BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 381-387 | DOI: 10.14421/biomedich.2025.141.381-387 ISSN 2540-9328 (online)

Antioxidant Activity Test of Ethanol Extract from The Leaves and Bark of Kapur (*Dryobalanops aromatica*) from Distillation Solid Waste Using the DPPH and ABTS Methods

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Manuscript received: 21 April, 2025. Revision accepted: 27 June, 2025. Published: 11 July, 2025.

Abstract

One of the endemic plants of East Kalimantan, kapur (*Dryobalanops aromatica*) belongs to the Dipterocarpaceae family and is typically used by the locals for essential oils. Solid waste is only used as a natural fertilizer, while the leaves and bark of Kapur (*Dryobalanops aromatica*) are often only used for the essential oil extracted. Research on solid waste from distilling kapur (*Dryobalanops aromatica*) has never been done. This investigation aimed to find the possible concentration of secondary metabolite chemicals and antioxidants in the ethanol extract of solid waste from distilling Kapur leaves and bark (*Dryobalanops aromatica*). The ethanol extract of Kapur leaves and bark (*Dryobalanops aromatica*) will undergo phytochemical testing utilizing a qualitative test method to ascertain the presence of secondary metabolite chemicals. The ethanol extract of Kapur leaves and bark (*Dryobalanops aromatica*) will be tested for antioxidant activity against DPPH and ABTS free radicals using a UV Spectrophotometer. The results of the phytochemical analysis showed that the ethanol extracts from the leaves and bark contained alkaloids, flavonoids and tannin. Triterpenoids are only found in the bark extracts, while saponins and steroids are only found in the leaves extracts. Antioxidant activity of *Dryobalanops atomatica* extract showed that ethanol extract displayed an ability to inhibit DPPH free radical in both the leaves and bark ethanol extracts, with percentages of 83.24% and 94.91% at 25 ppm concentration, respectively. Antioxidant activity of *Dryobalanops atomatica* extract showed that ethanol extract could inhibit DPPH free radical in the leaves and bark ethanol extracts, with percentages of 83.24% and 94.91% at 25 ppm concentration, respectively. Antioxidant activity of *Dryobalanops atomatica* extract showed that ethanol extract display an ability to inhibit ABTS free radical with a percentage of 84.23% at 50 ppm concentration in the leaves extracts, followed by 82.62% percentage of inhibition at 100 ppm concentration. According to the findings of the study, post-distillation solid waste from *Dryobalanops aromatica* leaves and bark had the potential to develop as a natural antioxidant.

Keywords: ABTS; DPPH; *Dryobalanops aromatica*; Kalimantan Timur; Phytochemical.

INTRODUCTION

Kapur (*Dryobalanops aromatica*) is one of the endemic species from East Kalimantan that produces borneol (camphor) in both crystal and oil forms. This plant is also known to contain bioactive compounds that have effects as antifungal, antioxidant, toxic, and capable of inhibiting the spread of HIV (RITONGA et al. 2018; Wibowo et al. 2011). The indigenous population in Kutai Barat Regency continues to depend on species that are today considered uncommon in Indonesia for their livelihoods. According to several investigations, camphor oil of the kapur variety (*Dryobalanops aromatica*) has been described and is suitable for use in aromatherapy (Kamariyah et al. 2012; Kuspradini et al. 2016; Le et al. 2016; Yakubu et al. 2020). Phytochemical screening of methanol extract from the bark of kapur (*Dryobalanops aromatica*) contains flavonoids, saponins, tannins, and coumarin. Borneol in this tree can be applied externally for wounds, burns, rheumatic pain, and skin diseases (Pasaribu et al. 2014). The branches and leaves of this plant are employed orally for gum swelling, cholera, and breast pain. It has been reported that this plant contains terpenoids such as D-borneol, terpinen-4-ol, alpha-terpineol, alphapinene, and caryophyllene, which is known for its antimicrobial, cytotoxic, and anti-inflammatory activities (Ali 2014; Le et al. 2016).

Numerous secondary metabolites, including terpenoids, lignans, flavonoids, saponins, secoiridoids, lactones, and alkaloids, are produced by plants and each have their distinct chemical structures and bioactivities.

The majority of significant pharmaceuticals are made from medicinal plants. With about 40,000 distinct plant species, including about 6000 medicinal plant species, Indonesia is the second-most biodiverse country in the world. As such, plants in Indonesia are predicted to be a great source of bioactive secondary metabolites for developing novel antiviral drugs (Aoki-Utsubo et al., 2023). One of the most physiologically varied groups of Indonesian plants is the Dipterocarpaceae family, which is found all around the nation. Oligostilbenes, phenolic compounds of resveratrol units ranging from monomers to octamers, are abundant in Dipterocarpaceae plants. From Dipterocarpaceae plants, resveratrol and its dimers (like ε-viniferin and laevifonol), trimers (like ampelopsin E, α-viniferin, and Malaysianol), and tetramers (like vaticaphenol B and C, hopeaphenol, and vaticaphenol B) have been identified. Because of their complex structures and wide range of biological functions, oligostilbenes have garnered much attention in recent decades. Nevertheless, little research has been done on their antiviral effectiveness (Abe et al. 2011; Ito 2020; ITO et al. 2000; Mattio et al. 2020).

Antioxidants are known to stop chain reactions to stabilize free radicals. Free radicals become highly reactive compounds in body cells due to the chain reaction caused by unpaired electrons. This can lead to various long-lasting degenerative diseases that can cause tissue or cell damage (Lulan 2022). An antioxidant is a chemical substance that lessens a free radical's oxidation action by giving an electron to an unpaired free radical. Numerous chemicals found in herbs are potent antioxidants in clinical settings and can be employed as natural exogenous antioxidants (Amorati and Valgimigli 2018). Phenolic chemicals, which are secondary metabolites, are among the chemical substances that shield plant organs from oxidation. Consequently, the term "natural antioxidant" refers to the phenolic component. A phenolic molecule found in plants is known to have anti-inflammatory, anti-carcinogenic, anti-microbial, anti-allergic, and anti-mutagenic qualities in addition to its antioxidant action (Johan Sukweenadhi et al. 2020). Flavonoids are among the several phytochemicals with antioxidant properties. Numerous plant species contain flavonoids, which are polyphenolic chemicals that have been shown to support human health. Regular consumption of fruits and vegetables that contain flavonoids can lower the risk of cardiovascular disease. Numerous investigations have shown that flavonoids influence various pharmacological actions, such as enzyme inhibitors, anti-inflammatory, anti-cancer, and antioxidant agents (Ivey et al. 2017; Kim et al. 2018; Kumar et al. 2019).

The strength of the evaluated *Dryobalanops aromatica* extract as a possible source of natural antioxidants for application in pharmaceutical, nutraceutical, and functional food compositions would undoubtedly be investigated with the aid of the current data. To further develop its uses in the food and pharmaceutical industries, more study is necessary to discover and purify the particular molecules that have antioxidant qualities.

MATERIALS AND METHODS

Study area

The raw materials used in this study are the leaves and bark of *Dryobalanops aromatica*, obtained from Nyaribungan Village, Laham District, Mahakam Ulu Regency, East Kalimantan Province. The materials used in this study are DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid), 96% ethanol, acetone, 1-naphthol, bismuth (III) nitrate, H2SO4 (sulfuric acid), HCl (hydrochloric acid), CH3COOH (acetic acid), NaOH, lead acetate (Pb CH3COO2), potassium iodide, ascorbic acid (Vitamin C). The equipment used includes test tubes, Erlenmeyer flasks, glass funnels, beakers, 1000 µL micropipettes, 200 µL micropipettes, yellow tips, blue tips, cuvettes, rotary vacuum evaporators, and UV spectrophotometers. This research was conducted at the Wood Properties and Product Analysis Laboratory (SKAP), Department of Environmental and Forestry, State Agricultural Polytechnic of Samarinda.

Procedures

Maceration

The raw materials, consisting of the leaves and bark of *Dryobalanops aromatica*, are first washed with running water to remove any adhering dirt, then dried in an air-conditioned room at a temperature of 20-25 ºC for 2-5 days until the samples are dry. The dried plant samples are then ground using a blender and are ready for maceration with ethanol solvent at room temperature and shaking on shaker about 48 hours. After filtration,the cride extract of the leaves and bark of *Dryobalanops aromatica* was evaporated using rotary vacuum evaporator at 38-40 ºC.

Preliminary Phytochemical Analysis

The ethanol extract of the leaves and bark of *Dryobalanops aromatica* was analyzed for secondary metabolites, including alkaloids, flavonoids, triterpenoids, steroids, tannins, and saponins, using qualitative testing methods (Sari et al. 2023).

Alkaloids determination: About 5 ml of the ethanol extracts from leaves and bark of *Dryobalanops aromatica* were added of 2 ml Hydrochloride Acid, then 1 ml of Dragendorff solution was added. The color changes in the solution was indicated the presence of alkaloids.

Flavonoids determination: About 1 ml of the ethanol extracts from leaves and bark of *Dryobalanops aromatica* were drops of 1% Sodium Hydroxide. The presence of yellow color at extracts solution and were

colorless after addition of 1 % Hydrochloride Acid was indicated the presence of flavonoids.

Triterpenoids determination: About 1 ml of the ethanol extracts from leaves and bark of *Dryobalanops aromatica* were drops of about 10 Acetic Acid Anhydride and 2 drops of Sulfuric Acid, sequentially. The red or purple color changes in the solution was indicated the presence of triterpenoids.

Steroids determination: About 1 ml of the ethanol extracts from leaves and bark of *Dryobalanops aromatica* were drops about 10 Acetic Acid Anhydride and 2 drops of Sulfuric Acid, sequentially. The green or blue color changes in the solution was indicated the presence of steroids.

Tannins determination: About 10 ml of the ethanol extracts from leaves and bark of *Dryobalanops aromatica* were added 1 % Lead II Acetate. The yellow precipitate reaction at solution was indicated the presence of tannin.

Saponins determination: About 10 ml of hot distilled water were added 1 ml of of the nethanol extracts from leaves and bark of *Dryobalanops aromatica*. The solution then cooled and shaken vigorously (10 seconds). A stable froth upon standing for 10 minutes after added 1 drops of Hydrochloride Acid 2N was indicated the presence of saponins.

Antioxidant Assay of DPPH

Antioxidant testing was conducted with 5 test sample concentrations into 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm. About 3 mg of the extract was weighed, then dissolved in 1 ml of ethanol solvent, and subsequently, a concentration dilution for testing was performed. 33 µl of the sample was placed into a test tube and 467 µl of ethanol was added. After homogenization, 500 µl of the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical solution was added (Sari et al., 2023). The mixing of the test sample is sufficient when it reaches a volume of 1000 µl (1 ml). The test sample was then incubated in a low-light room for 20 minutes at room temperature of 27-30 ºC. Antioxidant activity using DPPH decolorization was measured using a UV-Spectrophotometer at a wavelength of 517 nm. The testing was conducted in the triplicate repetitions. The percentage of DPPH free radicals is calculated using the formula:

$$Inhibitory\ of\ DPPH\ Radical\ (\%) = \frac{\Delta kontrol - \Delta sampel}{\Delta kontrol} \times 100$$

Antioxidant Assay of ABTS

Antioxidant testing was conducted with 5 test sample concentrations into 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm. About 5 mg of extract was weighed, then dissolved in 1 ml of methanol solvent, and then a dilution of the test concentration was performed. 50 µl of the sample was placed into a test tube and 450 µl of ethanol was added. After homogenization, 500 µl of the ABTS free radical solution (2,2-azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid) was added with slight modification (Sari et al. 2024). The mixing of the test sample is completed when it reaches a volume of 1000 µl (1 ml). The test sample was then incubated in a low-light room for 5 minutes at room temperature of 27-30 ºC. Antioxidant activity using ABTS decolorization was measured using a UV-Spectrophotometer at a wavelength of 734 nm. The testing was conducted in the triplicate repetitions. The percentage of DPPH free radicals is calculated using the formula:

$$Inhibitory\ of\ ABTSRadical\ (\%) = \frac{\Delta kontrol - \Delta sampel}{\Delta kontrol} \times 100$$

RESULTS AND DISCUSSION

Plant Extracts

Samples of leaves and bark of the kapur tree (*Dryobalanops aromatica*) have been macerated using ethanol at room temperature (Table 1). The extract is calculated based on the dry plant sample's weight divided by the sample's initial weight.

Table 1. Percentage of ethanol extract from the leaves and bark of kapur (*Dryobalanops aromatica*).

Plants parts	Yield of Extracts (g)	Percentage (%)
Leaves	2.87	9.89
Bark	16.36	19.05

Note: Percentage is calculated based on the weight of the dry sample.

Based on the results obtained, the weight of bark parts is 16.36 grams from a total of 85.91 grams of the extracted sample. Meanwhile, the extract of leaves parts weight is 2.87 grams from a total of 29.03 grams of the extracted sample. This also affect the yield produced by the samples, where the leaves parts have a yield of 9.89% and the bark parts has a yield of 19.05%.

A solvent with a high extraction rate, ethanol is readily available, effective, and safe for the environment (Chen, Xiao, and Pang 2020; Fan et al. 2020; Hakim and Saputri 2020). Ethanol is a polar, multipurpose solvent that works well for first extraction. The ability of ethanol solvent to permeate cell wall components allows it to perform cell diffusion and more quickly draw in bioactive substances (Prayitno and Rahim 2020; Yulianti et al. 2021).

Phytochemical Analysis

Ethanol extracts from the leaves and bark of the kapur tree (*Dryobalanops aromatica*) showed the presence of secondary metabolite compounds as presented in Table 2.

Alkaloids, phenolics, flavonoids, tannins, quinones, saponins, and terpenoids are among the chemical

components contributing to bioactivity as medicinal ingredients. Alkaloids serve as plant defenses against pathogens and herbivores as well as stimulants of the central nervous system, while tannins have been used as antidiarrheals and antihemorrhagic. Phenolics and flavonoids are classified as antioxidants and have been shown to have numerous health benefits, as well as the ability to prevent and cure several diseases (Mousavi, Salleh, and Murugaiyah 2018; Praptiwi et al. 2020; Tungmunnithum et al. 2018).

Table 2. Phytochemical analysis of ethanol extract from the leaves and bark of kapur (*Dryobalanops aromatica*).

No	Compounds	Presences	
		Leaves	Bark
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Triterpenoids	-	+
4	Tannin	+	+
5	Saponins	+	-
6	Steroids	+	-

Antioxidant Test using the DPPH Method

The ability of the ethanol extract of the leaves and bark of the kapur tree (*Dryobalanops aromatica*) to inhibit DPPH free radicals was demonstrated by antioxidant testing; the percentages of inhibition for the leaves and bark ranged from 27.30 to 83.24% and 82.32 to 94.91%, respectively. At a dosage of 25 ppm, the greatest inhibition was seen in the leaves and bark ethanol extracts, with percentages of 83.24% and 94.91%, respectively (Figure 1).

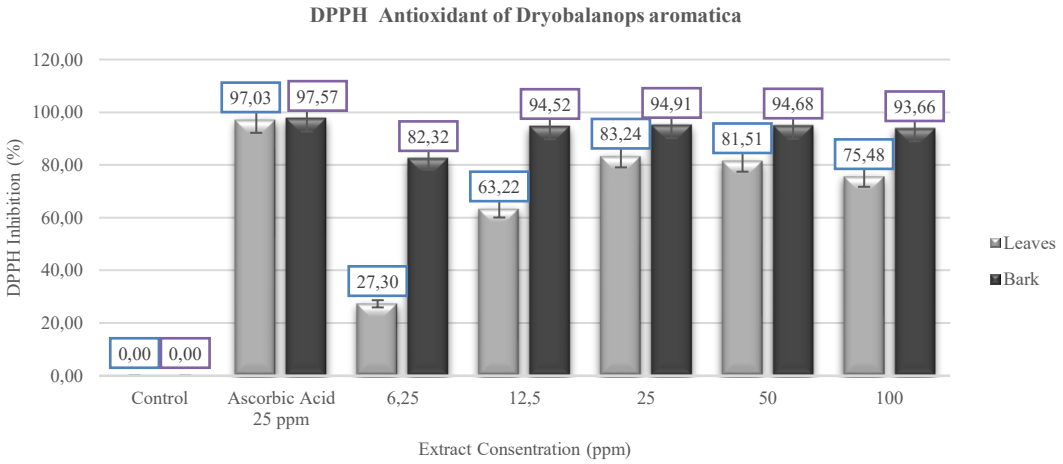

Figure 1. Inhibitory activity of ethanol extract of leaves and bark of the kapur tree (*Dryobalanops aromatica*) against DPPH free radicals.

Studies on the active ingredients in *Dryobalanops sp.* plant essential oils have revealed that α-pinene compounds and monoterpene derivatives may operate as natural antioxidants (Aswandi and Kholibrina 2020). Antioxidant qualities are known to be present in phenolic compounds. The oxidative activities of these substances are typically exhibited by many modes of action, such as metal ion chelation, hydrogen-donating antioxidant, free radical scavenger, and singlet oxygen quencher (Che Rozenan et al. 2021). This plant's potential for development as a natural antioxidant that can suppress DPPH free radicals is further demonstrated by alkaloids, flavonoids, and tannins in this plant extract.

Antioxidant test using ABTS Method

Inhibition percentages for the ethanol extract leaves and bark of the kapur tree (*Dryobalanops aromatica*) were from 27.84 to 81.62% in the leaves and 11.31 to 82.62% in the bark, respectively, indicating the extract's capacity to inhibit ABTS free radicals. The highest inhibition was observed in the leaves part of ethanol extracts with 84.23% at 50 ppm concentration, followed by the bark parts at 100 ppm concentration with a percentage of 82.62% inhibition (Figure 2).

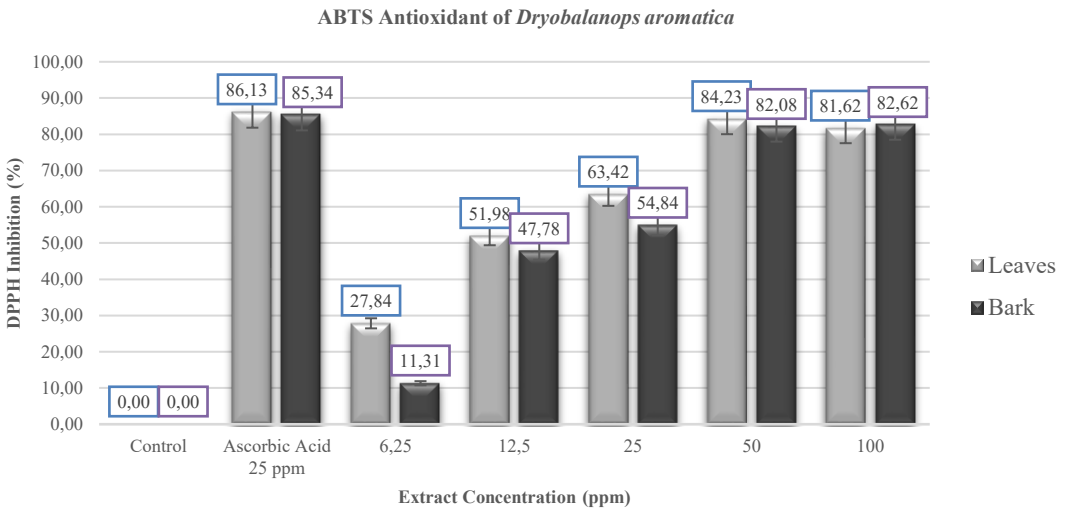

Figure 2. Inhibitory activity of ethanol extract of leaves and bark of the kapur tree (*Dryobalanops aromatica*) against ABTS free radicals.

Based on previous study, the crude extract of methanol from the leaves and bark of *Dryobalanops aromatica* showed the highest antioxidant activity with a percentage of 93.86% and 93.77%, respectively. The leaves and bark of *Dryobalanops aromatica* also have the highest ability in total phenolic content by 1084.13 and 972.71 GAE (Che Rozenan et al. 2021). *Dryobalanops aromatica* genus was reported to be a source of resveratrol oligimers such as trans-3,4',5-trihydroxystilbene and bergenin, which has many bioactive benefits such as antioxidants and antidiabetic activities (Wibowo et al. 2012, 2014). Research about GCMS from methanol extract of *Dryobalanops aromatica* found five similiar compounds such as performic acid, acetic acid, decane, glycerine and propanoic acid (Chang and Othman 2014). Meanwhile, these compounds suggested that both leaves and bark extracts contributed to the antioxidant and antidiabetic activities of *Dryobalanops aromatica* (Heinonen-Tanski and Miettinen 2010).

CONCLUSIONS

One of the endemics of East Kalimantan, Dryobalanops aromatica, belongs to the Dipterocarpaceae genus and has been researched for its antioxidant properties. Using DPPH and ABTS free radicals, the study's findings demonstrated that post-distillation solid waste from the leaves and bark extracted with ethanol gave useful information on the amount of active chemicals and antioxidants. This genus still requires much research, particularly to identify the active ingredients in Dryobalanops aromatica. The leaves and bark of Dryobalanops aromatica can be extracted and used as a natural antioxidant in the medical field, according to the study's findings.

Acknowledgements: This research was funded by the Samarinda State Agricultural Polytechnic (Politani Samarinda) through Penelitian Terapan Dosen Scheme (Lecturer Research Internal Grans) 2024 under contract number 141/PL21.G/PG/2024. We would also like to thank all parties involved in this research, who have been willing to provide information and plant specimens for research.

Authors' Contributions: In this study, Nur Maulida Sari designed the study, supervised all processes, and wrote the manuscript. Abdul Rasyid Zarta and Heriad Daud Salusu conducted observations and sample collection. Muhammad Fikri Hernandi supervised the samples and material preparation. Mohammad Ridho Ramadhan, Nur Indah Pragaloka, Rosalinda Kerawing and Pustitasari Muis supervised the research data analysis and manuscript. Siti Raihanah and Jasmawati supervised the research data analysis and manuscript writing. Farida Aryani supervised all research data analysis and manuscript writing.

Competing Interests: There is no conflict of interest in this research.

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