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In Silico Analysis of Kaempferol from *Foeniculum vulgare* and Coumarin from *Alyxia reinwardtii* Targeting Anti-Apoptotic Proteins: Potential Anticancer Agents

Honesty Nurizza Pinanti[1*], Nurul Jadid Mubarakati[2]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Jl. Ketintang Surabaya, 60231*

[*]*Corresponding author*
Email: honestypinanti@unesa.ac.id
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Abstract

Foeniculum vulgare and Alyxia reinwardtii are two herbal plants frequently co-utilized by Javanese people in Indonesia to treat various diseases, as evidenced by ancient manuscripts about traditional medicine (Jamu). However, the potential of the combination of kaempferol and coumarin, the major bioactive compounds derived from each plant, to inhibit BCL-2 and BCL-XL anti-apoptotic proteins in cancer has not yet been investigated. Therefore, this study aimed to identify the inhibitory activity of kaempferol, coumarin, and their combinations on BCL-2 and BCL-XL through in silico studies. The physicochemical properties of both compounds were predicted using the SwissADME web server. Meanwhile, the docking activity prediction of these compounds on BCL-2 and BCL-XL was performed using the molecular docking method with HEX 8.0.0 CUDA. Docking visualization was performed using BIOVIA Discovery Studio 2020 Client. The results of this study indicated that kaempferol could bind the binding groove of both BCL-2 (-246.40 kcal/mol) and BCL-XL (-245.76 kcal/mol), whereas coumarin only interacted with the binding groove of BCL-XL (-160.61 kcal/mol). The combination of the two compounds exhibited a stronger interaction with BCL-2 (-248.50 kcal/mol) and BCL-XL (-260.43 kcal/mol) compared to each compound individually. Therefore, the combination of these compounds is predicted to exhibit greater anticancer potential than either kaempferol or coumarin alone. Nevertheless, further extensive studies are required to validate the findings of this study.

1. INTRODUCTION

Cancer, a complex disease defined by uncontrolled growth and proliferation of cells in the body, caused 9.7 million deaths globally in 2022, with approximately one in every nine men and one in every twelve women dying from it [1]. The existing cancer therapy faces some obstacles, such as the emergence of toxicity in normal cells and resistance to conventional anticancer medicines [2]. Therefore, new strategies to effectively treat cancer are currently being developed. One of these strategies is utilizing complementary medicine involving bioactive compounds from medicinal plants. Bioactive compounds exhibit numerous benefits in cancer therapy, such as exhibiting multiple pharmacological effects by targeting many oncogene proteins, lowering the side effects of conventional cancer drugs, and strengthening the anticancer effects of conventional cancer therapies [3]. For example, resveratrol could target various cancer signaling pathways, including NF-κB, MAPK, TNF-α, and PI3K/Akt, demonstrate potential synergistic effects with chemotherapeutic agents, and reduce its side effects [4]. In addition, curcumin showed synergistic effects with several anticancer drugs such as cisplatin, doxorubicin, and 5-fluorouracil through multiple mechanisms, including activation of ERK1/2, inhibition of the ATPase activity of ABCB4, and downregulation of NF-κB pathways [5].

As one of the mega-biodiversity countries, Indonesia offers a wide variety of medicinal plants. For hundreds of years, Indonesian ancestors had used medicinal plants as traditional medicine called Jamu. According to the Serat Primbon Jampi Jawi and Serat Centhini, two ancient manuscripts explaining traditional Javanese medicines and recipes, *Foeniculum vulgare* or Adas (Fennel) and *Alyxia reinwardtii* or Pulasari are frequently used together in numerous herbal remedies to treat various types of diseases [6],[7]. *Foeniculum vulgare* exhibits antibacterial, antifungal, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic properties [7]. The major compound of *Foeniculum vulgare* is kaempferol, a compound from the flavonoid group possessing antioxidant, anti-inflammatory, and anticancer properties [8],[9]. Meanwhile, *Alyxia reinwardtii*, traditionally utilized to cure various illnesses, contains coumarin and its derivatives as the major compounds exerting antibacterial, antiviral, and anticancer activities [10]-[12]. However, the anticancer effectof the combination of these two major compounds, especially through inhibiting anti-apoptotic proteins, is still unclear.

This research is a preliminary study aimed to identify the anticancer potential of the combination of kaempferol and coumarin, which are dominant compounds in *Foeniculum vulgare* and *Alyxia reinwardtii*, respectively, through in silico methods. Since both compounds have anticancer effects by inducing apoptosis, we hypothesize that their combination will enhance the effect [13],[14]. Kaempferol, coumarin, and the kaempferol-coumarin complex were analyzed for their respective interactions toward anti-apoptotic proteins, such as BCL-2 and BCL-XL, through molecular docking studies. Molecular docking is a computational approach that can be used to identify the binding position of a compound (ligand) toward its particular target protein based on its structure, affinity, and interactions with amino acid residues of a protein [15]. Molecular docking can be used to predict the potential of a compound to inhibit its target protein. In this study, we used BCL-2 and BCL-XL, oncogene proteins involved in cancer's ability to evade apoptosis, a programmed cell death mechanism, as target proteins [16]. Both proteins are frequently reported to be overexpressed in various cancer cells, including glioma, breast cancer, and prostate cancer [17]. Therefore, inhibiting these two proteins with small molecules or bioactive compounds is a potential strategy in treating cancer.

2. MATERIALS AND METHODS

Physicochemical properties analysis of compounds

The oral bioavailability of kaempferol and coumarin was predicted based on Lipinski's rules using the SwissADME web server (http://www.swissadme.ch/). The canonical SMILE code of both compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Subsequently, the code was entered into the ADME web server to show several physicochemical properties of the compound. According to Lipinski's rule, a compound with at least one deviation from the following characteristics: MW ≤500, log P ≤ 5, H-bond donors ≤ 5, and H-bond acceptors ≤ 10, exhibits an excellent oral bioavailability [18].

Protein preparation

The crystal structures of BCL-2 (2W3L) and BCL-XL (2YXJ) protein complexes were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) in PDB format. The proteins were prepared with Biovia Discovery Studio 2020 software (Dassault Systèmes Biovia, San Diego, California, USA) to discard water molecules, ions, unnecessary chains, and native ligands. The respective A chain of BCL-2 and BCL-XL, saved in PDB format, was used for the docking simulation process.

Ligand preparation

Phenyl Tetrahydroisoquinoline Amide ($C_{34}H_{30}ClN_5O_2$), the native ligand of the BCL-2, was taken from the BCL-2 protein complex to be used as a positive control in the BCL-2 docking simulation. Meanwhile, ABT-737 ($C_{42}H_{45}ClN_6O_5S_2$), the native ligand of the BCL-XL, was isolated from the BCL-XL protein complex to be used as a positive control in the BCL-XL docking simulation. The structures of these native ligands were saved in PDB format. Meanwhile, the structures of kaempferol and coumarin were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. The kaempferol-coumarin complex was generated using HEX 8.0.0 CUDA software. Furthermore, all ligand structures were minimized using Open Babel in PyrX-Virtual Screening Tools and saved in PDB format.

Molecular docking simulation

Molecular docking of kaempferol, coumarin, and kaempferol-coumarin complex on BCL-2 and BCL-XL was performed using HEX 8.0.0 CUDA software with Shape + Electro + DARS mode [19]. The docking parameters included the grid dimension of 0.6 Å; the distance range of 40 Å; the translation step of 0.8 Å; the box size of 10 Å; and the generation of 2000 docking poses per compound. Additionally, the range of both receptor and ligand was set to 180° with a step size of 7.5°, whereas the twist range was set to 360° with a step size of 5.5°. As HEX uses a rigid-body docking approach, the flexibility of the ligand was not considered in this simulation. The validation of docking accuracy was conducted by redocking the native ligand into the original binding pocket of the protein, superimposing the native ligand and the docked ligand, and calculating the RMSD. Docking visualization and chemical interactions analysis were conducted using BIOVIA Discovery Studio 2020 Client software (Dassault Systèmes Biovia, San Diego, USA) [20].

3. RESULTS and DISCUSSION

In this study, the physicochemical properties of kaempferol and coumarin were first evaluated using SwissADME to predict the oral bioavailability of these compounds. The results of the physicochemical analysis revealed that kaempferol and coumarin fulfilled the criteria for compounds with excellent oral bioavailability, as they did not have any deviation from Lipinski's rule (Table 1). The rule states that a compound can be considered a good oral drug if it exhibits at least one deviation from the following characteristics: MW ≤500, log P ≤5, H-bond donors ≤5, and H-bond acceptors ≤10 [18].

Therefore, both compounds had great potential to be developed as oral drugs In the next step, molecular docking was performed to predict the inhibitory potential of kaempferol, coumarin, and the kaempferol-coumarin complex against BCL-2 and BCL-XL anti-apoptotic proteins by analyzing their binding affinities and chemical interactions. Before analyzing the docking results, the accuracy of the docking protocol was validated through a redocking of the native ligand, resulting in an RMSD value of 0.284 Å and 0.001 Å for BCL-2 and BCL-XL, respectively, indicating a high degree of accuracy and reliability of the docking method [21].

The results of the molecular docking simulation on the BCL-2 protein revealed that kaempferol bound BCL-2 via the TYR139 residue by forming a hydrogen bond (Table 2). Kaempferol also formed 3 hydrophobic interactions with PHE39 (a Pi-Pi T-shaped bond), VAL93 (a Pi-Alkyl bond), and ALA90 (a Pi-Alkyl bond). Meanwhile, coumarin interacted with BCL-2 through 5 hydrophobic interactions, consisting of 2 stacked Amide-Pi bonds and 3 Pi-Alkyl bonds. The amino acid residues involved in the interaction between coumarin and BCL-2 were ASP62, PHE63, ALA59, VAL107, and ARG66. Compared to the positive control, namely Phenyl Tetrahydroisoquinoline Amide, a selective BCL-2 inhibitor, all amino acid residues that interacted with kaempferol could also bind the positive control. Meanwhile, coumarin did not bind any amino acid residue that interacted with the positive control.

The binding position similarity between kaempferol and the positive control at BCL-2 protein indicates that both compounds may have a similar biological role, particularly as a BCL-2 inhibitor (Figure 1) [22].

Interestingly, the kaempferol-coumarin complex could also interact with several residues bound by the positive control, such as HIS143, ALA90, and PHE89, through 2 electrostatic bonds and 2 hydrophobic interactions. Furthermore, kaempferol (-246.40 kcal/mol), coumarin (-169.89 kcal/mol), and kaempferol-coumarin complex (-248.50 kcal/mol) had a higher binding energy than the positive control (-328.94 kcal/mol). A compound with a lower binding energy interacts more strongly with its protein targets [23].

This finding suggests that although kaempferol and the kaempferol–coumarin complex exhibited weaker interactions with BCL2 compared to the positive control, they can act as BCL2 inhibitors. Additionally, the kaempferol-coumarin complex interacted with BCL-2 more strongly than kaempferol alone. Therefore, the complex of these two compounds may function as a potential BCL-2 inhibitor. The visualization of positive control of BCL-2, kaempferol, coumarin, and kaempferol-coumarin complex interacting with amino acid proteins in BCL-2 is presented in Figure 2

Table 1. Physicochemical properties of kaempferol and coumarin

No.	Compound	PubChem ID	Physicochemical properties				Lipinski violation
			MW (g/mol)	mLogP	H-bond donor	H-bond acceptor	
1.	Kaempferol	5280863	286.24	-0.03	4	6	0
2.	Coumarin	323	146.14	1.65	0	2	0

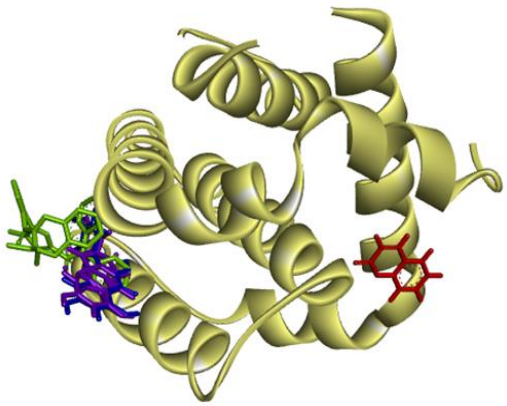


Figure 1. Binding position of kaempferol (blue), coumarin (red), the kaempferol-coumarin complex (purple), and positive control of BCL-2 inhibitor (green) at BCL-2 protein (yellow chain)

Table 2. Chemical Interactions of compounds with BCL-2

Compound	Binding Energy (kcal/mol)	Chemical Interactions	Types	Categories
Phenyl Tetrahydroisoquinoline Amide (Positive control of BCL-2)	-328.94	A:**ARG86**:NH2 - A:DRO1166	Pi-Cation	Electrostatic
		A:**GLU138**:OE1 - A:DRO1166	Pi-Anion	Electrostatic
		A:**TYR139** - A:DRO1166	Pi-Pi Stacked	Hydrophobic
		A:**PHE89** - A:DRO1166	Pi-Pi T-shaped	Hydrophobic
		A:DRO1166 - A:**ALA90**	Pi-Alkyl	Hydrophobic
		A:DRO1166 - A:**VAL93**	Pi-Alkyl	Hydrophobic
		A:DRO1166 - A:**ARG142**	Pi-Alkyl	Hydrophobic
		A:**HIS143**:ND1 - A:DRO1166:CAU	Unfavorable Bump	Unfavorable
		A:**HIS143**:CE1 - A:DRO1166:CAT	Unfavorable Bump	Unfavorable
		A:**HIS143**:HD1 - A:DRO1166:CAU	Unfavorable Bump	Unfavorable
Kaempferol	-246.40	:LIG1:H - A:**TYR139**:OH	Conventional Hydrogen Bond	Hydrogen Bond
		A:**PHE89** - :LIG1	Pi-Pi T-shaped	Hydrophobic
		:LIG1 - A:**VAL93**	Pi-Alkyl	Hydrophobic
		:LIG1 - A:**ALA90**	Pi-Alkyl	Hydrophobic
Coumarin	-169.89	A:ASP62:C,O;PHE63:N - :LIG1	Amide-Pi Stacked	Hydrophobic
		A:ASP62:C,O;PHE63:N - :LIG1	Amide-Pi Stacked	Hydrophobic
		:LIG1 - A:ALA59	Pi-Alkyl	Hydrophobic
		:LIG1 - A:VAL107	Pi-Alkyl	Hydrophobic
		:LIG1 - A:ARG66	Pi-Alkyl	Hydrophobic
Kaempferol-coumarin complex	-248.50	:LIG1:O - A:**HIS143**	Pi-Cation	Electrostatic
		:LIG1:O - A:**HIS143**	Pi-Cation	Electrostatic
		:LIG1:O - A:TRP135	Pi-Lone Pair	Other
		A:**ALA90** - :LIG1	Alkyl	Hydrophobic
		A:**PHE89** - :LIG1	Pi-Alkyl	Hydrophobic

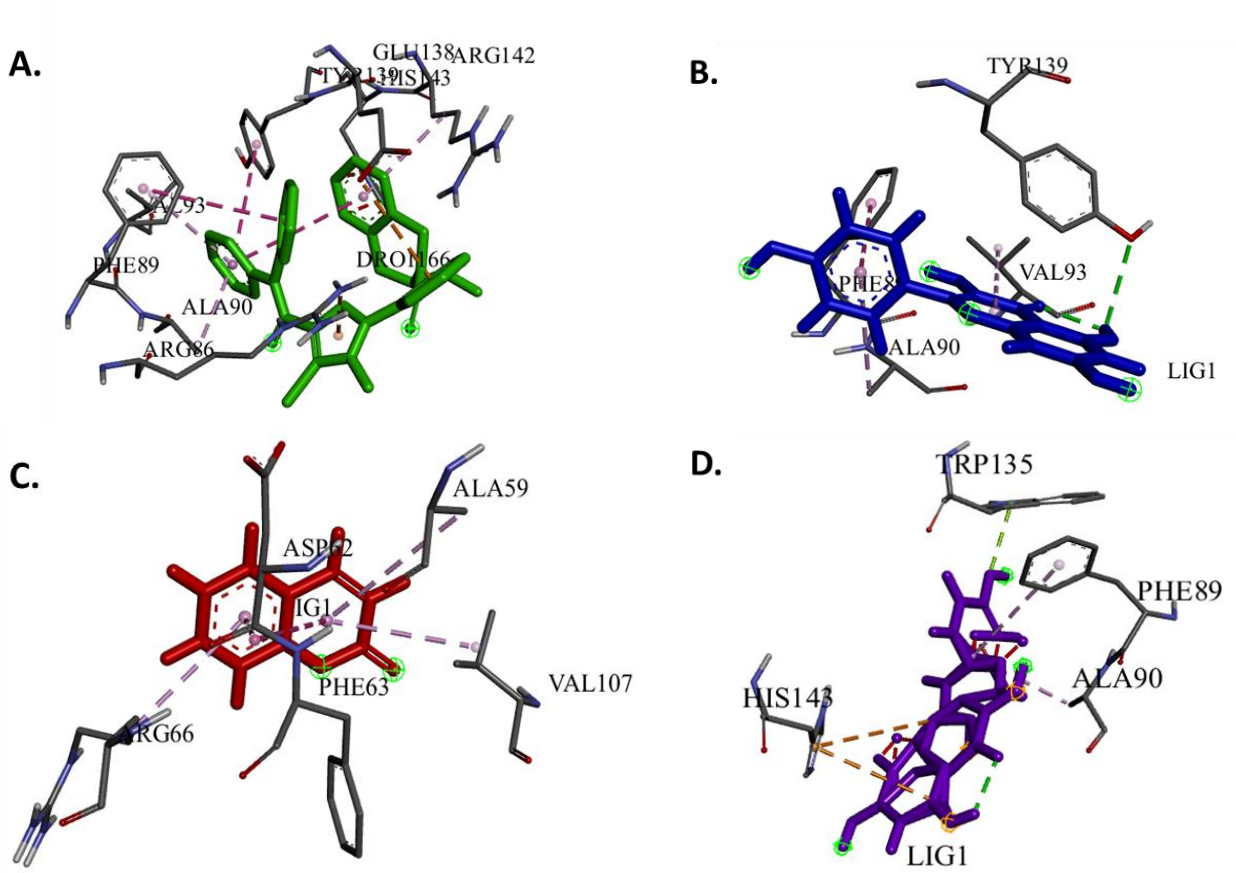


Figure 2. Interaction of compounds with BCL-2 protein. (A) positive control, (B) kaempferol, (C) coumarin, and (D) the kaempferol-coumarin complex interacted with amino acid residues in BCL-2 protein

Meanwhile, the docking study on the BCL-XL protein showed that kaempferol bound BCL-XL through a hydrogen bond, 5 hydrophobic interactions, an unfavorable interaction, and a Pi-lone pair interaction (Table 3). The residues involved in the interaction between kaempferol and BCL-XL were GLY138, ALA93, TYR195, VAL141, ALA93, and ASN197. Meanwhile, coumarin bound the protein via a hydrogen bond with GLU98 and four hydrophobic interactions with PHE105 (Pi-Pi T-shaped and Amide-Pi stacked bonds), ALA104 (Amide-Pi stacked bond), LEU108 (Pi-Alkyl bond), and ALA149 (Pi-Alkyl bond). Furthermore, all amino acid residues interacting with kaempferol were also bound by the positive control, ABT-737, a selective BCL-XL inhibitor. Meanwhile, coumarin only bound an amino acid residue that interacted with the positive control, namely LEU108, via a Pi-Alkyl bond. The binding mode similarity between kaempferol and coumarin to that of the positive control indicates that both compounds can function as a BCL-XL inhibitor (Figure 3). In addition, the kaempferol-coumarin complex could bind two residues that interacted with the positive control, namely LEU108 and PHE97, via Alkyl and unfavorable bonds, respectively. Kaempferol (-245.76 kcal/mol), coumarin (-160.61 kcal/mol), and the complex of the two compounds (-260.43 kcal/mol) had higher binding energy than the positive control (-492.76 kcal/mol). Therefore, this finding indicates that both

bioactive compounds and their combination may inhibit BCL-XL inhibitors, although with a lower affinity than the positive control. Kaempferol demonstrated a stronger interaction with BCL-XL than coumarin. Meanwhile, the formation of the two-compound complex strengthened its affinity toward BCL-XL. Therefore, the kaempferol-coumarin complex could be predicted as a promising candidate for BCL-XL inhibitor. The visualization of the positive control of BCL-XL, kaempferol, coumarin, and kaempferol-coumarin complex interacting with amino acid proteins in BCL-XL is presented in Figure 4.

Table 3. Chemical Interactions of compounds with BCL-XL

Compound	Binding energy (kcal/mol)	Chemical Interactions	Types	Categories
ABT-737 (Positive Control of BCL-XL)	-492.76	A:**GLY138**:HN - A:N3C1001:O29	Conventional Hydrogen Bond	Hydrogen Bond
		A:N3C1001:C40 - A:**TYR195**:O	Carbon-Hydrogen Bond	Hydrogen Bond
		A:N3C1001:C40 - A:**ASN197**:O	Carbon-Hydrogen Bond	Hydrogen Bond
		A:N3C1001:S42 - A:**ALA93**:O	Sulfur-X	Other
		A:**PHE97**:CB - A:N3C1001	Pi-Sigma	Hydrophobic
		A:**LEU130**:CD1 - A:N3C1001	Pi-Sigma	Hydrophobic
		A:**TYR195** - A:N3C1001	Pi-Pi Stacked	Hydrophobic
		A:**PHE97** - A:N3C1001	Pi-Pi T-shaped	Hydrophobic
		A:**TYR101** - A:N3C1001	Pi-Pi T-shaped	Hydrophobic
		A:**ALA93** - A:N3C1001	Alkyl	Hydrophobic
		A:**ALA142** - A:N3C1001	Alkyl	Hydrophobic
		A:N3C1001:CL1 - A:**LEU108**	Alkyl	Hydrophobic
		A:**PHE97** - A:N3C1001	Pi-Alkyl	Hydrophobic
		A:**TYR101** - A:N3C1001	Pi-Alkyl	Hydrophobic
		A:**PHE146** - A:N3C1001:CL1	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**ALA93**	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**VAL141**	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**ARG139**	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**LEU108**	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**VAL126**	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**LEU130**	Pi-Alkyl	Hydrophobic
		A:N3C1001 - A:**ALA142**	Pi-Alkyl	Hydrophobic
Kaempferol	-245.76	:LIG1:H - A:**GLY138**:O	Conventional Hydrogen Bond	Hydrogen Bond
		A:**ALA93**:CB - :LIG1	Pi-Sigma	Hydrophobic
		A:**TYR195**:O - :LIG1	Pi-Lone Pair	Other
		:LIG1 - A:**VAL141**	Pi-Alkyl	Hydrophobic
		:LIG1 - A:**ALA93**	Pi-Alkyl	Hydrophobic
		:LIG1 - A:**VAL141**	Pi-Alkyl	Hydrophobic
		:LIG1 - A:**ALA93**	Pi-Alkyl	Hydrophobic
		A:**ASN197**:O - :LIG1:O	Unfavorable Bump	Unfavorable
Coumarin	-160.61	A:GLU98:CA - :LIG1:O	Carbon-Hydrogen	Hydrogen Bond

			Bond	
		A:PHE105 - :LIG1	Pi-Pi T-shaped	Hydrophobic
		A:ALA104:C,O;PHE105:N - :LIG1	Amide-Pi Stacked	Hydrophobic
		:LIG1 - A:**LEU108**	Pi-Alkyl	Hydrophobic
		:LIG1 - A:ALA149	Pi-Alkyl	Hydrophobic
Kaempferol-coumarin complex	-260.43	:LIG1:O - A:GLU129:OE2	Attractive Charge	Electrostatic
		:LIG1:H - A:PHE105:O	Conventional Hydrogen Bond	Hydrogen Bond
		A:ALA104 - :LIG1	Alkyl	Hydrophobic
		A:**LEU108** - :LIG1	Alkyl	Hydrophobic
		A:**PHE97**:CZ - :LIG1:O	Unfavorable Bump	Unfavorable

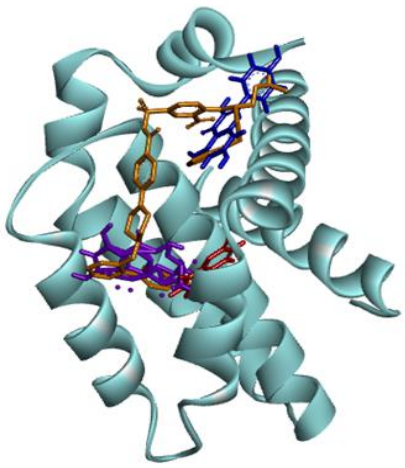
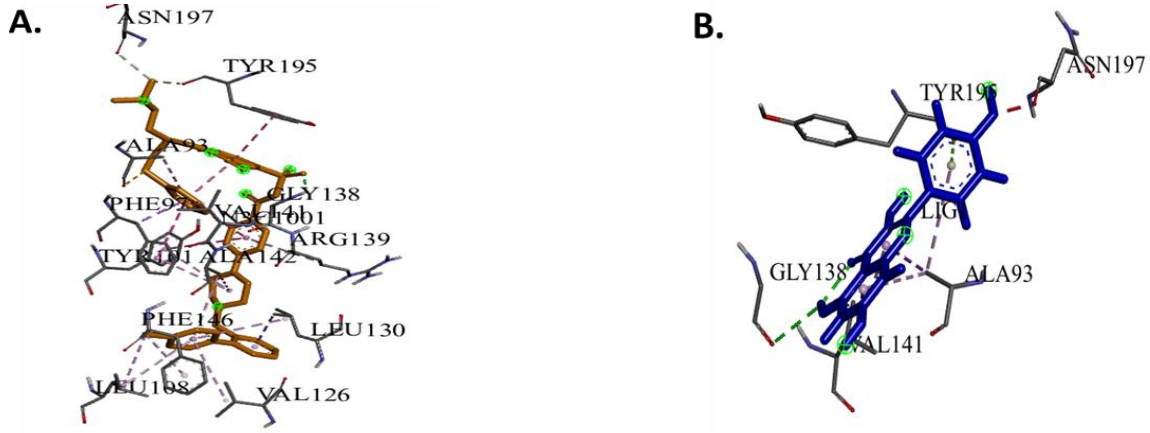


Figure 3. Binding position of kaempferol (blue), coumarin (red), the kaempferol-coumarin complex (purple), and positive control of BCL-XL inhibitor (brown) at BCL-XL protein (cyan chain)



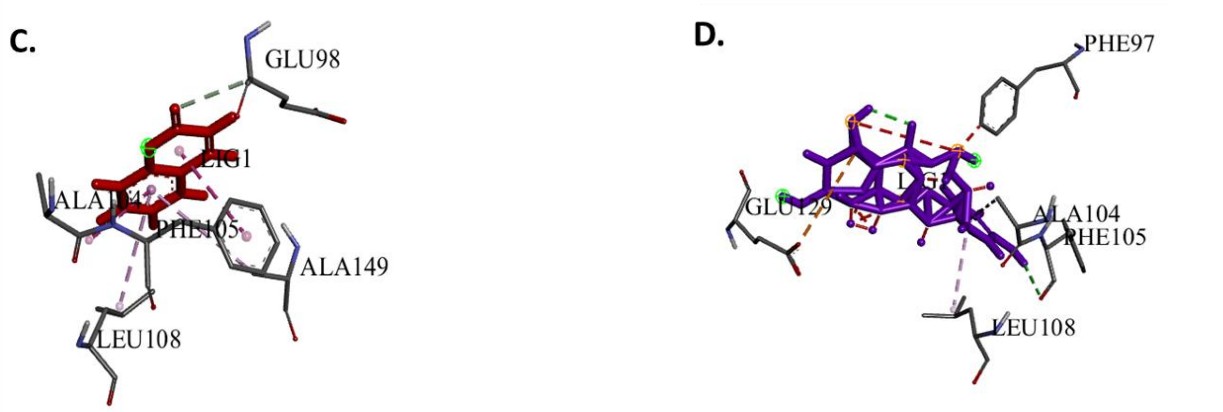


Figure 4. Interaction of compounds with BCL-XL protein. (A) positive control, (B) kaempferol, (C) coumarin, and (D) the kaempferol-coumarin complex interacted with amino acid residues in the BCL-XL protein

4. Discussion

Cancer cells are distinguished from normal cells by their ability to avoid apoptosis [24]. Apoptosis is a programmed cell death carried out by the body to maintain homeostasis. Cancer cells evade the apoptotic pathway, allowing them to survive and proliferate indefinitely in the body. Therefore, apoptosis becomes a promising target in cancer therapy [25]. Overexpressing antiapoptotic proteins, including BCL-2 and BCL-XL, is one of the strategies used by cancer cells to evade apoptosis [16]. Therefore, inhibition of these proteins' activity in cancer cells is an attractive way to treat cancer. This study aimed to identify the potency of kaempferol, coumarin, and kaempferol-coumarin complex in inhibiting the activity of BCL-2 and BCL-XL via an in silico study. Kaempferol is the major compound in Adas (*Foeniculum vulgare*), whereas coumarin is the major compound in Pulasari (*Alyxia reinwardtii*) [9], [11]. Both bioactive compounds were predicted to have potential as orally administered drugs due to their favorable oral bioavailability based on Lipinski's rule. Compounds or drugs with good oral bioavailability can provide optimal pharmacological effects to their target sites [26].

BCL-2 is one of the anti-apoptotic proteins involved in the intrinsic apoptosis pathway located in the mitochondria. BCL-2 forms heterodimers with BAX, a pro-apoptotic protein, thereby preventing BAX from binding to the mitochondrial membrane [27]. Therefore, it inhibits the development of membrane permeability, thus preventing the release of cytochrome c into the cytoplasm [27]. This event hinders the formation of apoptosomes, which can activate the initiator caspases (caspase 3, 6, or 7) and the executor caspases (caspase 3) cascades, thereby suppressing the apoptosis process [28]. However, the activity of the BCL-2 protein can be potentially inhibited using small molecules. The BCL-2 inhibition is considered to exert minimum adverse effects on normal cells because the expression of this protein in cancer cells is significantly greater than in normal cells [27]. Phenyl Tetrahydroisoquinoline Amide is a BCL-2 selective inhibitor, exhibiting a high affinity toward BCL-2 [29]. This compound interacts with the binding groove on BCL-2, where the BH3 domain of BAX binds, preventing BCL-2 from interacting with it and then triggering the intrinsic apoptosis cascade [29].

The results of this study indicated that kaempferol and the kaempferol-coumarin complex could be potential BCL-2 inhibitors in cancer therapy. The binding mode similarity of kaempferol, the kaempferol-coumarin complex, and Phenyl Tetrahydroisoquinoline Amide on BCL-2 implied that kaempferol and

the kaempferol-coumarin complex could also bind to the binding groove of the BH3 domain in BCL-2. The binding of kaempferol and the kaempferol-coumarin complex to BCL-2 protein could prevent the protein from interacting with BAX, thereby triggering intrinsic apoptosis in cancer cells. Kaempferol was reported to diminish the BCL-2 expression in head and neck cancer cells [13]. Therefore, this study predicts that kaempferol could induce apoptosis in cancer cells, not only by decreasing BCL-2 expression but also by inhibiting its activity. In addition, the kaempferol-coumarin complex was predicted to show better inhibitory potential toward BCL-2 than kaempferol alone. The interaction between the two compounds was expected to provide a higher apoptosis effect and stronger anticancer effects. Several previous studies showed that the combination of two anticancer agents might generate a synergistic anticancer effect to combat cancer cells more effectively [30]. However, the results of this research need to be validated by further comprehensive studies.

BCL-XL is a member of the anti-apoptotic protein family, alongside BCL-2. This protein shows 44% homology with the amino acid sequence that comprises BCL-2 [31]. Similar to BCL-2, this protein can trigger anti-apoptotic mechanisms in cancer cells by binding to pro-apoptotic proteins, for example BAX and BAK [32]. Additionally, BCL-XL contributes to cancer cell resistance, tumor cell progression, and low survival rates [31]**.** Therefore, inhibition of BCL-XL becomes a novel strategy to treat cancer. ABT-737 is a selective inhibitor of BCL-XL [33]. The structure of this molecule resembles the BH3 domain of pro-apoptotic proteins, allowing it to induce apoptosis through interaction with the BH3-binding groove of BCL-XL [33].

The findings of this study predict that kaempferol, coumarin, and the kaempferol-coumarin complex could be potential BCL-XL inhibitors in killing cancer. Kaempferol, coumarin, and the kaempferol-coumarin complex could bind the binding groove of the BH3 domain in BCL-XL due to their similar binding position to ABT-737, a positive control of BCL-XL. The interaction of kaempferol, coumarin, and the kaempferol-coumarin complex with BCL-XL protein might inhibit the ability of the protein to form a heterodimer with BAX or BAK, thereby promoting intrinsic apoptosis in cancer cells. Shahbaz et al. (2023) showed that kaempferol could lower the expression of BCL-XL in cancer [34]. Shahbaz et al. (2024) also discovered that coumarin could inhibit the expression of BCL-2 and BCL-XL in cancer cells [14]. Therefore, similar to BCL-2, both kaempferol and coumarin were predicted to promote intrinsic apoptosis in cancer cells, not only by decreasing the expression of BCL-XL protein but also by blocking its activity. Additionally, the kaempferol-coumarin complex showed a stronger binding affinity to the BCL-XL protein than kaempferol or coumarin alone, suggesting a possibility for greater inhibitory interaction. Further research is needed to confirm the findings of this preliminary study.

5. Conclusion

This study suggested that kaempferol and coumarin exhibited favorable predicted oral bioavailability. A combination of kaempferol, a major compound of *Foeniculum vulgare*, and coumarin, a major compound of *Alyxia reinwardtii*, exhibited a stronger binding affinity and interactions with BCL-2 and BCL-XL compared to each compound alone according to molecular docking studies. Therefore, this result suggested a potentially higher inhibitory effect of kaempferol-coumarin complex on BCL-2 and BCL-XL. However, this study requires validation through further comprehensive studies, including molecular dynamics simulation, in vitro assays, and in vivo experiments.

6. REFERENCES

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