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Comparison of Antioxidant Activity of Stem Bark, Stem Wood, and Leaf Extracts of Bayur Plant (*Pterospermum Subpeltatum* C.B.Rob)

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Abstract: Antioxidants play a role in preventing and repairing damage to human cells. Searches for antioxidant sources continue to be carried out including in *P. subpeltatum* plants. This study aims to compare the antioxidant activity of the bark, wood, and leaves of *P. subpeltatum* C.B. Rob plants. The materials were dried and then shaved to a fineness level of 80 mesh. Materials were extracted using the maceration method with ethanol solvent. The macerate was filtered and the filtrate obtained was concentrated with an evaporator. The three extracts were tested for antioxidants using the DPPH (1,1-diphenyl-2-picrylhydrazyl) Free radical scavengers method, ascorbic acid was used as a comparison. Absorbances was measured using UV-Vis spectroscopy. Each material was made in 5 concentrations. The IC_{50} value was calculated using the Blois regression equation. The results of the antioxidant test were obtained consecutively: stem wood with an IC_{50} value = 3.39 µg/mL, stem bark = 5.15 µg/mL, and leaf = 258.58 µg/mL, the IC_{50} value of ascorbic acid was 2.79 µg/mL. Based on these data, it shows that the antioxidant activity of stem wood and stem bark is in the high category, although still lower than ascorbic acid, while the leaf are included in the weak category.

Keywords: Antioxidant; Bayur; *P. subpeltatum*

Introduction

State People in developing countries, more than 80% utilize plants for medicinal purposes, and even 88% of the world's population today rely on traditional medicine (Marbun et al., 2024; Usman, 2012). Indonesia's diverse tropical plants are a potential source of wealth. Considering the potential of medicinal plants to play a major role in maintaining public health conditions from generation to generation (Valentino et al., 2022). In Indonesia, there are 31,750 plants, about 15,000 species of which have potential as drugs. Medicinal raw materials from plants have been found in around 7,000 species (Retnowati et al., 2019). The utilization of plants as raw materials for the drug industry has only reached 200 species (Susidarti, 2017).

The content of secondary metabolites is influenced by environmental factors such as light, temperature, water availability and soil fertility (Hafizah et al., 2024). Secondary metabolites obtained from plants are natural chemical compound products with diverse bioactivities: anti-inflammatory, antineoplasm, antioxidant (Zhang et al., 2023), antifungal (Hendra et al., 2024), and insecticide. Chemical compounds produced by plants are used for protection (Rumalolas et al., 2023) and defend themselves from climatic conditions and pest attacks (Achmad et al., 2013).

Pterospermum plant is a member of the Malvaceae family which is generally used as a traditional medicine

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(Paliwang et al., 2024). *Pterospermum subpeltatum* known as bayur is a tropical plant native to Indonesia that is widely distributed in Sumatera, Java, Kalimantan, and Sulawesi. This plant's height can reach 45 m with a trunk diameter of up to 120 cm so that it can be used as building material (Latif et al., 1997) and includes commercial-type plants (Hidayat, 2014). This plant's cellulose and hemicellulose content makes it a raw material for paper. According to Lemmens et al. (1995), the bark contains tannin, which is yellow and has the efficacy of treating the disease.

Pterospermum acerifolium is used as an itch medicine in Central Sulawesi. The leaves are heated to cure warts on the feet, and the leaf laceration is applied to the wounded body part. Its bark ash water is drunk to treat gonorrhea (Heyne, 1987). In India, the bark of *P. acerifolium* is used for wound medicine, anti-inflammation, ulcer medicine, antitumor, leprosy, and smallpox (Khare, 2007). The bark of *P. javanicum* can treat ulcers, diarrhea, toothache, and sprains. The root bark of *P. diversifolium* is used to poison fish, and the leaves for fish itch (Ogata, 1995). The people of Majene in West Sulawesi use the bark and stem wood slices of *P. subpeltatum* C.B. Rob as wound medicine. Salempa (2012) has isolated 2, 3, 23 - trihydroxy - 12 - oleanen - 28 - oat (45), which is active as an antibacterial from the root wood of *P. subpeltatum*.

Ethanol extract from *P. acerifolium* bark is an antioxidant and an essential factor in wound healing (Manna et al., 2009). *P. acerifolium* has antioxidant activity and contains phenolic, polyphenolic, and flavonoid compounds in its leaf methanol extract and bark ethyl acetate extract (Sannigrahi et al., 2010). According to Jaiganesh et al. (2011), preliminary phytochemical tests and antimicrobial potential of ethanol extracts of *P. canescens* Roxb leaves contain phenolic compounds that are active as antimicrobials and antioxidants. Research by Sapri (2011) showed that the methanol extract of *P. celebicum* Miq stems wood has potent antioxidant activity with an IC_{50} value of 180 ppm.

Methanol extract of *P. acerifolium* bark contains polyphenol compounds as antioxidants 92.02% (Saefudin et al., 2013). Antioxidant activity of the methanol fraction of *P. acerifolium* petiole shows 95% absorbance at a concentration of 100 ppm (Uddin et al., 2014). Deshwal et al. (2021) obtained an IC_{50} value of 9,329 µg/mL (firm) in the antioxidant test of n-hexane extract of *P. acerifolium* fruit seeds. The methanol extract of *P. acerifolium* flowers (Mazumder et al., 2011) states that it contains antioxidants and antihyperlipidemics tested on *W. albino*.

Alkaloid, flavonoid, phenolic, and steroid group compounds are found in chloroform and ethanol extracts of *P. canescens* Roxb leaves, which have antimicrobial activity (Jaiganesh et al., 2011). Methanol extract of stem wood respectively produced antioxidant activity of *P. javanicum* by 92.02%, *P. diversifolium* by 90.73%, and *P. celebicum* by 89.53% (Saefudin et al., 2013). Antioxidant activity was also found in *P. celebicum* wood with IC_{50} values of methanol extract 263 ppm, chloroform 240.95 ppm, ethyl acetate 172.9 ppm, and n-hexane 277.5 ppm (Sapri, 2011). *P. javacicum* stem bark contains flavonoid-derived compounds in ethanol extracts and has potent antioxidant power with an IC_{50} value of 76.87 ppm (Hapid, 2023).

Basically, free radicals are chemical molecules that do not have a free electron pair in their outermost orbital. This compound is very reactive so that it easily reacts with various organic chemicals. Antioxidant compounds have the ability to inhibit oxidation reactions of metabolic processes in living things (Nugrahani et al., 2020). Various studies of antioxidant activity that have been conducted on the genus *Pterospermum*, it turns out that there is no *P. subpeltatum* species.

Based on the ethnobotanical description of the genus Pterospermum, this study was conducted to determine the antioxidant power of the tissue of one of its species, *P. subpeltatum*, which the people of Majene have been using for building construction.

Method

Sample Preparation

Samples were obtained in Galung Village, Banggae District, Majene Regency, West Sulawesi, and identified at the Indonesian Institute of Sciences, Bogoriense Herbarium, Biology Research Center.

Materials

The *P. subpeltatum* parts studied were stem bark, stem wood, and leaf tissues. Using ethanol solvent, 1.5% cerium sulfate in 2N sulfuric acid, thin layer chromatography (TLC) with Merck Kiesegel 60 F254 0.25 mm Si gel coated plate, 2,2- diphenyl-1-picrylhydrazyl (DPPH) for antioxidant test (Lavlinesia et al., 2023), ascorbic acid as a comparator (Kumaradewi et al., 2021).

The tools used are distillation equipment, chamber, vacuum funnel, capillary tube, analytical balance, oven, evaporator, UV-visible spectrophotometry, and chromatography plate.

Procedure

Samples of stem bark, stem wood, and leaf tissue were dried at room temperature for five days, not exposed to direct sunlight. The dried samples were shaved, and the results were dried for two days. Furthermore, it was pulverized by grinding using a

grinding machine with a fineness level of 80 mesh. Each was extracted using the maceration method with ethanol solvent for 3x24 hours. The macerate was filtered, and the filtrate was concentrated by evaporation to obtain the total extract and then tested for antioxidant activity.

Extraction and chromatography using ethanol p.a. and technical solvents. Analysis of the separation of sample components using a TLC plate with cerium sulfate stain solution. DPPH for antioxidant test and ascorbic acid solution as a comparison. Each ethanol extract of stem bark, stem wood, and leaves of *P. subpeltatum* was tested for antioxidant activity. The IC_{50} value of each ethanol extract was calculated using a linear regression equation.

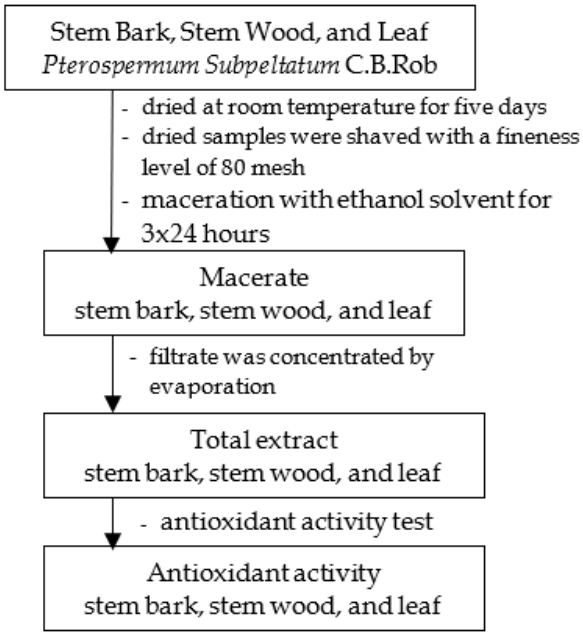

Figure 1. Procedure extraction and antioxidant test

DDPH absorption wavelength optimization was carried out by mixing 1000 µl ethanol p.a with 1000 µl DPPH solution of 100 µg/ml concentration in a test tube. The solution mixture was added with ethanol p.a. solvent until the volume reached 4 ml and homogenized. Then incubated for 30 minutes at 37 °C. Determination of the absorption spectrum of the solution using a UV-visible spectrophotometer.

The test series solution was prepared from 1000 µl extracts in five concentrations of 1, 2, 3, 4, and 5 µg/ml, each placed in five test tubes. Each concentration was added with 1000 µl of 100 µg/ml DPPH solution. The mixture was homogenized by shaking, which was then incubated for 30 minutes at 37 °C. After incubation, the absorption wavelength of the test solution was measured. Determination of antioxidant activity was done by Inhibition Concentration 50% (IC_{50}). IC_{50} is the concentration of sample solution required to reduce 50% of DPPH free radicals. The percentage of inhibition obtained at each concentration was calculated with the linear regression equation y = A + Bx.

Result and Discussion

According to the Blois method, all isolated extracts from the three tissues of *P. subpeltatum* were tested for antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method. UV-visible spectrophotometer (400-800 nm) was used to determine the optimum wavelength with λmax of 500 nm. Determination of the percent inhibition of DPPH using the formula:

$$\text{Antioxidant activity}(\%) = \frac{[\text{blank}]-[\text{sample}]}{[\text{blank}]}\ x\ 100\% \quad (1)$$

Table 1. Antioxidant Activity Test Results of Ethanol Extracts of Stem Bark, Stem Wood, and Leaves of *P. subpeltatum* C.B.Rob

Concentration (µg/mL)	Absorbance (A) λ = 500 nm	Antioxidant activity (%)	IC50 value (µg/mL)
Stem Bark			
1	0.263	2.23	
2	0.228	15.24	
3	0.205	23.79	5.14
4	0.174	35.32	
5	0.135	49.81	
Blank	0.269		
Stem Wood			
1	0.197	1.99	
2	0.138	31.34	
3	0.103	48.76	3.39
4	0.075	62.69	
5	0.057	71.64	
Blank	0.201		
Leaf			
1	1.020	10.53	
2	0.990	13.16	
3	0.950	16.67	258.58
4	0.925	18.86	
5	0.965	15.35	
Blank	0.269		
Ascorbic Acid (control)			
1	0.636	47.87	
2	0.700	42.62	
3	0.636	47.87	2.79
4	0.550	54.92	
5	0.480	60.66	
Blank	1.220		

Data from the measurement of antioxidant activity of the three *P. subpeltatum* tissues were processed using Microsoft Excel 2013 software to obtain a linear regression graph of the relationship between antioxidant activity and concentration.

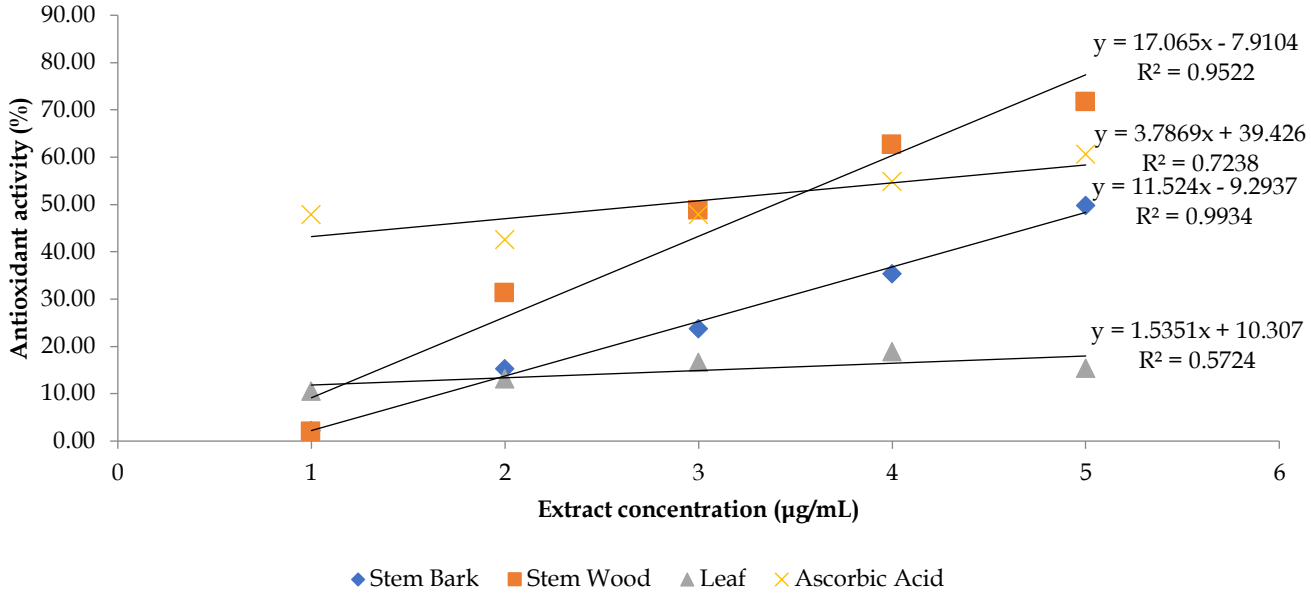

Figure 2. The relationship between extract concentration and antioxidant activity of three tissues of *P. subpeltatum*

According to the UV-visible spectrophotometer measurement results, each addition of extract sample concentration showed an increase in antioxidant activity. The antioxidant activity test results of *P. subpeltatum* stem bark, stem wood, and leaf extracts showed that stem bark (5.14 µg/mL) and stem wood (3.39 µg/mL) extracts are potent antioxidants because they have IC_{50} values of less than 50 µg/mL. In contrast, leaf tissue has an IC_{50} value of 258.58 µg/mL with a weak category. Although stem bark and wood have strong antioxidant activity, the IC_{50} value is still higher than the ascorbic acid. The test material sample is a crude extract with many compound components. The weak antioxidant activity of *P. subpeltatum* leaves followed the results of Rahmawati (2016) research conducted on the same tissue of *P. javanicum* Jungh, which amounted to 199.22 µg/mL.

Table 2. Antioxidant Strength Level (Jun et al., 2003)

IC_{50} Value (µg/mL)	Strength Level
<50	Strong
50-100	Active
101-250	Moderate
251-500	Weak
>500	Inactive

Pterospermum generally contains flavonoid compounds, including *P. Javanicum* (Rahmawati et al., 2016) leaf have an IC_{50} of 62.438 ppm (strong category) (Hapid, 2023), *P. celebicum* (Marzuki et al., 2016), and *P. acerifolium* (Saboo et al., 2022). Flavonoid compounds are derivatives of phenolic compounds that are found in many *P. subpeltatum* plants. The ability of flavonoids to donate hydrogen ions can reduce free radicals (Rheda, 2010).

Based on the content of flavonoid compounds in *Pterospermum*, it is estimated that *P. subpeltatum* also contains flavonoid compounds, which cause it to have strong antioxidant power in the stem bark and stem wood tissues, while in the leaves with weak antioxidant power due to the lack of phenol compounds.

Conclusion

Antioxidants play an important role in health. Searching for antioxidant sources needs to be done in various plants, including *P. subpeltatum* plants in the wood tissue of the stem wood, stem bark, and leaf. Comparison of antioxidants obtained from the three tissues of the *P. subpeltatum* plant in succession obtained: stem wood with an IC_{50} value = 3.39 µg/mL (strong category), stem bark = 5.15 µg/mL (strong category), and leaf = 258.58 µg/mL (weak category). Further research is needed on antioxidant tests of extract fractions and purification to find compounds in the bark and wood stem that cause strong antioxidant activity.

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Author Contributions

This research was technically conducted in the laboratory by Zakaria, responsible for writing the draft manuscript, and completing the manuscript and publication. Nunuk Hariani Soekamto was responsible for conceptualization. Suriani Nur

was responsible for research methodology. Ayun Dwi Astuti was responsible for reviewing the use of terms and language.

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Conflicts of Interest
The authors declare no conflict of interest.

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