

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

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### ABSTRACT

Breast cancer is the most prevalent cancer case in Indonesia and it 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 cajanin compound demonstrated binding affinity score with -9.9 and -9.8 kCal/mol, respectively, which also closely approximated to control and further proven its binding conformation 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-assay using breast cancer cell line MCF-7 is needed for confirmation and in vivo studies to validate and assess their therapeutic efficacy.

**Keywords:** Bajakah plant, breast cancer, cajanin, HER2.

### INTRODUCTION

Breast cancer is a significant global health issue with incidence rate about 23% of all cancer case in worldwide<sup>1</sup>. 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%<sup>2</sup>. It predicted that the occurrence and mortality would be increased by 2040<sup>3</sup>. Genetic factors, such as the HER2 gene, complicate breast cancer by promoting the

aggressive tumor behavior and poor prognosis<sup>4</sup>.

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<sup>5,6</sup>. However, the pursuit of more effective targeted therapies remains a priority, as seen in recent efforts to enhance cytotoxicity against MCF-7 breast cancer cell lines through nanoparticles<sup>7</sup>. Moreover, there is a growing impetus to explore the therapeutic potential and cytotoxic efficacy of indigenous medicinal flora as sustainable alternatives to costly synthetic regimens<sup>8</sup>.

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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<sup>9</sup>. 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<sup>10,11</sup>. The score with  $0.5 < Pa < 0.7$  indicated that the compounds are not too similar to known pharmaceutical agents<sup>12</sup>. 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                    |

| Compounds       | Pa score | Pi score | Biological Activity            |
|-----------------|----------|----------|--------------------------------|
| 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 hepatotoxicity (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<sup>13</sup>.

**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) |

| No | Compounds       | Toxicity Class | LD50 (mg/kg) | Accuracy (%) | Hepatotoxicity  |
|----|-----------------|----------------|--------------|--------------|-----------------|
| 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  $\leq 5$ , H-bond acceptors  $\leq 10$  and  $\log p \leq 5$ <sup>14</sup>.

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

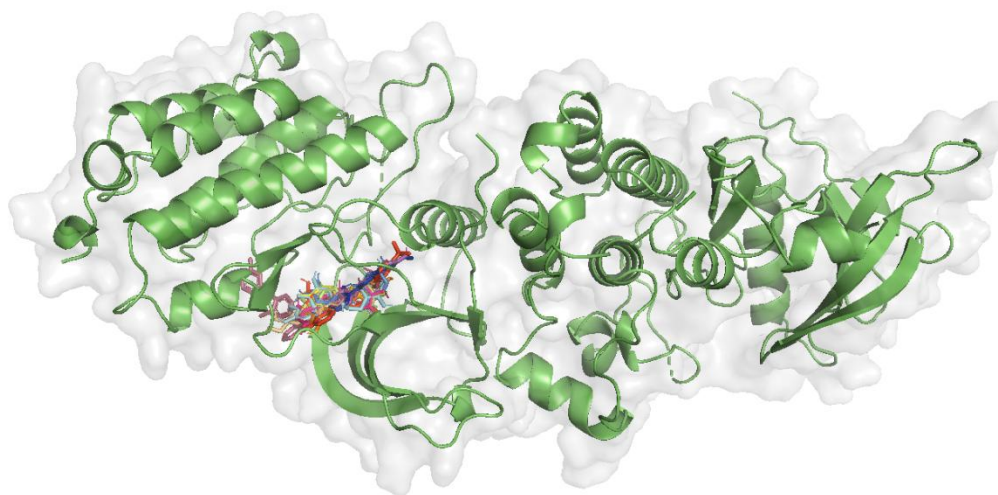
| Lipinski's Rule Of Five |                           |                               |                                      |                       |           |
|-------------------------|---------------------------|-------------------------------|--------------------------------------|-----------------------|-----------|
| Compounds               | H-Bond Donor ( $\leq 5$ ) | H-Bond Acceptor ( $\leq 10$ ) | Molecular Weight ( $\leq 500$ g/mol) | MLogP ( $\leq 4.15$ ) | 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 compound demonstrated approximately binding affinity score to the native ligand and control, they are butin and cajanin. Consequently, those two compounds are subjected to be main compound for

further analysis. Native ligand and control (lapatinib) are considered 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<sup>15</sup>.

**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                        |



**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 further 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 surpasses 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 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<sup>16,17</sup>.

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

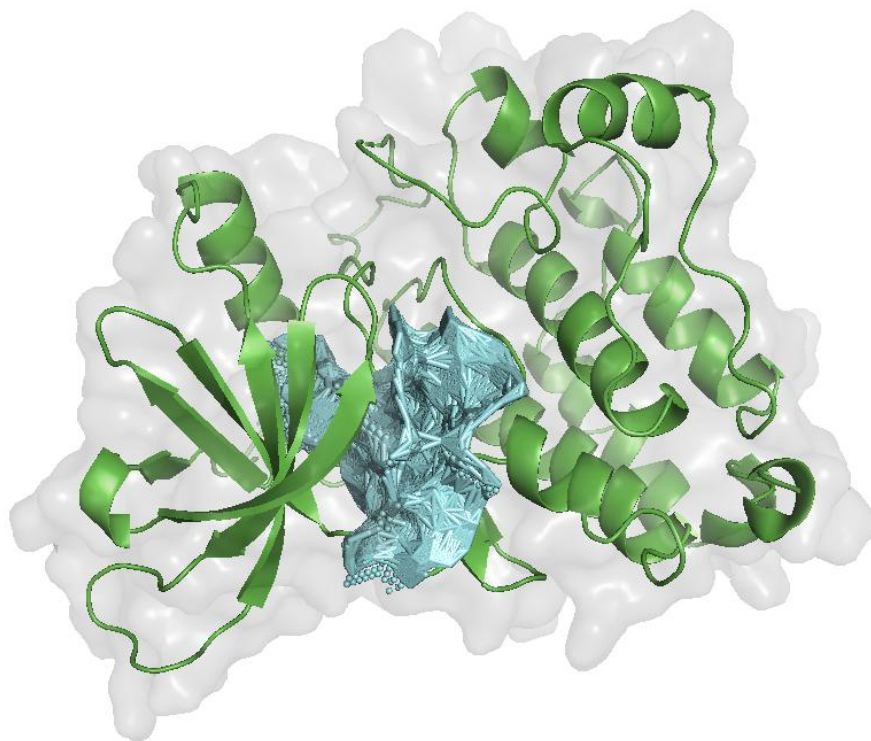
| No | Compound       | Cluster number | Cluster member | AC Score   | SwissParam Score |
|----|----------------|----------------|----------------|------------|------------------|
| 1  | Native Ligand  | 0              | 1              | 9.607319   | -10.9721         |
| 2  | Lapatinib      | 0              | 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 =  $\geq 600$ ; medium =  $600 > \text{drugscore} > -180$ ; weak =  $< -180$ <sup>18,19</sup>. With a surface area of 753.75 Å<sup>2</sup>, volume of 841.25 Å<sup>3</sup>,

and position which resemble approximately near our 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**

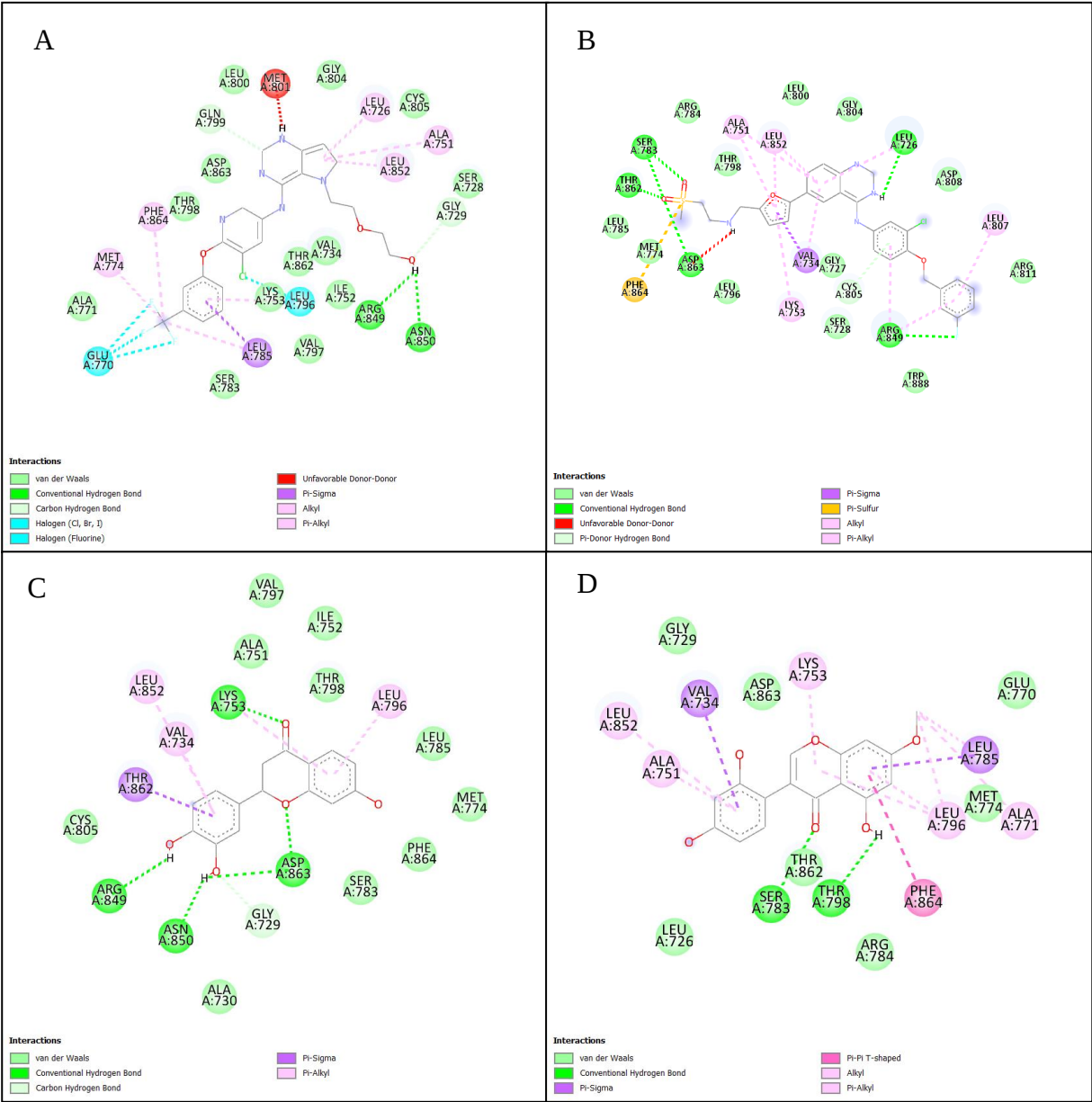
| No | Pred Max pKd | Pred Ave pKd | DrugScore | Druggability |
|----|--------------|--------------|-----------|--------------|
| 1  | 11.24        | 6.99         | 333.00    | Medium       |
| 2  | 11.70        | 6.63         | -325.00   | Weak         |
| 3  | 10.67        | 6.28         | 724.00    | 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         |



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

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)



**Figure 3.** Amino acid residue within complex of native ligand (A), lapatinib (B), butin (C), and cajanin (D) toward HER2 protein

Cajanin compound is considered as the most permeable for black lipid membranes (BLMs) with a score of -6.91 (Table 7). The negative  $\Delta G$  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<sup>20</sup>.

**Table 7. 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                      |

## 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<sup>21</sup>. This amplification correlates with aggressive tumor behavior and poor patient prognosis<sup>22</sup>. The HER2 kinase domain has a well-characterized structure when complexed with 03Q or SYR127063, a selective tyrosine kinase inhibitor<sup>23</sup>. Other known inhibitor is lapatinib, it works by specifically targeting the cytoplasmic tail, which is in the intracellular ATP binding site of HER2<sup>24,25</sup>.

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<sup>11</sup>. 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- $\kappa$ B pathway<sup>26–28</sup>. Toxicity analysis were assessed by using ProTox 3 (Table 2), which evaluates the potential toxicological risks of various substances<sup>29,30</sup>. It is necessary to avoid the adverse drug reaction and increased safety<sup>31</sup>. Most of the compound are included in toxicity class 4-6 which potentially safe. Moreover, all compound also had inactive activity for hepatotoxicity. 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<sup>32</sup>. 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<sup>31</sup>.

Molecular docking were conducted to evaluate the binding affinity 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. Further robust docking method of AC 2.0 also shows that butin and cajanin have a strong binding affinity and conformity against 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 further examination of amino acid interactions between these compounds and HER2 protein is conducted<sup>33,34</sup>. Furthermore, Figure 3 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.

Membrane permeability analysis were performed using PerMM webserver analyses which represent the permeability coefficient through lipid bilayer membrane. Result in Table 7 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<sup>35</sup> 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<sup>36</sup>.

The findings of this study are particularly significant when considered alongside the clinical limitations of current therapies. Our in silico analysis revealed that Butin and Cajanin exhibit binding affinities (-9.9 and -9.8 kCal/mol) that closely approximate those of established inhibitors like Lapatinib. Crucially, plant-derived flavonoids are frequently noted for their multi-modal biological activities, often possessing intrinsic anti-inflammatory and anti-platelet properties. Therefore, the Bajakah-derived compounds identified here may not only serve as potent HER2 inhibitors but could potentially offer a more balanced safety profile, avoiding the pro-thrombotic complications associated with some synthetic treatments. This makes them compelling candidates for the future in vitro and in vivo validations proposed in this research.

## 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<sup>37-39</sup>.

### 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 hepatotoxicity. 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 amino 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<sup>33,34</sup>. Visualization 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](https://permm.phar.umich.edu/permm_server_cgopm)). Binding energy of the membrane were 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<sup>40</sup>. 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<sup>16,17</sup>. Docking coordinate's of box centre (Å) : X = 16; Y = 17, Z = 26 and box size (Å) : X = 25; Y = 21; Z = 25.

### 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. 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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## مركبات نبات باجاكاه كعلاج محتمل لسرطان الثدي إيجابي البروتين (HER2)

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### ملخص

يعد سرطان الثدي من أكثر أنواع السرطانات انتشاراً في إندونيسيا، حيث يتسبب في 20.4% من وفيات الإناث. وعلى الرغم من أن سرطان الثدي إيجابي البروتين (HER2) أقل شيوعاً، إلا أنه أكثر عدوانية، مما يدفع المرضى للبحث عن علاجات بديلة بسبب الآثار الجانبية للعلاجات القياسية. يُستخدم نبات "باجاكاه" (*Spatholobus suberectus*) تقليدياً من قبل شعب "الداياك" لأغراض علاجية متنوعة. ومن هنا، هدف هذا البحث إلى تقييم مركبات من نبات باجاكاه ضد بروتين HER2. تم تحليل عشرة مركبات للنشاط البيولوجي باستخدام (PASS Online)، وتحديد مستوى السمية باستخدام (Protox-3)، وتحليل التشابه الدوائي باستخدام (SWISS ADME). علاوة على ذلك، تم إجراء محاكاة للالتحام الجزيئي (molecular docking) باستخدام برنامج (PyRx) و (SwissDock) عبر استخدام (attracting cavities 2.0 - AC 2.0) لتقييم تفاعل المركبات تجاه بروتين HER2 (PDB ID: 3PP0) مع استخدام عقار "لاباتينيب" (Lapatinib) كعينة ضابطة. حددت برامج (Cavityplus) و (Discovery Studio) مواقع الارتباط المحتملة وبقايا الأحماض الأمينية في معقد البروتين والمركب، بينما قام خادم الويب (PerMM) بتحليل نفاذية الغشاء. وأشارت النتائج إلى أن معظم المركبات آمنة، وأظهرت أنشطة مضادة للالتهابات، ومضادة للأورام، ومضادة للانبثاث، ومضادة للأكسدة، كما استوفت قاعدة "البيبيسكي" الخماسية. علاوة على ذلك، أظهر مركب "بوتين" (butin) و "كاجانين" (cajanin) طاقة ارتباط بلغت 9.9-9.8 كيلو كالوري/مول على التوالي، وهي نتائج تقترب بشكل وثيق من العينة الضابطة، وتم إثبات تأكيد ارتباطهما وإمكاناتهما بشكل أكبر عبر (AC 2.0). كما تشاركت هذه المركبات في بقايا أحماض أمينية مماثلة للعينة الضابطة. وفي الختام، أظهرت العينة الضابطة تفاعلاً أقوى تجاه بروتين HER2 مقارنة بمركبي "بوتين" و "كاجانين". وبالتالي، هناك حاجة إلى مزيد من التحليلات المخبرية (in vitro) باستخدام اختبار (MTT) على خط خلايا سرطان الثدي (MCF-7) للتأكيد، ودراسات حية (in vivo) لتقييم فعاليتها العلاجية.

الكلمات الدالة: نبات البجاكاه، سرطان الثدي، الكاجنين، HER2.

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