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Research Article

MOLECULAR DOCKING OF SELECTED COMPOUNDS FROM *CLINACANTHUS NUTANS* WITH BCL-2, P53, CASPASE-3 AND CASPASE-8 PROTEINS IN THE APOPTOSIS PATHWAY

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ABSTRACT

Clinacanthus nutans has been reported to interact with apoptosis protein in many cancer cells line. Thus, the objective of this study is to determine the interaction between selected compounds of *Clinacanthus nutans* reported from the literature with targeted proteins from the apoptosis pathway. The *C. nutans* compounds were selected based on drug-likeness and toxicity predictions using the Swiss ADME predictor and OSIRIS Property Explorer. Apoptosis proteins Bcl-2, p53, caspase-3, and caspase-8 were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank. The docking site was predicted using the Computed Atlas of Surface Topography of proteins online server. The docking analysis was performed using Auto Dock 4.2. Drug-likeness and toxicity predictions showed that the 18 *C. nutans* compounds evaluated had no toxicity risk factors. These selected compounds were used to analyse potential molecular docking with targeted apoptosis proteins. β-sitosterol had high binding affinities with caspase-3, p53 and Bcl-2 proteins and only stigmasterol had high binding affinities with caspase-8. This study showed the potential of compounds from *C. nutans* to interact with targeted apoptosis proteins. Considering increased number of experimental studies in cancer cell lines, this study can provide an insight prediction towards *C. nutans* compounds and targeted apoptosis protein in the structure analysis for compounds isolation and protein assays.

Keywords: *Clinacanthus nutans*, apoptosis protein, Lipinski's rule, molecular docking

INTRODUCTION

Cancer is a major health concern worldwide. Chemotherapy is the backbone of many cancer treatments, but chemotherapeutic resistance can result in therapeutic failure and eventually death.[1] Many studies have reported that intake of natural products that are rich in poly phenols and flavonoid compounds can reduce cancer risk because they contain abundant medicinal phytochemicals that have effective anticancer activity.[2] Thus, the use of natural products, especially medicinal plants, for cancer treatment and management has been growing, as conventional chemotherapy has enormous side effects.[3]

Clinacanthus nutans is gaining popularity as a medicinal plant in Malaysia because of claims of its anticancer properties. *C. nutans* is also known as Sabah Snake Grass or Belalai Gajah in Malaysia.[4] Studies have reported that *C. nutans* interacts with apoptosis proteins in many cancer cells line.[5-8] Thus, chemical compounds from *C. nutans* could potentially induce apoptosis by activating p53 tumour suppressors and regulating B-cell lymphoma 2 (Bcl-2) family and caspase proteins.

Cancer studies have shown that many cancer cells lack molecular targets, which makes it hard for chemotherapeutic drugs to be effective. These difficulties have led many researchers to pursue *in silico* studies to accelerate anticancer research. In cancer research, molecular docking studies have been used to identify protein-ligand interactions, elucidate mechanisms involved in ligand binding, and provide insight about the most stable complex of the ligand-protein interaction.[9] Targeting the apoptosis pathway using *in silico* studies is a promising approach to overcoming the drug resistance common to cancer chemotherapies.

Targeting apoptosis proteins such as p53 with drugs can trigger apoptosis when the drug suppresses anti-apoptotic proteins to enhance caspase activation.[10] The pro- and anti-apoptotic proteins from the Bcl-2 family provide the signal to release cytochrome c from the mitochondria and are part of the apoptosis pathway.[11] Initiator caspases -2, -8, -9 and -10 lead the apoptosis signal, whereas executioner caspases -3, -6 and -7 provide the mass proteolysis that contributes to apoptosis[12].

The objective of this study was to identify the interaction between selected compounds from *C. nutans* and targeted proteins in the apoptosis pathway (Bcl-2, p53, caspase-3, and caspase-8). The *C. nutans* compounds were selected based on the literature and the compounds were further validated through Lipinski's rule of five, drug-likeness, and toxicity. The selected compounds were further investigated through molecular docking studies to evaluate their binding affinities with the targeted proteins in the apoptosis pathway.

EXPERIMENTAL

Selection of compounds

Twenty-six chemical compounds from *C. nutans* were selected based on the isolation of compounds from reported *C. nutans* studies (Figure 1). Based on the literature review, the compounds were structured using ACD/Chem Sketch Freeware.[13] The generated structures were saved in .mol2 file format, which then was converted to .pdb file format using Open Babel: The Open Source Chemistry Toolbox.

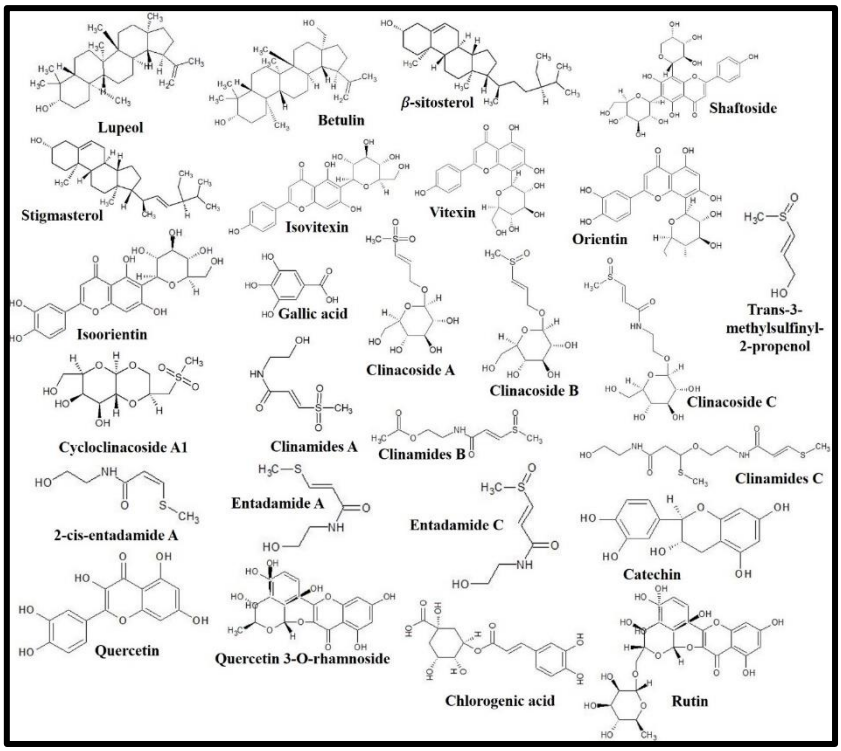

Figure 1: Two-dimensional chemical structures of selected compounds from *C. nutans*

Drug-likeness and toxicity predictions

Lipinski's rule of five was used to predict the drug-likeness in which this rule determines the consistency of orally active drug.[14,15] The Lipinski's rule predicts the permeation or absorption of a compound when the compound has more than 5 hydrogen bond donors, 10 hydrogen bond acceptors, calculated LogP (CLogP) greater than 5.37 and molecular weight greater than 500 g/mol.[16] Drug score was also used to determine the selection of the compounds. The compounds with higher drug score values are considered as a drug candidate.[17] In this study, the compounds were screened using Swiss ADME predictor[18] and OSIRIS Property Explorer[19]. Swiss ADME predictor gives the data on number hydrogen acceptor, hydrogen donors and rotatable bonds. The analyse compounds were screened and only compounds without violation based on Lipinski's rule were used to evaluate the mutagenic, reproductive risks, irritant and tumorigenic, hydrophilicity, molecular weight, solubility, drug-likeness and drug score by using OSIRIS Property Explorer. Then, analyse compounds without any violation were used for the docking study.

Protein model and active site prediction

Proteins structures of p53 (PDB ID: 1YCR), Bcl-2 (PDB ID: 4MAN), caspase-3 (PDB ID: 6CKZ), and caspase-8 (PDB ID: 1QDU) were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank. All data files were saved in .pdb file format. Water and ligand molecules from the proteins were removed using Discovery Studio Visualizer 4.1 Client.[20] The Computed Atlas of Surface Topography of proteins (CASTp), which is an online server that predicts the active sites present in the protein structure, then was used to evaluate the proteins.[21]

Molecular docking

Molecular docking analysis was conducted using the automated docking tool Auto Dock 4.2[22]. Polar hydrogenatoms and Gasteiger partial charges were added to the 3Dprotein structure. The protein structure was written in .pdbqt file format for further analysis. The grid boxes were set according to the amino acid active sites predicted by CASTp.

In this study, Lamarckian Genetic Algorithm 4.2 was used in the docking analysis[23] and the protein macromolecules were kept rigid throughout the docking simulation. Genetic algorithm runs were set at 100 and the other parameters for docking analyses were left at default settings. The best protein-compound conformations were chosen from the Auto Dock 4.2 scoring function, and they were ranked according to their binding affinities. Discovery Studio Visualizer 4.1 Client[20], Chimera 1.14[24], and LigPlot[25] were used for post-docking analyses.

RESULTS

Drug-likeness prediction studies

Table 1 shows the physiochemical parameters of the ligands predicted using Swiss ADME. After screening and filtration using Lipinski's rules, 21 potential active compounds were retained. Generally, Lipinski's rule states that an orally active drug can have no more than one violation of the Lipinski's rule parameters.[17] Twenty-one of the original 26 selected compounds satisfied Lipinski's rule; the ones that did not were shaftoside, orientin, isoorientin, quercetin 3-O-rhamnoside, and rutin. Shaftoside and rutin violated three parameters (number of hydrogen bond acceptors > 10, number of hydrogen bond donors > 5 and molecular weight > 500 g/mol). The three other *C. nutans* compounds significantly violated two parameters from Lipinski's rule (number of hydrogen bond acceptors > 10 and number of hydrogen bond donors > 5).

The remaining 21 compounds were subjected to OSIRIS Property Explorer to predict whether they have no, low, or high toxicity risk factors. Only vitexin, gallic acid, and quercetin had toxicity risk factors (Table 2). For vitexin the risk was high mutagenic factor and for gallic acid it was high mutagenic and high reproductive effects. Quercetin demonstrated high mutagenic and tumorigenic effects. After this analysis, 18 compounds were retained for use in the docking analyses.

Table 1: Lipinski's rule for *C. nutans* compounds by Swiss ADME

Ligands	Lipinski's Rule of Five					
	MW (g/moL)	Log P	H Donor	H Acceptor	Violation	Drug-Likeness
	Less than 500 g/mol	Less than 5	Less than 5	Less than 10		Lipinski's Rule Follows
Lupeol	426.72	6.92	1	1	1	Yes
Betulin	442.72	6.00	2	2	1	Yes
β-sitosterol	414.71	6.73	1	1	1	Yes
Stigmasterol	412.69	6.62	1	1	1	Yes
Shaftoside	564.49	-3.97	10	14	3	No
Vitexin	432.38	-2.02	7	10	1	Yes
Isovitexin	432.38	-2.02	7	10	1	Yes
Orientin	448.38	-2.51	8	11	2	No
Isoorientin	448.38	-2.51	8	11	2	No
Gallic acid	170.12	-0.16	4	5	0	Yes
Clinacoside A	298.31	-2.57	4	8	0	Yes
Clinacoside B	282.31	-2.50	4	7	0	Yes
Clinacoside C	339.36	-2.78	5	9	0	Yes
Cycloclinacoside A1	298.31	-2.47	3	8	0	Yes
Clinamides A	193.22	-1.18	2	4	0	Yes
Clinamides B	219.26	-0.49	1	4	0	Yes
Clinamides C	322.44	-0.43	3	4	0	Yes
2-cis-entadamide A	161.22	-0.12	2	5	0	Yes
Entadamide A	161.22	-0.12	2	2	0	Yes
Entadamide C	177.22	-1.07	2	3	0	Yes
trans-3-methylsulfinyl-2-propenol	120.17	-0.43	1	2	0	Yes
Catechin	290.27	0.24	5	6	0	Yes
Quercetin	302.24	-0.56	5	7	0	Yes
Quercetin 3-O-rhamnoside	448.38	-1.84	7	11	2	No
Rutin	610.52	-3.89	10	16	3	No
Chlorogenic acid	354.31	-1.05	6	9	1	Yes

MW: molecular weight, Log P: Lipophilicity, H Donor: Hydrogen bond donors, H acceptor: Hydrogen bond acceptors

Table 2: Drug-likeness and toxicity as predicted using OSIRIS Property Explorer

Ligands	Pharmacodynamics				Pharmacokinetics				
	Mutagenic	Tumorigenic	Eye and skin irritation	Reproductive effect	Solubility	Absorption	TPSA	Drug-likeliness	Drug score
Lupeol	No	No	No	No	-6.80	7.65	20.23	-22.17	0.13
Betulin	No	No	No	No	-6.30	6.72	40.46	-29.93	0.15
β-sitosterol	No	No	No	No	-6.67	7.86	20.23	-4.48	0.13
Stigmasterol	No	No	No	No	-6.44	7.60	20.23	1.22	0.25
Vitexin	High	No	No	No	-2.27	-0.08	177.10	-1.26	0.30
Isovitexin	No	No	No	No	-2.27	-0.08	177.10	-1.26	0.50
Gallic acid	High	No	No	High	-0.74	0.11	97.99	0.12	0.27
Clinacoside A	No	No	No	No	-0.21	-2.95	141.90	-3.47	0.49
Clinacoside B	No	No	No	No	0.32	-3.39	135.6	-3.78	0.49
Clinacoside C	No	No	No	No	0.55	-4.30	164.7	-3.72	0.48
Cycloclinacoside A1	No	No	No	No	-0.35	-3.20	130.9	-3.34	0.49
Clinamides A	No	No	No	No	-0.28	-2.03	91.85	0.58	0.81
Clinamides B	No	No	No	No	-0.16	-1.98	91.68	0.17	0.75
Clinamides C	No	No	No	No	-1.31	-0.89	138.2	0.48	0.76
2-cis-entadamide A	No	No	No	No	-0.83	-0.55	74.63	0.20	0.76
Entadamide A	No	No	No	No	-0.83	-0.55	74.63	0.20	0.76
Entadamide C	No	No	No	No	0.25	-2.47	85.61	0.38	0.79
trans-3-methylsulfinyl-2-propenol	No	No	No	No	0.02	-1.55	56.51	-1.09	0.62
Catechin	No	No	No	No	-1.76	1.51	110.3	1.92	0.87
Quercetin	High	High	No	No	-2.49	1.49	127.4	1.6	0.30
Chlorogenic acid	No	No	No	No	-1.5	-0.77	164.7	0.17	0.7

TPSA: Topological Polar Surface Area

Active site prediction

Table 3 and Figure 2 show the active site prediction (area and volume) of the four targeted proteins from CASTp 3.0. Bcl-2, caspase-3, p53, and caspase-8 had 166, 477, 53, and 1067 amino acid residues, respectively, at the predicted active sites. The grid box was centred at the predicted active site.

Table 3 a: Active site prediction of targeted proteins from CASTp 3.0

Protein	Area (SA)	Volume (SA)	Total amino acid residue
Bcl-2	204.21	158.41	166
Caspase-3	78.64	106.69	477
p53	25.46	10.58	53
Caspase-8	999.76	1264.56	1067

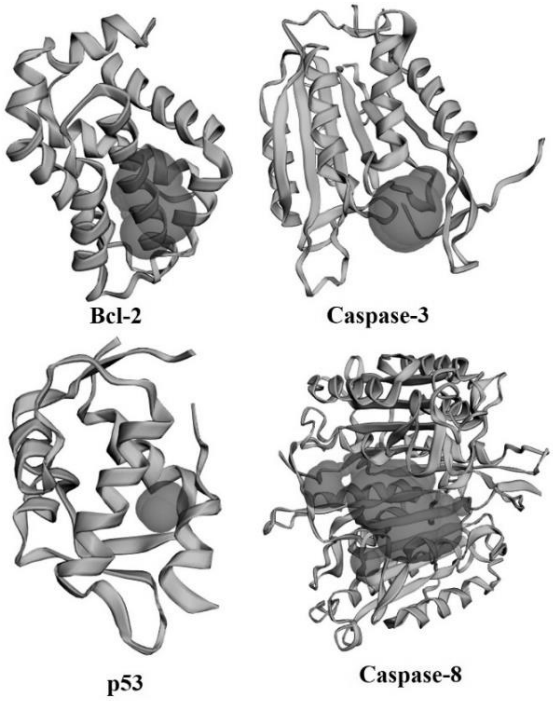

Figure 2: The predicted binding pocket of the four targeted apoptosis proteins as computed using CASTp 3.0

Molecular docking

The proteins p53, caspase-3, bcl-2, and caspase-8 were docked with the 18 selected compounds evaluated by OSIRIS Property Explorer. Table 3 shows the best results for each tested compound and targeted proteins. The molecular docking and interaction pattern of the compounds with targeted proteins were analysed using Chimera and the interaction analysis of the compounds with active site residues was performed using Lig Plot + and Discovery Studio Visualizer 4.1 Client (Figure 3). The compounds exhibited varying degrees of favourable interactions with each of the protein targets. β-sitosterol had high binding affinity towards Bcl-2 (–7.86 kcal/mol), caspase-3 (–5.14 kcal/mol), and p53 (–7.11 kcal/mol), whereas stigmasterol had high binding affinity towards caspase-8 (–7.55 kcal/mol) (Table 3). The lowest binding affinity detected was –1.98 kcal/mol for isovitexin towards caspase-3. Trans-3-methylsulfinyl-2-propenol had the lowest binding affinity towards Bcl-2 (–3.08 kcal/mol). Clinamides B and clinamides C exhibited the lowest binding affinity towards p53 (–3.20 kcal/mol) and caspase-8 (–3.37 kcal/mol), respectively.

Table 3 b : Energy-based interactions for selected compounds and targeted proteins.

Compounds	Lupeol	Betulin	β-sitosterol	Stigmasterol	Isovitexin	Clinacoside A	Clinacoside B	Clinacoside C	Cyclocl inacosi l-1-1	Clinamides A	Clinamides B	Clinamides C	2-cis-entada	Entadamide A	Entadamide C	Ttrans-3-	Catechin	Chlorogenic
Receptors	**Binding affinity (kcal/mol)**																	
Bcl-2	-4.97	-4.17	**-7.86**	-7.72	-5.51	-4.48	-4.66	-5.24	-6.19	-4.57	-4.16	-4.40	-3.84	-3.75	-4.17	-3.08	-5.62	-5.86
Caspase-3	-4.05	-3.91	**-5.14**	-2.90	-1.98	-4.34	-4.29	-3.94	-4.25	-3.53	-3.20	-2.53	-2.35	-2.77	-3.32	-2.31	-4.17	-5.08
p53	-4.70	-3.83	**-7.11**	-6.87	-4.59	-5.98	-5.64	-5.38	-5.95	-5.08	-3.20	-4.08	-4.41	-4.31	-5.10	-3.68	-5.91	-6.51
Caspase-8	-7.07	-6.45	-6.95	**-7.55**	-5.95	-5.13	-5.35	-5.54	-5.29	-4.32	-4.46	-3.37	-4.36	-4.21	-4.40	-3.88	-5.43	-5.32

The bold values indicate the highest binding affinity of compounds and targeted proteins.

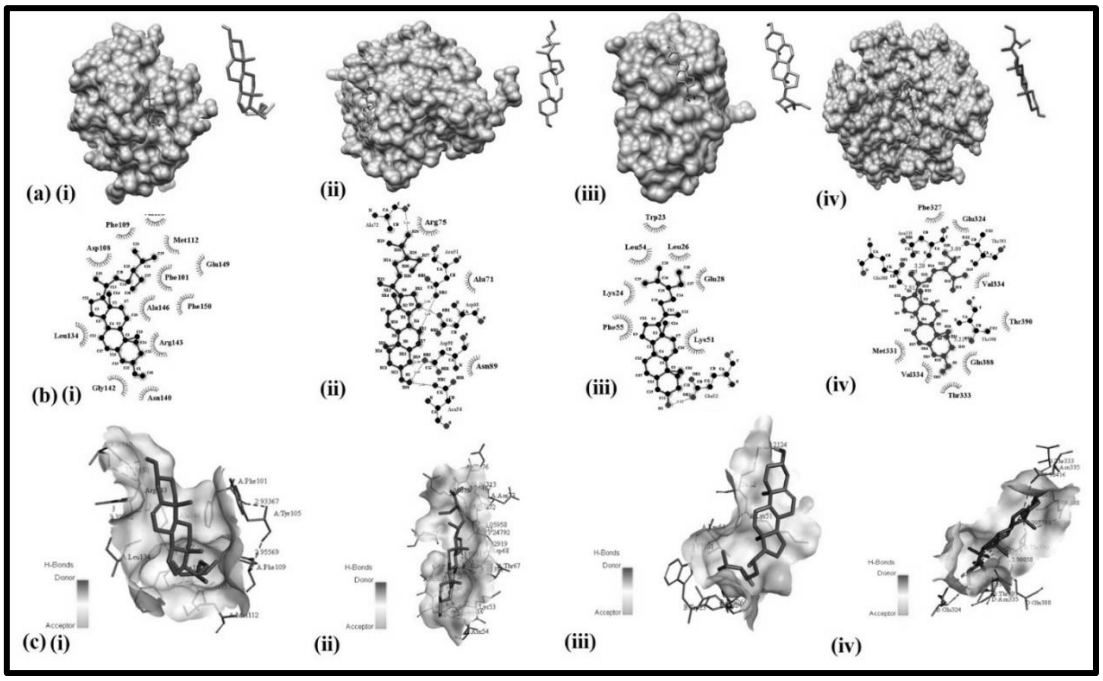

Figure 3: Molecular docking and interaction pattern analysis of the compounds with targeted protein (a) The best binding affinities of the compounds with targeted proteins;
(b) Interaction of the compounds and active site residues using Lig Plot+;
(c) Interaction of the compounds with active site residues using Discovery Studio Visualizer 4.1 Client.
Here in the compounds and proteins corresponds to (i) β-sitosterol with Bcl-2, (ii) β-sitosterol with caspase-3, (iii) β-sitosterol with p53 and (iv) stigmasterol with caspase-8

Protein-docked analysis showed that β-sitosterol had a good binding affinity towards Bcl-2; no hydrogen bonding was observed and there were 18 hydrophobic interactions formed between β-sitosterol and Bcl-2 (Table 4). Ala-146, Arg-143, and Leu-134 formed two Alkyl type interactions with β-sitosterol. β-sitosterol also formed two Pi-Alkyl interactions with Phe-101 and Phe-109, respectively. Other residues that formed one Pi-Alkyl interaction were hydrophobic Tyr-105 and Phe-150. The molecular interactions of β-sitosterol and caspase-3 residues also are shown in Table 4. β-sitosterol formed 11 hydrogen bonds with 6 residues, namely Asn-51, Asn-87, Arg-75, Asn-54, Ala-71, and Asp-90. β-sitosterol also had the highest binding affinity when it was docked with p53. β-sitosterol formed 12 hydrophobic interactions with Trp-23, Lys-51, Lys-24, Leu-54, Leu-26 and Glu-52. Lys-51, Lys-24, Leu-54 and Leu-26 formed Alkyl interactions, while Trp-23 formed Pi-Orbitals and Pi-Alkyl interactions. Two hydrogen bonds formed when β-sitosterol was docked with p53. Stigmasterol showed highest affinity when docked with caspase-8; no hydrophobic bonding was found, but stigmasterol formed 17 hydrogen bonds when it was docked with p53.

Table 4: Residues interaction of targeted proteins with selected compounds.

Protein	Compounds	Interacting residues	No. of hydrophobic interaction	No. of hydrogen bond
Bcl-2	β-sitosterol	Leu-134	3	-
		Arg-143	2	-
		Ala-146	2	-
		Met-112	3	-
		Val-153	1	-
		Phe-101	2	-
		Tyr-105	1	-
		Phe-109	3	-
		Phe-150	1	-
Caspase-3	β-sitosterol	Asn-51	-	4
		Asn-87	-	2
		Ala-71	-	2
		Arg-75	-	2
		Asp-90	-	1
p53	β-sitosterol	Glu-52	-	2
		Trp-23	3	-
		Lys-51	5	-
		Lys-24	1	-
		Leu-54	2	-
		Leu-26	1	-
Caspase-8	Stigmasterol	Glu-324	-	2
		Asn-335	-	4
		Gln-388	-	4
		Thr-390	-	4
		Thr-393	-	2
		Thr-333	-	1

DISCUSSION

Lipinski's rule of five states that molecular compounds should be < 500 g/mol and have lipophilicity < 5 and numbers of hydrogen bond acceptors and donors of 10 and 5, respectively.[26] These parameters are significantly associated with aqueous solubility and intestinal permeability in the first step of oral bioavailability. If a ligand fails to fulfil these parameters, it will cause problems if ingested. These parameters explain the molecular properties for a drug's pharmacokinetics in the human body, such as absorption, distribution, metabolism, and excretion (ADME).[18]

Table 1 shows that lupeol, betulin, β-sitosterol, and stigmasterol had lipophilicity > 5, while vitexin, isovitexin and chlorogenic acid had more than 5 hydrogen donors; these represent only one violation of Lipinski's rule. However, these compounds still followed Lipinski's rule, because Lipinski *et al*.[17] reported that an orally active drug still meets the requirements if it has no more than one violation of the criteria. Toxicity is a significant factor that often surpasses the ADME parameters. Drugs usually fail at the clinical trial stage due to adverse effects created from their toxicity.[27]

Table 2 shows the drug-likeness properties of *C. nutans* compounds identified using OSIRIS Property Explorer. This tool measures the compounds' hydrophilicity, with higher hydrophilicity indicating good absorption and permeation. A low log S value indicates higher solubility that can boost absorption. Moreover, lower molecular weight can enhance the absorption rate.[28] Topological Polar Surface Area (TPSA) is a characteristic of the polar atoms in the compound, and lower TPSA is a favourable for drug-like property. This tool also predicted toxicity factors such as tumorigenicity, irritability, mutagenicity, and reproductive toxicity.

Binding energy is essential parameters that can be generated from the result of molecular docking.[29] Binding energy data provide information about the strength and binding affinity of ligand and protein receptor interactions. The lowest binding energy provides the highest binding affinity and interaction. In this study, we determined the binding energies of the targeted apoptosis proteins with *C. nutans* compounds and identified the amino acid residues involved in the binding interactions through docking analysis; one bioactive compound, β-sitosterol, exhibited good binding affinities with Bcl-2, caspase-3, and p53. For caspase-8, stigmasterol had the best binding affinity, followed by β-sitosterol. These data agree with results from cell culture studies. For example, a previous study reported that β-sitosterol and stigmasterol were the most active compounds promoting cell death in *in vitro* studies of cancer cells such as breast cancer (MCF-7) and cervical cancer (HeLa) cells.[30] An *in silico* study also showed that β-sitosterol was an active compound in cancers when it docked with estrogen receptor α.[31]

In summary, our results indicated that stigmasterol and β-sitosterol had good interactions with the targeted proteins, whereas the other compounds showed poor affinities towards the targets. However, use of a different software program may yield different outcomes because different applications use different algorithms.[32] In addition, different protein structures retrieved from the database might result in different results.[33] Further studies should be conducted to compare various protein structures and different experimental models and software. Additionally, gene and protein expression from *in vitro* and *in vivo* studies should be evaluated to support the *in silico* findings.

CONCLUSION

This study determined the binding affinities of selected chemical compounds from *C. nutans* towards targeted apoptosis proteins (p53, Bcl-2, caspase-3, and caspase-8). β-sitosterol had high affinity towards Bcl-2, caspase-3, and p53, while stigmasterol showed high affinity towards caspase-8. These results provide valuable information about compounds from *C. nutans* that may be useful for cancer treatment.

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