https://doi.org/10.48048/tis.2023.5350

Flavonoids from Extract Butanol of Twigs *Erythrina crista-galli* Against the Breast Cancer Cell Line Within *In Silico* Method

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Received: 22 July 2022, Revised: 24 September 2022, Accepted: 1 October 2022, Published: 18 March 2023

Abstract

Breast cancer is one of the most serious health problems in the world. The common treatments for breast cancer have side effects and resistance, so the search for cancer drugs from natural products that are more effective is still a concern for researchers. Flavonoids from *Erythrina crista-galli,* which is one of the natural products, have the potential as anticancer drugs based on their phytoestrogen activity that has been reported. The aim of this research is to isolate flavonoids from extract butanol of *E. crista-galli* with column chromatography and determine their anticancer activity by the *in silico* molecular docking method. Two flavonoid compounds with the names 6,8-diprenylgenistein from the isoflavone group and phaseolin from the pterocarpan group were isolated. Molecular docking analysis showed that the highest binding affinity values for compounds 1 and 2 were to CDK-2 protein with values of −10.14 and −8.8 kcal/mol, respectively. However, it is also possible to suggest inhibition through ER protein, especially in compound 1, with a value of −10.66 kcal/mol. Therefore, inhibition of the target CDK-2 enzyme is the most promising for the development of new drugs.

Keywords: Flavonoid, Butanol extract, *Erythrina crista-galli*, *In silico*, Breast cancer

Introduction

Breast cancer is a serious health problem in the world, especially for women. Word Health Organization (2020) reported that breast cancer patients in these women occupy the highest percentage of cancer cases [1]. Many cancer treatments have been developed, including surgery, chemotherapy, radiotherapy, hormone therapy, and immunomodulatory therapy [2]. However, this treatment has several disadvantages, namely causing resistance, side effects and allergic reactions, requiring high costs, and being less effective [3,4]. More effective treatments need to be developed. Natural products are the most suitable alternative for new cancer drug candidate agents [5].

Flavonoids are natural products that are proven to have many biological activities, one of which is anticancer [6]. Fahmi *et al.* reported that flavonoids in the *Erythrina* genus have potential anticancer breasts activity [7]. Echrenone b10, erythraddison II, warangalone (scandenone), erysenegalinsein M, and eryvarin B from the isoflavone group have cytotoxic activity against MCF-7 breast cancer cells with IC_{50} values respectively were 1.82, 4.63, 2.83, 3.34 and 2.8 µg/mL [8,9]. Another flavonoid from the pterocarpan group, namely erivarin, has an IC_{50} value of 7.51 µg/mL [10]. The activity belongs to the high category [11].

The species *E. crista-galli* is one of the genus *Erythrina* which has little exploration for flavonoids. No studies are reporting anticancer or cytotoxic activity against breast cancer cells of the flavonoids in this plant, but that is potentially based on its phytoestrogen activity against estrogen receptor alpha (ERα) within *in silico* which has been reported. Apigenin, luteonin, apigenin-7-O-ramnosyl-6-C-glucoside, isovitexin, and isoorientin are flavonoids that are reported to have phytoestrogen activity with binding affinity values (ΔG) of −43.33, −40.97, −44.7, −39.11 and −41.91 kcal/mol [12]. So, based on what has been reported, we tried to test the breast anticancer activity of flavonoids isolated by molecular docking. Molecular docking is a promising method and can be used in the early stages of drug design because the process is fast, affordable, and effective [13]. In addition, computational methods have been widely used to predict and design of anticancer drugs [14].

In this study, we report 2 flavonoids isolated from the plant *E. crista-galli*, namely 6, 8-diprenylgenistein and phaseolin. These flavonoids were then tested on breast cancer cells within *in silico*

by molecular docking method. This molecular docking method studies the interaction of proteins with 6,8-diprenylgenistein and phaseolin ligands based on the binding affinity value (G) produced [15]. Based on suggestions from *Swiss Target Prediction*, we employed ERα, Bcl-2, CDK-2 and EGFR as selected proteins, known to play a role in the apoptosis, proliferation, differentiation, and survival process of cells [16-21].

Materials and methods

Materials

The twigs of *E. crista-galli*, were collected from Jl. Sersan Bajuri, Bandung, West Java, Indonesia. These plant materials were determined under the voucher specimen number 1,020 at the Agricultural Production Technology and Services Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Universitas Padjadjaran. For extraction and isolation, distilled water, n-hexane, ethyl acetate, n-butanol, methanol, and chloroform were employed. ODS RP-18 and Silica G-60 (63 - 200 and 200 - 500 m, Merck) were used for separations in column chromatography (40 - 63 m, Merck). Silica G 60 F_{254} plate and staining reagent $AlCl_3$ in ethanol (v/v) used for thin-layer chromatography (TLC). The protein used were ERα (ID: 1A52), Bcl-2 (ID: 6QGG), CDK-2 (1FVT) and EGFR (ID: 3W32). The ligands used were 6.8 diprenylgenistein (CID 480783) and phaseolin (CID 92572) with the comparison ligand estradiol (EST) bound to the ERα, J1H binds to Bcl-2, oxindole (106) binds to CDK-2, and W32 bound to EGFR. Tamoxifen (CID 2733526), doxorubicin (CID 31703), roniciclib (45380979), navitoclax (CID 24978538), and canertinib (CID 156414) were used as positive controls for each protein. The Research Collaboratory for Structural Bioinformatics (RSCB) (https://www.rscb.org/) provided the 3D structure of the receptor, while PubChem (https://pubchem.ncbi.mlm.nih.gov/) provided the information on ligands.

Instrument

Vilbert Luomart UV light detectors (254 and 365 nm) were used to guide the separation in thin layer chromatography. Fractionation and purification were carried out using a separating funnel and chromatography. For analysis and characterization of isolates, UV-Vis spectrophotometer (Hp 8452 A Shimadzu), FTIR spectrometer (One Perkin Elmer) and 500 MHz JEOL delta NMR spectrometer (1H-NMR, 13C NMR, and 2D NMR) were used. (HR-TOF-MS).

Isolation compounds 1 and 2 form *E. crista-galli* twigs

E. crista-galli twig powder (4 kg) was extracted with methanol redistillation for @10×24 h at room temperature using the maceration technique. The filtrate from the maceration was then concentrated with a rotatory evaporator at a temperature of ± 40 °C to obtain a concentrated methanol extract (115 g). The concentrated methanol extract was partitioned with n-hexane, ethyl acetate, and butanol to yield extracts of n-hexane (20.86 g), ethyl acetate (34.6 g), and n-butanol (6.6 g). Butanol extract (4.5 g) was separated by column chromatography (the column size is 3.2×81.5 cm^2 with a flow rate of 2.23 mL/min for 3 h) with the normal phase eluted by chloroform: Methanol gradient 10 % to yield 10 fractions (A - J). A typical coloumn size may be Fraction B was separated by ODS column chromatography (the column size is 0.7×77 cm^2 with a flow rate of 1.73 mL/min for 8 h) with the reverse phase using methanol: Water (8:2) as eluent, resulting in 9 fractions (B1 - B9). The fractions B6 (24.8 mg) and B3 (22.7 mg) were then purified with n-hexane: Chloroform: Ethyl acetate as eluent (7:2:1) to yield compounds 1 (6.5 mg) and 2 (5, 6 mg).

Characterization of the isolated compound from *E. crista galli* twigs

6,8-diprenylgenistein (1). UV: Wavelengths 341 and 272 nm. IR: Wavenumber 836, 1,220, 1,363, 1,514, 1,646, 1,913, 2,968 and 3,368 cm^{-1}. ^{1}H-NMR: H 1.72 (3H, s), 1.75 (3H, s), 1.81 ppm (3H, s), 1.83 ppm (3H, s), 3.45 (4H, t, J = 6 Hz), 5.07 (1H, s), 5.23 ppm (2H, m), 6.35 (1H, s), 6.87 ppm (2H, dd, J = 2.5 and 7 Hz), 7.38 (2H, dd, J = 2.5 and 7 Hz), 13.13 (1H,s). ^{13}C-NMR: C 17.9, 18.0, 25.8, 25.9, 21.7, 105.5, 105.8, 110.2, 115.6, 121.3, 121.6, 123.1, 123.4, 130.4, 134.2, 135.7, 152.6, 153.4, 155.8, 157.6, 159.6. MS: (m/z) of 1 was 407.1855.

Phaseolin (2). UV: Wavelengths 325 and 279 nm. IR: Wavenumber 850, 1,211, 1,622, 2,925 and 3,379 cm^{-1}. ^{1}H-NMR: H 1.38 (3H, s), 1.41 (3H, s), 3.48 (1H, m), 3.51 (1H, t, J = 11), 4.22 (1H, dd, J = 5 and 11), 5.46 (1H, d, J = 7), 5.56 ppm (1H, d, J = 10 Hz), 6.33 (1H, d, J = 8), 6.40 (1H, d, J = 2), 6.49 ppm (1H, d, J = 10 Hz), 6.55 (1H, dd, J = 2 and 8.5), 6.94 (1H, 8, J = 8 Hz), 7.40 (1H, d, J = 8.5). ^{13}C-NMR: C 27.7, 27.8, 39.7, 66.6, 76.2, 78.7, 103.7, 106.3, 108.2, 109.7, 112.6, 116.5, 119.1, 123.9, 129.7, 132.4, 152.7, 155.4, 156.7, 157.1. MS: (m/z) of 2 was 323.1283.

Molecular docking analysis of compounds 1 and 2

The protein crystal structure was retrieved from the RCSB protein database (www.rcsb.org/) in PDB format. The receptor was separated from the native ligand by the BIOVIA program and then the method validation process was carried out. Molecular docking was performed using AutoDockTools-1.5.6. The validation of the molecular docking method through re-docking of the crystallized ligand around the protein binding site was carried out. The grid box parameters are set for molecular docking of 6,8-diprenylgenistein, phaseolin, and positive controls based on the results of the coordinates obtained from the validation method. The structures of 6,8-diprenylgenistein (CID 480783), phaseoline (CID 92572), tamoxifen (CID 2733526), doxorubicin (CID 31703), roniciclib (45380979), navitoclax (CID 24978538), and canertinib (CID 156414) were obtained from PubChem (https://www.ncbi.nlm.nih.gov/pccompound). The docking results were analyzed by Biovia discovery studio 202054 to show the docking position between proteins and ligands in 3D molecules and determine the type of interaction.

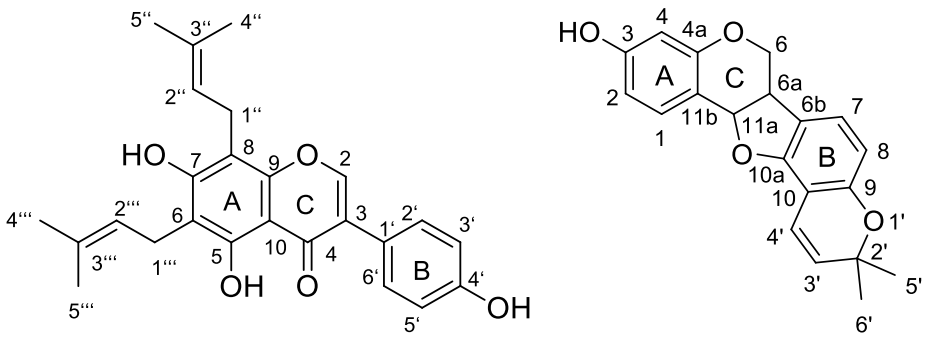
Results and discussion

Characterization of compounds 1 and 2

Compound 1 has a characteristic yellow color, is oily, and is soluble in acetone, methanol, or chloroform. The molecular formula is $C_{25}H_{26}O_5$ with a value of $[M+H]^+$ 407.1855 and m/z_{cald} 407.1858 measured using HR-TOF-MS mass spectrometry. The UV spectrum showed 2 peaks at wavelengths of 272 and 341 nm. Absorption ranges of 245 - 275 nm in band II and 310 - 340 nm in band I are types of isoflavones. The functional groups in the flavonoid ring can be analyzed by adding a shifting reagent, NaOH. The results show that there is a red shift of 13 nm in band II (272→285 nm) and by 2 nm in the I band (341→343 nm). The red shift indicates the presence of an auxochrome group, such as −OH group [22].

The IR spectrum on compound 1 showed a group −OH at 3368 cm^{-1}, CH stretch vibration at 2,968 and 2,913 cm^{-1}, there is a C - O at 1,646 cm^{-1}, stretch vibration C = C sp^2 at 1,514 cm^{-1}, gem dimethyl at 1,363 cm^{-1}, stretch vibration CO (ether) at 1,220 cm^{-1}, and CH bending vibration (aromatic) at 836 cm^{-1}. The ^{13}C-NMR and DEPT spectra of compound 1 showed 25 carbon signals consisting of 1 carbonyl carbon signal at δ_C 181.3 ppm (C - 4), 4 oxygenated quaternary carbons at δ_C 159.6 (C - 7); 157.6 (C - 5); 155.8 (C - 4'); and 153.4 ppm (C - 9), 1 carbon of methine oxygenated at δ_C 152.6 ppm (C - 2), 6 methine carbons at δ_C 2×130.4 (C - 6'); 121.6 (C - 2'''); 121.3 (C - 2''); and 2×115,6 ppm (C - 3' and C - 5'), 7 quaternary carbons at δ_C 135.7 (C - 3''); 134.2 (C - 3'''); 123.4 (C - 3); 123.1 (C - 1'); 110.2 (C - 6); 105.8 (C - 10) and 105.5 ppm (C - 8), 2 methylene carbons at δ_C 2×21.7 (C - 1'' and C - 1'''), and 4 methyl carbons at δ_C 25.9 (C - 4''); 25.8 (C - 4'''); 18.0 (C - 5'') and 17.9 ppm (C - 5''').

The H-NMR spectrum of compound 1 showed 12 proton signals consisting of 4 methyls at δ_H 1.72; 1.75; 1.81 and 1.83 ppm (H - 4'', H - 4''', H - 5''' and H - 5''; 3H, s), 2 methylene at δ_H 3.45 ppm (H - 1' and H - 1'''; 4H, t, J = 6 Hz) and 2 methine sp^2 at δ_H 5.23 (H - 2'' and H - 2'''; 2H, m) which is a prenyl function group. The methine protons at δ_H 6.87 (H - 3' and H - 5'; 2H, dd, J = 2.5 and 7 Hz) and 7.38 (H - 2' and H - 6'; 2H, d, J = 2.5 and 7 Hz) show the ortho position at J = 7 and the meta position at J = 2.5 in the aromatic ring B. One oxygenated methine proton at δ_H 7.88 ppm (1H, s) is a characteristic of the isoflavonoid group at position H - 2. There are 3 hydroxyl groups at δ_H 5.07, 6,35 and 13.13 (4' - OH, 7 - OH and 5 - OH; 1H, s). At δ_H 13.13 ppm, it is typical of the H - 5 position in ring A because it can chelate with the carbonyl at the C - 4 position. Comparison to the literature values showed compound 2 as the known compound 6,8-diprenylgenistein (**Figure 1**) [23,24].



(1) (2)

Figure 1 Structure compound of 6,8-diprenilgenistein (1) and phaseolin (2).

Compound 2 has a characteristic orange color, is oily, and is soluble in acetone, methanol, or chloroform. The molecular formula is $C_{20}H_{18}O_4$ with a value of $[M+H]^+$ 3231.282, m/z_{cald} 3231.282 measured using HR-TOF-MS mass spectrometry. The UV spectrum showed 2 peaks at wavelengths of 279 and 325 nm and there is no shift when the NaOH shift reagent is used.

The IR spectrum on compound 2 showed a −OH group at 3379 cm^{-1}, CH stretching vibration at 2,925 cm^{-1}, C = C stretching vibration (aromatic) at 1,622 cm^{-1}, CO stretching vibration (ether) at 1,211 cm^{-1}, and CH bending vibration (aromatic) at 850 cm^{-1}. The ^{13}C-NMR and DEPT spectra of compound 2 show 20 carbon signals consisting of 5 oxygenated quaternary carbons at δ_C 76.2 (C - 2'); 153.7 (C - 9); 155.4 (C - 10a); 156.7 (C - 4a) and 157.1 ppm (C - 3), 1 oxygenated methine carbon sp^3 at δ_C 78.7 ppm (C - 11a), 7 methane carbons at δ_C 103.7 (C - 4); 106.3 (C - 8); 109.7 (C - 2); 116.5 (C - 4'); 123.9 (C - 7); 129.7 (C - 3') and 132.4 ppm (C - 1), 3 quaternary carbons at δ_C 108.2 ppm (C - 10), 112.6 (C - 11b) and 119.1 (C - 6b), 1 oxygenated methylene carbon at δ_C 66.6 (C - 6), 1 methane carbon sp^3 at δ_C 39.7 (C - 6a) and 2 methyl carbons at δ_C 27.6 (C - 6') and 27.8 ppm (C - 5').

The H-NMR spectrum of compound 2 showed 13 proton signals. The protons in the pyran group consist of 2 methyls at δ_H 1.38 and 1.41 ppm (H - 6' and H - 5'; 3H, s) and 2 methines at δ_H 5.56 (H - 3'; 1H, d, J = 10 Hz) and 6.50 ppm (H - 4'; 1H, d, J = 10 Hz). The same value of J = 10 at δ_H 5.56 and 6.49 ppm indicates that the proton is in an ortho position. There are 2 methines sp^2 that are in ortho position at δ_H 6.33 (H - 8; 1H, d, 8) and 6.94 ppm (H - 7; 1H, d, 8) in ring B. The protons in ring C consist of 4 methines sp^3 at δ_H 3.48 (H - 6a; 1H, m), 3.51 (H - 6; 1H, t, J = 11), 4.22 (H - 6; 1H, dd, J = 5 & 11) and 5.46 (H - 11a; 1H, d, J = 7). The other 3 protons are methine signals at δ_H 6.40 (H - 4; 1H, d, J = 2), 6.55 (H - 2; 1H, dd, J = 2 & 8.5) and 7.40 (H - 1; 1H, d, J = 8 ,5) which is the A ring on flavonoids. Comparison to the literature values showed compound 2 as the known compound phaseolin [25].

Molecular docking studies within *in silico* method

The first step of molecular docking is the validation of the method at each receptor by redocking the native ligand with the receptor. Method validation is carried out to prove and ensure that the method used meets the validity requirements and can be used for other molecular testing and can minimize errors [25]. The results of molecular docking of ERalfa, Bcl-2, CDK-2, and EGFR receptors with native ligands had RMSD values of 0.650, 1.872, 0.711 and 1.168 Å. Therefore, all receptors used were validated because they have an RMSD value below 2 Å [25].

Compounds 1 and 2 were tested with *in silico* method for their ability to inhibit human estrogen receptor- (hER-), B-cell lymphoma 2 (Bcl-2), cyclin dependent kinase (CDK-2), ikappaB kinase (IkB), and growth factor receptor epidermal layer (EGFR). The docking was also carried out on 5 FDA-approved drugs for breast cancer, as well as their native ligands, so that the results obtained were more convincing.

This anti-breast cancer activity was evaluated based on its intermolecular interaction and binding affinity value. Binding affinity (G) is a parameter of the strength of the interaction between the binding of the ligand and the receptor. The high binding affinity (value of G minus) indicates that the intermolecular forces are greater and the interaction between the receptor and the ligand is more stable [26]. The binding energy values between compounds 1, 2, native ligands, and positive controls with selected proteins can be seen in **Table 1**.

Table 1 Binding affinity of the protein-ligands.

Ligand	ERα (Kcal/mol)	Bcl-2 (Kcal/mol)	CDK-2 (Kcal/mol)	EGFR (Kcal/mol)
6,8-diprenylgenistein	−10.66	−7.61	−10.14	−9.51
Phaseolin	−9.22	−6.92	−8.03	−9.06
17β- Estradiol	−10.40	-	-	-
J1H	-	−14.15	-	-
106 (Oxindole)	-	-	−9.24	-
W32	-	-	-	−12.99
Tamoxifen	−11.35	-	-	-
Doxorubicin	-	−9.19	-	-
Roniciclib	-	-	−7.86	-

Ligand	ERα (Kcal/mol)	Bcl-2 (Kcal/mol)	CDK-2 (Kcal/mol)	EGFR (Kcal/mol)
Navitoclax	-	-	-	-
Canertinib	-	-	-	−11.22

Protein-ligand interactions are very diverse, such as hydrogen bonds, ionic interactions (salt bridges), metal complexes, hydrophobic interactions, and cation-∏ interactions [27]. One of the intermolecular interactions that can impact the stability of the contact between the ligand and protein and the affinity of the ligand bond is hydrogen bonding [26]. Apart from hydrogen bonding, hydrophobic interactions are also considered to be the main driving force behind the conformational change of the target protein [21]. Intermolecular interaction is shown in **Table 2**. All interaction proteins between ligands will be explained below.

Table 2 Interaction of proteins with ligands.

No	Compound	Interaction type	
		Hydrophilic (hydrogen bound)	**hydrophobic**
		ER	
1	6,8-diprenylgenistein	-	Leu349A, Leu387A, Phe404A, His524A, Ile424A, Met421A, Ala350A, Leu525A
2	Phaseoline	Met343A, Thr347A,	Leu525A, Leu346A, Leu384A, Met388A, Ala350A, Leu391A, Phe404A, Leu387A, Leu349A
3	*Native Ligand* (Estradiol)	His524A, Arg394A, Glu 353A	His524A, Met421A, Ile424A, Met388A, Leu387A, Ala350A, Leu391A, Phe 404A, Leu525A
4	Tamoxifen (+)	Arg394A, Glu353A, Leu387A, Asp351A, Thr347A	Met421A, Leu525A, Trp383A, Ala350A, Leu428A, Phe404A, Leu391A
		Bcl-2	
1	6,8-diprenylgenistein	Arg146A, Tyr108A	Ala100A, Val148A, Tyr202A, Ala149A, Arg146A, Tyr108A
2	Phaseolin	Ala100A, Asn143A	Tyr108A, Val148A, Tyr202A, Phe198A, Ala100A
3	*Native Ligand* (J1H)	Arg107A, Gly145A, Asp111A, Tyr108A	Asp103A, Ala100A, Val148A, Leu137A, Val133A, Met115A, Phe112A, Val156A, Ala149A, Tyr202A
4	Doxorubicin (+)	Gly145A, Tyr108A, Arg107A	Ala100A
		CDK-2	
1	6,8-diprenylgenistein	Thr14A, Lys129A, Leu83A	Leu134A, Ile10A,Ala31A, Phe80A, Val64A, Val18A, Ala144A
2	Phaseolin	Gu12A	Val18A, Leu134A, Ile10A, Ala31A, Phe82A
3	106, Oxindole	Leu111A, Gln110A, Leu103A, Ile104A	Leu108A, Ser106A, Ile141A, Ile135A
4	Roniciclib	Lys89A, Leu83A, Glu81A, Asp86A	Ile10A, Ala31A, Leu134A, Phe82A
		EGFR	
1	6,8-diprenylgenistein	Lys745A	Val726A, Leu844A, Leu792A, Phe997, Leu718A, Leu1001A, Thr790A

No	Compound	Interaction type	
		Hydrophilic (hydrogen bound)	**hydrophobic**
2	Phaseolin	-	Val726A, Ala743A, Leu777A, Met766A, Phe856A, Cys775A, Leu788A, Lys745A, Thr 854A
3	*Native Ligand (W32)*	Lys745A,Thr854A, Leu777A, Gln791A	Cys775A, Arg776A, Met766A, Leu788A, Val726A, Ala743A, Leu844A, Cys797A
4	Canertinib	Lys745A, Gly796A	Leu792A, Met793A, Cys797A, Leu844A, Thr853A, Val72A, Met766A, Phe856A, Leu777A, Leu788A

ERα

Estrogen receptors alpha (ERα) play a role in breast development and activation of pro-proliferation in normal or cancer-affected breast cells [28]. Compound 1-ERα has an ΔG value of −10.66 kcal/mol, where the stability of the interaction is stronger than 17β-estradiol (−10.4 kcal/mol) but still weaker than tamoxifen (−11.35 kcal/mol). Estrogen hormones (17β-estradiol) are hormones that play a role in activating the work of ERα while tamoxifen is a drug commonly used for ERα targeting therapy [29]. Compound 2-ERα shows the weakest interaction with the ΔG value of −9.22 kcal/mol. The key activity of ERα is in 3 hydrogen bonds with the residues Arg 394, Glu 353 and His 524 [12]. All ligands can interact with the active site of the protein at Leu525A and Ala350A with the same type of interaction, hydrophobic [30]. Compound 1 shows interactions with several other active sites of ERα namely with Leu387A and Phe404 via hydrophobic interactions. As for compound 2, it shows a hydrophobic interaction with the active site on Leu525A, Ala350A, Leu391A, Phe404A and Leu387A. No hydrogen interaction occurred between compound 1 or compound 2 with the key residue. However, based on the ΔG value, compound 1 is thought to be able to compete with estradiol in its binding to ERα.

Bcl-2

Bcl-2 is an anti-apoptotic protein that functions to maintain survival and inhibit cell death/cell apoptosis in the intrinsic or mitochondrial-mediated pathway [31]. The first docking analysis was carried out on Ligand J1H, which is an analog of the compound ABT-737, an inhibitor known as a Bcl-2 inhibitor [32]. The isolated compound is expected to replace the role of the protein bound to Bcl-2 so that the action of Bcl-2 is inhibited and cancer cell apoptosis can take place.

The result of docking analysis for compound 1-Bcl-2 protein has ΔG values of −7.61 kcal/mol and for compound 2-Bcl-2 is −6.92 kcal/mol, where the value is weaker than the native ligand J1H (−14.15 kcal/mol) and the positive control, namely doxorubicin (−9.19 kcal/mol). Although the binding of compounds 1 and 2 to Bcl 2 is less stable, these compounds can interact with several active sites of the protein. Compound 1 can interact hydrophilically (hydrogen bonds) with the active site of bcl-2 on residues Tyr108A and hydrophobically on Val148A, Tyr202A, Ala149A and Tyr108A. Compound 2 interacts with the active site on residues Phe104A and Tyr202A via hydrophobic interactions and Tyr108A via hydrophobic interactions [33]. Thus, compounds 1 and 2 have not been able to replace the role of J1H inhibitors.

CDK-2

Cyclin-dependent kinases (CDKs), which are kinase enzymes, are involved in the cell cycle's transition from the G1 to the S phases, during which time cells manufacture the proteins necessary for mitosis and DNA replication [34]. Overproduction of cyclins or CDKs, including CDK1, CDK2, CDK4 and CDK6, can impair normal regulatory control and ultimately result in cancer. Using CDK inhibition as a therapeutic method is a significant business opportunity and a promising plan for speedy drug discovery. In this investigation, roniciclib (BAY 1000394, **Figure 1**), one of the CDK-2 inhibitors that has undergone clinical testing, is employed as a positive control [35].

The binding activity of the compound 1-CDK-2 resulted in the highest ΔG value compared to other ligands, −10.14 kcal/mol which indicated that the compound was the most active. While the value of binding energy on compound 2 is −8.03 kcal/mol where the interaction is more stable than roniciclib (−7.86 kcal/mol) which is a positive but less stable control of the native ligand, oxindole (−9.24 kcal/mol). CDK-

2 has an active site on the amino acid residues Ala31A, Leu134A, Leu83A, Asp86A, Gln85A, Ile10A, Val64A and Asp145A [36].

This compound 1 successfully interacted with several active sites of CDK-2, namely with Leu134A and Ala31A through hydrophobic interactions and Leu83A through hydrophilic interactions. **Figure 2** shows the C - 7 hydroxyl group can interact with Leu83A via hydrogen bonding, which is the key to CDK-2 activity [37]. Compound 2 can interact with active site residues Leu134A and Ala31A. Compounds 1 and 2 are thought to be able to compete with CDK-2 inhibitors and with their native ligands in inhibiting enzyme activity.

EGFR

The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a transmembrane protein of the receptor tyrosine kinase that triggers several biological processes, including cell proliferation, differentiation, and survival. These receptors are also essential for the growth of breast cancer. Poor prognosis is associated with high expression levels of EGFR and HER2 which have been found in 15 - 30 % of breast tumors. HER3 expression, which is present in 18 % of cancers, is associated with lower overall cell survival and may confer resistance to treatments that target EGFR or HER2 [19].

Compounds 1 (−9.51 kcal/mol) and 2 (−9.06 kcal/mol) that interact with EGFR have less stable bonds than W32 (−12.99 kcal/mol kcal/mol) and canernitib (−11.22) based on the value of the binding energy. However, compounds 1 and 2 can interact with the active site of EGFR protein hydrophilically at residue Lys745 and hydrophobically at residue Leu844A and Thr790A for compound 1. In compound 2, the interaction that occurs is hydrophobic with residues Ala743A, Leu777A, Met766A, Phe856A, Cys775A, Leu788A and Thr 854A. The interaction with the active site of EGFR also occurs hydrophilically and hydrophobically in the native ligand (W32) and its positive control (Cabertinib) [38]. The targeting of inhibition of this protein is not possible to suggest.

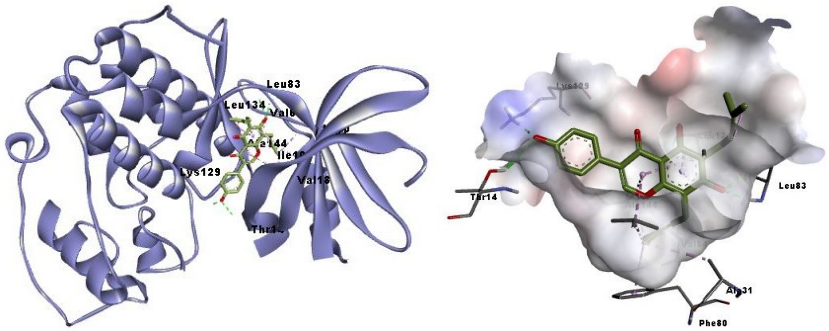


Figure 2 Molecular docking visualization of complex CDK-2 and 6,8-diprenylgenistein.

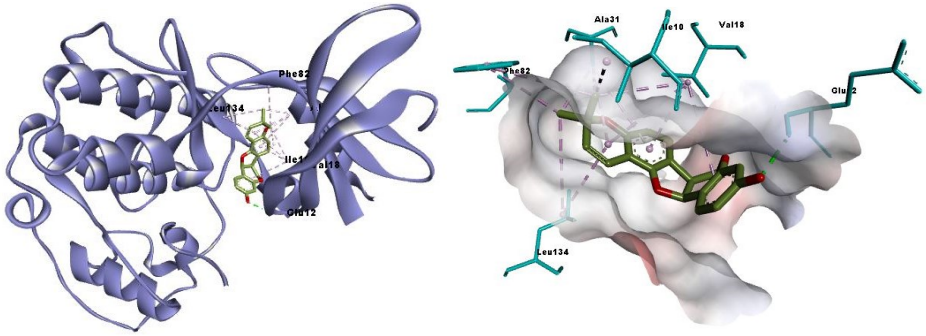


Figure 3 Molecular docking visualization of complex CDK-2 and phaseolin.

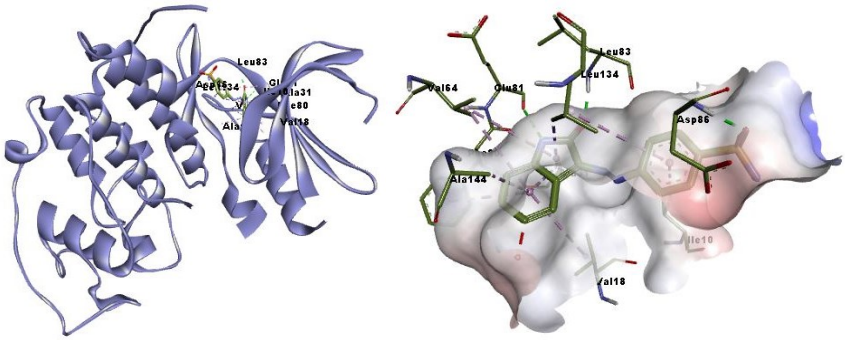


Figure 4 Molecular docking visualization of complex CDK-2 and native ligand (106, Oxindole).

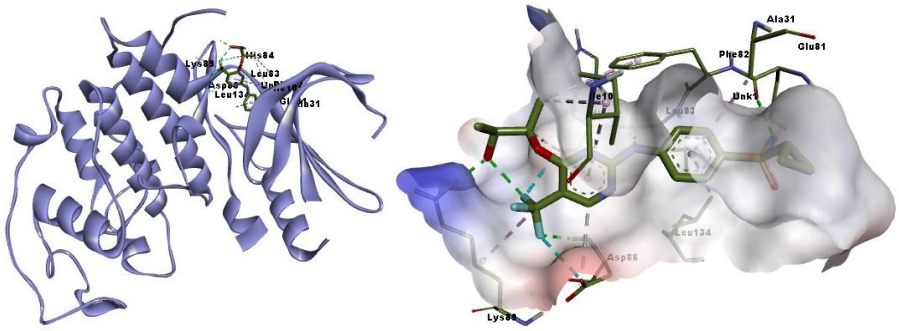


Figure 5 Molecular docking visualization of complex CDK-2 and roniciclib.

Figures 2 - **5** is an example of that visualization of compounds 1, 2, native ligand, and roniciclib with CDK-2 because of the highest activity compared to targeting other proteins, where 6,8-diprenylgenistein has higher activity than phaseollin. It can also be suspected that the isoflavone group has higher anticancer activity than the pterocarpan group. This is supported by *in vitro* testing of MCF-7 breast cancer cells that has been carried out by previous researchers [8-10].

Conclusions

6,8-diprenylgenistein (1) from the isoflavone group and phaseolin (2) from the pterocarpan group were isolated from the twigs of *E. crystal-galli* and this was the first time reported to be present in this species. Prediction of the target of compounds 1 and 2 as breast cancer drug candidates is through the cell cycle process by replacing the role of CDK-2 inhibitors. Inhibition through proteins is also possible to be suggested. The isoflavones are thought to have a higher activity potential than pterocarpans, so further testing *in vitro* and *in vivo* is necessary.

Acknowledgments

The authors are grateful for the facilities from Universitas Padjadjaran, Indonesia by Academic Leadership Grant (ALG) scheme by Prof Tati Herlina (No. 094/E5/PG.02.00.PT/2022).

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