e-ISSN xxxx-xxxx
p-ISSN xxxx-xxxx

URANIUM
Journal of Sciences Research

Publisher: Fermora Education
Available online at https://fermoraedu.com/index.php/ujsr/

Antioxidant Activity Comparison of Ethyl Acetate Extract of Gandaria Stem Bark Using DPPH and ABTS Methods

Nurhayati Bialangi*[1] and Weny JA Musa[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo, Indonesia

*Corresponding author: nurhayatibialangi@yahoo.co.id

Article Information:

Submited: 4 August 2025
Revised: 5 August 2025
Accepted: 5 August 2025

Keywords:
Antioxidant; *Bouea macrophylla;* Gandaria

Abstract

This study aimed to evaluate and compare the antioxidant activity of ethyl acetate extract of *Bouea macrophylla* (Gandaria) stem bark using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) free radical scavenging methods. The extraction was performed by maceration using ethyl acetate as the solvent, yielding 16.7 g of concentrated extract with an extraction yield exceeding 10%, indicating efficient solvent penetration and metabolite recovery. The antioxidant activity was measured at concentrations of 0, 2, 4, 6, 8, and 10 ppm, and the IC50 value was calculated for each method. The DPPH method showed a higher antioxidant activity with an IC_{50} value of 5.837 ± 0.060 ppm, while the ABTS method yielded an IC_{50} of 9.645 ± 0.697 ppm. These results indicate that the extract possesses strong antioxidant potential, likely due to the presence of secondary metabolites such as flavonoids, tannins, and polyphenols. The findings suggest that Gandaria stem bark extract could serve as a promising natural antioxidant source for pharmaceutical or nutraceutical applications.

1. INTRODUCTION

Oxidative stress caused by an imbalance between free radicals and antioxidants in the body has been linked to the development of various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions (Mikkili et al., 2023). Antioxidants are compounds that can neutralize free radicals, thereby reduce oxidative damage and protect cellular components (Musdalipah et al., 2021). As a result, the search for natural antioxidants, particularly from plant sources, has gained considerable attention in recent years (Idowu et al., 2023).

Gandaria (*Bouea macrophylla* Griff.), a tropical fruit-bearing plant commonly found in Southeast Asia, has been traditionally used in local medicine for its health-promoting properties (Andina & Musfirah, 2017). Various parts of the plant, including the leaves, fruits, and bark, are believed to contain bioactive compounds such as flavonoids, phenolics, and tannins that exhibit antioxidant potential (Hardinsyah et al., 2019). Among these, the stem

bark is of particular interest due to its rich phytochemical content, which may serve as a valuable source of natural antioxidants (Rudiana et al., 2018).

In order to evaluate the antioxidant capacity of plant extracts, several in vitro methods are commonly employed. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay are two widely used methods that measure the ability of antioxidants to scavenge free radicals (Situmeang et al., 2024). Each method has its own advantages and sensitivity, and the use of both can provide a more comprehensive assessment of antioxidant activity (Kabré et al., 2023).

This study aims to compare the antioxidant activity of the ethyl acetate extract of *Bouea macrophylla* stem bark using both DPPH and ABTS methods. The findings are expected to contribute to the growing body of knowledge on natural antioxidants and highlight the potential use of Gandaria bark as a source of functional bioactive compounds.

2. METHOD

Materials and equipments

The sample used in this study was the stem bark of Gandaria (*Bouea macrophylla*). The chemicals used included analytical grade ethyl acetate (pro analysis), methanol (pro analysis), DPPH, ABTS, and distilled water. The equipment utilized in this research consisted of Erlenmeyer flasks, filter paper, a UV-Vis spectrophotometer, micropipettes, pipette tips, volumetric flasks, beakers, test tubes, and a test tube rack.

Sample preparation

The stem bark samples of Gandaria (*Bouea macrophylla*) were collected from Mancak District, Serang Regency, Banten Province. A total of 1 kg of bark was collected and then dried for 14 days under ambient conditions. The dried samples were cut into small pieces and ground using a blender to obtain a powdered form (simplicia). A total of 200 g of dried Gandaria stem bark simplicia was used for extraction by maceration method using ethyl acetate as a solvent.

Sample extraction

The extraction of Gandaria (*Bouea macrophylla*) stem bark was carried out using the maceration method. A total of 100 g of dried powdered sample was used, with 1 L of ethyl acetate as the extraction solvent (10 times the sample weight). The maceration process was performed for 48 hours (2 × 24 hours) with occasional stirring every 6 hours. After maceration, the mixture was filtered using filter paper. The ethyl acetate solvent was then removed from the filtrate using a rotary evaporator to obtain a concentrated ethyl acetate extract of Gandaria stem bark.

Antioxidant activity assay using the DPPH method

The antioxidant activity test began with the preparation of a stock solution of the ethyl acetate extract of Gandaria stem bark. The stock solution was prepared at a concentration of 1000 ppm by dissolving 50 mg of the extract in a 50 mL volumetric flask. The extract was completely dissolved using methanol as the solvent. For the antioxidant activity assay, working solutions were prepared at various concentrations: 0, 2, 4, 6, 8, and 10 ppm (Musa et al., 2025).

Antioxidant activity assay using the ABTS method

The antioxidant activity assay of the ethyl acetate extract of Gandaria stem bark was conducted following the method described by Mikkili et al. (2023). Sample concentrations were prepared by diluting the 1000 ppm stock solution to obtain final concentrations of 0, 2,

4, 6, 8, and 10 ppm. In each test, 0.7 mL of ABTS solution was added to the extract solution. The absorbance was measured at a wavelength of 715 nm (Mikkili Indira et al., 2023; Liu et al., 2023).

3. RESULT AND DISCUSSION

Extraction was carried out using the maceration method with ethyl acetate as the solvent, which was selected due to its moderate polarity and effectiveness in extracting various phenolic and flavonoid compounds from plant tissues (V. et al., 2011). The presence of secondary metabolites such as tannins, flavonoids, and polyphenols in Gandaria (*Bouea macrophylla*) stem bark contributes significantly to its antioxidant activity. A total of 16.7 g of concentrated ethyl acetate extract was obtained. The extraction yield exceeded 10% of the initial dried simplicia weight. According to Musa et al. (2022), a yield greater than 10% is considered high, indicating that the solvent had strong efficiency in extracting secondary metabolites from the sample (Musa et al., 2022).

The antioxidant activity of the ethyl acetate extract of Gandaria (*Bouea macrophylla* Griff.) stem bark was evaluated using two different methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)). The antioxidant activity results are expressed as IC_{50} values, which represent the extract concentration required to inhibit 50% of the DPPH and ABTS free radicals. Sample absorbance was measured at a wavelength of 517 nm for DPPH and 715 nm for ABTS (Ozdemir et al., 2024). The selection of these wavelengths was based on previous studies. The percentage of inhibition was calculated by subtracting the sample absorbance from the blank absorbance (Nur et al., 2023). The calculated % inhibition values and IC_{50} results are presented in Table 1.

Table 1. Inhibition values (%) and IC_{50} in DPPH and ABTS radical scavenging

Methods	Concentrations (ppm)	% Inhibitions			X ± SD	IC_{50} (ppm)
		1	2	3		
DPPH	0	0	0	0	0	5.837 ± 0.060
	2	19.568	20.772	18.136	19.492 ± 1.319	
	4	34.660	35.265	35.274	35.066 ± 0.352	
	6	48.258	46.054	44.592	46.301 ± 1.845	
	8	70.978	69.565	70.382	70.308 ± 0.709	
	10	86.567	85.024	85.357	85.649 ± 0.811	
ABTS	0	0	0	0	0	9.645 ± 0.697
	2	22.703	14.185	18.243	18.377 ± 4.261	
	4	28.478	25.983	30.270	28.244 ± 2.153	
	6	36.745	31.882	36.486	35.038 ± 2.736	
	8	43.569	39.045	50.405	44.34 ± 5.719	
	10	51.312	49.297	51.486	50.699 ± 1.217	

The antioxidant activity of the ethyl acetate extract of *Bouea macrophylla* stem bark was evaluated using DPPH and ABTS radical scavenging methods. The results are presented as percentage inhibition values at various concentrations (0–10 ppm) and expressed as mean ± standard deviation (SD) from three replicates. The IC_{50} values were also calculated for each method. The DPPH assay results revealed a clear dose-dependent antioxidant activity of the extract, with increasing concentration leading to higher inhibition percentages. At the lowest test concentration of 2 ppm, the extract exhibited 19.49 ± 1.32% inhibition. This value increased to 35.07 ± 0.35% at 4 ppm and continued to rise significantly, reaching 70.31 ± 0.71% at 8 ppm and 85.65 ± 0.81% at 10 ppm (Figure 1). The IC_{50} value, which represents the

concentration of extract required to inhibit 50% of DPPH radicals, was calculated to be 5.837 ± 0.060 ppm. The result calculated from regression linear curve showed in figure 2. This relatively low IC_{50} value indicates that the extract possesses strong antioxidant properties, as lower IC_{50} values are associated with higher antioxidant potency (Olutayo & Doyinsola, 2013).

The high DPPH scavenging activity can be attributed to the presence of phenolic and flavonoid compounds in the stem bark extract. These secondary metabolites are known to donate hydrogen atoms or electrons to neutralize free radicals, thereby terminating radical chain reactions (Eisa et al., 2025). The ethyl acetate solvent used in the extraction process is moderately polar, making it effective in isolating semi-polar antioxidant compounds such as flavonoids, phenolic acids, and tannins (Pham et al., 2024).

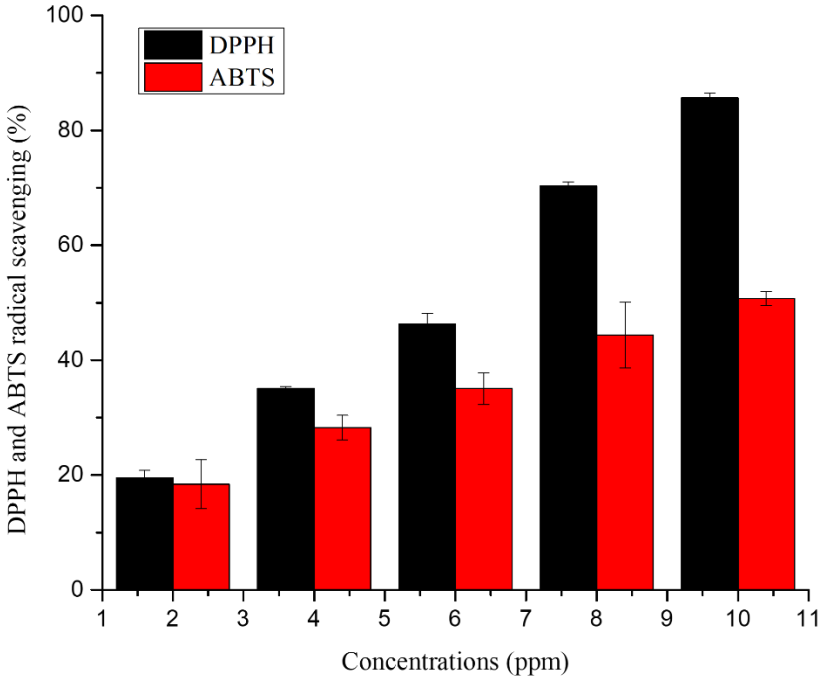

Figure 1. DPPH and ABTS radical scavenging in various concentrations

Similar to the DPPH assay, the ABTS assay also demonstrated a concentration-dependent antioxidant activity. The percentage of radical inhibition at 2 ppm was 18.38 ± 4.26%, which increased steadily with higher concentrations. At 4 ppm, the inhibition reached 28.24 ± 2.15%, followed by 35.04 ± 2.74% at 6 ppm, and 50.70 ± 1.22% at the highest tested concentration of 10 ppm (Figure 1). The IC_{50} value obtained from the ABTS assay was 9.645 ± 0.697 ppm, which, although slightly higher than the DPPH result, still reflects good antioxidant potential. The result calculated from regression linear curve showed in figure 3.

The difference in IC_{50} values between the two methods may be due to the different mechanisms by which the radicals react with antioxidant compounds. DPPH is a nitrogen-centered stable free radical, while ABTS forms a more reactive radical cation (Tajammal et al., 2022). Additionally, the ABTS assay can detect both hydrophilic and lipophilic antioxidant activities, whereas the DPPH assay is more selective for lipophilic compounds (Liu et al., 2023). This may explain why the extract showed slightly lower scavenging efficiency in the ABTS assay.

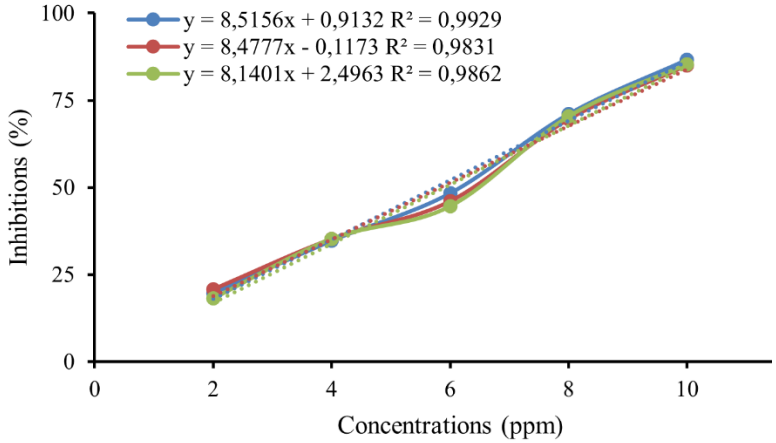

Figure 2. Regression linear curve of ethyl acetate extract in DPPH

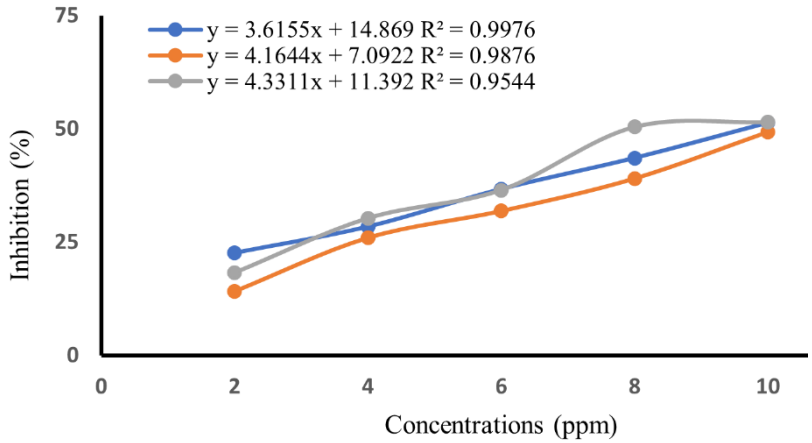

Figure 2. Regression linear curve of ethyl acetate extract in ABTS

Both DPPH and ABTS assays confirmed the significant antioxidant activity of the ethyl acetate extract of Gandaria stem bark. However, the extract demonstrated greater potency in the DPPH assay, as evidenced by the lower IC_{50} value. This suggests that the active compounds in the extract may have stronger hydrogen-donating abilities or better affinity toward the DPPH radical than the ABTS cation radical.

The strong antioxidant activity observed in this study supports the traditional use of Gandaria in herbal medicine and highlights its potential as a natural source of antioxidants. The findings also emphasize the importance of extraction solvent selection, as ethyl acetate proved effective in isolating bioactive constituents with radical scavenging properties. These results align with previous studies on plant-based antioxidants, which have shown that extracts rich in flavonoids and phenolic compounds generally exhibit high radical scavenging activity (Musa et al., 2022). Further phytochemical analysis and in vivo studies are recommended to identify specific compounds responsible for the antioxidant activity and to evaluate their therapeutic potential.

4. CONCLUTION

The DPPH method showed a higher antioxidant activity with an IC_{50} value of 5.837 ± 0.060 ppm, while the ABTS method yielded an IC_{50} of 9.645 ± 0.697 ppm. These results indicate that the extract possesses strong antioxidant potential, likely due to the presence of secondary metabolites such as flavonoids, tannins, and polyphenols. The findings suggest that

Gandaria stem bark extract could serve as a promising natural antioxidant source for pharmaceutical or nutraceutical applications.

REFERENCES

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.

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

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

Idowu, D. O., Aiyelaagbe, O. O., & Idowu, P. A. (2023). Chemical composition and biological activities of volatile oil of the stem of Dombeya buettneri K. Schum. (Sterculiaceae). *Scientific African*, *20*, 1–10. https://doi.org/10.1016/j.sciaf.2023.e01624

Kabré, P., Ouattara, L., Sanou, Y., Ouédraogo, R. J., Ouoba, P., Zanté, A. A., Zoungo, D., Somda, M. B., & Ouédraogo, G. A. (2023). Comparative study of polyphenols, flavonoids content, antioxidant and antidiabetic activities of Lophira lanceolata Tiegh.ex Keay (Ochnaceae) extracts. *Scientific African*, *22*(October). https://doi.org/10.1016/j.sciaf.2023.e01922

Liu, L., Chen, J., Chang, X., Qin, J., Lai, H., & Zhang, X. (2023). Phytochemical profiles and bioactivities of Parnassia palustris L. *European Journal of Integrative Medicine*, *57*(September 2022), 102207. https://doi.org/10.1016/j.eujim.2022.102207

Mikkili Indira, Karlapudi Abraham Peele, Srirama Krupanidhi, Kodali Vidya Prabhakar, K.B.S. Vimala, P. Satya kavya, Isani Sravya, & T. C. Venkateswarulu. (2023). In Vitro Assessment of The Bioactive Compounds and Anticancer Potential of Citrus medica Leaf Extract. *Tropical Life Sciences Research*, *34*(3). https://doi.org/10.21315/tlsr2023.34.3.11

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.

Musa, W. J. A., Bialangi, N., Kilo, A. K., Lamangantjo, C. J., Situmeang, B., & Ibrahim, A. M. (2022). *Flavonoid Glycoside Compound from Tombili Seed (Caesalpinia bonducella) And Its Antioxidant*. *15*(4), 2237–2242.

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

Nur, S., Setiawan, H., Hanafi, M., & Elya, B. (2023). Phytochemical composition, antioxidant, in vitro and in silico studies of active compounds of Curculigo latifolia extracts as promising elastase inhibitor. *Saudi Journal of Biological Sciences*, *30*(8), 103716. https://doi.org/10.1016/j.sjbs.2023.103716

Olutayo, O., & Doyinsola, I. (2013). Phytochemical and antioxidant properties of some Nigerian medicinal plants. *American Journal of Scientific and Industrial Research*, *4*(3), 328–332. https://doi.org/10.5251/ajsir.2013.4.3.328.332

Ozdemir, M., Gungor, V., Melikoglu, M., & Aydiner, C. (2024). Solvent selection and effect of extraction conditions on ultrasound-assisted extraction of phenolic compounds from

galangal (Alpinia officinarum). *Journal of Applied Research on Medicinal and Aromatic Plants*, *38*(July 2023), 100525. https://doi.org/10.1016/j.jarmap.2023.100525

Pham, T. L., Ha Nguyen, T. T., Nguyen, T. A., Le-Deygen, I., Hanh Le, T. M., Vu, X. M., Le, H. K., Van, C. B., Usacheva, T. R., Mai, T. T., & Tran, D. L. (2024). Antioxidant activity of an inclusion complex between rutin and β-cyclodextrin: experimental and quantum chemical studies. *RSC Advances*, *14*(26), 18330–18342. https://doi.org/10.1039/d4ra02307b

Rudiana, T., Fitriyanti, F., & Adawiah, A. (2018). Aktivitas Antioksidan dari Batang Gandaria (Bouea macrophylla Griff). *EduChemia (Jurnal Kimia Dan Pendidikan)*, *3*(2), 195. https://doi.org/10.30870/educhemia.v3i2.3328

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

Tajammal, A., Siddiqa, A., Irfan, A., Azam, M., Hafeez, H., Munawar, M. A., & Basra, M. A. R. (2022). Antioxidant, molecular docking and computational investigation of new flavonoids. *Journal of Molecular Structure*, *1254*, 132189. https://doi.org/10.1016/j.molstruc.2021.132189

V., V. P., G., N., N., C., & S., S. K. (2011). Evaluation of anti inflammatory and analgesic activity of Tridax procumbens Linn against formalin, acetic acid and CFA induced pain models. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(2), 126–130. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L361759865%0Ahttp://www.ijppsjournal.com/Vol3Issue2/2021.pdf