▲
Clinacoside A	Degradable	Nonmutagen	Irritant	Irritant	Nonsensitizer	2.27
Clinacoside B	Degradable	Nonmutagen	Irritant	Irritant	Nonsensitizer	ND◊
Clinacoside C	Degradable	Nonmutagen	Irritant	Irritant	Nonsensitizer	ND◊
Cycloclinacoside A1	Nondegradable	Nonmutagen	Irritant	Irritant	Nonsensitizer	2.34
Shaftoside	Degradable	Nonmutagen	Irritant	Nonirritant	Nonsensitizer	2.23
Vitexin	Degradable	Nonmutagen	Irritant	Nonirritant	Nonsensitizer	1.16
Orientin	Degradable	Nonmutagen	Irritant	Nonirritant	Nonsensitizer	1.43
Isovitexin	Degradable	Nonmutagen	Irritant	Nonirritant	Nonsensitizer	1.06
Isoorientin	Degradable	Nonmutagen	Irritant	Nonirritant	Nonsensitizer	1.31
Lupeol	Degradable	Nonmutagen	Irritant	Irritant	Nonsensitizer	2.2
β-sitosterol	Degradable	Nonmutagen	Irritant	Irritant	Nonsensitizer	1.63

*AB: Aerobic biodegradability; **AM: Ames mutagenicity; #OI: Ocular irritancy; ##SI: Skin irritancy; ♦SS: Skin sensitization; ▲Oral toxicity: Oral toxicity in rat (LD50 in g/kg of body weight); ◊ND: Not determined

cellular signaling molecule playing a vital role in various cellular processes.[21] Table 6 shows the docking studies and binding free energy calculations in which the highest interaction energy (−61.31 kcal/mol) was exhibited by shaftoside with that of NOS. Clinacoside B, on the contrary, showed the least interaction energy (−38.67 kcal/mol). Possibly due to the general poor binding phenomenon,[16] lupeol and *β*-sitosterol could not dock with NOS. In this study, six ligands namely clinacoside B, clinacoside C, shaftoside, vitexin, orientin, and isovitexin exhibited interaction with the Glu377 amino acid residue of NOS as shown in Table 6. Lin *et al.*[22] reported that isovitexin suppressed lipopolysaccharide mediated NOS in mouse macrophage cells.

As for the docking studies and binding free energy calculations with HNE, shaftoside and clinacoside B exhibited the highest (−43.89 kcal/mol) and the least interaction energy (−30.15 kcal/mol), respectively, compared to other ligands [Table 7]. In this study, six ligands namely clinacoside A, clinacoside B, cycloclinacoside A1, orientin, isovitexin, and lupeol exhibited interaction with the Ser195 amino acid residue of HNE as shown in Table 7. Lupeol as HNE inhibitor was also reported by Mitaine-offer *et al.*[23] Wanikiat *et al.*[24] reported that acetone extract of

Table 5: The interaction energy analysis of 11 ligands with that of XO using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Clinacoside A	32.41	Thr101	3.0
Clinacoside B	F*	Nil	Nil
Clinacoside C	43.08	Arg880	1.9 and 2.6
		Thr101	2.4 and 2.9
Cycloclinacoside A1	25.60	Ser876	1.4, 2.3 and 3.0
		Thr101	2.9
Shaftoside	F*	Nil	Nil
Vitexin	F*	Nil	Nil
Orientin	F*	Nil	Nil
Isovitexin	46.95	Leu648	2.2
		Lys771	2.3
		Arg880	2.9
		Phe914•	3.1
Isoorientin	49.80	Lys771	2.3
		Arg880	2.6
		Ser876	3.0
		Phe914•	3.1
		MOS•1328	1.9 and 2.5
Lupeol	F*	Nil	Nil
β-sitosterol	F*	Nil	Nil

*F: Failed to dock; •π: π interaction; •MOS: Molybdenum-oxygen-sulfur; XO: Xanthine oxidase

Table 6: The interaction energy analysis of 11 ligands with that of NOS using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Clinacoside A	49.40	Tyr373	2.2
		Asp382	2.0
Clinacoside B	38.67	Cys200	2.3
		Glu377	1.8 and 1.9
Clinacoside C	45.28	Ile201	2.3
		Trp372	1.7
		Glu377	1.5
Cycloclinacoside A1	38.56	Trp194	2.3
		Cys200	2.2
		Asn370	1.8
Shaftoside	61.31	Gln263	2.1
		Ile201	1.9
		Trp372	2.3
		Glu377	1.9 and 2.3
		Trp463	1.9
Vitexin	55.93	Trp194•	3.8
		Phe369•	5.4
		Glu377	1.7 and 1.7
Orientin	57.81	Trp194•	3.7
		Gly371◊	2.4
		Phe369•	4.8
		Glu377	1.5 and 1.6
Isovitexin	48.71	Glu377	2.4
		Pro350	2.2
Isoorientin	55.00	Trp372	2.1 and 2.5
		Tyr373	2.3
		Asp382	1.8 and 1.9
		Arg388	2.5
Lupeol	F*	Nil	Nil
β-sitosterol	F*	Nil	Nil

*F: Failed to dock; •π: π interaction; ◊π: Sigma interaction; NOS: Nitric oxide synthase

Table 7: The interaction energy analysis of 11 ligands with that of HNE using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Clinacoside A	36.42	Arg147	2.5 and 2.6
		Ser195	1.7, 2.2, 2.3, 2.6 and 3.1
		Ser214	2.2
		Cys220	2.9
Clinacoside B	30.15	Ser195	2.9 and 3.1
		Ser214	1.7
Clinacoside C	35.95	Arg147	3.0
		Ser214	1.6
		Gly218	2.9
		Gly219	2.5
Cycloclinacoside A1	31.76	Phe192	2.6
		Ser195	3.2
		Gly219	2.8
Shaftoside	43.89	Val99	2.1
		Arg147	1.6
		Arg177	2.6
		Gly218	2.8
		Phe215•	5.6
Vitexin	43.02	Arg147	2.4
		Phe192	2.6
		Ser214	1.9
		Val216	2.9
Orientin	44.22	Arg177	2.6
		Ser195	1.9 and 2.4
		Phe215•	6.0 and 6.5
		Gly218	2.3
Isovitexin	40.79	Leu99B◊	2.9
		Pro98	2.3
		Phe192	2.5
		Ser195	2.2, 2.4, 2.6 and 3.0
		Ser214	1.9
Isoorientin	41.24	Phe41	1.7 and 2.1
		Arg177	3.0
		Gly193	2.7
		Phe215•	2.3
		Val216	3.0
Lupeol	30.21	Ser195	2.4
β-sitosterol	39.54	Arg177	2.5

•π: π interaction; ◊π: Sigma interaction; •Sigma: π interaction; HNE: Human neutrophil elastase

C. nutans exhibited dose-dependent inhibition of *N*-formyl-methionyl-l eucyl-phenylalanine induced HNE release.

The maximum interaction energy in the docking studies and binding free energy calculations with that of MMP 2 was exhibited by isovitexin (−58.59 kcal/mol), whereas clinacoside B showed the least interaction energy of – 46.90 kcal/mol. In contrast, the other six ligands; clinacoside A, shaftoside, vitexin, orientin, lupeol and *β*-sitosterol, could not dock with MMP 2 which might be due to the general poor binding phenomenon.[17] Four ligands namely clinacoside B, clinacoside C, isovitexin, and isoorientin exhibited interaction with the Glu202 amino acid residue of MMP 2 as shown in Table 8.

Table 8: The interaction energy analysis of 11 ligands with that of MMP 2 using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Clinacoside A	F*	Nil	Nil
Clinacoside B	46.90	Leu164	1.4
		Glu202	1.8
Clinacoside C	56.71	Ala167	1.2, 1.7 and 1.9
		Glu202	1.6
Cycloclinacoside A1	50.28	His201	1.3
		Pro221	2.5
Shaftoside	F*	Nil	Nil
Vitexin	F*	Nil	Nil
Orientin	F*	Nil	Nil
Isovitexin	58.59	Leu164	1.8
		Ala165	2.2
		Glu202	1.9
Isoorientin	57.28	Leu164	1.6
		Ala165	1.2, 1.4 and 1.7
		Glu202	1.6
Lupeol	F*	Nil	Nil
β-sitosterol	F*	Nil	Nil

*F: Failed to dock; MMP: Matrix metalloproteinase

MMP 9 is another target protein which its docking studies and binding free energy calculations showed isoorientin having the maximum interaction energy of – 60.45 kcal/mol. Clinacoside B, on the other hand, showed the very least interaction energy (−43.57 kcal/mol). Four of other ligands which are shaftoside, vitexin, orientin, and lupeol could not dock with MMP 9. Th s might be due to the general poor binding phenomenon as suggested by Akdogan *et al.*[17] Among the ligands, clinacoside A, cycloclinacoside A1, isovitexin, and isoorientin exhibited interaction with Leu188 amino acid residue of MMP 9 as shown in Table 9. Although *C. nutans* has been known to have anti-inflammatory activity, until at the present, there is no available reported investigation for its MMP 2 and 9 inhibitory activity.

Finally the docking studies and binding free energy calculations with that of SQS in which isoorientin exhibited the largest interaction energy (−49.07 kcal/mol) while cycloclinacoside A1 showed smallest interaction energy (−34.16 kcal/mol) [Table 10]. Shaftoside, vitexin, orientin, lupeol, and *β*-sitosterol were the ligands which could not dock with SQS, which were suggested to be due to the general poor binding phenomenon.[17] Other three ligands namely clinacoside A–C exhibited interaction with Asp80 amino acid, the residue of SQS. To our best of knowledge, there is no report yet available with regard to these ligands' SQS inhibitory activity.

CONCLUSION

In the present study, it was found that isovitexin and isoorientin has the potential to dock and bind with all of the six targeted enzymes, whereas vitexin and orientin failed to dock and bind with four enzymes except NOS and HNE. As for *β*-sitosterol, it could dock and bind with only HNE and MMP 9. Interestingly, all of the selected ligands from *C. nutans* showed the potential to dock and bind with HNE. Hence, it is strongly suggested that the results of this present study has paved better understanding of these 11 ligands of *C. nutans* as potential XO, NOS, HNE, MMP 2, MMP 9, and SQS inhibitors in relation to the prevention of associated disorders of hyperuricemia, wound healing, and hyperlipidemia.

Table 9: The interaction energy analysis of 11 ligands with that of MMP 9 using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Clinacoside A	51.80	Leu188	3.2
		Ala189	1.9
		His230	2.8
Clinacoside B	43.57	Ala191	2.9
		Gln227	2.2 and 3.2
Clinacoside C	55.38	Ala191	1.8, 2.9 and 3.0
		His226♦	3.0
Cycloclinacoside A1	43.81	Gly186	2.2
		Leu188	2.4
		His226	2.0
Shaftoside	F*	Nil	Nil
Vitexin	F*	Nil	Nil
Orientin	F*	Nil	Nil
Isovitexin	59.20	Leu188	3.0
		Gln227	2.7
Isoorientin	60.45	Leu188	2.9
		Gln227	1.5
		Tyr245	2.3
		Met247	3.1
Lupeol	F*	Nil	Nil
β-sitosterol	28.91	His226♦	2.8

*F: Failed to dock; ♦Sigma: π interaction; MMP: Matrix metalloproteinase

Table 10: The interaction energy analysis of 11 ligands with that of SQS using Discovery Studio® 3.1

Ligand name	-CDOCKER interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Clinacoside A	40.22	Asp80	2.3
		Gln212	1.6 and 2.2
		Gln293	2.1
Clinacoside B	36.19	Asp80	1.8 and 1.9
		Val175	2.4
		Gln212	1.4
Clinacoside C	44.82	Asp80	2.1 and 2.4
		Gln212	1.2
Cycloclinacoside A1	34.16	Met207	2.3
		Asn215	1.7
		Gln293	1.9
Shaftoside	F*	Nil	Nil
Vitexin	F*	Nil	Nil
Orientin	F*	Nil	Nil
Isovitexin	44.41	Asp84	1.5
		Ala176	2.3
Isoorientin	49.07	Asp84	1.5 and 2.3
		Met207	2.3
		Gln293	2.2
Lupeol	F*	Nil	Nil
β-sitosterol	F*	Nil	Nil

*F: Failed to dock; SQS: Squalene synthase

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Conflicts of interest

There are no confli ts of interest.

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