Bajakah Plant Compounds as Potential Therapy for HER2-Positive Breast Cancer

ABSTRACT

Breast cancer is the most prevalent cancer ~~cases~~ case in Indonesia and it ~~caused~~ causes 20.4% of female deaths. HER2-positive breast cancer is less common but more aggressive, leading patients to seek alternative treatments due to side effects from standard therapies. The Bajakah plant (*Spatholobus suberectus*) is traditionally used by the Dayak people for treating various healthy treatment. Therefore, this research aimed to evaluate compounds from Bajakah plant against HER2 protein. Ten compounds were analyzed for biological activity using PASS Online, toxicity level was identified with Protox-3, and drug-likeness was analyzed by using SWISS ADME. Moreover, molecular docking simulation with Pyrx software and SwissDock using attracting cavities 2.0 (AC 2.0) assessed interaction of compounds toward HER2 (PDB ID: 3PP0) with Lapatinib as control. Cavityplus and Discovery Studio software identified potential binding sites and amino acid residues in the compound-protein complex, while PerMM webserver analyzed membrane permeability. Results indicated that most compounds are safe, exhibited anti-inflammatory, antineoplastic, antimetastatic, and antioxidant activities, also fulfilled the Lipinski's rule of five. Furthermore, ~~butin and~~ butin and cajanin compound demonstrated binding affinity score with -9.9 and -9.8 kCal/mol, respectively~~cajanin compound demonstrated binding affinity score with -9.9 and -9.8 kCal/mol~~, ~~respectively~~ which also closely approximated to control and futher proven its binding confomation and potential by AC 2.0. ~~.~~ Those compounds also shared similar amino acid residue as control. In conclusion, control had stronger interaction toward HER2 protein as compared to butin and cajanin compound. Hence, further in vitro analysis with MTT-

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assay using breast cancer cell line MCF-7 is needed for confirmation and in vivo studies to validate and assess their therapeutic efficacy.

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KEYWORDS: attracting cavities 2.0, bajakah plant, breast cancer~~, butin~~, cajanin, HER2

INTRODUCTION

Breast cancer is a significant global health issue with incidance rate about 23% of all cancer case in worldwide[1]. In Indonesia, breast cancer became the most prevalent cancer in 2022, representing 19.2% of all cancers, with most patients diagnosed in late stages about 60-70%[2]. It predicted that the occurrence and mortality would be increased by 2040[3]. Genetic factors, such as the HER2 gene, complicate breast cancer by promoting the aggressive tumor behavior and poor prognosis[4]. Traditional treatments often involve combined therapies, including drug-antibody conjugates (ADCs) like sacituzumab govitecan (SG) and trastuzumab deruxtecan (T-DXd), which is targeting the protein like Trop-2 and HER2[5,6]. Despite their effectiveness, these treatments sometimes cause significant side effects, prompting interest in alternative therapies.

The bajakah plant (*Spatholobus suberectus*) is traditionally used by the indigenous Dayak people in Kalimantan, Indonesia. It considered rich in bioactive compounds, such as flavonoids, tannins, and alkaloids. Therefore, it is commonly used to treat various health conditions, including diabetes and hypertension[7]. Based on those potential therapeutic properties, this research aimed to investigate the potency of compounds derived from the bajakah plant for inhibiting the HER2-activity in breast cancer by using bioinformatics method.

RESULT

The biological activity prediction of compounds used the metric known as probability of activity (Pa) and probability of inactivity (Pi) score, which indicates its likelihood to exhibit biological activity. Higher score of Pa means that compounds had more probability to be active for certain biological activity[8,9]. The score with 0.5<Pa<0.7 indicated that the compounds are not too similar to known pharmaceutical agents[10]. In this research, aromadendrin is suggested for possessing high activity as anti-inflammatory which indicated by Pa score 0.691. For antineoplastic activity, the (+/-)-eriodictyol compound had the highest score with Pa score 0.644. Moreover, the highest antimetastatic activity is exhibited by prunasin with Pa score 0.578 and the highest antioxidant activity is demonstrated by aromadendrin with Pa score 0.946 (Table 1).

Table 1. Biological activity prediction result of the 10 Bajakah plant compound

Compounds	Pa score	Pi score	Biological Activity
Butin	0,634	0,025	Anti-inflammatory
	0,607	0,010	Antineoplastic (Breast Cancer)
	0,232	0,124	Antimetastatic
	0,721	0,004	Antioxidant
Cajanin	0,608	0,030	Anti-inflammatory
	0,594	0,011	Antineoplastic (Breast Cancer)
	-	-	Antimetastatic
	0,681	0,004	Antioxidant
(+/-)-Eriodictyol	0,691	0,017	Anti-inflammatory
	0,644	0,008	Antineoplastic (Breast Cancer)
	0,196	0,165	Antimetastatic
	0,817	0,003	Antioxidant
Liquiritigenin	0,616	0,028	Anti-inflammatory
	0,601	0,010	Antineoplastic (Breast Cancer)

	0,250	0,109	Antimetastatic
	0,678	0,004	Antioxidant
Formononetin	0,517	0,052	Anti-inflammatory
	0,526	0,016	Antineoplastic (Breast Cancer)
	-	-	Antimetastatic
	0,557	0,005	Antioxidant
Taxifolin	0,628	0,026	Anti-inflammatory
	0,475	0,022	Antineoplastic (Breast Cancer)
	0,305	0,079	Antimetastatic
	0,938	0,002	Antioxidant
(-)-Epicatechin	0,548	0,044	Anti-inflammatory
	0,486	0,020	Antineoplastic (Breast Cancer)
	0,290	0,086	Antimetastatic
	0,810	0,003	Antioxidant
Afromosin	0,563	0,040	Anti-inflammatory
	0,546	0,015	Antineoplastic (Breast Cancer)
	-	-	Antimetastatic
	0,591	0,005	Antioxidant
Aromadendrin	0,722	0,013	Anti-inflammatory
	0,452	0,024	Antineoplastic (Breast Cancer)
	0,328	0,070	Antimetastatic
	0,946	0,002	Antioxidant
Prunasin	0,410	0,090	Anti-inflammatory
	0,157	0,130	Antineoplastic (Breast Cancer)
	0,578	0,007	Antimetastatic
	0,426	0,010	Antioxidant

Furthermore, the toxicity prediction showed that most of the compounds are classified as toxicity class 4, except for (-)-Epicatechin which is belong to toxicity class 6. Moreover, all of compounds are considered inactive for hepatoxicity (Table 2). The toxicity classes followed the labelling classification of chemicals (GHS) with toxicity class 1 is the most toxic with low LD50; toxicity class 2 had LD50 value between 5 to 50 mg/kg which is categorized as fatal; toxicity class 3 had the LD50 value between 50 to 300 mg/kg that identified as toxic; toxicity class 4 had the value of LD50 between 300 to 2000 mg/kg categorized as harmful if swallowed, while toxicity

class 5 had the value of LD50 between 2000 to 5000 mg/kg that may harmful if swallowed, and lastly, toxicity class 6 with LD50 value greater than 5000 mg/kg is considered as non-toxic[11].

Table 2. Toxicity prediction of the ten bajakah compounds

No	Compounds	Toxicity Class	LD50 (mg/kg)	Accuracy (%)	Hepatotoxicity
1	Butin	4	2000	69.26	0.65 (Inactive)
2	Cajanin	5	2500	69.26	0.73 (Inactive)
3	(+/-)-Eriodictyol	4	2000	69.26	0.67 (Inactive)
4	Liquiritigenin	4	2000	69.26	0.64 (Inactive)
5	Formononetin	5	2500	70.97	0.73 (Inactive)
6	Taxifolin	4	2000	100	0.69 (Inactive)
7	(-)-Epicatechin	6	10000	100	0.72 (Inactive)
8	Afromosin	5	2500	69.26	0.71 (Inactive)
9	Aromadendrin	4	2000	70.97	0.68 (Inactive)
10	Prunasin	4	560	100	0.88 (Inactive)

Druglikeness analysis of the ten bajakah plant compounds showed that none of compound has a violation toward Lipinski's rule of five (Table 3). It demonstrated that all of the compounds are considered safe and potentially used as drug candidate as for further analysis. In brief, parameters of Lipinski's rule of five (RO5) stated that potential drug should fulfil the molecular weight which not exceed 500 Da, H-bond donor's ≤5, H-bond acceptors ≤10 and log p ≤ 5[12].

Table 3. Druglikeness prediction based on Lipinski's rule of five

Lipinski's Rule Of Five					
Compounds	**H-Bond Donor (≤5)**	**H-Bond Acceptor (≤10)**	**Molecular Weight (≤500 g/mol)**	**MLogP (≤4.15)**	**Violation**
Butin	3	5	272.25	0.71	0
Cajanin	3	6	300.26	0.22	0
Eriodictyol	4	6	288.25	0.16	0
Liquiritigenin	2	4	256.25	1.27	0
Formononetin	1	4	268.26	1.33	0
Taxifolin	5	7	304.25	-0.64	0
(-)-Epicatechin	5	6	290.27	0.24	0

Afromosin	1	5	298.29	1.01	0
Aromadendrin	4	6	288.25	-0.10	0
Prunasin	4	7	295.29	-1.58	0

According to the molecular docking result, all of bajakah compound bind to the same location of active site in HER2 protein (Figure 1). Among all ten Bajakah compound, two ~~there are several~~ compound ~~that~~ demonstrated approximately binding affinity score to the native ligand and control, ~~such as~~ they are butin and~~,~~ cajanin~~, and (+/-) Eriodictyol~~. Consequently, those two compounds are subjected to be main compound for further analysis. ~~, Consequently, those three compounds are subjected to be main compound for further analysis.~~ Native ligand and control (lapatinib) are condidered for having stronger interaction toward HER2 protein since it had lower binding affinity score as compared to those compound (Table 4). Low binding energy score are considered for indicating a strong interaction of ligand toward protein[13].

Table 4. Binding affinity score of bajakah plant compounds toward HER2 protein

Compounds	Binding Affinity (kCal/mol)
03Q (Native Ligand)	-11,0
Lapatinib (Control)	-10.8
Butin	-9.9
Cajanin	-9.8
(+/-)-Eriodictyol	-9.7
Liquiritigenin	-9.7
Formononetin	-9.4
Taxifolin	-9.3
(-)-Epicatechin	-9.2
Afromosin	-8.9
Aromadendrin	-8.9
Prunasin	-8.1

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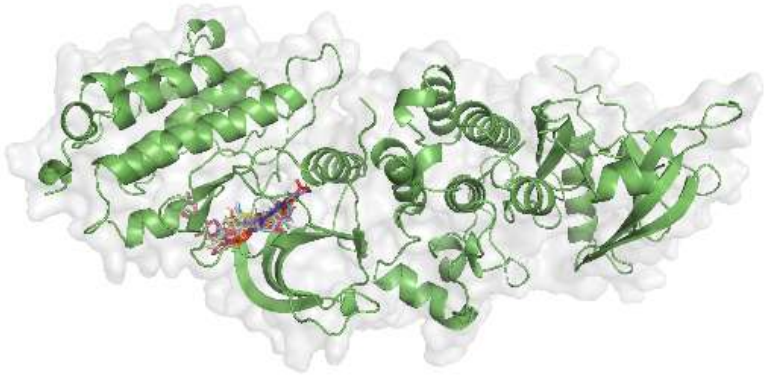

Figure 1. Complex of HER2 protein (green) with native ligand (red), Lapanitib (raspberry), Cajanin (blue), Butin (marine), Liquiritigenin (yellow), (+/-)-Eriodictyol (limon), Formononetin (magenta), Taxifolin (hotpink), (-)-Epicatechin (cyan), Afromosin (aquamarine), Aromadendrin (orange), and Prunasin (light orange).

To futher compliment the docking results, SwissDock docking by using Attracting Cavities 2.0 method (AC 2.0) resulted in butin, cajanin and liquiritigenin with the closest AC score to control (lapatinib) and liquiritigenin even surprasses control, in terms of SwissParam score afromorsin, cajanin, formononetin are the closest to control and native ligand. Just like molecular docking rules, in AC 2.0, the lower the score in AC score indicates a more favorable binding interaction between the ligand and the target protein (better binding conformation). As for

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SwissParam score, it suggest a better binding affinity (stronger binding potential). This score approximates the binding free energy, and lower (or more negative) values indicate stronger interactions between the ligand and the target[14,15].

Table 5. AC score and SwissParam score by using Attracting Cavities 2.0 method.

No	Compound	Cluster number	Cluster member	AC Score	SwissParam Score
1	Native Ligand	0	1	9.607319	-10.9721
2	Lapatinib	0	1	-18.664506	-10.3774
3	Afromosin	0	1	16.265074	-8.0908
4	Aromadendrin	0	1	-7.884233	-7.6632
5	Butin	0	1	-18.724123	-7.517
6	Cajanin	0	1	-12.383866	-7.9881
7	Epicatechin	0	1	-10.638112	-7.5512
8	Eriodictyol	0	1	-30.64509	-7.5614
9	Formononetin	0	1	6.866445	-7.7489
10	Liquiritigenin	0	1	-23.447768	-7.3496
11	Prunasin	0	1	68.24473	-7.5797
12	Taxifolin	0	1	-1.068924	-7.7442

Detecting potential cavity within the target protein resulted in several potential sites, only site no 3 provides a strong druggability potential with a drugscore of 724.00. The degree of druggability : strong = > 600; medium = 600 > drugscore > -180; weak = < -180[16,17]. With a surface area of 753.75 $Å^2$, volume of 841.25 $Å^3$, and position which resemble approximately near our

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molecular docking site with a coordinate box centre (Å) : X = 15.0; Y = 15.25; Z = 27.25 and box size (Å) : X = 18.0; Y = 21.5; Z = 16.5, it is the closes site with the strongest potential druggability.

Table 6. Cavity detection of target HER2 protein.

No	Pred Max pKd	Pred Ave pKd	DrugScore	Druggability
1	11.24	6.99	333.00	Medium
2	11.70	6.63	-325.00	Weak
3	10.67	6.28	724.00	Strong
4	10.59	6.25	28.00	Medium
5	8.16	5.41	-888.00	Weak
6	8.07	5.38	-941.00	Weak
7	6.81	4.95	-774.00	Weak
8	6.58	4.87	-1112.00	Weak
9	6.21	4.75	-1371.00	Weak
10	6.21	4.75	-932.00	Weak
11	5.88	4.63	-1120.00	Weak
12	5.63	4.55	-1239.00	Weak

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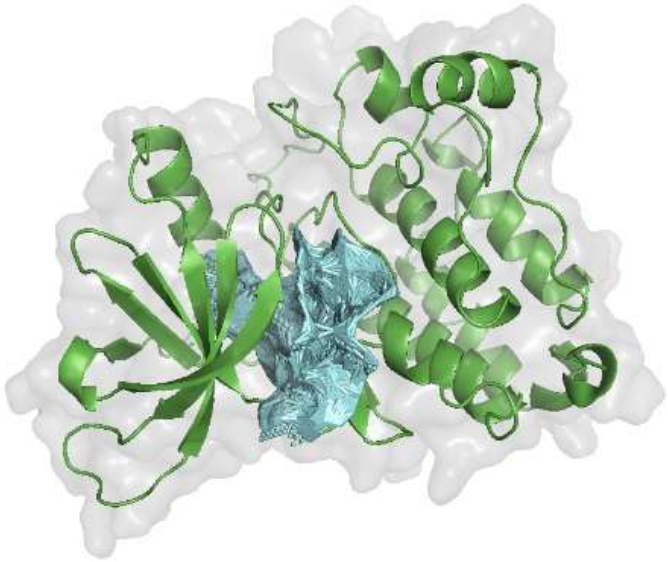

Figure 2. Target protein (green) with its cavity site no 3 (cyan) which approximately located near the coordinates determined by molecular docking.

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Amino acid interaction depicted that butin has similar hydrogen bond in Arg849 with native ligand and lapatinib, while Gly729 and Asn850 amino acid residues are only found in native ligand and Asp863 amino acid residue only found in lapatinib. The hydrophobic of butin showed a similar bond with native ligand and lapatinib, while Val734 and Lys753 are only depicted in lapatinib. The Van der Waals bond interaction in butin compound had similar bond with native ligand in Ile752, Ser783, Val797, Thr798, and Cys805. Whilst, the similar bond toward lapatinib are exhibited by Met774 and Leu785. Moreover, for cajanin compound, the hydrogen bond has similarity with lapatinib in Ser783 amino acid residue. The similar hydrophobic bond for both native ligand and lapatinib are Ala751, Leu852, and Phe864 (Figure 3~~2~~)

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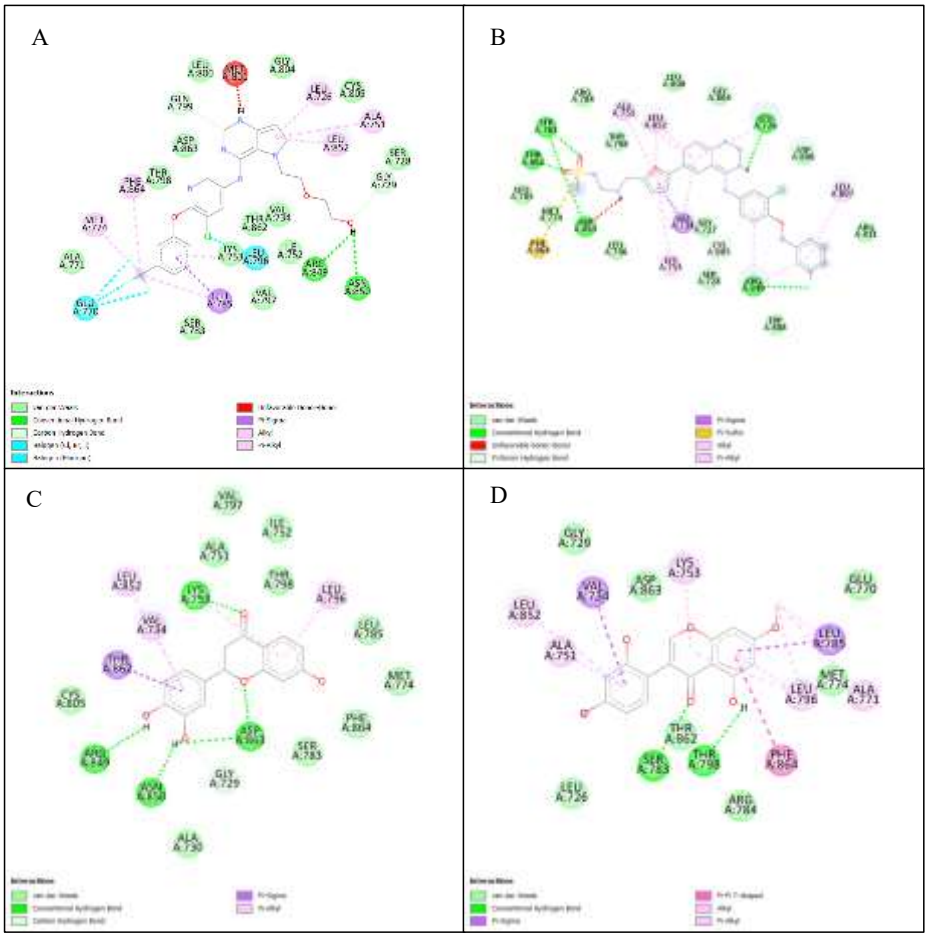

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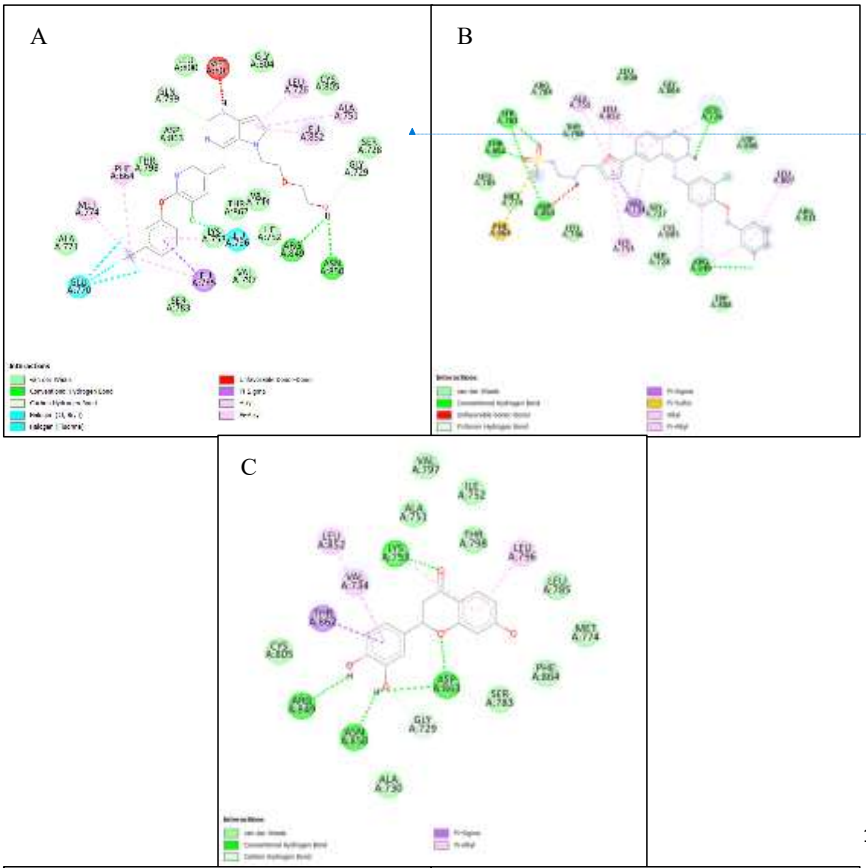

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Figure 32. Aamino acid residue within complex of native ligand (A), lapatinib (B), butin (C), and cajanin (D)), and (+/-)-eriodictyol (E) toward HER2 protein

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~~The~~ Cajanin~~(+/-) Eriodictyol~~ compound is considered as the most permeable for black lipid membranes (BLMs) with a score of -6.91~~8.50~~ (Table 7~~5~~). The negative ΔG bind or membrane binding energy value determined that the binding process is thermodynamically favorable. It considered that the compound can easily transition from an aqueous environment to a lipid bilayer environment[18].

Table 7~~5~~. Membrane permeability of bajakah plant compounds

No	Compounds	Log of perm. Coeff. - BLMs
1	03Q (Native Ligand)	-3.91
2	Lapatinib (Control)	-2.07
3	Butin	-6.24
4	Cajanin	-6.91
~~5~~	~~(+/-) Eriodictyol~~	~~-8.50~~

DISCUSSION

This research presents an in silico analysis as an initial phase, laying the foundation for subsequent investigations, including both in silico and in vivo analyses. Initial phase research is essential as it provides the essential knowledge base for further exploration. In brief, the bioactive compounds from the bajakah plant (*Spatholobus suberectus*) was specifically targeting HER2 protein. HER2 protein overexpression is a prominent characteristic of breast cancer with certain tumors displaying amplification of the HER2 gene, resulting in the presence of 25–50 copies of the gene and up to 2 million HER2 receptors on the surface of each tumor cell[19]. This amplification correlates with aggressive tumor behavior and poor patient prognosis[20]. The HER2 kinase domain has a well-characterized structure when complexed with 03Q or SYR127063, a selective tyrosine kinase inhibitor[21]. Other known inhibitor is lapatinib, it works by specifically targeting the cytoplasmic tail, which is in the intracellular ATP binding site of HER2[22,23].

Selected ten compounds of Bajakah plant are used for screening of biological activity, toxicity level, and drug-likeness. Biological activity was predicted using Pass Online, which

assesses various pharmacological effects [9]. Among the selected compounds, (+/-)-Eriodictyol exhibited significant antineoplastic properties, inducing apoptosis in various cancer cell types by modulating the Bcl-2/Bax signaling pathway and halting the cell cycle at the G2/M phase. It also inhibited proliferation and metastasis in glioma cells through the PI3K/Akt/NF-κB pathway[24–26]. Toxicity analysis were assessed by using ProTox 3 (Table 2), which evaluates the potential toxicological risks of various substances[27,28]. It is necessary to avoid the adverse drug reaction and increased safety [29]. Most of the compound are included in toxicity class 4-6 which potentially safe. Moreover, all compound also had inactive activity for hepatoxicity. Among the compounds, prunasin was found to be particularly toxic. It has potential to release hydrogen cyanide upon hydrolysis, posing risks of liver damage and cellular asphyxiation[30]. Whilst, all compounds fulfilled the Lipinski's Rule of Five (RO5) parameters and considered as potential to be developed as a drug (Table 3). Those physicochemical parameters play a crucial role in drug absorption as it influence the compound's aqueous solubility and intestinal permeability[29].

Molecular docking were conducted to evaluate the binding affinitiy of the compounds toward HER2 protein (Table 4). The docking results indicated that butin and cajanin had binding affinity score that similar to the native ligand (control) and lapatinib which suggesting their strength interaction against HER2 that almost similar to control. Futher robust docking method of AC 2.0 also shows that butin and cajanin have a strong binding affinity and conformity againts control and native ligand. While there are some compounds that excel more in certain aspects like liquiritigenin in AC score and afromosin in swissparam score, in overall, butin and cajanin still has better results, however, cajanin supersedes butin in all of the docking results.

Detection of cavity (Figure 2) provided potential amino acid residues that can interact with ligand and f~~F~~urther~~r analysis is involved~~ examination~~ning~~ of amino acid interactions between

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these compounds and HER2 protein is conducted[31,32]. Furthermore, Figure 32 showed that butin formed more hydrogen bonds with the native ligand than cajanin, although cajanin displayed a critical interaction with Ser783. The hydrophobic interactions were also assessed, highlighting that both compounds interacted with key residues within the HER2 binding pocket. Butin has only one common connection with Leu796 while cajanin has 3 similar connections with Leu785, Leu796, and Phe864.

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Membrane permeability analysis were performed using PerMM webserver analyses which represent the permeability coefficient through lipid bilayer membrane. Result in Table 75 showed that control considered for having better permeability than cajanin, butin, and (+/-)-Eriodictyol compounds. In brief, negative value suggested poor permeability and positive value considered better permeability. It essential for predicting drug absorption[33] and suggested a favorable pharmacokinetic profile for these bajakah compounds. Moreover, a higher chance to be absorbed and orally bioavailable could be linked to 90% of orally active drugs that have reached phase II clinical trials [34].

EXPERIMENTAL SECTION

Preparation of sample compound

Ten compounds derived from the bajakah plant, such as butin (92775), cajanin (5281706), (+/-)-eriodictyol (11095), liquiritigenin (114829), formononetin (5280378), taxifolin (439533), (-)-epicatechin (72276), afromosin (5281704), aromadendrin (122850), and prunasin (119033) are selected as sample compound. Structure preparation of the test compounds are carried out using SMILES code for each compound was collected from the database PubChem

(https://pubchem.ncbi.nlm.nih.gov/) and 3D structure are obtained in sdf. format. In addition, the Lapatinib is selected as control.

Preparation of target protein

In this study, HER2 protein (PDB ID: 3PP0) was downloaded from the PDB database (http://www.rcsb.org/) in .pdb format which in complex with 03Q or SYR127063 (2-{2-[4-({5-chloro-6-[3-(trifluoromethyl)phenoxy]pyridin-3-yl}amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl]ethoxy}ethanol) as native ligand. The water molecules were eliminated using PyMOL. Additionally, polar hydrogen atoms were added to the protein structure for further analysis.

Analysis of biological activity

Compound was addressed for biological activity analysis use PASS Online (http://way2drug.com/passonline/). Various biological activities related to cancer was selected, such as anti-inflammatory, antineoplastic, antimetastatic, and antioxidant. The Probability of Activity (Pa) score and Probability of Inactivity (Pi) score are measured with scoring system between 0-1.0. The score Pa>0.7 was considered as high probability, 0.5<Pa<0.7 demonstrated moderate probability, and Pa<0.5 reflected poor probability of the biological activity[35–37].

Toxicity analysis

Compounds were analyzed using ProTox 3.0 (https://tox.charite.de/protox3/index.php?site=home) to determine their toxicity levels. Several parameters were selected, such as LD50 (mg/kg), toxicity class, accuracy, and hepatoxicity. Compound which considered safe were used for further analysis.

Drug-likeness analysis

Drug-likeness was assessed using SWISS ADME webserver (http://www.swissadme.ch/index.php) to identify the Lipinski's rule of five (RO5) parameters. By using several parameters, like H-bond donor <5, H-bond acceptor <10, molecular weight (MW) <500 g/mol, and calculated LogP (MlogP) <4.15. Compound that successfully possessed the parameter of Lipinski's rule of five was considering as potential drug compound and subjected for further analysis.

Cavity detection and a~~A~~mino acid interaction

Protein cavity is analyzed by using CAVITY tool from CavityPlus webserver to detect potential binding sites and to evaluate its ligandability and druggability. By using three representative protein-ligand complexes based on their binding affinities (highest, medium and lowest, with a minimum of 90% similarity in PDBind). Sample of 70 cluster collected totalling to 210 complexes, generating a linear relationship to calculate the experimental binding affinity (pred max pKd), pred ave pKd is the same as pred max pKd but uses the average binding affinity of each cluster, the drugscore is an inner score used in the calculation of pKd[31,32]. V~~v~~isualization of amino acid interaction with ligand compound is depicted by using Discovery Studio software. The similarity of the hydrogen, hydrophobic, van der Waals, were compared between samples and control.

Membrane permeability analysis

Permeability analysis of membrane was identified by using the perMM webserver (https://permm.phar.umich.edu/permm_server_cgopm). Binding energy of the membrane were

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predicted through black lipid membranes (BLMs) parameters. The condition were set in 310 K of temperature and pH 7.4. The optimization method for calculating the transmembrane pathway were set to optimize and estimate the BLM permeability including deionization energy for ionizable molecules. The binding energy values were used to determine the membrane permeability of potential compounds.

Molecular docking simulation

Molecular docking simulations were conducted by using PyRx software. It aimed to observe the interaction between HER2 protease and bajakah plant compounds. The molecular docking was conducted by using specific docking with specific coordinate of center point: X = 15.7674; Y = 16.8217; Z = 25.8257 and dimensions (Angstrom): X = 25.3906; Y = 21.4296; Z = 25.2588.

Futher robust docking use Attracting Cavities 2.0 method in the webserver SwissDock, it has higher confidence predictions and allows for a more flexible docking approach, accommodating various ligand conformations and protein dynamics[14,15]. Docking coordinate's of box centre (Å) : X = 16; Y = 17, Z = 26 and box size (Å) : X = 25; Y = 21; Z = 25.

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CONCLUSION

Selected ten bajakah compounds demonstrated anti-inflammatory, antineoplastic, antimetastatic, and antioxidant activities. Among them, (+/-)-Eriodictyol showed the highest antineoplastic activity but has a lower binding affinity. Both butin and cajanin exhibited promising

binding affinities with score about -9.9 kCal/mol and -9.8 kCal/mol, respectively. These findings are further supported by their AC 2.0 docking results, which are comparable to the control., ~~respectively.~~ It suggested as potential candidates as inhibitor of HER2 protein activity in breast cancer treatment. However, further in vitro analysis with MTT-assay using breast cancer cell line MCF-7 is needed for confirmation and in vivo studies are necessary to validate these findings and assess their therapeutic efficacy comprehensively.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest during the research.

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REFERENCE

1. Zhang C. A brief research progress of breast cancer. *E3S Web Conf*. 2021;308:02022. doi:10.1051/e3sconf/202130802022

2. Gautama W. Breast Cancer in Indonesia in 2022: 30 Years of Marching in Place. *Indones J Cancer*. 2022;16(1):1-2. doi:10.33371/ijoc.v16i1.920

3. Ng B, Puspitaningtyas H, Wiranata JA, et al. Breast cancer incidence in Yogyakarta, Indonesia from 2008–2019: A cross-sectional study using trend analysis and geographical information system. *PLOS ONE*. 2023;18(7):e0288073. doi:10.1371/journal.pone.0288073

4. Zhou L, Zheng Y. A brief introduction to Her2 in breast cancer. In: *Third International Conference on Biological Engineering and Medical Science (ICBioMed2023)*. Vol 12924. SPIE; 2024:115-119. doi:10.1117/12.3012985

5. Dowling GP, Toomey S, Bredin P, et al. Neoadjuvant trastuzumab deruxtecan (T-DXd) with response-directed definitive therapy in early stage HER2-positive breast cancer: a phase II study protocol (SHAMROCK study). *BMC Cancer*. 2024;24(1):91. doi:10.1186/s12885-024-11851-4

6. Shastry M, Jacob S, Rugo HS, Hamilton E. Antibody-drug conjugates targeting TROP-2: Clinical development in metastatic breast cancer. *Breast Edinb Scotl*. 2022;66:169-177. doi:10.1016/j.breast.2022.10.007

7. Triwibowo DR. Bajakah, between the Dayak Myth and the Hope of Being Free from Cancer. kompas.id. March 25, 2024. Accessed October 10, 2024. https://www.kompas.id/baca/english/2024/03/25/en-bajakah-antara-mitos-dayak-dan-harapan-terbebas-dari-kanker

8. Parasuraman S. Prediction of activity spectra for substances. *J Pharmacol Pharmacother*. 2011;2(1):52-53. doi:10.4103/0976-500X.77119

9. Filimonov DA, Lagunin AA, Gloriozova TA, et al. Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. *Chem Heterocycl Compd*. 2014;50(3):444-457. doi:10.1007/s10593-014-1496-1

10. Basanagouda M, Jadhav VB, Kulkarni MV, Rao RN. Computer Aided Prediction of Biological Activity Spectra: Study of Correlation between Predicted and Observed Activities for Coumarin-4-Acetic Acids. *Indian J Pharm Sci*. 2011;73(1):88-92. doi:10.4103/0250-474X.89764

11. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2024;52(W1):W513-W520. doi:10.1093/nar/gkae303

12. Waring MJ. Defining optimum lipophilicity and molecular weight ranges for drug candidates—Molecular weight dependent lower log *D* limits based on permeability. *Bioorg Med Chem Lett*. 2009;19(10):2844-2851. doi:10.1016/j.bmcl.2009.03.109

13. Idrees S, Ashfaq UA. Discovery and design of cyclic peptides as dengue virus inhibitors through structure-based molecular docking. *Asian Pac J Trop Med*. 2014;7(7):513-516. doi:10.1016/S1995-7645(14)60085-7

14. Bugnon M, Röhrig UF, Goullieux M, et al. SwissDock 2024: major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic Acids Res*. 2024;52(W1):W324-W332. doi:10.1093/nar/gkae300

15. Röhrig UF, Goullieux M, Bugnon M, Zoete V. Attracting Cavities 2.0: Improving the Flexibility and Robustness for Small-Molecule Docking. *J Chem Inf Model*. 2023;63(12):3925-3940. doi:10.1021/acs.jcim.3c00054

16. Yuan Y, Pei J, Lai L. Binding site detection and druggability prediction of protein targets for structure-based drug design. *Curr Pharm Des*. 2013;19(12):2326-2333. doi:10.2174/1381612811319120019

17. Yuan Y, Pei J, Lai L. LigBuilder 2: a practical de novo drug design approach. *J Chem Inf Model*. 2011;51(5):1083-1091. doi:10.1021/ci100350u

18. Lomize AL, Hage JM, Schnitzer K, et al. PerMM: A Web Tool and Database for Analysis of Passive Membrane Permeability and Translocation Pathways of Bioactive Molecules. *J Chem Inf Model*. 2019;59(7):3094-3099. doi:10.1021/acs.jcim.9b00225

19. Hou Y, Nitta H, Parwani AV, Li Z. The assessment of HER2 status and its clinical implication in breast cancer. *Diagn Histopathol*. 2020;26(2):61-68. doi:10.1016/j.mpdhp.2019.10.014

20. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. *Mol Biol Int*. 2014;2014:852748. doi:10.1155/2014/852748

21. Aertgeerts K, Skene R, Yano J, et al. Structural Analysis of the Mechanism of Inhibition and Allosteric Activation of the Kinase Domain of HER2 Protein. *J Biol Chem*. 2011;286(21):18756-18765. doi:10.1074/jbc.M110.206193

22. Hutchinson L. Lapatinib is effective in patients with p95HER2-positive tumors. *Nat Rev Clin Oncol*. 2010;7(7):358-358. doi:10.1038/nrclinonc.2010.93

23. Tsang RY, Sadeghi S, Finn RS. Lapatinib, a Dual-Targeted Small Molecule Inhibitor of EGFR and HER2, in HER2-Amplified Breast Cancer: From Bench to Bedside. *Clin Med Insights Ther*. 2011;3:CMT.S3783. doi:10.4137/CMT.S3783

24. de Luna FCF, Ferreira WAS, Casseb SMM, de Oliveira EHC. Anticancer Potential of Flavonoids: An Overview with an Emphasis on Tangeretin. *Pharmaceuticals*. 2023;16(9):1229. doi:10.3390/ph16091229

25. Li W, Du Q, Li X, et al. Eriodictyol Inhibits Proliferation, Metastasis and Induces Apoptosis of Glioma Cells via PI3K/Akt/NF-κB Signaling Pathway. *Front Pharmacol*. 2020;11. doi:10.3389/fphar.2020.00114

26. Zhang Y, Zhang R, Ni H. Eriodictyol exerts potent anticancer activity against A549 human lung cancer cell line by inducing mitochondrial-mediated apoptosis, G2/M cell cycle arrest and inhibition of m-TOR/PI3K/Akt signalling pathway. *Arch Med Sci AMS*. 2019;16(2):446-452. doi:10.5114/aoms.2019.85152

27. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2024;52(W1):W513-W520. doi:10.1093/nar/gkae303

28. Nemala SK, Prava P, Vedula GS. Discovery of Structural prospects of novel bis di hydro pyrazole derivatives as Antitubercular agents: A Computational approach. *Res J Pharm Technol*. 2023;16(7):3239-3244. doi:10.52711/0974-360X.2023.00532

29. Tungary E, Ongko J, Sukweenadhi J, Antonius Y. Molecular Docking of Active Compounds from Traditional Medicinal Plants as ACE-2 protein (1R4L) inhibitor in searching for COVID-19 drug. *Res J Pharm Technol*. 2022;15(9):4235-4240. doi:10.52711/0974-360X.2022.00712

30. Grant E. Attempted Quantification of the Cyanogenic Glycosides Prunasin and Sambunigrin in the Sambucus L. (Elderberry). *Honors Coll*. Published online 2016. Accessed September 9, 2024. https://digitalcommons.library.umaine.edu/honors/389

31. Wang S, Xie J, Pei J, Lai L. CavityPlus 2022 Update: An Integrated Platform for Comprehensive Protein Cavity Detection and Property Analyses with User-friendly Tools and Cavity Databases. *J Mol Biol*. 2023;435(14):168141. doi:10.1016/j.jmb.2023.168141

32. Xu Y, Wang S, Hu Q, et al. CavityPlus: a web server for protein cavity detection with pharmacophore modelling, allosteric site identification and covalent ligand binding ability prediction. *Nucleic Acids Res*. 2018;46(W1):W374-W379. doi:10.1093/nar/gky380

33. Legiawati L, Fadilah F, Bramono K, Indra Pratama A. In Silico Study of Centella asiatica Derivatives as Antioxidant: Enhancer of Superoxide Dismutase and Glutathione Peroxidase Activity. *Res J Pharm Technol*. Published online January 27, 2023:399-403. doi:10.52711/0974-360X.2023.00068

34. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol*. 2004;1(4):337-341. doi:10.1016/j.ddtec.2004.11.007

35. Khurana N, Ishar MPS, Gajbhiye A, Goel RK. PASS assisted prediction and pharmacological evaluation of novel nicotinic analogs for nootropic activity in mice. *Eur J Pharmacol*. 2011;662(1-3):22-30. doi:10.1016/j.ejphar.2011.04.048

36. Paul A, Kabir MSH, Mojumdar M, et al. Molecular docking and PASS prediction for antinociceptive activity of some isolated compounds from Anisomeles indica (L.) Kuntze. and ADME/T property analysis of the compounds International Journal of Pharmacy. In: ; 2016. Accessed September 19, 2024. https://www.semanticscholar.org/paper/Molecular-docking-and-PASS-prediction-for-activity-Paul-Kabir/857bf4f527c96354c4bf1734b48f39afcade6dee

37. Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products. *Med Chem Res*. 2011;20(9):1509-1514. doi:10.1007/s00044-010-9398-y