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Comparison of the Antioxidant Activity of *n*-Hexane Extract of Gandaria Stem Bark Using DPPH and ABTS Assays

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ABSTRACT

This study aimed to evaluate and compare the antioxidant activity of the n-hexane extract of gandaria (Bouea macrophylla) stem bark using DPPH and ABTS methods. This research was conducted as a laboratory-based quantitative study. The extraction was performed using the maceration method with n-hexane as a solvent, yielding 6.2% extract. Antioxidant activity was determined from IC_{50} values obtained by linear regression of concentration versus percentage inhibition. The results showed that the n-hexane extract exhibited strong antioxidant activity, with IC_{50} values of 26.695 ± 0.892 ppm for the DPPH method and 17.28 ± 0.352 ppm for the ABTS method. The lower IC_{50} value obtained in the ABTS assay indicates a higher radical-scavenging capacity than in the DPPH assay. These differences are likely due to variations in radical types and reaction mechanisms between the two assays. In conclusion, the n-hexane extract of Gandaria stem bark demonstrates strong antioxidant potential. The ABTS method showed greater sensitivity than the DPPH method in evaluating antioxidant activity. These findings suggest that Gandaria stem bark has potential as a natural antioxidant source, and further research is required to elucidate its active constituents.

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1. INTRODUCTION

Oxidative stress caused by an imbalance between free radicals and antioxidants in the body is associated with various degenerative diseases, including cancer, cardiovascular disorders, and aging-related conditions (Eisa et al., 2025). Free radicals are highly reactive molecules that can damage cellular components such as lipids, proteins, and DNA (Jain et al., 2019). Therefore, antioxidants play a crucial role in neutralizing these radicals and preventing cellular damage (Amparo et al., 2022). In recent years, there has been increasing interest in natural antioxidants derived from plants due to their safety, availability, and potential therapeutic benefits (Musdalipah et al., 2021).

One plant that has attracted attention is gandaria (*Bouea macrophylla*), a tropical species widely found in Indonesia (Andina & Musfirah, 2017). Various parts of this plant, including its leaves, fruits, and stem bark, are known to contain bioactive compounds such as phenolics, flavonoids, and other secondary metabolites that exhibit antioxidant properties (Hardinsyah et al., 2019). However, studies focusing specifically on the antioxidant activity of the *n*-hexane extract of gandaria stem bark are still limited. The use of *n*-hexane as a non-polar solvent allows the extraction of non-polar compounds, which may contribute uniquely to antioxidant activity (da Nóbrega et al., 2022).

To evaluate antioxidant capacity, several in vitro methods are commonly employed, including the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assays (Situmeang, Bialangi, et al., 2025). These methods are widely used due to their simplicity, sensitivity, and reliability in measuring the ability of compounds to scavenge free radicals (Musa et al., 2025). Although both methods assess antioxidant activity, they differ in terms of radical sources, solubility, and reaction mechanisms, which may result in variations in the measured activity (Warinthip et al., 2023).

Although several studies have reported the biological activities of plant extracts obtained using polar and semi-polar solvents, information regarding the bioactive constituents present in the n-hexane fraction remains limited. This represents an important research gap, particularly for non-polar compounds that may contribute significantly to the observed biological activities. Therefore, n-hexane was selected as the extraction solvent due to its ability to selectively extract non-polar constituents, such as terpenoids, steroids, fatty acids, and other lipophilic compounds, allowing a more comprehensive evaluation of their potential biological effects. Therefore, this study aims to compare the antioxidant activity of the *n*-hexane extract of gandaria (*Bouea macrophylla*) stem bark using DPPH and ABTS methods. The findings of this research are expected to provide scientific information regarding the potential of gandaria stem bark as a natural antioxidant source and contribute to the development of plant-based antioxidant agents.

2. METHODS

This study is a Laboratory based research conducted at the Research Laboratory of the School of Chemical Analysis Cilegon. The research was carried out in 2024 over a period of one year.

A. Type of Research

This study is quantitative research. The data obtained are IC_{50} values from antioxidant activity assays using the DPPH and ABTS methods. Linear regression curves were constructed using Microsoft Excel.

B. Instruments and Materials

a. Instruments

The instruments used in this research were filter paper, an analytical balance, a drop pipette, micropipette, beakers, volumetric flasks, and an UV–Visible spectrophotometer shimadzu.

b. Materials

The material used in this study was gandaria (*Bouea macrophylla*) stem bark. The stem bark was collected from Mancak Village, Serang Regency, Banten. Chemicals used were n-hexane pro analysis, methanol pro analysis, DPPH (0,1 mM), ABTS (7 mM).

C. Working Procedure

a. Sample Preparation

A total of 1 kg of fresh gandaria (*Bouea macrophylla*) stem bark was collected. The sample was washed, cut into small pieces, and then dried for 14 days. The dried sample was ground into a fine powder using a blender. The powdered sample was weighed and subsequently extracted using *n*-hexane by the maceration method.

b. Extraction of Gandaria Stem Bark

A total of 100 g of powdered gandaria sample was extracted using *n*-hexane. The volume of *n*-hexane used was 1 L. The extraction was carried out for 3 × 24 hours with stirring every 12 hours. The extract was then filtered and concentrated using a rotary evaporator. The concentrated extract was placed in a desiccator for 24 hours prior to further analysis.

c. Preparation of Antioxidant Test Solutions

The concentrated *n*-hexane extract of gandaria stem bark was prepared into a stock solution at a concentration of 1000 ppm using methanol as the solvent. Subsequently, a series of test solutions were prepared from the stock solution at concentrations of 2, 4, 6, 8, and 10 ppm by appropriate dilution with methanol. All solutions were freshly prepared prior to use in the antioxidant activity assays.

d. DPPH Test

The antioxidant activity assay using the DPPH method was performed by adding 0.6 mL of DPPH solution to the sample. The total volume of the reaction mixture (sample and DPPH) was adjusted to 3 mL. The procedure was carried out based on the method of Situmeang et al. (2024) with slight modifications (Situmeang, Swasono, et al., 2024). All measurements were conducted in triplicate. The absorbance was measured at a wavelength of 517 nm.

e. ABTS Radical Scavenging Test

The ABTS solution was prepared by weighing 30 mg of ABTS and adding 6 mg of potassium permanganate. The mixture was homogenized and incubated for 16 hours. The absorbance was measured at a wavelength of 450 nm, and the solution was diluted to obtain an absorbance of 0.6–0.7. For the antioxidant activity assay, 1.8 mL of ABTS solution was added to 0.4 mL of the sample. The measurements were performed in triplicate. The ABTS antioxidant activity assay was conducted based on the method of Situmeang et al. (2025) with slight modifications (Situmeang, Swasono, et al., 2025).

3. RESULTS AND DISCUSSIONS

A. Sample Extraction

The extraction of gandaria (*Bouea macrophylla*) stem bark using *n*-hexane as a solvent produced a thick extract with a yield of 6.2%. This yield indicates the percentage of compounds successfully extracted from 100 g of powdered sample, resulting in 6.2 g of concentrated extract. The obtained yield reflects the efficiency of the extraction process as well as the solubility of the target compounds in the solvent used.

The use of *n*-hexane, a non-polar solvent, plays an important role in determining the type of compounds extracted. Non-polar solvents such as *n*-hexane are known to selectively extract non-polar compounds, including lipids, terpenoids, steroids, and certain non-polar secondary metabolites (Abuzaid et al., 2020). Therefore, the yield of 6.2% suggests that gandaria stem bark contains a moderate amount of non-polar constituents that are soluble in *n*-hexane (Negm El-Dein et al., 2024).

Several factors may influence the extraction yield, including solvent type, extraction time, particle size, and extraction method (Kusuma et al., 2022). In this study, the maceration method was carried out for 3 × 24 hours with periodic stirring, allowing sufficient contact between the solvent and the plant matrix. The relatively fine particle size of the powdered sample also increases the surface area, enhancing solvent penetration and mass transfer of compounds into the solvent (Nogueira et al., 2022) .

Compared to polar solvents such as methanol or ethanol, the yield obtained using *n*-hexane is generally lower. This is because polar solvents can extract a wider range of compounds, including phenolics and flavonoids, which are usually present in higher quantities in plant materials. However, the use of *n*-hexane remains important for targeting specific non-polar bioactive compounds that may contribute to biological activities, including antioxidant activity (Situmeang et al., 2024).

The yield obtained in this study (6.2%) can be categorized as moderate and indicates that the gandaria stem bark has potential as a source of non-polar bioactive compounds. This result also serves as a preliminary basis for further analysis, particularly in evaluating the antioxidant activity of the extract using DPPH and ABTS methods.

B. DPPH and ABTS Radical Scavenging

The results of the antioxidant activity assay using the DPPH method were indicated by a color change from purple to yellow. In the ABTS assay, the color change was observed from a bluish-green to a light blue color. These color changes were measured using a UV-Visible Spectrophotometer. The inhibition values from each measurement, along with the averages of inhibition, are presented in Table 1.

Table 1. The inhibition (%) of DPPH and ABTS scavenging result

Methods	Concentrations (ppm)	Inhibition (%)			X±SD
		1	2	3	
ABTS	0	0	0	0	0
	2	9.145	8.957	8.272	8.791 ± 0.46
	4	13.717	13.509	12.998	13.408 ± 0.37
	6	19.912	19.089	20.089	19.697 ± 0.533
	8	23.009	24.082	22.157	23.083 ± 0.965
	10	30.826	31.571	31.237	31.235 ± 0.379
DPPH	0	0	0	0	0
	2	13.516	12.674	14.661	13.617 ± 0.997
	4	16.395	14.955	15.664	15.671 ± 0.719
	6	18.773	17.490	18.170	18.144 ± 0.641
	8	23.529	21.039	21.553	22.040 ± 1.314
	10	25.281	25.095	25.939	25.438 ± 0.443

The intensity of these color changes reflects the ability of the extract to neutralize free radicals (Jiangseubchatveera et al., 2023). The greater the antioxidant activity, the more significant the color fading observed in both methods. These changes were quantitatively measured using a UV-Visible spectrophotometer at their respective maximum wavelengths. To determine the IC_{50} value, a linear regression curve was constructed with concentration as the X-axis and percentage inhibition (% inhibition) as the Y-axis. Calibration curve of DPP shown in Figure 1, and calibration curve of ABTS shown in Figure 2.

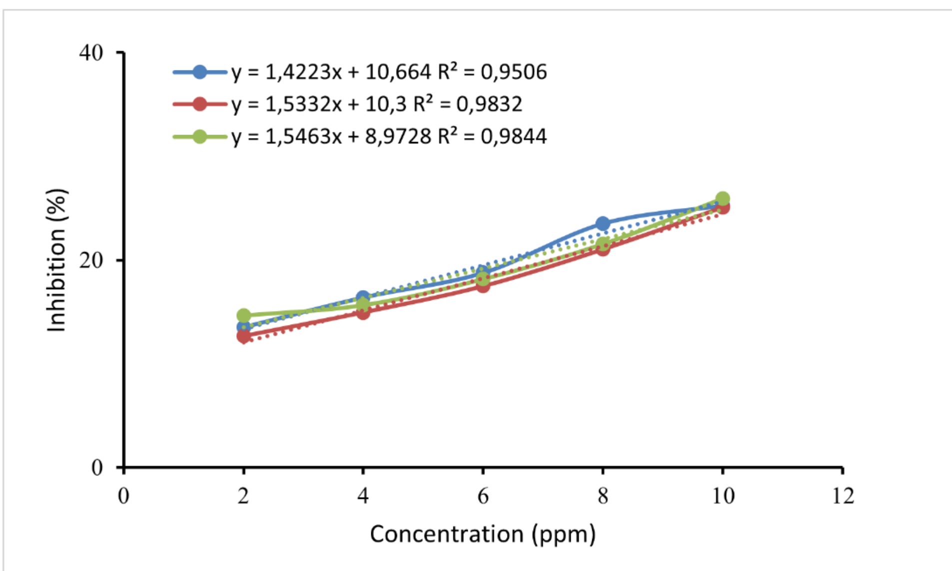

Figure 1. Linear regression curve of DPPH radical scavenging activity

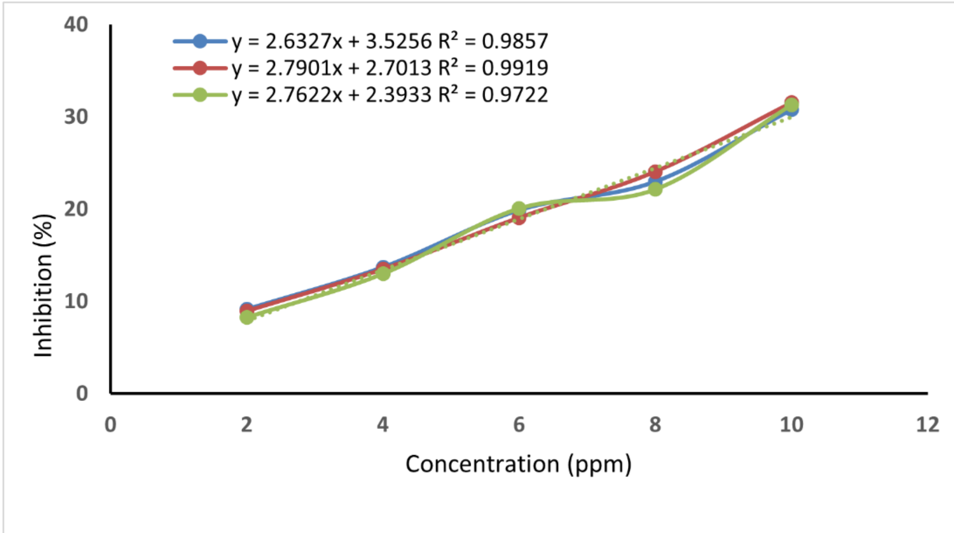

Figure 2. Linear regression curve of ABTS radical scavenging activity

The antioxidant activity of the *n*-hexane extract of gandaria (*Bouea macrophylla*) stem bark was evaluated using DPPH and ABTS methods, yielding IC_{50} values of 26.695 ± 0.892 ppm and 17.28 ± 0.352 ppm, respectively. These results indicate that the extract exhibits strong antioxidant activity in both assays, as IC_{50} values below 50 ppm are generally categorized as strong antioxidants (Adeshina et al., 2021).

The lower IC_{50} value obtained in the ABTS assay compared to the DPPH method suggests that the extract has a higher (ability) to scavenge ABTS radicals. This difference can be attributed to the distinct mechanisms and characteristics of the two assays. The DPPH method primarily measures the ability of antioxidants to donate hydrogen atoms to stabilize DPPH radicals, which are more soluble in organic solvents (Yakoubi et al., 2021). In contrast, the ABTS method involves both hydrogen atom transfer (HAT) and electron transfer (ET) mechanisms and can be applied in both aqueous and organic systems. As a result, ABTS is often more sensitive in detecting a broader range of antioxidant compounds.

The stronger activity observed in the ABTS assay may also indicate that the compounds present in the *n*-hexane extract, although predominantly non-polar, still possess significant radical scavenging through electron transfer mechanisms. Compounds such as terpenoids, steroids, and other lipophilic constituents extracted by *n*-hexane may contribute to this activity (Gündüz et al., 2023).

Furthermore, the relatively low IC_{50} values obtained in this study suggest that gandaria stem bark has promising potential as a natural antioxidant source. The difference in IC_{50} values between the two methods highlights the importance of using multiple assays to obtain a more comprehensive evaluation of antioxidant capacity.

Overall, these findings demonstrate that the *n*-hexane extract of gandaria stem bark possesses strong antioxidant activity, with the ABTS method showing higher sensitivity compared to the DPPH method. This study supports the potential utilization of gandaria stem bark as a source of bioactive compounds for applications in pharmaceutical, cosmetic, and food industries.

4. CONCLUSIONS

The antioxidant activity of the *n*-hexane extract of gandaria stem bark was evaluated using DPPH and ABTS methods, yielding IC_{50} values of 26.695 ± 0.892 ppm and 17.28 ± 0.352 ppm, respectively. These results indicate that the extract exhibits strong antioxidant activity in both assays, as IC_{50} values below 50 ppm are generally categorized as strong antioxidants. *Further research is required to elucidate the active constituents.*

REFERENCES

Abuzaid, H., Amin, E., Moawad, A., Usama Ramadan, Abdelmohsen, Hetta, M., & Mohammed1, R. (2020). Liquid Chromatography High-Resolution Mass Spectrometry Analysis, Phytochemical and Biological Study of Two Aizoaceae Plants Plants: A New Kaempferol Derivative from Trianthema portulacastrum L. *Pharmacognosy Research*, *10* (October), 24–30. https://doi.org/10.4103/pr.pr

Adeshina, I., Abdel-Tawwab, M., Tijjani, Z. A., Tiamiyu, L. O., & Jahanbakhshi, A. (2021). Dietary Tridax procumbens leaves extract stimulated growth, antioxidants, immunity, and resistance of Nile tilapia, Oreochromis niloticus, to monogenean parasitic infection. *Aquaculture*, *532*(August 2020), 736047. https://doi.org/10.1016/j.aquaculture.2020.736047

Andina, L., & Musfirah, Y. (2017). Total Phenolic Content of Cortex and Leaves Of Ramania (Bouea macrophylla Griffith) And Antioxidant Activity Assay by DPPH Method. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *8*(1), 134–140.

da Nóbrega, A. B., Motta Lessa, B. da, Henrique Brasil Bizarri, C., Ribeiro de Paiva, S., & Alves Slana, G. B. C. (2022). Comparison of betulinic acid extraction techniques from Eugenia florida DC (Myrtaeae) leaves. *Journal of Applied Biotechnology & Bioengineering*, *9*(4), 120–125. https://doi.org/10.15406/jabb.2022.09.00298

Eisa, N., Mohamed, A., Ismail, A. A. A., & Eisa, A. (2025). *Phytochemical Profiling , Antimicrobial , and Antioxidant Activities of Tamarindus indica Pulp Extracts : A Comprehensive Evaluation*. *14*(1), 51–56. https://doi.org/10.14421/biomedich.2025.141.51-56

Gündüz, M., Çiçek, Ş. K., & Topuz, S. (2023). Extraction and optimization of phenolic compounds from butterbur plant (Petasites hybridus) by ultrasound-assisted extraction and determination of antioxidant and antimicrobial activity of butterbur extracts. *Journal of Applied Research on Medicinal and Aromatic Plants*, *35*(December 2022). https://doi.org/10.1016/j.jarmap.2023.100491

Hardinsyah, H., Windardi, I. P., Aries, M., & Damayanthi, E. (2019). Total phenolic content, quercetin, and antioxidant activity of gandaria (Bouea Macrophylla Griff.) leaf extract at two stages of maturity. *Jurnal Gizi Dan Pangan*, *14*(2), 61–68. https://doi.org/10.25182/jgp.2019.14.2.61-68

Jain, P., Haque, A., Islam, T., Alam, M. A., & Reza, H. M. (2019). Comparative evaluation of Ziziphus mauritiana leaf extracts for phenolic content, antioxidant and antibacterial activities. *Journal of Herbs, Spices and Medicinal Plants*, *25*(3), 236–258. https://doi.org/10.1080/10496475.2019.1600627

Jiangseubchatveera, N., Saechan, C., Petchsomrit, A., Treeyaprasert, T., Leelakanok, N., & Prompanya, C. (2023). Phytochemicals and Antioxidant Activities of Red Oak, Red Coral and Butterhead. *Tropical Life Sciences Research*, *34*(1), 1–17. https://doi.org/10.21315/tlsr2023.34.1.1

Kusuma, G. F., Mahardika, R. G., & Sari, F. I. P. (2022). Ekstrak Batang Pelawan (Tristaniopsis merguensis Griff.) sebagai Antibakteri pada Staphylococcus aureus dan Escherichia coli. *Stannum : Jurnal Sains Dan Terapan Kimia*, *4*(2), 40–46. https://doi.org/10.33019/jstk.v4i2.3063

Musa, W. J. A., Bialangi, N., & Kilo, A. K. (2025). *Evaluation of Polyphenolic Content , Antioxidant and Anti-diabetic Activity of Different Solvent Extracts of Sauauria vulcani Korth . Leaves*. *24*(2), 1–12.

Musdalipah, M., Tee, S. A., Karmilah, K., Sahidin, S., Fristiohady, A., & Yodha, A. W. M. (2021). Total Phenolic and Flavonoid Content, Antioxidant, and Toxicity Test with BSLT of Meistera chinensis Fruit Fraction from Southeast Sulawesi. *Borneo Journal of Pharmacy*, *4*(1), 6–15. https://doi.org/10.33084/bjop.v4i1.1686

Negm El-Dein, S., Hussein, A., Abu-Bakr, M. S., Negm El-Dein, A., Salem, H. M., & Ragab, E. A. (2024). Rutin and luteolin purified from Gerbera jamesonii and Chrysanthemum 'dante purple' flowers exert remarkable protection against the enteropathogen, E. coli O78, in poultry. *Discover Food*, *4*(139), 1–6. https://doi.org/10.1007/s44187-024-00217-1

Nogueira, L. A., Figueiredo, Y. G., Ramos, A. L. C. C., Correia, V. T. da V., Nunes, B. V., Ribeiro, L. V., Franco, A. O., Ferreira, R. B., Sousa, I., Mota, J., Batista-Santos, P., Araújo, R. L. B. de, & Melo, J. O. F. (2022). The Presence of Flavonoids in Some Products and Fruits of the Genus Eugenia: An Integrative Review. *Frontiers in Food Science and Technology*, *2*(May), 1–12. https://doi.org/10.3389/frfst.2022.899492

Roquete Amparo, T., Cherem Peixoto da Silva, A., Brandão Seibert, J., dos Santos da Silva, D., Martins Rebello dos Santos, V., Melo de Abreu Vieira, P., Célio Brandão, G., Henrique Bianco de Souza, G., & Aloise Maneira Corrêa Santos, B. (2022). In vitro and in silico investigation of the photoprotective and antioxidant potential of Protium spruceanum leaves and its main flavonoids. *Journal of Photochemistry and Photobiology A: Chemistry*, *431*(June 2021). https://doi.org/10.1016/j.jphotochem.2022.114037

Situmeang, B., Bialangi, N., Musa, W. J. A., Kilo, A. K., Widiyanto, H., Susvira, D., & Yulianti, N. (2025). *Perbandingan Aktivitas Antioksidan Ekstrak Metanol Kulit Batang Gandaria Dengan Metode DPPH dan ABTS. Jurnal Redoks, Jurnal Pendidikan Kimia dan Ilmu Kimia. 8*(2), 63–70.

Situmeang, B., Musa, W. J., Mulyadi, A., Ibrahim, A. M., Widiyanto, H., & Bialangi, N. (2024). Perbandingan Aktivitas Antioksidan Ekstrak Etil asetat dan Metanol Daun Tumbuhan Matoa (Pometia pinnata). *Jurnal Penelitian Sains*, *26*(1), 65. https://doi.org/10.56064/jps.v26i1.919

Situmeang, B., Swasono, R. T., & Raharjo, T. J. (2024). Antioxidant, Photoprotective, and Cytotoxic Activities of Tristaniopsis merguensis Leaf Fractions With Molecular Docking Study of Potential Fraction. *Karbala International Journal of Modern Science*, *10*(4), 406–417. https://doi.org/10.33640/2405-609X.3365

Situmeang, B., Swasono, R. T., & Raharjo, T. J. (2025). Evaluation of phytochemical composition, antioxidant, cytotoxic and in silico studies of ethyl acetate fractions of Tristaniopsis merguensis leaves. *Toxicology Reports*, *14*(January), 101911. https://doi.org/10.1016/j.toxrep.2025.101911

Warinthip, N., Liawruangrath, B., Natakankitkul, S., Rannurags, N., Pyne, S. G., & Liawruangrath, S. (2023). Chemical Constituents Antioxidant and Antibacterial Activities of the Leaves and Flowers from Gardenia carinata Wallich. *Natural and Life Sciences Communications*, *22*(1). https://doi.org/10.12982/NLSC.2023.003

Yakoubi, R., Megateli, S., Hadj Sadok, T., & Gali, L. (2021). Photoprotective, antioxidant, anticholinesterase activities and phenolic contents of different Algerian Mentha pulegium extracts. *Biocatalysis and Agricultural Biotechnology*, *34*(May), 102038. https://doi.org/10.1016/j.bcab.2021.102038