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Dioscorea alata Extract as Anti-Cancer Agent by Regulating Genetic Mutations, Apoptosis, and Cell Proliferation: *in vitro* and *in silico* Studies

(Ekstrak *Dioscorea alata* sebagai Agen Anti-Kanser dengan Mengawal Mutasi Genetik, Apoptosis dan Pembiakan Sel: Kajian *in vitro* dan *in silico*)

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ABSTRACT

Dioscorea alata (DA) tubers contain some compounds of potential anti-cancer agents. However, despite its potential, there has been limited investigation on the potential anti-cancer agent of DA, especially in breast cancer cells. Therefore, this study aims to investigate the anti-cancer effect of ethanolic extract of DA tubers against the MCF-7 cell lines *in vitro* and *in silico*. The *in vitro* study was carried out by cytotoxicity effect using MTT assay. The *in silico* study examined active ingredients, determined anti-cancer activity, analyzed the protein target, protein-protein interactions, and molecular docking. The study results showed that the high-concentration ethanolic extract of DA tubers exhibited the highest decrease in MCF-7 cell viability with an IC_{50} value of 50.98 g/mL and can be categorized as a promising anti-cancer agent. As many as 34 active compounds belonging to anthocyanins, saponins, and flavonoids were screened. Active compounds of DA tubers with a violation score < 2 were analyzed for their biological potentials. Ten compounds of DA with the highest potency were selected for docking analysis with the targeted protein EGFR. The *in-silico* study showed that 21 bioactive compounds contribute to the anti-cancer activity of DA. Chlorogenic acid is the most promising bioactive compound as an anti-cancer since it is the most similar to the anti-cancer drug. Meanwhile, dihydroquercetin has a low binding affinity value, requiring less energy to bind to proteins in cancer signaling indicated that this compound is promising as anti-cancer. This study suggested that the ethanolic extract of DA tubers can be considered an anti-cancer agent against the MCF-7 cell line *in vitro* by regulating genetic mutations, apoptosis, and cell proliferation.

Keywords: Anti-cancer; chlorogenic acid; dihydroquercetin; *Dioscorea alata; in silico*

ABSTRAK

Ubian *Dioscorea alata* (DA) mengandungi beberapa sebatian agen anti-kanser yang berpotensi. Walaupun dengan potensinya, terdapat penyelidikan yang terhad mengenai potensi agen anti-kanser DA, terutamanya dalam sel kanser payudara. Oleh itu, kajian ini bertujuan untuk mengkaji kesan anti-kanser ekstrak etanol ubian DA terhadap garisan sel MCF-7 secara *in vitro* dan *in silico*. Kajian *in vitro* telah dijalankan melalui kesan ketoksikan menggunakan asai MTT. Kajian *in silico* mengkaji bahan aktif menentukan aktiviti anti-kanser, menganalisis sasaran protein, interaksi protein dan dok molekul. Keputusan kajian menunjukkan bahawa ekstrak etanol berkepekatan tinggi tuber DA mengeluarkan penurunan tertinggi dalam daya maju sel MCF-7 dengan nilai IC_{50} sebanyak 50.98 g/mL dan boleh dikategorikan sebagai agen anti-kanser yang berpotensi. Sebanyak 34 sebatian aktif kepunyaan antosianin, saponin dan flavonoid telah disaring. Sebatian aktif ubian DA dengan skor pelanggaran < 2 telah dianalisis untuk potensi biologinya. Sepuluh sebatian DA dengan potensi tertinggi telah dipilih untuk analisis dok dengan protein yang disasarkan EGFR. Kajian *in silico* menunjukkan bahawa 21 sebatian bioaktif menyumbang kepada aktiviti anti-kanser DA. Asid klorogenik adalah sebatian bioaktif yang paling berpotensi sebagai anti-kanser kerana ia adalah yang paling sama dengan ubat anti-kanser. Sementara itu, dihidrokuersetin mempunyai nilai pertalian pengikatan yang rendah, memerlukan kurang tenaga untuk mengikat protein dalam isyarat kanser menunjukkan bahawa sebatian ini berpotensi sebagai anti-kanser. Penyelidikan ini mencadangkan bahawa ekstrak etanol ubian DA boleh dianggap sebagai agen anti-kanser terhadap garisan sel MCF-7 secara *in vitro* dengan mengawal mutasi genetik, apoptosis dan percambahan sel.

Kata kunci: Anti-kanser; asid klorogenik; dihidrokuersetin; *Dioscorea alata; in silico*

Introduction

Breast cancer is aggressive cancer in women with the greatest prevalence of all malignancies worldwide (Łukasiewicz et al. 2021; Smolarz, Nowak & Romanowicz 2022). According to Globocan data in 2020, breast cancer is presently one of the most frequently diagnosed malignancies, with an estimated 2.3 million new cases yearly and the fifth cause of cancer-related deaths. Of the 396,914 new cancer cases in Indonesia, 68,858 (or 16.6%) were new breast cancer cases. Meanwhile, there were more than 22,000 incidents of fatalities (Sung et al. 2021). Regarding the number of cancer patient mortalities, breast cancer is the most prevalent in Indonesia (Kemenkes Republik Indonesia 2022).

Delays in handling and treating breast cancer cases are common, resulting in an advanced stage or greater difficulty to cure. The current chemotherapeutic agents have several limitations, such as resistance, side effects, and inadequate efficacy (Pearce et al. 2017), resulting in inefficient therapy. The chemopreventive agents can stop the formation of aberrant cells, decrease the development of cancer cells, and inhibit the initiation of preneoplastic lesions by carcinogens or reverse cancer progression (Ko & Moon 2015). Therefore, it is necessary to explore more effective and efficient chemopreventive agents. The current trend for exploring chemopreventive compounds is using natural products from potential plants (Naeem et al. 2022).

Dioscorea alata (DA) contains flavonoids, steroidal saponins, purines, allantoin, mucin, polysaccharides, dioscorin, and diosgenin. According to Mirulaini and Shahira (2011), diosgenin has antioxidant properties by increasing the damage resistance of lymphocyte DNA from oxidative stresses and inhibiting cancer cell growth. The biological activities of diosgenin, steroidal saponins, and alkaloids were investigated *in vitro* to determine their impacts on apoptosis, cell cycle dispersion, and proliferation ratio. The anti-cancer properties of diosgenin are associated with hetero-sugar bonds and the 5,6-double bonds in its structure. The biological activity of diosgenin is also significantly influenced by the structural alignment of the carbon atoms at positions C-5 and C-25 (Raju & Rao 2012). Olayemi and Ajaiyeoba (2007) proved that *Dioscorea esculenta* extract contains dioscin and diosgenin. Diosgenin activates the tumor suppressor gene TP53, inhibiting breast cancer proliferation. DA also contains flavonoid compounds. Numerous cancer cell growth can be prevented by flavonoids (Mardiyaningsih & Ismiyati 2014). The ability of flavonoids to inhibit cell proliferation is influenced by the ability to modulate estrogen receptor alpha (ER-α) (Virgili et al. 2004).

Another study proved that the proliferation of prostate cancer cells (DU145) and lung cancer cells could be decreased by white yam (DA) acetone extract (A549) (Wallace, Asemota & Gray 2021). More investigations on the potential of purple yam (DA) as an anti-cancer agent, especially in breast cancer cells, were still limited. Therefore, this study aimed to investigate the cytotoxic potential of purple yam ethanolic extract (DA) against MCF-7 breast cancer cells *in vitro* and *in silico.*

Materials and Methods

Ethical approval

The toxicity test of DA ethanolic extract against the MCF-7 cell line has received approval from the Research Ethics Committee of the Faculty of Medicine and Health Sciences UMY with a certificate number: 076/EC-EXEM-KEPK FKIK UMY/IX/2020.

Plant collection and extract preparation

D. alata tubers were obtained from Sumberrahayu, Moyudan, Sleman, Yogyakarta. The plant was identified and authenticated by the Laboratory of Plant Taxonomy, Faculty of Biology, Gadjah Mada University, Indonesia, with an accession number 0368/S.Tb./XI. Ten kilograms of DA tubers were cleaned, diced, and sun-dried. The dried materials were then crushed into a fine powder, and a 70% ethanol solution was used to macerate them. The samples were then evaporated to obtain a thick fraction. The extraction process was held according to Supriatno et al. (2018).

Cell culture

The Michigan Cancer Foundation-7 (MCF-7) cell line of human breast cancer cells was obtained from the Laboratory of Cancer Chemoprevention Research Center (CCRC), Gadjah Mada University, Indonesia. Cells were cultured in Dulbecco's Minimum Essential Medium (DMEM, Sigma-Aldrich®, Merck KGaA, Darmstadt, Germany), which contains 10% fetal bovine serum (FBS) and 100 g/mL penicillin-streptomycin (Gibco™, ThermoFisher Scientific, US). They were then incubated for 48 h at 37 °C with 5% CO_2 in the incubator.

Cytotoxic assay

The cells were planted in 96-well plates at 3 × 103 cells/mL density and were incubated for 48 h. After 48 h of

incubation, cells were exposed to different concentrations of DA ethanolic extracts at 15.625, 31.25, 62.5, 125, 250, and 500 µg/mL for 24 h. Then, the treatment medium was removed and cleaned with PBS. Various amounts of previously dissolved samples in DMSO were added, and the mixture was then incubated once more for 48 h. MTT assays were used to determine the cytotoxic effect of plant extract (Amir & Murcitro 2017). After incubation, the MTT reagent was added to each well, and the wells were then incubated for 4 h. The wells were filled with 10% SDS in 0.1N HCl and then incubated for 24 h. The absorbance was determined at 550 nm using a microplate reader. The number of surviving cells was observed, and the IC_{50} value was calculated by plotting the % live cells with % control (PBS and DMSO only). The concentration that could stop 50% of cell development was determined as the Inhibitory Concentration 50 (IC_{50}) value. Data were presented as mean ± standard deviation with 3 repetitions.

in silico ANALYSIS

DA TUBER ACTIVE COMPOUND

Active compounds extracted from the DA tuber were obtained from previous studies (Makiyah et al. 2022). A total of 21 active compounds that do not violate the two stipulations of the Lipinski Rule of 5 (Ro5) were selected and analyzed to predict their general bioactivity using the Molinspiration web server (https://www.molinspiration.com/) (Molinspiration Cheminformatics 2010; Pollastri 2010).

QSAR ANALYSIS OF ACTIVE COMPOUNDS BY Way2Drug

A Quantitative Structure-Activity Relationship (QSAR) analysis was performed using Way2Drug/PASS online (www.way2drug.com) to predict the anti-cancer activity of each active compound by assessing the similarity of the observed compound to the database (Stasevych, Zvarych & Novikov 2020). *In silico* QSAR analysis concentrates on determining how similar the structure of the input is to the database (Filimonov et al. 2014). The accuracy of the analysis is indicated by the score, which ranges from 0-1, displayed by the webserver. The parameters used in the analysis were related to the anti-cancer prediction.

PROTEIN TARGET ANALYSIS

Similarity Ensemble Approach (SEA) target (https://sea.bkslab.org/) was used to suggest protein targets that may interact with the active component of DA. Like QSAR, SEA Target is a similarity-based method to forecast interactions with protein targets. The minimum cut-off used was 0.57, as suggested by Keiser et al. (2007). In addition, the Search Tool for Interactions of Chemicals database (STITCH DB) V.5 (http://stitch.embl.de/), with a high confidence score of 0.7 as the input belongs to Homo sapiens, was also utilized (Petricca et al. 2022).

PROTEIN-PROTEIN INTERACTION ANALYSIS

The protein target analysis was then followed by determining the interactions of the protein targets using STRING DB V.11 (https://string-db.org//) with a high confidence score of 0.7 as the input belongs to Homo sapiens organisms. After the 14 protein targets were obtained, the betweenness centrality of each protein was analyzed using Cytoscape version 3.8.2. BC is an analysis to see the role of the most dominant protein in the pathway being analyzed. STRING DB is used to observe protein interactions for further analysis in Cytoscape version 3.8.2. Cytoscape is widely used to determine pathway visualization and analysis (Shannon et al. 2003).

MOLECULAR DOCKING

Docking is an analysis to determine the interaction of receptor proteins and ligands based on the binding affinity value. Protein selection is crucial for reliable docking results since docking is always measurable regardless of the docking compounds. Therefore, the receptor proteins were selected based on the QSAR results and protein target predictions. In this study, the molecular docking process was carried out with four conditions: 1) the compounds have been predicted to interact, 2) the structure of the compounds is available in the database, 3) the binding site between the ligand and the protein is known, and 4) the results should be compared with the control.

The ten most-potential compounds were selected from the predicted protein for docking analysis. The protein target was epidermal growth factor receptor (EGFR) chain A (PDB ID: 4HJO), which binds to control erlotinib. The compound was prepared to minimize its energy with PyRx 0.8 software with the Open Babel plug-in. The Biovia Discovery Studio Software produced the protein target by eliminating water molecules and their ligands. Then, the active site and binding site for EGFR (PDB ID: 4HJO) were obtained from the crystallographic results, including VAL702, ALA719, LYS721, VAL745, LEU764, THR766, GLN767, LEU768, MET769, PRO770, PHE771, GLY772, CYS773, LEU820,

THR830, and ASP831. In addition, the active site of EGFR (P00533) refers to UniProt, i.e., LEU837, and the binding site refers to VAL745. Then, the control compound for EGFR (PDB ID: 4HJO) was erlotinib (PubChem ID: 176870), which binds to the A chain of EGFR located intracellularly, wherein phosphorylation occurs.

EGFR was docked with these ten compounds using a specific docking that includes the active and binding sites, referring to the crystallographic results of 4HJO and UniProt. Autodock Vina was used to dock the PyRx 0.8 software with the following grid positions: Center, X: 29.7427, Y:13.6581, Z: -0.5906; Dimensions, X: 22.0890, Y: 30.9692, Z: 21.8476 (Dallakyan & Olson 2015). The broad stages of this research are presented in Figure 1.

Results and Discussion

Viability of MCF-7 cells

MCF-7 cells were exposed to ethanolic extracts of DA tubers at various concentrations (15.625, 31.25, 62.5, 125, 250, and 500 µg/mL) to assess their cytotoxicity effect. The findings showed that ethanolic extract of DA tubers could decrease the percentage of MCF-7 viability in a dose-dependent manner. The high concentration of ethanolic extract of DA tubers (500 µg/mL) exhibited the highest decrease in cell viability (1.57%). Furthermore, doxorubicin decreased MCF-7 viability at 0.375 to 5 µg/mL. However, the cell viability of MCF7 cells was elevated after receiving 6 µg/mL doxorubicin.

The IC_{50} value of doxorubicin (3.98 µg/mL) was also higher than the IC_{50} value of ethanolic extract of DA tubers (50.98 µg/mL) (Table 1). A strong cytotoxicity effect was indicated by an IC_{50} value of less than 100 µg/mL (Ueda et al. 2002). According to these findings, the ethanolic extract of DA tubers has a potent cytotoxic effect on MCF-7 cells due to IC_{50} value of less than 100 µg/mL.

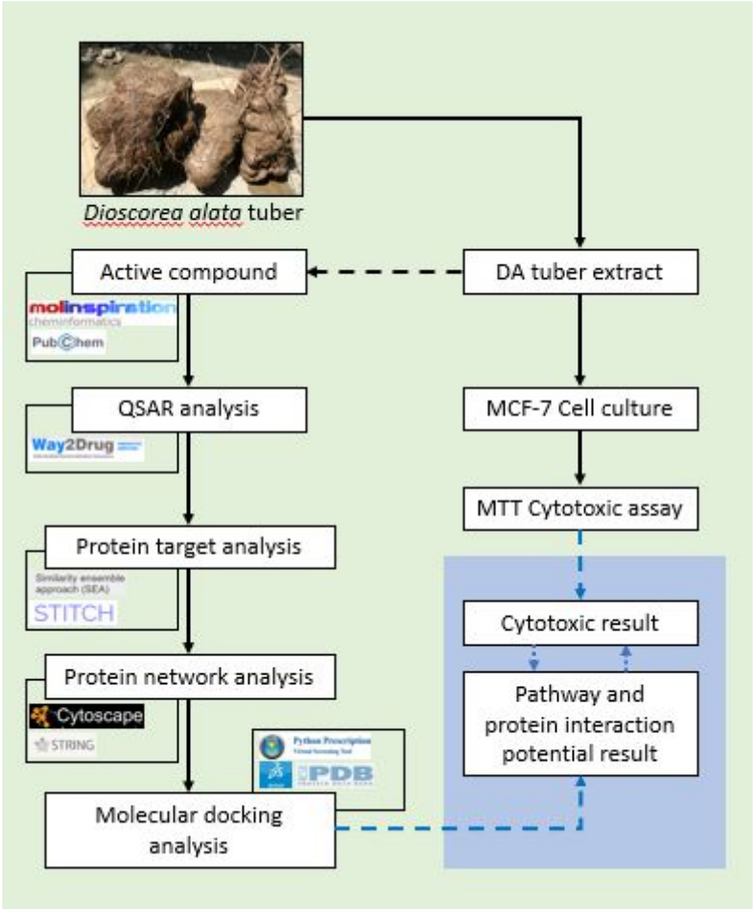


FIGURE 1. The study design for the analysis work. Blue color: the result; DA: *Dioscorea alata*; MCF7: the Michigan Cancer Foundation-7 cell line; MTT: colorimetric assay with MTT dye; QSAR: Quantitative structure–activity relationship

TABLE 1. The cell viability of the MCF-7 line after being treated with ethanolic extract of DA tubers and Doxorubicin

Concentration (µg/mL)	Cell viability (%) (Mean ± SD)	IC_{50} value (µg/mL)
Dioscorea alata		
15.625	68.90 ± 0.68	50.98
31.25	56.35 ± 3.31	
62.5	49.52 ± 2.41	
125	37.96 ± 1.61	
250	25.86 ± 0.83	
500	1.57 ± 0.09	
Doxorubicin		
0.375	94.62 ± 1.40	3.98
0.75	78.58 ± 4.71	
1.5	68.62 ± 2.75	
3	41.87 ± 4.44	
6	49.96 ± 7.00	

Based on Wallace, Asemota and Gray (2021), DA tuber acetone extract has good anti-cancer potential against A549 and DU145 cells with IC_{50} values of 22.28 and 31.45 g/mL, respectively. This finding was in line with the potential anti-cancer activity of DA extract using ethanol found in the study. In addition, DA acetone extract was also reported to have an anti-proliferative activity by inducing G2/M cell arrest in DaJa-3 cells and G1 arrest in A549 cells. Another study showed that hydro-methanol extract of DA tuber possessed anti-inflammatory activity through inhibition of NO and TNF-α expression with IC_{50} values of 134.51 ± 6.75 and 113.30 ± 7.44 µg/mL. This extract also exhibited total inhibition of cyclooxygenase (COX), COX-1, COX-2 activities, and Prostaglandin E2 (PGE2) levels (Dey et al. 2016). However, research on anti-cancer activity of DA tubers is still limited despite its potential. Thus, this study was conducted to provide complete information on one of the potential anti-cancer agents of DA tubers. According to Aumsuwan et al. (2016), the IC_{50} of 5-Azacitidine (5-aza) in MCF-7 cells after 72 h of treatment is 2.25 µM, but dioscin extracted from *Dioscorea villosa* root extract has an IC_{50} value of 13.70 µM after 24 h exposure and 3.85 µM after 72 h exposure to MCF-7 cells. The results suggested that the MCF-7 cell line had a considerable and time-dependent sensitivity to dioscin.

SCREENING OF BIOACTIVE COMPOUND

The screening compound analysis found 41 active compounds of DA tubers. Following Lipinski rule analysis, 21 bioactive compounds were discovered, as indicated in Table 2. In detail, the 21 bioactive compounds found were: 1 steroidal saponin (gracillin), 1 triterpenes saponin (prosapogenin), 1 phytosterol (γ-sitosterol/fucosterol), 1 glyoxylic(acid) diureide (allantoin), 6 flavonoid ((+)-catechin, dihydroquercetin, naringenin, chalcone naringenin, dihydrokaempferol, and myricetin), 5 anthocyanins (peonidin, pelargonidin, leucopelargonidin, leucocyanidin, and cyanidin), 1 essential nutrient (choline), 1 protein (mucin), 1 polyphenol (chlorogenic acid), 1 saponin (sapogenin), 1 diterpenoid lactones (diosbulbin B) and 1 phyto steroid sapogenin (diosgenin).

Compound computational analysis was carried out to find and develop drug-candidate compounds. Computational analysis provides efficiency in the discovery and development of drug compounds by knowing what compounds have solubility and permeability that cells can absorb. Based on the Rule of Five (RoF), compounds with molecular weights of > 500 Da, logP > 5, hydrogen donor bonds > 5, and acceptor hydrogen bonds > 10 are predicted to have poor absorption and permeation by cells (Lipinski et al. 2001).

In addition Ghose, Viswanadhan and Wendoloski (1999) have provided 80% of pharmacological compounds in the Comprehensive Medical Chemistry (CMC) database, fall within a qualifying range for their physicochemical structure, which includes LogP values between 0.4 and 5.6, Molecular Weight (MW) values between 160 and 480, molar refractivity values between 40 and 130, and an atom count of 20 to 70. Furthermore, RoF is intended for drugs that are taken orally, and its relevance to small injected compounds is still being questioned. However, almost 90% of drugs currently available meet the RoF requirements, meaning that, in general, the requirements are still relevant (Benet et al. 2016).

TABLE 2. DA tubers bioactive compounds (Makiyah et al. 2022) with bioactive prediction by Molinspiration

No	Metabolite	Category	Bioactivity prediction					
			GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	gracillin	streoidal saponin	-0.239	-0.205	-1.086	0.184	-0.367	0.488
2	prosapogenin	triterpen saponin	0.163	-0.389	-0.588	0.233	0.157	0.481
3	γ-sitosterol	phytosterol	0.136	0.045	-0.51	0.734	0.071	0.509
4	Allantoin	senyawa	-1.284	-0.704	-2.011	-1.947	-1.108	-0.959
5	(+)-catechin	flavonoid	0.409	0.137	0.087	0.599	0.26	0.467
6	peonidin	anthocyanin	-0.157	-0.176	0.013	0.033	-0.337	-0.044
7	pelargonidin	anthocyanin	-0.181	-0.114	-0.071	0.034	-0.33	-0.022
8	leucopelargonidin	anthocyanin	0.225	0.135	0.074	0.395	0.07	0.369
9	leucocyanidin	anthocyanin	0.249	0.126	0.097	0.423	0.083	0.364
10	cyanidin	anthocyanin	-0.128	-0.094	0.017	0.091	-0.297	0.012
11	dihydroquercetin	flavonoid	0.086	0.025	-0.039	0.293	0.046	0.292
12	naringenin	flavonoid	0.032	-0.203	-0.264	0.419	-0.12	0.209
13	chalcone naringenin	flavonoid	-0.107	0.017	-0.206	0.106	-0.214	0.134
14	dihydro-kaempferol	flavonoid	0.054	0.029	-0.068	0.259	0.032	0.294
15	choline	essential nutrient	-2.643	-2.207	-3.627	-3.886	-3.659	-2.179
16	mucin	protein	-0.403	-0.088	-0.766	-0.267	-0.295	0.125
17	chlorogenic acid	polyphenol	0.286	0.143	-0.004	0.738	0.269	0.617
18	sapogenin	saponin	0.051	0.058	-0.479	0.369	-0.025	0.461
19	myricetin	flavonoid	-0.06	-0.182	0.284	0.315	-0.205	0.299
20	diosbulbin B	senyawa	0.518	-0.234	-0.396	0.344	0.01	0.591
21	diosgenin	phytosteroid sapogenin	0.052	-0.14	-0.568	0.582	-0.058	0.612

The most bioactive compounds found in DA tubers were flavonoids and anthocyanins. Numerous epidemiological, short-term randomized controlled trials, and pre-clinical studies have shown evidence for the potential health benefits of flavonoids, particularly in lowering the incidence of cancer. Quercetin, rutin, apigenin, naringenin, kaempferol, myricetin, epigallocatechin-3-gallate, and catechin are flavonoids with exceptional anti-cancer effects (Carocho & Ferreira 2013; Hazafa et al. 2020; Sharma et al. 2022). Numerous studies show that flavonoids' antioxidant, anti-inflammatory, and anti-cancer characteristics significantly impact human health (Ullah et al. 2020).

Evidence of possible anti-cancer effects of anthocyanins on breast carcinogenesis (BC) *in vitro* and *in vivo* has made it conceivable to employ anthocyanins as a natural chemopreventive strategy in BC. Anthocyanins alter a variety of receptor families to have anti-cancer and angiogenic effects. Anthocyanins also boost the activities of detoxifying enzymes while inhibiting proinflammatory, signal transducer and activator of transcription 3 (STAT3), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B) pathways. Moreover, anthocyanins also inhibit tumor invasion and metastatic processes in laboratory conditions by reducing metalloproteinases (MMPs) (Li et al. 2022).

BIOLOGICAL ACTIVITY PREDICTION OF DA ACTIVE COMPOUNDS

The bioactive compounds from DA were compared with compounds with anti-cancer potential recorded in the database. A high structural similarity will be reflected by a high prediction score with a cut-off above 0.5 (Filimonov et al. 2014). According to the outcomes of the bioactivity prediction study from Molinspiration, the nuclear receptor ligand bioactivity of choline received the lowest score (-3.89), while that of chlorogenic acid (0.74) received the highest. The active compound's bioactivity as an enzyme inhibitor and nuclear receptor ligand is indicated by the highest probability score, with more than nine metabolites having a predictive score of > 0.3. Thus, based on these results, the 21 compounds have the probability of being enzyme inhibitors, nuclear receptor ligands, or GPCR ligands (Table 2). Following that, we performed a more specific bioactivity prediction analysis on all active compounds using Way2Drug PassOnline. The Pa value demonstrated the active potentiality of the compound, with a score close to 1 indicating a high potential. Figure 2 shows that chlorogenic acid had the highest drug-likeness score, followed by dihydroquercetin, dihydrokaempferol, and myricetin.

Cancer is a multifactorial disease caused by various factors. These cancer-causing factors can also be called Hallmarks of cancer. There are 10 new Hallmarks of cancer, such as continuous proliferation, avoiding growth suppression, avoiding destruction by immune cells, continuing to replicate due to active telomerase, inducing inflammation, invasion, and metastasis, angiogenesis, genome mutations, avoiding cell death, and energetic cellular deregulation (Hanahan & Weinberg 2000). Based on these factors, 15 active compounds of DA tubers showed their potential as anti-cancer agents by avoiding growth suppression, reducing the potential for mutations due to genomic instability, and avoiding the induction of cell death (Figure 2).

By boosting Tumor Protein 53 (TP53) expression, lowering proliferative, and increasing anticarcinogenic and antineoplastic activity, the active compound of DA tubers has great potential to inhibit the proliferation of cancer cells. TP53 protein and retinoblastoma-associated protein (RB) play an important role in regulating cell growth, but most cancers show inactivation of these proteins (Hanahan & Weinberg 2000). The prediction score for preventing genomic mutations activity is 0.50, which means that it is predicted to have the potential and structural similarity to compounds that have been shown to maintain genome stability. The antioxidant activity and free radical scavenging play an important role in overcoming excessed ROS that can damage DNA structure and cause mutations. In addition, damage to the components of DNA repair was observed in cancer cells. Damage to the repairment system of the genome will affect the detection of DNA damage and activation of repair, direct DNA repair, inactivation, and prevent mutagen molecules before damaging DNA (Negrini, Gorgoulis & Halazonetis 2010).

The predicted score for the induction of apoptosis of the active compound DA tubers was 0.49. The score was calculated based on the average parameter, Caspase 3 and Caspase 8 stimulation, TP53 expression enhancer, apoptosis agonist, anti-cancer activity, and antineoplastic. Based on these calculations, the active compound can prevent apoptotic resistance in cancer cells because it is similar to compounds with the same role in the database. Since cancer cells develop due to uncontrolled cell proliferation and inhibition of apoptosis, apoptosis is crucial in cancer pathophysiology (Chen et al. 2012).

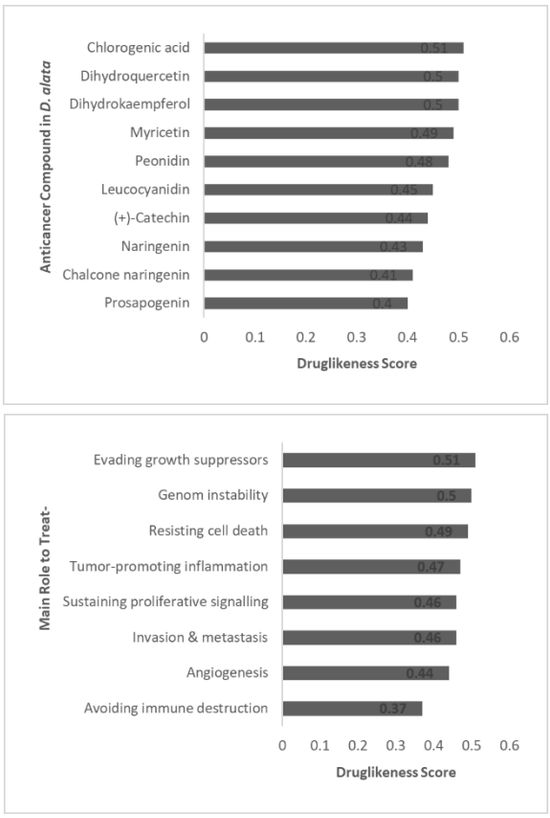


FIGURE 2. Prediction of anticancer of the active compound of the DA tubers. Note: the x-axis is the Pa score (Probability to be active). The effectiveness of an anticancer drug will be impacted by the action potential, which increases with Pa score

PROTEIN INTERACTION ANALYSIS

A protein target prediction study was performed using SEA Target and protein-protein interactions (PPIs) analysis using STITCH to learn more about the potential bioactivity of the 21 compounds that were chosen and listed in Table 1. Based on SEA Target and STITCH, the bioactive compounds of DA tubers could interact with proteins involved in cancer mechanisms. The calculation of betweenness centrality found that EGFR, Caspase-8 (Cas8), epidermal growth factor (EGF), mitogen-activated protein 2 kinase 1 (MAP2K1), fibroblast growth factor 2 (FGF2), and interleukin-2 (IL-2) have a high betweenness centrality: 0.54, 0.32 0.31, 0.18, 0.07, and 0.07, respectively, as seen in Table 3. This study used STITCH and SEAtarget analysis to determine the protein targets. The results of the STRING analysis were not considered for molecular docking analysis because they are indirect targets used to study pathway development. Therefore, this study used EGFR as a protein target based on STITCH analysis.

KEGG databases were then used to analyze the protein-protein interaction (PPI) pathway. The results showed protein-protein interactions on 13 proteins, of which 12 proteins could act as oncoproteins, and 4 of these proteins regulate apoptosis, as seen in Figure 3. EGFR has a high score of betweenness centrality. Based on the protein interactions that result (Figure 4), seven of ten proteins interact with the phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and Ras signaling pathways, while the EGFR pathway is constructed from the interactions of six of ten proteins only. On the other hand, the estrogen and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways are generated from the interactions of 5 and 3 proteins only, showing that the interactions between the 10 proteins tend to express the PI3K, MAPK, Ras, and EGFR signaling pathways.

Based on its protein, EGFR is known to play a role in all signaling pathways, whereas its ligand (EGF) cannot induce Estrogen signaling pathways. The

interaction with MAP1K2 and FGF is known to form the PI3K, MAPK, Ras, EGFR, and estrogen signaling pathways. In contrast, the interaction with IGFR1 forms the same signaling pathway except for the estrogen signaling pathway. Along with the MAPK signaling route in FLT3 and the JAK/Stat signaling pathway in IL-2, FLT3 and IL-2 contribute to regulating the PI3K and Ras signaling pathways. Lastly, proteins that form the least number of signaling pathways are BRAF, which forms signaling in the MAPK and EGFR pathways, and ESR1/2, which only forms signaling in the Estrogen signaling pathway.

TABLE 3. A protein's betweenness centrality score in the cancer pathway

No	Protein name	Betweenness centrality score	Note
1	EGFR	0.54	Direct target analyzed by STITCH
2	CASP8	0.32	Indirect target analyzed by STRING
3	EGF	0.31	Indirect target analyzed by STRING
4	MAP2K1	0.18	Indirect target analyzed by STRING
5	FGF2	0.07	Direct target analyzed by SEA
6	IL2	0.07	Direct target analyzed by SEA
7	ESR1	0.06	Direct target analyzed by SEA
8	IGF1R	0.01	Indirect target analyzed by STRING
9	ESR2	0.00	Direct target analyzed by SEA
10	FLT3	0.00	Direct target analyzed by SEA
11	CASP6	0.00	Direct target analyzed by SEA
12	CASP7	0.00	Direct target analyzed by SEA
13	BRAF	0.00	Direct target analyzed by SEA

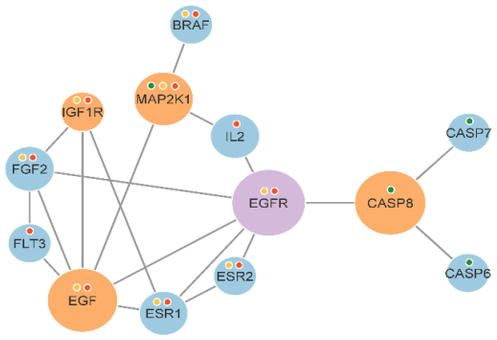


FIGURE 3. Protein-protein interaction on 13 proteins, of which 12 proteins can act as oncoproteins and 4 of them are apoptotic regulators. Dot annotations, red: pathways in cancer, green: Apoptotic proteins, and yellow: breast cancer oncoproteins. The size of the circle represents the betweenness centrality score. Protein color meaning, orange: indirect target by STRING, blue: direct target by SEA target, light purple: direct target by STITCH

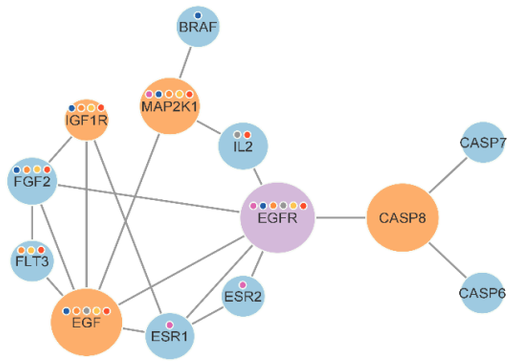


FIGURE 4. PPIs network is related to metabolic and cancer signaling pathways. Dot annotations, red: PI3K signaling pathway, yellow: MAPK signaling pathway, grey: JAK/STAT signaling pathway, orange: Ras signaling pathway, blue: EGFR signaling pathway, and violet: Estrogen signaling pathway. The size of the circle represents the betweenness centrality score. Protein color meaning, orange: indirect target by STRING, blue: direct target by SEA target, light purple: direct target by STITCH

The results of the PPI interaction indicate that EGFR has an important role in the protein network in this study. EGFR is known to have a role in all predicted signaling pathways; this agrees with the findings of previous investigations. EGFR upregulation was generally found in patients with Glioblastoma, head and neck cancer, pancreatic cancer, breast cancer, metastatic colorectal cancer, and non-small-cell lung cancer. Molecularly, mutations and truncation of EGFR are known to cause EGFR to be upregulated and activate two signaling pathways, RAS-RAF-MEK-ERK MAPK and AKT-PI3K-mTOR (Wee & Wang 2017). In order to investigate the potential of the DA active substance as an EGFR inhibitor, molecular docking on EGFR was performed.

MOLECULAR DOCKING RESULTS

Ten compounds with the highest potency (Figure 2) were selected with the targeted protein (EGFR) for docking analysis. The selected compounds include chlorogenic acid from the polyphenolic compound; dihydroquercetin, dihydrokaempferol, myricetin, (+)-catechin, naringenin, and chalcone naringenin from flavonoid compound; peonidin and leucocyanidin from anthocyanin; and prosapogenin from the triterpenoid compound. Erlonitib was used as a control.

Binding affinity (BA) is a value to refer to the amount of energy needed in an interaction. The lower the BA value, the more likely the interaction will occur. Based on docking results, dihydroquercetin has a lower binding affinity value (-9 kcal/mol) than erlotinib and other compounds (Table 4). Root Mean Square Deviation (RMSD) was utilized to assess the degree of correspondence between the docking ligands and the crystallographic findings. The more closely the docked ligand matches the crystallographic ligand, the lower the RMSD value. Some bonds formed between compounds and amino acids are hydrogen bonds (HB), electrostatic bonds, and hydrophobic bonds. Based on these results, myricetin and catechins were compounds with similar hydrogen bonds and hydrophobicity to erlotinib as control.

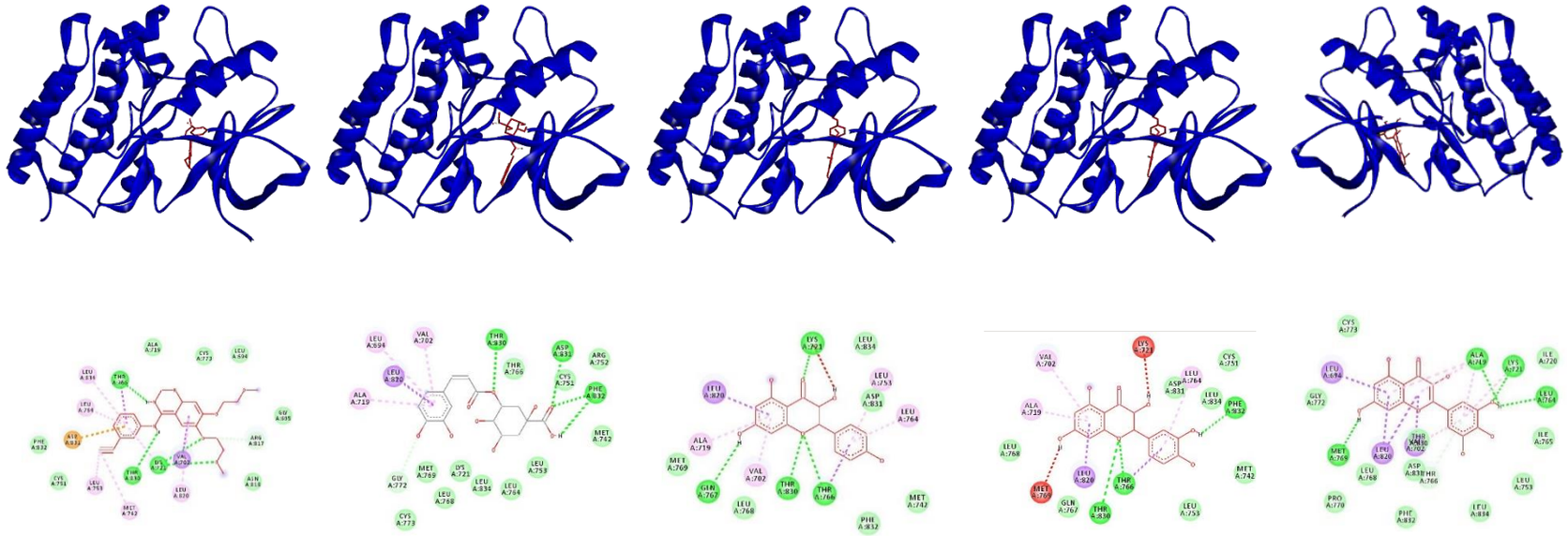
TABLE 4. Docking result between EGFR and 11 active compounds of DA tubers

No	Compound	PubChem ID	BA (kcal/mol)	HB	Electrostatic	Hydrophobic	Van Der Waals
0	Control (Erlotinib)	176870	-8.3	5	1	9	7
1	Dihydroquercetin	439533	-9	3	0	5	7
2	Myricetin	5281672	-9	5	0	9	11
3	Peonidin	441773	-8.9	4	0	11	8
4	Dihydrokaempferol	122850	-8.7	4	0	6	6
5	(+)-Catechin	9064	-8.7	5	0	8	7
6	Naringenin	439246	-8.6	4	0	6	7
7	Chlorogenic acid	1794427	-8.3	6	0	4	11
8	Leucocyanidin	71629	-7.8	5	0	5	8
9	Chalcone naringenin	5658754	-7.6	1	0	4	12
10	Prosapogenin	21626435	-5.9	3	0	9	9

TABLE 5. Amino acid interactions

Compounds	Hydrogen	Van Der Waals	Hydrophobic Bond	BA (kcal/mol)
Control (Erlotinib)	LYS721 LYS721 THR766 ARG817 THR830	LEU694 GLY695 ALA719 CYS751 CYS773 ASN818 PHE832	VAL702 VAL702 THR766 MET742 LEU753 LYS721 LEU764 LEU834 LEU820	-8.3
Chlorogenic acid	GLY772 THR830 ASP831 PHE832 PHE832	**CYS773** MET769 LEU768 LYS721 LEU834 LEU764 LEU753 MET742 ARG752 **CYS751** THR766	**LEU820** LEU694 **VAL702** ALA719	-8.3
Dihydroquercetin	**THR766** THR380 PHE832	MET742 **CYS751** LEU753 GLN767 LEU768 ASP831 LEU834	**THR766** **LEU820** **VAL702** ALA719 LEU764	-9
Dihydrokaempferol	**LYS721** **THR766** GLN767 **THR830**	MET742 LEU768 MET769 ASP831 **PHE832** LEU834	THR766 **LEU820** **VAL702** ALA719 LEU753 **LEU764**	-8.7
Myricetin	ALA719 **LYS721** LEU764 **THR766** MET769	ILE720 LEU753 ILE765 LEU768 PRO770 GLY772 **CYS773** THR830 ASP831 **PHE832** LEU834	LEU694 **VAL702** **LEU820** **LEU820** ALA719 ALA719 ALA719 **VAL702** **LYS721**	-9

*Amino acids in bold are the same as control (Erlotinib)



(A) Erlotinib – EGFR (chain A) | Chlorogenic acid – EGFR (chain A) | Dihydrokaempferol – EGFR (chain A) | Dihydroquercetin – EGFR (chain A) | E) Myricetin – EGFR (chain A)

FIGURE 5. Docking Results (chain A) and amino acid residues, visualized with Biovia Discovery Studio v20.1.0, were interacting with (A) Erlotinib in 2D and 3D; and 2D interactions of amino acids with ligands (B) Chlorogenic acid (C) Dihydroquercetin (D) Dihydrokaempferol and (E) Myricetin. The bond is dominated by hydrophobic and van Der Wall (green). Blue: protein, red: ligand

The molecular docking results between EGFR and ten compounds of *DA* were demonstrated in Figure 5. Based on the number of bonds formed, myricetin had the most bonds, followed by erlotinib. Threonine 766 (THR766) was found as an active site that can interact with all three compounds based on the hydrogen bond. According to van der Wall binding, dihydroquercetin binds to Cysteine 751 (CYS751) similarly to ER, and M binds to Cysteine 773 (CYS773) and Phenylalanine 832 (PHE832) to ER. While based on the hydrophobic bonds, the amino acids Valin702 (VAL702) and Leucine (LEU820) were active sites that can be donated with all ligands (Table 5 & Figure 5). Thus, the ethanolic extract of DA is potentially an anti-cancer and requires further *in vitro* and *in vivo* investigation.

Furthermore, research has been carried out on myricetin as an anti-cancer compound (Ha et al. 2017; Sun et al. 2012). Myricetin has been shown to inhibit Cyclin B1 and cyclin-dependent kinase cdc2 to cause cell arrest in G2/M against the cell line T24 both *in vitro* and *in vivo*. Additionally, myricetin can influence the Bcl-2 family of proteins and cause apoptosis in T24 bladder cancer cells by activating caspase-3 (Sun et al. 2012). Other investigations have also demonstrated myricetin's anti-cancer efficacy against human papillary thyroid cancer (HPTC) cells. Myricetin showed cytotoxic activity, induces DNA condensation and upregulates and activates the caspase cascade and Bax-Bcl-2 expression ratio in the cell line SNU-790 HPTC. Another known activity is that myricetin can induce the expression of apoptosis-inducing factor (AIF) and eliminate the potential of the mitochondrial membrane, resulting in cell death of SNU-790 HPTC (Ha et al. 2017). Finally, the ethanolic extract of DA can be considered an anti-cancer agent *in vitro* against the MCF-7 cell line. Specifically, the high-concentration ethanolic extract of DA exhibited low viability (IC_{50}: 50.98 g/mL) in MCF-7 cells, and an *in-silico* study showed 21 bioactive compounds playing a role in anti-cancer activity. Furthermore, chlorogenic acid is a bioactive compound with the greatest potential as an anti-cancer drug because it has the most similar value to the drug. The low binding affinity value of dihydroquercetin indicated that this compound is promising because it requires less energy to bind to proteins in cancer signaling.

Conclusions

The ethanolic extract of *Dioscorea alata* (DA) can be considered an anti-cancer agent against MCF-7 cells with an IC_{50} value of 50.98 g/mL. The ethanolic extract of DA tuber decreased the MCF-7 cell viability in a dose-dependent manner. The *in-silico* study showed 21 bioactive compounds playing a role in the anti-cancer activity of DA tuber. Two of the best active compounds obtained as anti-cancer agents are chlorogenic acid and dihydroquercetin, where chlorogenic acid has similar bioactivity to anti-cancer drugs and dihydroquercetin has the potential to interact highly with oncoproteins as inhibitors.

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