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Antioxidant and Cytotoxic Potential of Ethyl Acetate Fraction of Gandaria Stem Bark (*Bouea macrophylla*) Against MCF-7 Cell Line

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Abstract

The stem bark of *Bouea macrophylla* (gandaria) represents a promising subject for further scientific investigation. Previous studies have reported that the bark contains high concentrations of total phenolic and flavonoid compounds, associated with potent antioxidant activity. This study evaluates the antioxidant and cytotoxic potential of the ethyl acetate fraction of *B. macrophylla* stem bark. Antioxidant activity was assessed using the DPPH and ABTS radical scavenging methods, while cytotoxic activity against MCF-7 breast cancer cells was determined using the MTT assay. The ethyl acetate fraction exhibited strong antioxidant activity with IC_{50} values of 5.837 ± 0.060 ppm (DPPH) and 9.645 ± 0.697 ppm (ABTS). The cytotoxicity assay revealed an IC_{50} value of 99.55 ppm, indicating moderate cytotoxic potential. These findings suggest that the ethyl acetate fraction of *B. macrophylla* stem bark possesses significant antioxidant activity and potential anticancer properties, supporting its possible application in natural therapeutic development.

Keywords: *Bouea macrophylla*; antioxidant; cytotoxic; MCF-7; gandaria.

INTRODUCTION

Breast cancer is one of the leading causes of cancer-related morbidity and mortality among women globally (Amalina et al., 2020). The MCF-7 cell line, derived from human breast adenocarcinoma, is widely utilized as a model system for investigating estrogen receptor-positive breast cancer and screening potential anticancer agents (Yeniçeri et al., 2024). In recent years, natural products have gained increasing attention as a source of bioactive compounds with therapeutic potential, particularly in developing antioxidant and anticancer agents (Herdiana et al., 2022).

One of the plants known to contain antioxidant compounds is *Bouea macrophylla* Griff., commonly referred to as gandaria (Rudiana et al., 2018). This species is indigenous to Indonesia and is widely distributed across the islands of Java, Sumatra, Maluku, and Kalimantan. According to a study by Rudiana et al. (2018), the ethyl acetate extract of the stem of *Bouea macrophylla* Griff. exhibited a high content of phenolic and flavonoid compounds, both recognized for their antioxidant properties. Furthermore, these compounds were found to be positively correlated with anticancer activity.

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system, plays a critical role in carcinogenesis (Situmeang et al., 2024). Therefore, evaluating antioxidant activity is essential in identifying compounds with potential anticancer properties. In this study, the antioxidant activity of the ethyl acetate fraction of *B. macrophylla* stem bark was assessed using two in vitro methods: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. Both assays are widely employed for their sensitivity in determining plant-derived compounds' free radical scavenging capacity.

In addition, the cytotoxic potential of the ethyl acetate fraction was evaluated against the MCF-7 breast cancer cell line using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. This colorimetric assay measures cell metabolic activity to indicate cell viability, proliferation, and cytotoxicity. The outcomes of this study aim to provide scientific evidence supporting the potential use of *B. macrophylla* stem bark as a source of antioxidant and anticancer agents, thereby contributing to the ongoing search for effective and safer alternatives to conventional chemotherapeutic drugs.

MATERIALS AND METHODS

Material

The equipment used in this study consisted of glassware such as maceration flasks, Erlenmeyer flasks, volumetric flasks, test tubes, graduated pipettes, beakers, and separatory funnels. Supporting instruments included an evaporator, filter paper, micropipettes, and a spectrophotometer. The sample used in this study was the bark of the *Bouea macrophylla* (gandaria) tree, collected from the 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), MCF-7 cells, Trypsin-EDTA solution, DMEM medium, PrestoBlue cell viability reagent, and cisplatin.

Sample Preparation

A total of 0.8 kg of fresh gandaria bark was collected as the sample. The sample was then cleaned, cut into smaller pieces, and air-dried at room temperature. The drying process was carried out for 14 days. Sample identification (determination) was conducted at the Herbarium Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

Extraction and Partition

The extraction of gandaria bark samples was carried out using the maceration method, with methanol as the extraction solvent. The extraction process lasted 3 × 24 hours, stirring every 12 hours. Fractionation was conducted by partitioning using n-hexane and ethyl acetate-water as solvents.

Antioxidant Activity Test Using DPPH

The antioxidant activity assay using the DPPH method was conducted concerning the study by Kirmani et al. (2024), with several modifications (Kirmani et al., 2024). 50 mg of the sample was dissolved in 50 mL of methanol to obtain a stock solution with a concentration of 1000 ppm. Subsequently, ethyl acetate fraction solutions with varying concentrations (0, 2, 4, 6, 8, and 10 ppm) were prepared.

Antioxidant Activity Test Using ABTS

The antioxidant activity assay using the ABTS method was conducted concerning the study by Jiangseubchatveera et al. (2023), with slight modifications (Jiangseubchatveera et al., 2023). A volume of 20 µL of the sample was added to 180 µL of $ABTS^{\bullet+}$ solution in a test tube and allowed to stand at room temperature for 5 minutes. The absorbance was then measured at a wavelength of 734 nm. Trolox was the standard (positive control) (Idowu et al., 2023).

Cytotoxic Activity Test Against MCF-7 Cell Line

The anticancer activity assay was conducted concerning the studies by Herdiana et al. (2022) and Sirait et al. (2019). MCF-7 cells were seeded into microplates at a density of 3×10^4 cells cm^{-3}, treated with 1 mL of Trypsin-EDTA solution, and incubated for 5 minutes (Sari Sirait et al., 2019). The cells were then transferred into tubes containing culture medium and centrifuged at 3000 rpm for 5 minutes. The resulting pellet was resuspended in fresh medium. The cells were cultured in 96-well plates and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. Subsequently, 100 µL of each sample and the positive control (cisplatin) were transferred from microtubes into the respective wells and incubated for another 24 hours. After incubation, the medium in each well was removed and replaced with 100 µL of a mixture of 10% PrestoBlue Cell Viability Reagent in culture medium (prepared by mixing 10 µL reagent with 90 µL medium). The plate was then incubated for 1 hour until a visible color change occurred. Finally, absorbance was measured at a wavelength of 750 nm using a multimode reader. The IC_{50} value was determined from a graph of the percentage of viable cells relative to the control. IC_{50} is the concentration required to inhibit 50% of cell growth (Matulja et al., 2022).

Data analysis

The one-way ANOVA test was used in statistical evaluation and data representation using the GraphPad Prism 10.1.2 and origin 9 software. The data were reported as the mean ± standard deviation.

RESULTS AND DISCUSSION

Sample preparation

Stem bark samples of *Bouea macrophylla* were collected from the Mancak District, Serang Regency, Banten Province, Indonesia. Approximately 2 kg of fresh stem bark was harvested and air-dried for two weeks under ambient conditions. Botanical authentication confirmed the species as *Bouea macrophylla*, with a registered identification number of 00683/s.Tb./VII/2024. Following the drying process, the final dry weight of the sample was 750 g.

Extraction and Fractionation

A total of 750 g of dried sample was subjected to maceration using 3 L of methanol, following a sample-to-solvent ratio of 1:4 (w/v). This ratio was selected based on its suitability for achieving optimal extraction efficiency, ensuring that the entire sample was fully immersed and adequately extracted. The resulting methanolic crude extract yielded a final concentrated extract weight of 125.0 g. The extraction yield, calculated based on the weight of the dried plant material (simplicia), was 16.667%. Fractionation of the methanolic extract was carried out using a liquid-liquid partitioning method. The first solvent used was a non-polar solvent (n-hexane), followed by a semi-polar solvent (ethyl acetate). A total of 125.0 g of methanolic

extract was subjected to partitioning, using 125 mL of each solvent (1:1, w/v) for each fractionation step. The ethyl acetate fraction yielded 39.50 g, corresponding to an extraction yield of 31.60%.

Antioxidant test Result

The antioxidant activity of the ethyl acetate fraction was evaluated using DPPH and ABTS radical scavenging assays. For the DPPH assay, 2.4 mL of the sample was mixed with 0.6 mL of DPPH solution. The mixture was then incubated in the dark for 30 minutes. Following incubation, a color change from pink to yellowish-brown was observed, indicating radical scavenging activity, which intensified with increasing sample concentration. The absorbance was subsequently measured at 517 nm (Aldayel, 2023). The percentage of inhibition (% inhibition) calculated from the DPPH assay is presented in Table 1.

The ABTS solution was prepared by mixing ABTS with potassium persulfate, followed by incubation at room temperature for 16 hours to generate the $ABTS^{\bullet+}$ radical cation. Test solutions were prepared at concentrations of 0, 2, 4, 6, 8, and 10 ppm, with 0.3 mL of each sample placed into test tubes. Subsequently, 2.7 mL of the ABTS solution was added to each tube, and the mixture was incubated for 15 minutes. The reaction between the sample and ABTS was indicated by a color change from bluish-green to light blue (Roubi et al., 2023). Absorbance was then measured at 714 nm (Benslama et al., 2023). The percentage of inhibition (% inhibition) and antioxidant activity of the fraction, as determined by the ABTS assay, are presented in Table 1.

Table 1. % inhibition and antioxidant activity result using DPPH and ABTS method of ethyl acetate fraction

Methods	Concentrations (ppm)	Inhibition (%) replications			IC_{50} (ppm) ±SD
		1	2	3	
DPPH	0	0	0	0	5.837 ± 0.060
	2	19.568	20.772	18.136	
	4	34.660	35.265	35.274	
	6	48.258	46.054	44.592	
	8	70.978	69.565	70.382	
	10	86.567	85.024	85.357	
ABTS	0	0	0	0	9.645 ± 0.697
	2	22.703	14.185	18.243	
	4	28.478	25.983	30.270	
	6	36.745	31.882	36.486	
	8	43.569	39.045	50.405	
	10	51.312	49.297	51.486	

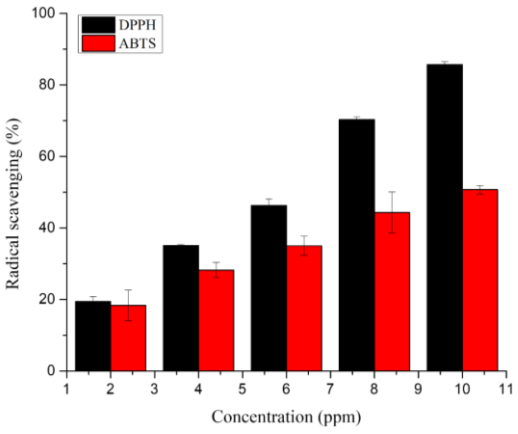

Figure 1. Correlation of DPPH and ABTS radical scavenging with various concentrations of sample.

The IC_{50} value was obtained from the linear regression equation. The higher the percentage of inhibition, the lower the IC_{50} value, indicating a stronger ability to scavenge ABTS free radicals (Figure 1). The results of the antioxidant activity test of the ethyl acetate fraction of gandaria stem bark using the DPPH and ABTS methods showed very strong antioxidant activity. According to the study by Nwude et al. (2024), antioxidant activity below 50 ppm is classified as very strong (Nwude et al., 2024). The ethyl acetate fraction had an IC_{50} value of 5.837 ± 0.060 ppm using the DPPH method and 9.645 ± 0.697 ppm using the ABTS method. This is by the study by Rudiana et al. (2018), which revealed that the ethyl acetate fraction has the highest total phenolic and total flavonoid contents (Rudiana et al., 2018).

The antioxidant activity of the ethyl acetate fraction using the ABTS method is nearly comparable to the IC_{50} value of the positive control (Trolox), which has an IC_{50} of 9.417 ± 0.577. Based on the results of antioxidant activity tests using the DPPH and ABTS methods, it can be concluded that the ethyl acetate fraction has a very high potential to be further developed as a source of natural antioxidants.

Cytotoxic activity test result against MCF-7 cell line

The cytotoxicity test of the ethyl acetate fraction of gandaria stem bark against MCF-7 cells was carried out using serial dilutions, starting from a concentration of 1000 ppm down to 7.81 ppm. The linear regression curve

and the IC50 value of the cytotoxic activity of the ethyl acetate fraction against MCF-7 breast cancer cells are shown in Figure 2.

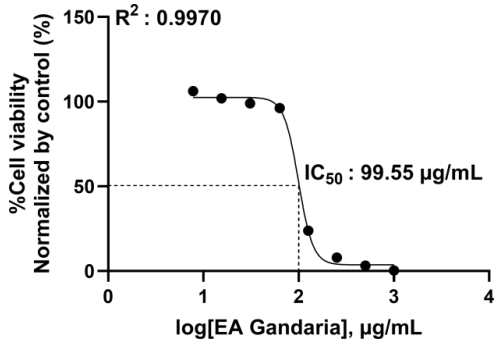

Figure 2. Regresion linear curve and IC_{50} of ethyl acetate fraction of gandaria.

The cytotoxic activity of samples targeting cancer cells can be classified into three categories. An IC_{50} value below 100 ppm is considered active (Haryanti & Widiyastuti, 2017). The ethyl acetate fraction of gandaria stem bark, with an IC_{50} value of 99.55 µg/mL, falls into the active category. Cells treated with the ethyl acetate fraction of gandaria stem bark showed morphological changes indicative of apoptosis (Figure 3), such as cell shrinkage, cytoplasmic condensation, and extracellular matrix degradation (Nurmaulawati, 2021). Therefore, the cytotoxic activity of the gandaria stem bark fraction is likely mediated through apoptosis induction and inhibition of cell migration, suggesting potential antimetastatic properties (Asefian & Ghavam, 2024).

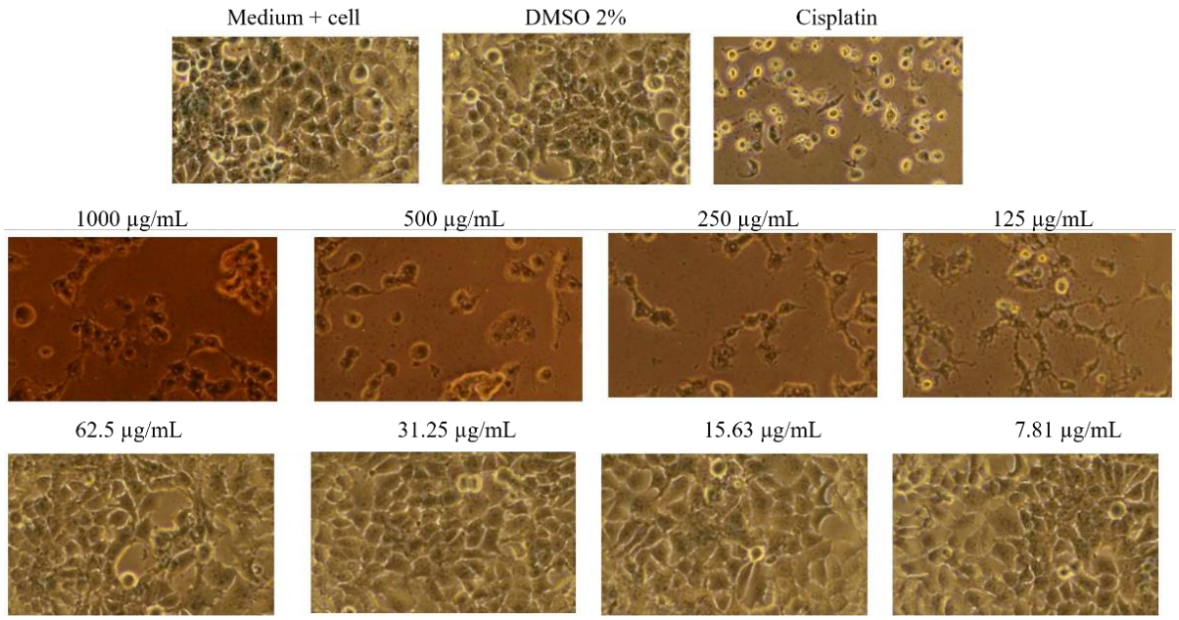

Figure 3. The morphology of MCF-7 cell lines.

This toxic property is presumed to be caused by the presence of antioxidant compounds in the gandaria stem bark fraction, which play a role in reducing the number of cancer cells, as reported by Rudiana et al. (2018). This is in line with previous studies showing that gandaria stem bark extract contains various chemical compounds, including flavonoids, phenolics, tannins, and terpenoids.

The ethyl acetate fraction of *B. macrophylla* demonstrated strong antioxidant activity, as indicated by its low IC_{50} values in both DPPH (5.837 ± 0.060 ppm) and ABTS (9.645 ± 0.697 ppm) assays, suggesting a high capacity to scavenge free radicals. This potent antioxidant activity is likely attributed to phenolic and flavonoid compounds typically extracted by semi-polar solvents like ethyl acetate. Furthermore, the same fraction exhibited cytotoxic activity against MCF-7 breast cancer cells, with an IC_{50} value of 99.55 ppm, meeting the National Cancer Institute's criteria for potential anticancer agents (IC_{50} < 100 ppm). Previous studies have reported similar correlations, where high antioxidant activity in plant extracts, particularly those rich in polyphenols, is often associated with significant anticancer potential. These findings support the therapeutic promise of gandaria, particularly its ethyl acetate fraction, as a natural source of both antioxidants and anticancer agents.

CONCLUSIONS

The ethyl acetate fraction of *B. macrophylla* stem bark demonstrated strong antioxidant activity, as evidenced by

low IC_{50} values obtained from the DPPH (5.837 ± 0.060 ppm) and ABTS (9.645 ± 0.697 ppm) assays. Additionally, the cytotoxicity test using the MTT assay against MCF-7 breast cancer cells showed moderate cytotoxic potential, with an IC_{50} value of 99.55 ppm. These results indicate that the ethyl acetate fraction possesses significant antioxidant properties and promising cytotoxic activity, highlighting its potential as a natural source for developing antioxidant and anticancer agents.

Authors' Contributions: Boima Situmeang designed the study. Junia Salsha and Ismi Oktafiani carried out the laboratory work. Weny JA Musa analyzed the data. Boima Situmeang and Ahmad Kadir Kili 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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