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Potential Role of Bioactive Compounds of Purple Corn as Breast Cancer Drug Candidates Through Inhibition of Cyclin-Dependent Protein Kinase 6 (Cdk6): An In-Silico Approach

Ayu Tri Agustin[1*], Amalia Wardatul Firdaus[2], Anas Fadli Wijaya[1]
[1]Medical Laboratory Technology Study Program, Faculty of Health Sciences, Universitas dr. Soebandi, Indonesia, 68111
[2]Study Program of Pharmacy, Faculty of Health Sciences, Universitas dr. Soebandi,Indonesia, 68111

ABSTRACT: Cancer is characterized by disorderly cell proliferation. Upregulation and activation of the Cyclin-Dependent Protein Kinase 6 (CDK6) signalling pathway can induce uncontrolled proliferation of breast cancer cells. Therefore, inhibition of CDK6 persists to be developed as a target for the design and development of potential drugs to treat breast cancer. This study aims to predict the biofunction of anthocyanin compounds from purple corn extract as Cyclin-Dependent Protein Kinase 6 (CDK6) inhibitors in silico. The research methods used include data mining, ligand and receptor preparation, molecular docking, docking visualization, and data analysis. Our results show that six compounds of purple corn extract (cyanidin, cyanidin 3-glucoside, pelargonidin-3-glucoside, pelargonidin, peonidin, and peonidin-3-glucoside) canbind CDK6 at the C-terminal and N-terminal domains. The binding pattern indicated that cyanidin, cyanidin 3-glucoside, pelargonidin-3-glucoside, pelargonidin, peonidin, and peonidin-3-glucoside bind to the identical site CDK6 residues as Palbociclib (control). This finding indicated that compounds from purple corn are most likely competitive inhibitors of CDK6. Peonidin-3-glucoside showed the lowest binding energy of -282.5 kcal/mol, close to palbociclib (-329.4 kcal/mol).

KEYWORDS: anthocyanidins, anthocyanins, breast cancer, CDK6, palbociclib.

INTRODUCTION

Cancer is a multifactorial and multiphase disease, where the cell cycle plays an essentialrole in regulation at the cellular level. Dysregulation of the cell cycle can affect the imbalanceof proto-oncogenes, tumour suppressors, and cell cycle-related proteins (1). Thus, triggering unlimited division and the capacity to metastasize, leading to the development of cancer stages(2). Breast cancer is a significant health problem experienced by women due to high mortalityand morbidity. The leading causes are genetic factors and hormonal factors (3). Currently, morethan 85% of breast cancer patients survive more than five years, and about 80% survive more than ten years after diagnosis (4). In addition, an estimated 2.3 million new cases of breast cancer are diagnosed globally each year. Current projections suggest that by 2030, the numberof new cases diagnosed worldwide could reach 2.7 million annually, while the number of deathscould reach 0.87 million (5).

Treatment alternatives for breast cancer include local therapies such as surgery and radiotherapy and systemic therapies such as chemotherapy, hormonal therapy and targeted therapy. Chemotherapy improves tumour control, increases the chance of cure and prolongs the life of breast cancer patients (6). Anthracycline-based chemotherapy (ABC) (a combinationof epirubicin or adriamycin with cyclophosphamide) has shown superior benefits. However, ithas cytotoxic effects that disrupt the cell cycle. As a result, adverse side effects such as gastrointestinal disturbances, bone marrow suppression, neuropathy, hair loss, fatigue and skindisorders are often experienced by patients as a result of the treatment received (6–8).

The potential of phytomedicines is a preferred alternative because of its minimal side effects compared to chemotherapy drugs. Although natural treatments cannot cure breast cancer, maintaining a proper diet, frequent exercise, and adequate sleep will help fight cancer (9). The bioactive compound anthocyanin has shown a potential to inhibit triple-negative breastcancer (TNBC) in preclinical studies (10). In addition, anthocyanins also have anticancer chemopreventive effects related to increased apoptosis, decreased cell proliferation, and cell cycle arrest (11).

Purple corn is one of the food yields rich in anthocyanins. Anthocyanins are water- soluble compounds responsible for the presence of coloured pigments in grains. Pigmented corn varieties like purple corn are highly valued due to their high phenolic compound content,such as flavonoids and anthocyanins. The phenolic compound content in purple corn is the totalproanthocyanin (TPA) content of 1381 µg/g, DW), total anthocyanin (TAC) (780 µg/g DW), total flavonoid (TFC) (1998 µg/g DW) and total polyphenol (TPC) (4047 µg/g DW) (12). In Indonesia, purple corn has also begun to be cultivated since its potential as a functional food source for

body health was discovered. Several studies have shown that anthocyanin compounds are beneficial as antioxidants, anti-diabetic, anti-hypoglycemic, anti-hypertensive,anti-cancer, anti-inflammatory, anti-mutagenic, anti-microbial, anti-obesity, preventing liver dysfunction and other degenerative diseases (13,14). Cyanidin, pelargonidin, peonidin, cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside are the main anthocyanins contained in purple corn extract (15–18).

Previous studies on the anti-cancer potential of purple corn extract in vitro have been evaluated. Pigmented purple corn inhibits LNCaP cell proliferation by decreasing Cyclin D1 expression and inhibiting the G1 phase of the cell cycle. In addition, in vivo tests have been conducted on 36 male transgenic mice induced with prostate adenocarcinoma. The results showed that the administration of pigmented corn can reduce the incidence of adenocarcinomain the lateral prostate and slow the development of prostate cancer. Lower Ki67 positive levelsdecreased Cyclin D1 expression, and downregulation of Erk1/2 and p38 MAPK activation wereobserved in the group consuming purple corn in their diet (18). In vitro and in silico studies have also been conducted and showed that purple and red corn extracts exhibit anti-proliferative effects on colon cancer cells as indicated by increased apoptosis markers, namely BAX, Bcl-2, cytochrome c, and TRAILR2/D5.

Furthermore, the results of in silico analysis showed the binding energy of cyanidin-3-glucoside complex with non-receptor tyrosine kinase and peonidin with receptor tyrosine kinase of −7.86 and −7.76 kcal/mol, indicating the potential of purple corn compounds in inhibiting colon cancer cell proliferation (19). So far, the potential of purple corn extract as ananti-breast cancer agent and its mechanism is still unknown. Therefore, it is necessary to studythe bio-function of compounds in purple corn to inhibit target genes involved in the breast cancer gene cascade.

This study aims to predict the biological function of anthocyanin compounds from purple corn extract as inhibitors of molecular markers of breast cancer cells, namely Cyclin- Dependent Protein Kinase 6 (CDK6) in silico. CDK6 is critical in the cell cycle and intercellular communication in the oncogenic signalling pathway. CDK6 binds to D-type cyclins (cyclin D1, cyclin D2, and cyclin D3). It is specifically involved in promoting the cellular transition from the G1 phase to the S phase of the cell cycle when DNA synthesis occurs. It is responsible for the initiation, growth, and survival of many types of cancer (20). Upregulation of cyclin-dependent kinases (CDKs) 6 causes uncontrolled cell division, which leads to cancer (21). If CDK6 is inhibited, cancer cells' cell cycle and proliferation activity willbe disrupted. Thus, CDK6 inhibitors are needed for the discovery of potent and effective drugsin treating malignant diseases, especially breast cancer.

METHOD

Materials

The materials used in this study were six main compounds from purple corn, namely cyanidin (CID_128861), cyanidin 3-glucoside (CID_441667), pelargonidin-3-glucoside (CID_443648), pelargonidin (CID_440832), peonidin (CID_441773), and peonidin-3-glucoside (CID_443654) which act as ligands. The breast cancer cell marker used in this studywas Cyclin-Dependent Protein Kinase 6 (CDK6), which also acts as a receptor with PDB ID 5l2i. In addition, the synthetic drug Palbociclib (CID_5330286) was used as a comparative control in this study. Palbociclib is a first-class synthetic oral drug and is highly selective in advanced breast cancer (22).

Data Mining

The NCBI PubChem database (https://www.ncbi.nlm.nih.gov/) was used to obtain theligand structures (cyanidin, pelargonidin, and peonidin) and the structure of Palbociclib in PDFformat. The receptor (Cyclin-Dependent Protein Kinase 6) and its structure were obtained fromRSCB PDB (https://www.rcsb.org/).

Ligand and Receptor Preparation

The ligand was conditioned using PyRx 0.8 software to underrate energy. Meanwhile,the receptor was designed using Discovery Studio software. After the preparation was concluded, all were earmarked in .pdb format using Open Babel (23).

Docking Molekuler

Molecular docking between ligands (cyanidin, pelargonidin, peonidin, palbociclib) andCDK6 receptors was performed using Hex 8.0 software. The setting column was set in Shape+Electro+DARS mode (24). Grid dimension was set to 0.6; solution range 180, twist range 360, distance range 40, translation step 0.8 and score threshold 0.0.

Data Analysis

The successfully docked ligand and receptor complex is then examined for its amino acid residues. The type and binding energy of receptor-ligand interactions are identified usingDiscovery Studio software (25). The binding site of amino acid residues, type of chemical bond, and binding energy are analyzed using Microsoft Excel 2013 software.

RESULTS AND DISCUSSION

The results of molecular docking between bioactive compounds of purple corn extract(cyanidin, cyanidin 3-glucoside, pelargonidin-

3-glucoside, pelargonidin, peonidin, and peonidin-3-glucoside) with cyclin-dependent protein kinase 6 (CDK6) are shown by the binding pattern of amino acid residues, types of chemical bonds, and binding energy. X-ray crystallography studies show that the overall structure of CDK6 is a bilobal fold consisting ofN-terminal and C-terminal domains. The N-terminal lobe consists of residues 1-100 composedof 5 antiparallel b-sheet strands and aC helices. The C-terminal domain is an a-helix and consists of residues 101–326 (26).

The molecular interaction of the cyanidin-CDK6 complex involves six amino acid residues, namely PHE283, ILE235, LEU278, ALA259, LEU278, and ILE235. The binding of the cyanidin-CDK6 complex is maintained by hydrogen and hydrophobic interactions. Cyanidin 3-glucoside is able to bind CDK6 at amino acid residues LEU281, ASP275, ILE235,ILE235, ASP242, LEU278, and LYS279. These interactions (LEU281, ASP275, ILE235,

ILE235, ASP242, LEU278, and LYS279) are stabilized by hydrogen and hydrophobic bonds and electrostatic interactions. Pelargonidin 3-glucoside is able to bind CDK6 domain at aminoacid residues ARG214, ARG220, PHE265, ARG220, and LYS230. Hydrogen and hydrophobicinteractions stabilize these interactions (ARG214, ARG220, PHE265, ARG220, and LYS230).There are six amino acid residues of CDK6 bound by pelargonidin, including ASP242, PHE283, ILE235, LEU278, and ALA259. The interaction of the pelargonidin-CDK6 complexis maintained by two hydrogen bonds and four hydrophobic interactions (Figure 1).

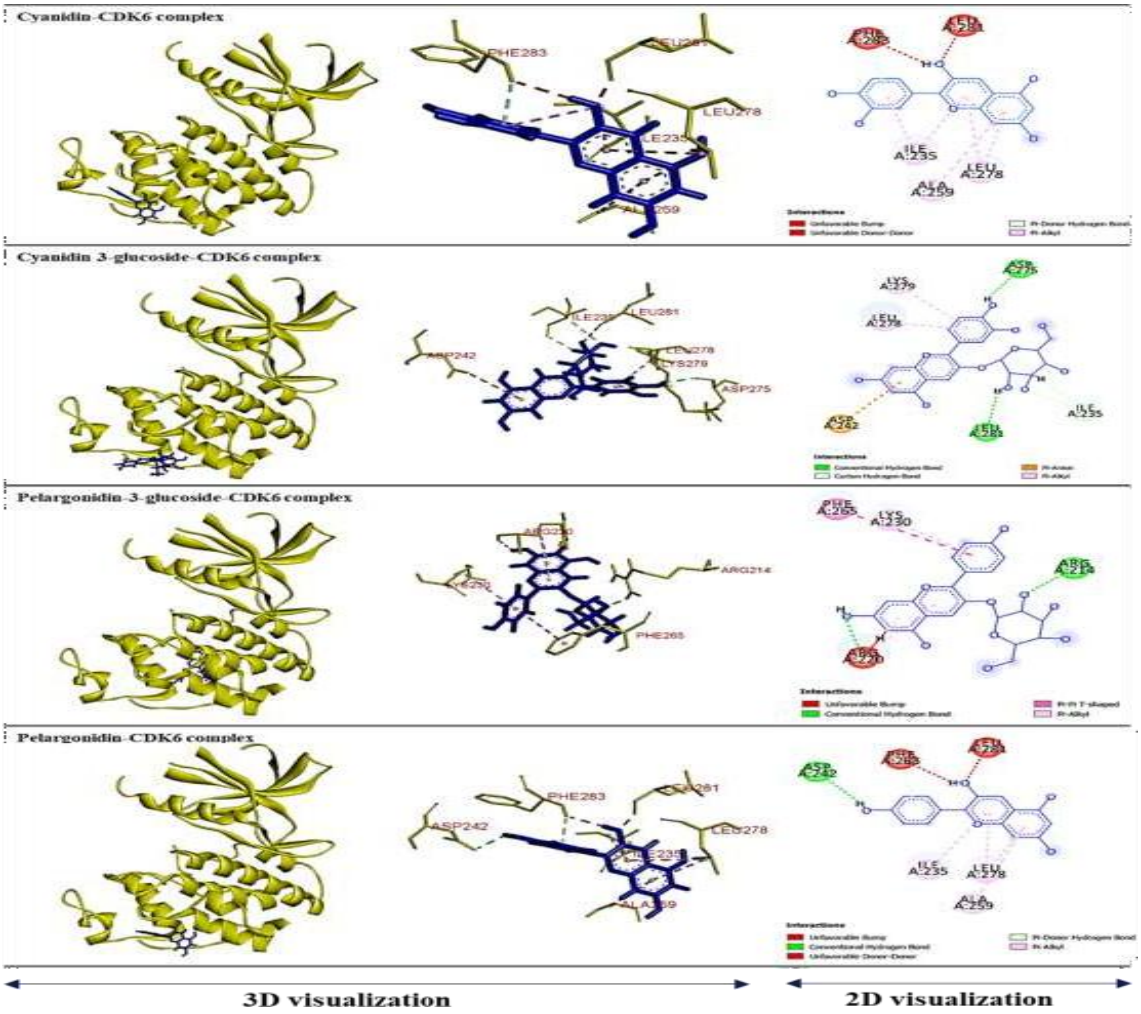

Figure 1. Molecular docking results between cyanidin compounds, cyanidin 3-glucoside, pelargonidin 3-glucos ide, and pelargonidin with Cyclin-Dependent Protein Kinase6 (CDK-6). The ball and stick indicate the ligand, while the yellow ribbon indicatesthe receptor (CDK6).

The results of molecular docking (Figure 2) show four amino acid residues of CDK6 bound by peonidin, including LYS93, VAL62, LEU94, and VAL82. The interaction of the peonidin-CDK6 complex is stabilized by one hydrogen bond and five hydrophobic interactions. Peonidin-3-glucoside is able to bind CDK6 at amino acid residues LEU281, ASP275, ILE235, LYS287, ASP242, LEU278, and LYS279. Hydrogen bonds, hydrophobic, and hydrophobic interactions stabilize this peonidin-3-glucoside-CDK6 complex. Our study used Palbociclib (a synthetic drug) as a control. The docking results showed that Palbociclib interacts with CDK6 through binding to amino acid residues ASP275, GLU271, LEU278, ILE235, and LYS279. Interestingly, the ASP275 residue in the Palbociclib-CDK6 complex wasalso found in the Cyanidin 3-glucoside-CDK6 complex and the Peonidin-3-glucoside-CDK6

complex. In addition, five bioactive compounds from purple corn extract (cyanidin, cyanidin 3-glucoside, pelargonidin-3-glucoside, pelargonidin, and peonidin-3-glucoside) were found tobind to the same amino acid residue as the control (Palbociclib), namely the LEU278 residue.The ILE235 residue in the Palbociclib-CDK6 complex was also found in the interaction of cyanidin, cyanidin-3-glucoside, pelargonidin, peonidin-3-glucoside with CDK6.

Cyanidin-3-glucoside-CDK6 complex and peonidin-3-glucoside-CDK6 complex showed the same binding site as the control Palbociclib-CDK6 complex, namely at the LYS279residue (Table 1). This interaction indicates that five compounds from purple corn extract (cyanidin, cyanidin 3-glucoside, pelargonidin-3-glucoside, pelargonidin, and peonidin-3- glucoside) act as competitive CDK6 inhibitors through hydrogen bond formation and hydrophobic interactions. As a result, the catalytic activity of the enzyme is inhibited. Interestingly, these four CDK6 amino acid residues (ASP275, LEU278, ILE235, and LYS279)are located at the C-terminal, which is the catalytic site of CDK6 (27).

CDK6 is essential in cancer development through (RB)-E2F signalling. Uncontrolled regulation of the cyclin D-CDK4/6-INK4-RB pathway causes uncontrolled cell cycle and cell growth (28). CDK 6 in a complex with cyclin D and CDK2/cyclin E can induce phosphorylation of RB so that proliferation and the initiation of cell division occur. If CDK6 is inhibited, Rb hyperphosphorylation does not ensue, so Rb is inhibited from initiating cell division, which is called the G1 restriction point (29).

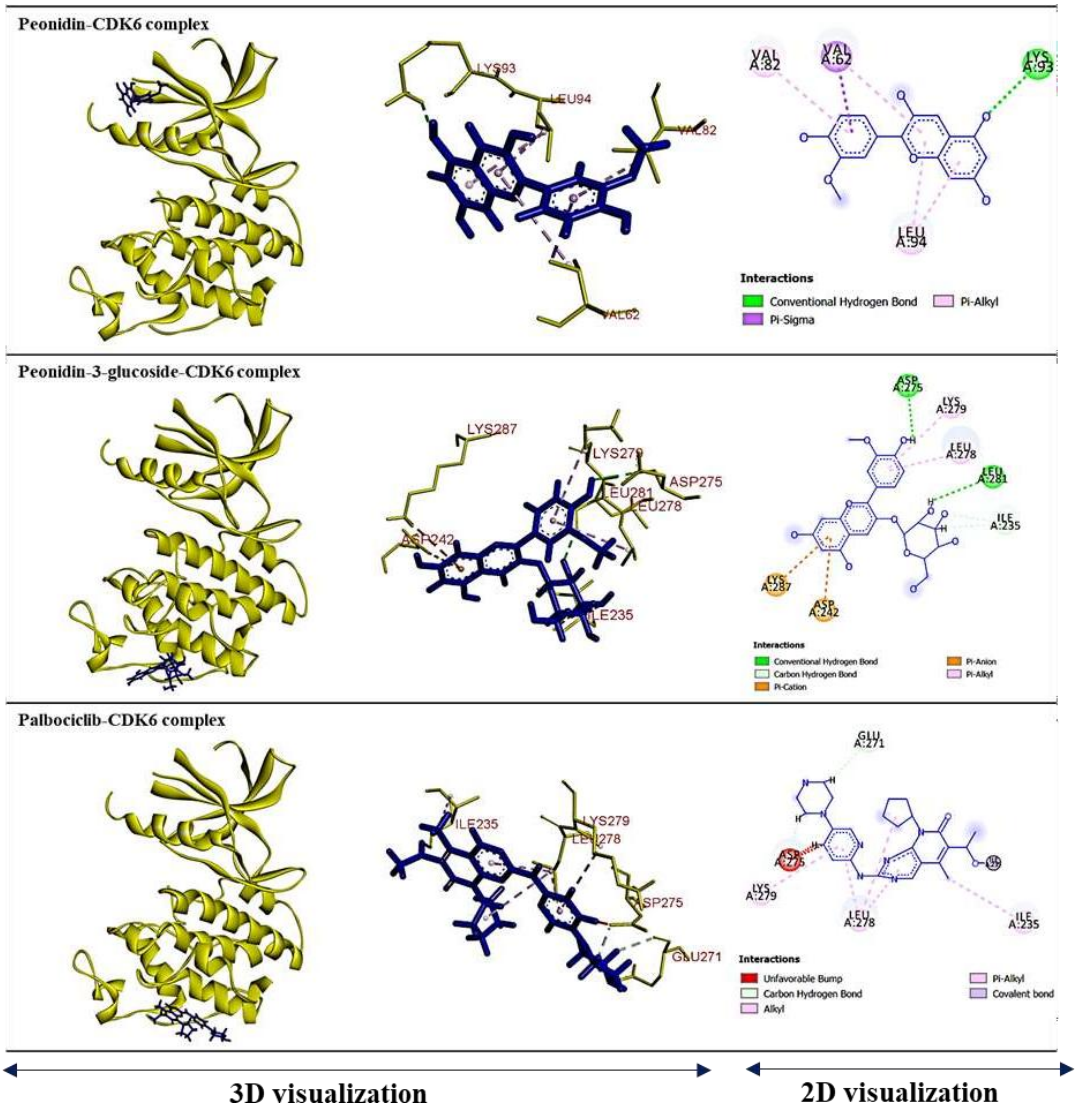

Figure 2. Molecular docking results between peonidin, peonidin-3-glucoside, and palbociclib (as a control) with Cyclin-Dependent Protein Kinase 6 (CDK-6). The ball and stick indicate the ligand, while the yellow ribbon indicates the receptor (CDK6).

Table 1. Interactions of anthocyanidins and major anthocyanins from purple corn withCDK6

Compounds	Point interaction	Chemistry bond	Type	Energy binding (kcal/mol)
Cyanidin-CDK6 complex	A:PHE283:HN - :LIG1	Hydrogen Bond	Pi-Donor Hydrogen Bond	-238.8
	:LIG1 - A:ILE235	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ALA259	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ILE235	Hydrophobic	Pi-Alkyl	
Cyanidin 3-glucoside-CDK6 complex	:LIG1:H - A:LEU281:O	Hydrogen Bond	Conventional Hydrogen Bond	-270
	:LIG1:H - A:ASP275:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ILE235:CA - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:ILE235:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:ASP242:OD2 - :LIG1	Electrostatic	Pi-Anion	
	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LYS279	Hydrophobic	Pi-Alkyl	
Pelargonidin 3-glucoside-CDK6 complex	A:ARG214:HH11 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-280.8
	:LIG1:H - A:ARG220:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:PHE265 - :LIG1	Hydrophobic	Pi-Pi T-shaped	
	:LIG1 - A:ARG220	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ARG220	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LYS230	Hydrophobic	Pi-Alkyl	
Pelargonidin-CDK6 complex	:LIG1:H - A:ASP242:OD2	Hydrogen Bond	Conventional Hydrogen Bond	-248.1
	A:PHE283:HN - :LIG1	Hydrogen Bond	Pi-Donor Hydrogen Bond	
	:LIG1 - A:ILE235	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ALA259	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl	
Peonidin-CDK6 complex	A:LYS93:HZ3 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-241.3
	A:VAL62:CG2 - :LIG1	Hydrophobic	Pi-Sigma	
	:LIG1 - A:VAL62	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LEU94	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LEU94	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:VAL82	Hydrophobic	Pi-Alkyl	
Peonidin-3-glucoside-CDK6 complex	:LIG1:H - A:LEU281:O	Hydrogen Bond	Conventional Hydrogen Bond	-282.5
	:LIG1:H - A:ASP275:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ILE235:CA - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:ILE235:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:LYS287:NZ - :LIG1	Electrostatic	Pi-Cation	
	A:ASP242:OD2 - :LIG1	Electrostatic	Pi-Anion	
	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LYS279	Hydrophobic	Pi-Alkyl	
Palbociclib-CDK6 complex (as a	:LIG1:H - A:ASP275:OD1	Hydrogen Bond	Carbon Hydrogen Bond	-329.4
	:LIG1:H - A:GLU271:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:LEU278 - :LIG1	Hydrophobic	Alkyl	
	:LIG1:C - A:ILE235	Hydrophobic	Alkyl	

control)	:LIG1 - A:LEU278	Hydrophobic	Pi-Alkyl
:LIG1 - A:LEU278		Hydrophobic	Pi-Alkyl
:LIG1 - A:LYS279		Hydrophobic	Pi-Alkyl

The molecular docking method helps predict the binding orientation of a compound inthe receptor binding domain and its binding energy. The lower binding energy indicates that the molecule will bind more easily to other molecules and vice versa (30). The high binding energy of the compound to CDK6 causes a significant decrease in enzyme activity (28). The results of this study showed the lowest binding energy in sequence, namely peonidin-3- glucoside (-282.5 kcal/mol), pelargonidin-3-glucoside (-280.8 kcal/mol), cyanidin 3-glucoside(-270 kcal/mol), pelargonidin (-248.1 kcal/mol), peonidin (-241.3 kcal/mol), and cyanidin (-238.8 kcal/mol). Interestingly, peonidin-3-glucoside showed the lowest binding energy (-282.5 kcal/mol), approaching the control (palbociclib, at -329.4 kcal/mol) (Table 1). This binding shows that peonidin-3-glucoside can act as a competitive CDK6 inhibitor.

CONCLUSION

In conclusion, five bioactive compounds from purple corn extract (cyanidin, cyanidin 3-glucoside, pelargonidin-3-glucoside, pelargonidin, and peonidin-3-glucoside) bind to the active site of CDK6 with the same binding pattern as the drug Palbociclib, namely at residuesASP275, LEU278, ILE235, and LYS279. The strong binding energy of peonidin-3-glucoside shows the most potential inhibitory activity against CDK6. Inhibition of CDK6 by compoundsfrom purple corn extract can reduce the accessibility of enzyme substrates by acting as competitive inhibitors, eventually inhibiting enzymes. This finding led to the discovery of anti-breast cancer drugs based on natural compounds from purple corn extract.

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