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LC-MS Analysis of Ethyl Acetate Fraction of Gandaria (*Bouea macrophylla* Griff.) Stem Bark and Molecular Docking Study against HER2 Protein

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Abstract

Breast cancer is a disease that has long been a source of concern for the public due to its high mortality rate. Excessive exposure to free radicals has been identified as a potential carcinogen, thus necessitating the utilization of antioxidants to counteract the damaging effects of these molecules. Gandaria (*Bouea macrophylla* Griff.) is a plant that is commonly found in Java, Sumatra, Maluku, and Kalimantan. *B. macrophylla* is known to have strong antioxidant activity, which is correlated with anticancer activity. A part of *B. macrophylla* that has not been extensively researched is its stem bark. The objective of this study was to analyze the potential of the ethyl acetate fraction of *B. macrophylla* stem bark as an anticancer agent using in silico method. The ethyl acetate fraction of *B. macrophylla* stem bark was characterized by LC-MS, which revealed four major compounds: 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one, 1-(2,5-dihydroxyphenyl)-propan-1-one, 3-ethoxy-4-hydroxybenzaldehyde, and 2-nitrobenzoic acid. Molecular docking was performed between the compounds against the HER2 protein with PDB ID 3RCD. The four compounds demonstrated binding energy values of -8.29 kcal/mol, -5.04 kcal/mol, -4.66 kcal/mol, and -4.28 kcal/mol, respectively.

Keywords: anticancer; *Bouea macrophylla*; gandaria; HER2 protein; molecular docking.

INTRODUCTION

Breast cancer is included in the list of diseases with a high risk of death for those affected, making it a disease of concern to the public, as it can cause death in a significant number of people. In 2022, data from the Global Cancer Observatory (GLOBOCAN) indicated that Indonesia had 66.271 (16.2%) cases of breast cancer out of a total of 408.661 cancer cases, with a mortality rate of 22.598 deaths, which is expected to continue increasing annually (GLOBOCAN, 2022). High levels of environmental pollution, exposure to radiation and chemicals, and poor lifestyle choices can be sources of unstable and highly reactive free radicals in the body. When present in excessive amounts, these free radicals can oxidize normal cells into abnormal cells, causing a chain reaction that continues uncontrollably. The oxidation of normal cells by free radicals is also known as oxidative stress, which can lead to various diseases, including cancer. Some treatment options, such as surgery, chemotherapy, and radiation therapy, which aim to kill and stop the growth of cancer cells, are generally expensive and can cause side effects in patients, including fatigue, nausea, weight loss, and hair loss (Mayo Clinic, 2023).

The exploration of natural materials for use as anticancer drugs is the focus of current research. The antioxidant compounds found in natural materials, such as phenolic, alkaloid, terpenoid, flavonoid, and tannin compounds, are known to neutralize and inhibit the activity of free radicals by donating electrons, thereby stabilizing free radical molecules and preventing oxidative stress in the body (Gusungi et al., 2020; Yohanes, 2020). *Bouea macrophylla* Griff. with the local name Gandaria is commonly found in Java, Sumatra, Maluku, and Kalimantan. *B. macrophylla* is known to contain antioxidant compounds that are correlated with anticancer activity. According to a study by Situmeang et al. (2025), the ethyl acetate fraction of *B. macrophylla* stem bark shows strong antioxidant and potential anticancer activity, supporting its possible application in natural therapeutic development (Situmeang et al., 2025).

HER2 (Human Epidermal Growth Factor Receptor 2) is a tyrosine kinase receptor, which is one of the genes that produce transmembrane proteins from the Epidermal Growth Factor (EGF) group that play a role in controlling the growth, proliferation, and repair of breast

cells under normal conditions. However, in some cases of breast cancer, the HER2 gene can mutate and produce additional copies of the gene, leading to HER2 overexpression. This causes breast cancer cells to grow and spread more rapidly due to higher levels of HER2 protein than normal. This condition is referred to as HER2-positive (Cheng, 2024). In this study, the anticancer activity of the ethyl acetate fraction of *B. macrophylla* stem bark was assessed using in silico method. The structure of bioactive compounds from the ethyl acetate fraction of *B. macrophylla* stem bark, which were obtained from LC-MS analysis, was conducted molecular docking against the HER2 protein. The results of this study aim to reveal the anticancer activity of the ethyl acetate fraction of *B. macrophylla* stem bark based on in silico analysis, therefore contributing to further research as a cancer prevention therapy or an anticancer agent with minimal side effects and reduced costs.

MATERIALS AND METHODS

Materials

The equipment used in this study consisted of laboratory glassware, analytical balance, rotary evaporator, LC-MS, and a computer with Chimera® 1.10, Discovery Studio® 3.1, Gaussian 09W, and AutoDock4. The sample used in this study was the stem bark of *Bouea macrophylla* Griff. (Gandaria) obtained from Mancak District, Serang Regency, Banten Province. The chemical reagents utilized in the research included methanol (p.a), n-hexane (p.a), ethyl acetate (p.a), aquadest. The HER2 protein with PDB ID 3RCD was downloaded from RCSB Protein Data Bank (www.rcsb.org).

Sample Preparation and Determination

B. macrophylla stem bark was collected as the sample, then cleaned, cut into smaller pieces, and air-dried at room temperature for 14 days. The sample was identified in the Laboratory of Plant Systematics at Gadjah Mada University, Special Region of Yogyakarta, Indonesia, with specimen number: No. 00683/S.Tb./VII/2024.

Extraction and Fractionation

B. macrophylla stem bark was extracted using the maceration (solid-liquid) method with methanol as the solvent for 3 × 24 hours and stirred every 24 hours. The methanol extract of *B. macrophylla* stem bark was fractionated using the partition (liquid-liquid) method with n-hexane and ethyl acetate as the solvents.

LC-MS Analysis

The method of LC-MS analysis refers to the research by Amelia et al. (2024). The sample will be injected into the LC-MS system using a closed glass capillary ± 10 cm and analyzed using the APCI (Atmospheric Pressure Chemical Ionization) method. Helium as the carrier gas with heating temperature 350-400ºC and nitrogen as the nebulization gas with heating temperature ± 400ºC.

Molecular Docking

The method of molecular docking study refers to the research by Situmeang et al. (2024). HER2 protein with PDB ID 3RCD was prepared using Chimera® 1.10 to remove water molecules and native ligand, then hydrogen atoms were added to the protein. The 3D structure of bioactive compounds from the ethyl acetate fraction of *B. macrophylla* stem bark as the ligands was drawn using and optimized using Gaussian 09W. The optimized data was saved in PDB format. Redocking was performed using AutoDock4 with 100 runs of the Lamarckian Genetic Algorithm (LGA) and the grid box sized 40 × 40 × 40 cm. The docking analysis using the same parameters (LGA and grid box) could be continued if the RMSD value of redocking was less than 2 Å.

RESULTS AND DISCUSSION

Sample Preparation and Determination

One kg of clean *B. macrophylla* stem bark samples obtained from Mancak District, Serang Regency, Banten Province, were air-dried for 14 days at room temperature, producing 250 g dry weight of the sample. The purpose of air-dried sample using temperature room was to reduce the moisture content without damaging the bioactive compounds in the sample. The determination analysis confirmed the sample with specimen number of No. 00683/S.Tb./VII/2024 as *Bouea macrophylla* Griff based on botanical authentication.

Extraction and Fractionation

Two hundred and fifty g of *B. macrophylla* stem bark was macerated using 1 L of methanol. The sample-to-solvent ratio for maceration was 1:4 (w/v) because it is the ideal ratio based on its ability to immerse and well-extract the sample (Musa et al., 2023). The weight of the methanol extract was 30 g, with the extraction yield of 12% based on calculations. Partition method for fractionating the methanol extract using non-polar solvent (n-hexane) as the first solvent and semi polar solvent (ethyl acetate) as the second solvent. Twenty five g of methanol extract was dissolved in 125 mL of methanol, then partition was carried out using 125 mL of each solvent for each fractionation step (1:1 v/v). The weight of the ethyl acetate fraction was 4.26 g with the extraction yield of 17.04% based on calculations.

LC-MS Analysis

The results of LC-MS analysis using the APCI method revealed a chromatogram (Figure 1) and 4 major compounds that were detected in the ethyl acetate fraction of *B. macrophylla* stem bark (Figure 2). The compounds were identified as 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one, 1-(2,5-dihydroxyphenyl)-

propan-1-one, 3-ethoxy-4-hydroxybenzaldehyde, 2-nitrobenzoic acid. The biological activities of the plant were significantly retrieved by the identified active compounds (Üst et al., 2024).

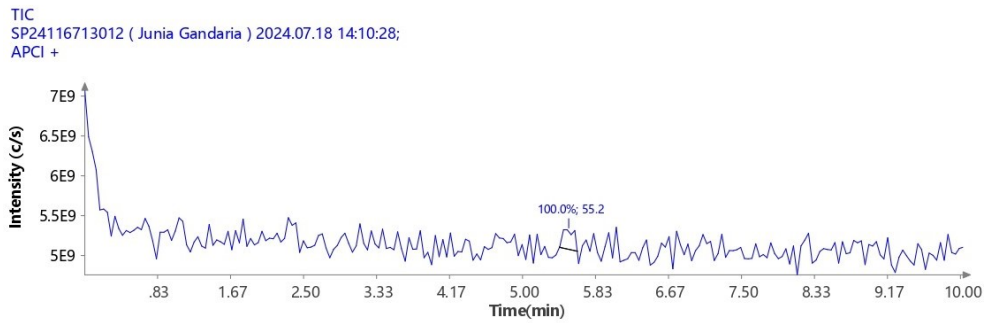

Figure 1. Chromatogram of ethyl acetate fraction of B. macrophylla stem bark.

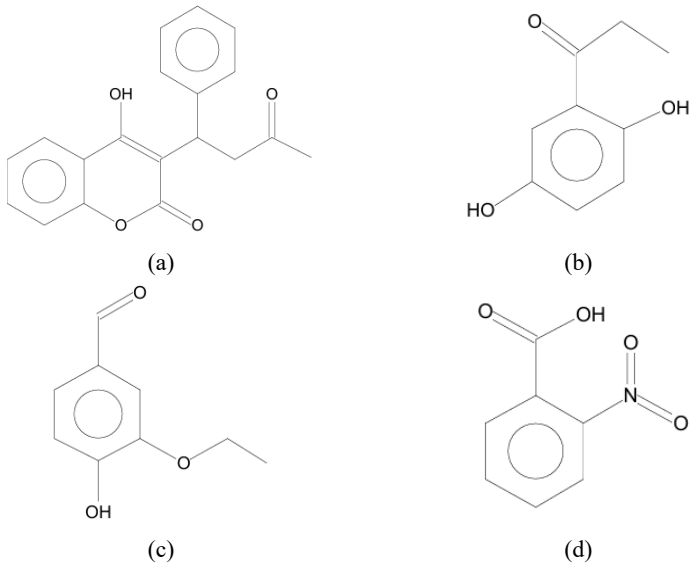

Figure 2. The compound structure of the ethyl acetate fraction of B. macrophylla stem bark. (a) 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one, (b) 1-(2,5-dihydroxyphenyl)-propan-1-one, (c) 3-ethoxy-4-hydroxybenzaldehyde, (d) 2-nitrobenzoic acid.

Molecular Docking

The molecular docking study revealed binding energy value, amino acid residues, and chemical bonds. The RMSD value of redocking analysis was 1.05 Å, which indicates that the method is already valid. Figure 3 shows the visualization before and after redocking analysis of the native ligand and the HER2 protein.

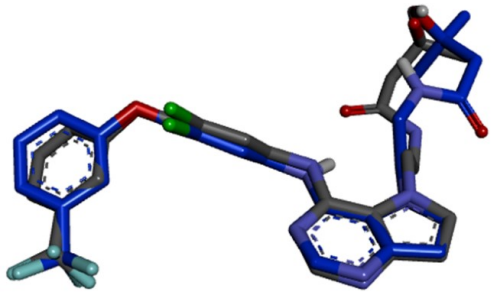

Figure 3. The visualization of before (black) and after (blue) redocking analysis.

The results of molecular docking can be observed in Table 1. The more negative the binding energy value, the more stable the complex had formed (Priatna et al., 2025). From the 4 bioactive compounds, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one had the lowest binding energy of -8.29 kcal/mol, lower than the native ligands (TAK-285) of -5.08 kcal/mol. On the other hand, 1-(2,5-dihydroxyphenyl)-propan-1-one had a similar binding energy value of -5,04 kcal/mol compared to the native ligands. Meanwhile, the other compounds, 2-nitrobenzoic acid and 3-ethoxy-4-hydroxybenzaldehyde, had the binding energy values of -4,28 kcal/mol and -4,66 kcal/mol. The amino acid residues that are located in the active side of the HER2 protein with the native ligand were Asp863, Met801, Phe864, Ala751, Leu852, Leu800, Lys753, Leu796, Leu785, Ser783, and Gln799 through the chemical bonds such as hydrogen bond, Van der Waals, hydrophobic bond, etc. can be observed in Figure 4. The native ligand and the bioactive compounds

had some similar interactions of the amino acid residues of each chemical bond.

Table 1. The result of molecular docking.

Compounds	RMSD (Å)	Binding Energy (kcal/mol)	H-Bond	Other Interaction
TAK-285	1.05	-5,08	Asp863, Met801	Phe864, Ala751, Met801, Leu852, Leu800, Lys753, Leu796, Leu785, Ser783, Gln799
4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one	0.19	-8.29	Asp863	Ala751, Val734, Lys753, Leu796
1-(2,5-dihydroxyphenyl)-propan-1-one	0.4	-5.04	Asp863, Thr862, Ala751	Lys753, Leu796, Met774, Phe864, Leu785
3-ethoxy-4-hydroxybenzaldehyde	0.32	-4.66	Asp863, Leu785	Leu785, Lys753, Asp863
2-nitrobenzoic acid	0.19	-4.28	Met801, Thr862, Ser783, Gln799	Ala751, Val734, Leu852, Leu800

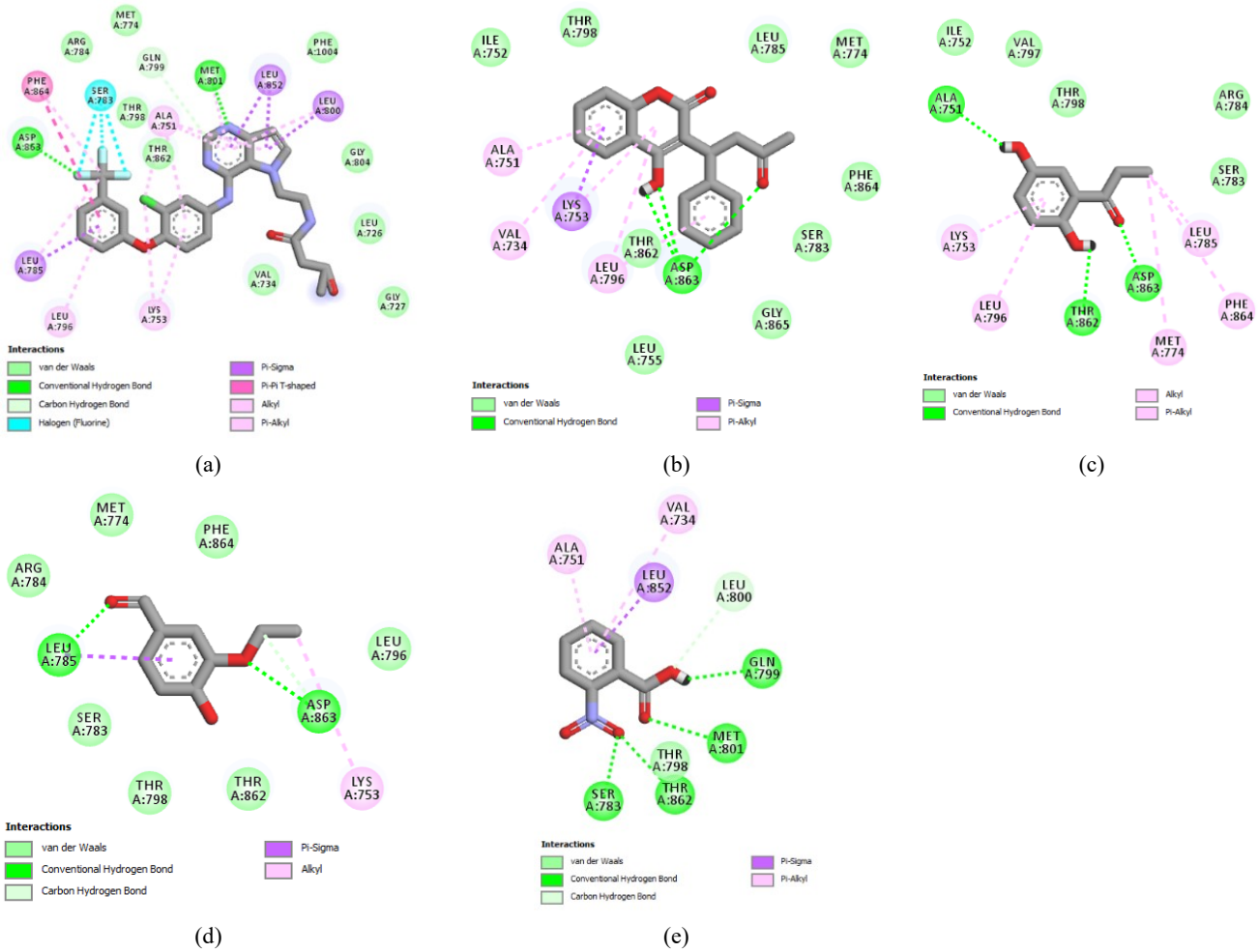

Figure 4. 2D molecular docking interactions between the compounds and the HER2 protein. (a) TAK-285 (native ligands), (b) 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one, (c) 1-(2,5-dihydroxyphenyl)-propan-1-one, (d) 3-ethoxy-4-hydroxybenzaldehyde, (e) 2-nitrobenzoic acid.

CONCLUSIONS

The ethyl acetate fraction of *B. macrophylla* stem bark revealed 4 major compounds, which were identified by LC-MS. Those compounds were 4-hydroxy-3-(3-oxo-1-phenylbutyl)-chromen-2-one, 1-(2,5-dihydroxyphenyl)-propan-1-one, 3-ethoxy-4-hydroxybenzaldehyde, and 2-nitrobenzoic acid, which had binding energy values of -8.29 kcal/mol, -5.04 kcal/mol, -4.66 kcal/mol, and -4.28 kcal/mol. 4-hydroxy-3-(3-oxo-1-phenylbutyl)chromen-2-

one was the only compound that had the binding energy lower than the native ligands (TAK-285) of -5,08 kcal/mol. Nevertheless, the bioactive compounds that were detected should be reconfirmed for further analysis using LC-MS/MS (tandem mass) and further validation for the in silico method using molecular dynamics to confirm inhibitor potential of 4-hydroxy-3-(3-oxo-1-phenylbutyl)chromen-2-one against the HER2 protein.

Authors' Contributions: Boima Situmeang designed the study and analyzed the data. Junia Salsha Primawati, Siti Safuroh, and Yuke Agustin carried out the laboratory work. Sriwijayanti and Junia Salsha Primawati wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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