

Original Article

Molecular Docking Study of Naturally Derived Flavonoids with Antiapoptotic BCL-2 and BCL-XL Proteins toward Ovarian Cancer Treatment

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ABSTRACT

The naturally derived flavonoids are well known to have anticarcinogenic effects. Flavonoids could be an alternative strategy for ovarian cancer treatment, due to existing platinum-based drugs are reported to develop resistance with low survival rates. Inhibition of antiapoptotic proteins, namely B-cell lymphoma (Bcl-2) and B-cell lymphoma-extra large (Bcl-xl), is the key target to stimulate apoptosis process in cancer cells. This study aimed to determine the binding interaction of five naturally derived flavonoids (biochanin A, myricetin, apigenin, galangin, and fisetin) with potential antiapoptotic target proteins (Bcl-2 and Bcl-xl). The molecular docking study was conducted using AutoDock Vina program. The binding affinity and the presence of hydrogen bonds between the flavonoids and target proteins were predicted. Our findings showed that all the flavonoids showed better binding affinity with Bcl-xl than that of Bcl-2 proteins. The highest binding affinity was recorded in fisetin-Bcl-xl protein complex (−8.8 kcal/mol). Meanwhile, the other flavonoids docked with Bcl-xl protein showed binding affinities, ranging from −8.0 to −8.6 kcal/mol. A total of four hydrogen bonds, four hydrophobic contacts, and one electrostatic interaction were detected in the docked fisetin-Bcl-xl complex, explaining its high binding affinity with Bcl-xl. The present results indicate that all flavonoids could potentially serve as Bcl-xl protein inhibitors, which would consequently lead to apoptotic process in ovarian cancers.

KEYWORDS: Antiapoptotic proteins, docking, flavonoid, ovarian cancer

INTRODUCTION

Ovarian cancer is the highest fatal type of gynecological cancer and ranked as the third of global top mortality rate among females.^[1] Meanwhile, ovarian cancer in Malaysia was ranked 12th for both incidence and mortality rate in 2018.^[2] Patients with ovarian cancer generally suffer from a recurrence and progressive disease due to the resistance with the existing chemotherapy treatment.^[3] Most of the prescribed chemotherapy drugs are synthetically derived and have shown toxicity effects not only against the cancer cells, but also against the normal cells.^[4] In contrast, the recent findings indicate that naturally

extracted phytochemicals from plants significantly show selective cytotoxicity to cancer cells and minimal toxicity to normal cells.^[5] This could be an alternative treatment for cancer therapy. One of the interesting phytochemical compounds are flavonoids that are considered to exert anticarcinogenic effects. Flavonoids will induce apoptosis in the cancer cells, in which the

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apoptosis induction can occur in B-cell lymphoma (Bcl-2) family members.^[6]

Bcl-2 family members comprise both proapoptotic and antiapoptotic proteins, which play an important role in controlling cellular apoptosis.^[6] Antiapoptotic proteins (Bcl-2 and B-cell lymphoma-extra large [Bcl-x] l) are involved in preserving mitochondrial integrity, preventing the loss of mitochondrial membrane potential, and preventing cell death.^[7] In addition, the intrinsic apoptotic mechanism will be triggered by the suppression of Bcl-2 and Bcl-xl proteins in tumor cells, resulting from strong antagonizing apoptosis signals.^[8] Antiapoptotic proteins are abundant in most cancer cells as compared with proapoptotic proteins which could be absent or less expressed.^[9] Inhibition of Bcl-2 and Bcl-xl proteins is the key target to induce apoptosis process in cancer cells. The small molecules that could inhibit the function of Bcl-2 or Bcl-xl proteins have gathered attention in recent years to be studied as potential new anticancer drugs.^[10]

In this study, we aimed to investigate the binding interaction between a total of five selected naturally derived flavonoids (biochanin A, myricetin, apigenin, galangin, and fisetin) and potential antiapoptotic target proteins (Bcl-2 and Bcl-xl). The selection of these flavonoids is based on the commonality of their structures with only the difference in hydroxyl group position. Besides, the availability of each flavonoid is required for further investigation. These flavonoids are known to exist naturally in plants and possess potential pharmacological properties in many reported studies. Docking studies are performed to examine the binding affinities and the presence of non-covalent interactions between the selected flavonoids and Bcl-2 and Bcl-xl proteins.

MATERIALS AND METHODS

All the molecular docking functions in this study were performed using AutoDock Vina.^[11] This docking software has been reported to provide better results and explanations in docking analyses. The AutoDock Vina software is also recognized as a trusted and accurate methods due to its rapid operation.^[12]

Preparation of ligands

The chemical structures of biochanin A, myricetin, apigenin, galangin, and fisetin were obtained from the Pubchem compound database (<http://pubchem.ncbi.nlm.nih.gov/>) with their respective IDs as follows: 5280373, 5281672, 5280443, 5281616, and 5281614 [Figure 1]. Polar hydrogen atoms were added to the

ligands, whereas nonpolar hydrogen atoms were merged. The downloaded three-dimensional chemical structures in the .sdf format were then converted into the .pdbqt format using the AutoDock Tool (ADT) program, version 1.5.6. Local minimization of ligands was executed using Biovia Discovery studio to reduce any possible bad contacts, as well as to sustain the conformation ligand.

Preparation of proteins

Crystal structures of the Bcl-2 and Bcl-xl proteins with PDB IDs 4IEH^[13] and 3ZK6,^[14] respectively, were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The crystal water molecules, ligand atoms, and ions that were bound to the proteins were removed. Hydrogen atoms were subsequently added using ADT.

Molecular docking

Molecular docking between flavonoids and antiapoptotic proteins (Bcl-2 and Bcl-xl) was performed using AutoDock Vina. The grid box for docking was positioned at the active binding site. The docking exhaustiveness was set up to 100 and repeated for 10 times. The results of hydrogen bond, hydrophobic, and electrostatic interactions were obtained using Biovia Discovery Studio Visualizer.^[15]

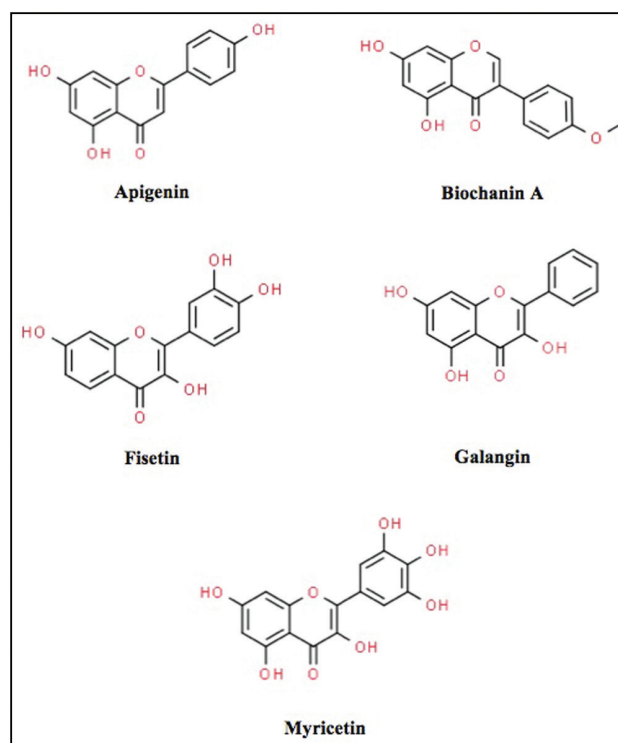


Figure 1: Two-dimensional structures of flavonoids

RESULTS

Docking results were analyzed based on the binding affinity and non-covalent interactions that were present between the flavonoids and Bcl-2 or Bcl-xl proteins. The docking results were ranked according to the binding affinity of flavonoids–protein complex as presented in Table 1.

Docked with B-cell lymphoma 2 protein

The docked results showed that myricetin and galangin were found to have the highest binding affinities of -7.3 kcal/mol. In contrast, biochanin A showed the lowest binding affinity of -6.9 kcal/mol compared with other flavonoids. Hydrogen bonds were observed in the Bcl-xl–galangin complex but not in myricetin of two residues, that is Arg66 and Tyr67 [Table 1]. Based on the binding interactions, one critical residue (Phe63) of Bcl-2 protein interacted with all flavonoids except biochanin A either via hydrophobic or electrostatic interactions.

Docked with B-cell lymphoma-extra-large protein

Analyses of the docked flavonoids with Bcl-xl protein suggested that all ligands had good interaction with

Bcl-xl protein with a binding affinity of less than -8.0 kcal/mol. Among them, fisetin showed the highest binding affinity (-8.8 kcal/mol), whereas galangin showed the lowest binding affinity (-8.0 kcal/mol). Four hydrogen bonds were formed in the Bcl-xl–fisetin complex of the Asp133, Arg139, Glu129, and Gly138 residues [Figure 2]. There were also four hydrophobic contacts contributed to the fisetin binding. Similarly, hydrogen bonds and hydrophobic contacts were also detected in other flavonoids with Bcl-xl protein. Two residues, namely, Phe105 and Ala104, were found to hydrophobically contacted with all flavonoids. Additional electrostatic interactions with Arg139 residue were observed in the docked myricetin and fisetin.

DISCUSSION

The molecular docking in this study shows a vital role in predicting molecular interactions of flavonoids with targeted protein. This application is widely used in the pharmaceutical industry as a powerful tool, particularly in the analysis of structure–activity relationship.^[16] The analysis of molecular docking outputs, such as binding

Table 1: Docking results for Bcl-2 and Bcl-xl proteins with flavonoids

Protein	Ligand	Binding affinity (kcal/mol)	Hydrogen bonding	Hydrophobic interaction	Electrostatic interaction
Bcl-2	Myricetin	−7.3	—	PHE63 GLY104	—
	Galangin	−7.3	ARG66 TYR67	PHE63	—
	Apigenin	−7.2	ARG105	PHE63	—
	Fisetin	−7.1	—	—	PHE63
	Biochanin A	−6.9	—	TYR161	ASP62
Bcl-xl	Fisetin	−8.8	ASP133 ARG139 GLU129 GLY138	PHE105 ARG139 ALA142 ALA104	ARG139
	Apigenin	−8.6	ARG139	ALA104 PHE105	—
	Biochanin A	−8.5	SER106 GLU129 ASN136	LEU130 ALA104 PHE105 LEU108 ARG139 ALA142	—
	Myricetin	−8.1	ASP133 ARG139 GLU129	PHE105 ARG139 ALA104	ARG139
	Galangin	−8.0	LEU108	LEU130 PHE105 ALA142 ARG139 ALA104 ARG102 LEU108	—

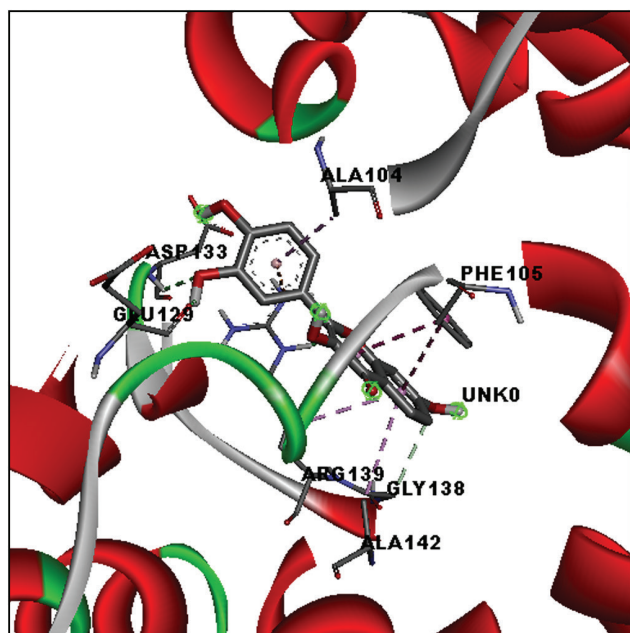


Figure 2: Three-dimensional illustration of docked Bcl-xL-fisetin complex. Green dashed lines represent hydrogen bonds. The hydrophobic interaction is depicted by purple dashed lines

affinity, are frequently applied in the determination of potential ligands.^[17] Molecular docking also has the ability to predict small-molecule ligands binding toward appropriate target binding site.^[18]

The targeted antiapoptotic proteins in this study are Bcl-2 and Bcl-xL, which are involved in the regulation of intrinsic apoptotic pathway. Bcl-2 and Bcl-xL proteins are said to be more crucial in apoptotic signaling pathway compared with other Bcl-2 family members.^[19] Bcl-2 and Bcl-xL proteins have been investigated in various molecular modeling studies, including for apoptosis induction in cancer cells.^[20-22] Those studies also involved small molecules, such as phenothiazine^[21] and piperine,^[22] which docked into Bcl-2 and Bcl-xL proteins, respectively.

According to our results, all flavonoids appear to have good docking interactions with both proteins. Similarly, two compounds as previously reported, ABT-737^[23] and ABT-263,^[24] interacted with both Bcl-2 and Bcl-xL proteins.^[25] However, Bcl-xL protein in this study possessed better binding affinity with flavonoids, ranging from -8.0 to -8.8 kcal/mol, when compared with Bcl-2 protein, ranging from -6.9 to -7.3 kcal/mol [Table 1]. The binding interactions into the antiapoptotic binding pocket cause inhibitory activity and it eventually induces cell apoptosis via mitochondrial signaling pathway.^[18,19]

The presence of hydrophobic interaction and hydrogen bond in the docked flavonoids with Bcl-xL protein was observed to contribute to the higher binding affinities

compared with that of Bcl-2 protein [Table 1]. In the process of protein-ligand interaction, water forces the hydrophobic groups to aggregate and disrupt the hydrogen bond in water. This is known as the hydrophobic effect.^[26] The presence of water molecules in hydrophobic site increases the binding affinity of protein and ligand.^[27] These statements are also supported by Qian *et al.*,^[28] where binding affinity values could associate with the hydrophobic interaction and the hydrogen bond. Although the water molecules were removed during preparation of proteins in this study, the chances of getting stronger binding affinities could possibly occur in a physiological condition.

In the docking between flavonoids and Bcl-2 protein, hydrophobic contact was detected at the same residue (Phe63) for myricetin, apigenin, and galangin. Meanwhile, the hydrophobic interaction between the docked flavonoids and Bcl-xL protein was facilitated via two critical residues, namely, Ala104 and Phe105. Phe105 gained contact with flavonoids due to the unfolding of α -helix 3 of Bcl-xL. It was also observed in A-1155463 along with other residues, such as Ser106 and Leu108.^[25] In addition, Phe105 is located at the Bcl-xL region with BH3 peptide binding to it that causes a major conformational change in Bcl-xL.^[29] Therefore, interaction with a critical residue, Phe105 as well as Ala104 in this study, is anticipated to be the key for the high binding affinities of flavonoid toward Bcl-xL protein. These findings suggest possible inhibitory interaction of flavonoids with essential residues in Bcl-xL located in the same binding site employed by the Bcl-xL inhibitors.

CONCLUSION

Molecular docking predicts the best orientation and conformation of flavonoids in Bcl-2 and Bcl-xL protein binding site to form stable complex for the inhibitory reaction. Our findings conclude that all flavonoids are possibly able to act as potential inhibitors for the targeted Bcl-2 and Bcl-xL proteins, supported by the high binding affinities. The binding of the flavonoids could, therefore, stimulate the apoptotic process in ovarian cancer cells. However, further confirmation, as well as more experiment studies, is required to validate the docking results in the development of anticancer drugs.

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Conflicts of interest

There are no conflicts of interest.

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