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INVESTIGATION OF *Melaleuca leucadendron* L. FROM SOUTH SUMATRA: Phytochemicals and IC50-BASED ANTIOXIDANT POTENTIAL

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ABSTRACT

Melaleuca leucadendron L. (commonly known as Gelam wood) is a plant that thrives in the swampy regions of South Sumatra. The utilization of this plant has historically been limited to its wood, leaving the potential of its leaves and seeds largely unexplored. Therefore, this study aimed to identify the species of Gelam wood and investigate the phytochemical content and antioxidant potential of its leaves and seeds.The methodology involved methanol extraction of the plant material, followed by comprehensive phytochemical screening and an antioxidant activity assay using the DPPH method.The phytochemical analysis confirmed the presence of several key compounds, with the Gelam extracts testing positive for Alkaloids, Flavonoids, Steroids, Saponins, Tannins, and Triterpenoids. Furthermore, the extract from the leaves and seeds demonstrated potent antioxidant activity, evidenced by an IC50 value of 47.56 ppm, which classifies it as a strong antioxidant (<50 ppm).This research highlights the significant therapeutic potential of *Melaleuca leucadendron* leaves and seeds, suggesting they are valuable sources of natural antioxidants.

Introduction

Exploration into the potential of plant resources within Indonesia continues actively to this day. This potential is strongly supported by the country's rich biodiversity, which means that every plant species in a given region possesses unique characteristics and harbors many undiscovered benefits (Setiawan, 2022). Nowadays, natural antioxidants of plant is a focus as alternatives to synthetic counterparts that is for improving oxidation stability (Lankanaya A, 2025). Often, these valuable plants grow right in our surroundings without their potential being fully realized. One such plant commonly used by the people of South Sumatra primarily for its timber, yet whose full benefits are unknown, is Gelam wood (*Melaleuca leucadendron*). Gelam wood is easily found in swampy regions (Basyaruddin et al., 2019; Istanto & Saputra, 2013). Its presence is significant in South Sumatra due to the ideal habitat for its growth Currently, Gelam wood in South Sumatra is exploited mainly for its high-

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quality timber, while its leaves and seeds have not been widely utilized (Pramono, 2016). Therefore, successfully utilizing the leaves and seeds of Gelam wood would enhance the plant's economic value and foster new economic activities for local communities.

Gelam wood is recognized as belonging to the same family as Cajeput wood (*Melaleuca cajuputi*), a plant famously utilized in the production of essential oils (Aryani, 2020). While the essential oil derived from the cajeput tree has been well-known and used by communities for a long time, the potential of the closely related Gelam tree has not yet been fully explored. To address this gap and determine the potential of Gelam wood, particularly in its leaves and seeds, this research was specifically conducted to investigate the phytochemical content and antioxidant capacity of methanol extracts derived from these parts of the Gelam plant.

Materian and Methods

Extraction Method

The extraction was performed using the maceration method. A 500 gram sample of the dried powder was submerged in ethanol at a 1:1 ratio (powder:solvent). This mixture was allowed to stand for 72 hours in a cool place, protected from direct light, with stirring performed every 3 hours for 5 minutes throughout the period. After 72 hours, the resulting macerate was filtered using a funnel lined with Whatman No. 1 filter paper. The filtrate was collected and subjected to rotary evaporation at a temperature of 55 ∘C to separate the solvent from the crude extract. Finally, the remaining extract was concentrated further using a water bath to yield a viscous extract (Hakim et al., 2019).

Phytochemical Screening of Leaf and Seed Simplicia of *Melaleuca leucadendron*

Tannin testing was initiated by dissolving 5 g of the dried leaf and seed material (simplicia) in a beaker containing 20 mL of distilled water (a q u a d e s), followed by five minutes of heating and subsequent filtration. The resulting filtrate was then treated with three drops of 10% Iron(III) Chloride ($FeCl_3$) solution, with a positive result indicated by the formation of a blackish-green color. The Saponin test used the same initial preparation; however, the filtrate was transferred to a tightly capped test tube and shaken vigorously. The test was considered positive if a stable foam (emulsion) was produced.

For the Flavonoid test, 5 g of the dried leaf and seed material (simplicia) was dissolved in a beaker containing 20 mL of distilled water (a q u a d e s). After heating for five minutes, the resulting filtrate was transferred to a test tube, followed by the addition of Magnesium powder, a 1:1 mixture of HCl (hydrochloric acid) and EtOH (ethanol), and finally, amyl alcohol. A positive result was indicated if an orange layer formed in the amyl alcohol phase. Conversely, for the Steroid test, 1 g of the sample was extracted with hot EtOH and filtered. The filtrate was heated to dryness, and the residue was treated with 1 mL of diethyl ether, one drop of concentrated H_2SO_4 (sulfuric acid), and one drop of concentrated anhydrous CH_3COOH (acetic acid). The test was considered positive upon the formation of a green or blue color. For the Triterpenoid test, 1 g of the sample was placed in a test tube, extracted with hot EtOH (ethanol), and filtered. The filtrate was evaporated to dryness, and the residue was then treated with 1 mL of diethyl ether. This solution was subsequently treated with one drop of H2SO4 (sulfuric acid) and one drop of concentrated CH_3COOH (acetic acid). A positive result was indicated by the formation of a red or purple color. Separately, the Alkaloid test was initiated by grinding 1 g of the sample in a mortar with a few drops of NH_3 (ammonia). The resulting paste was extracted with 5 mL of $CHCl_3$ (trichloromethane) and filtered. The filtrate was mixed with 2 M H_2SO_4 and shaken regularly, then allowed to settle

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until three distinct layers formed. The test was considered positive if the following precipitates were observed in the first layer: a reddish-brown precipitate with Wagner's reagent.

Antioxidant Activity Assay

The assay began with the preparation of a 40 ppm DPPH solution by dissolving 0.01 g of DPPH in a 250 mL volumetric flask using methanol, which was then kept cold and protected from light. The solution's maximum wavelength (λmax) was determined across 490−534 nm using methanol as a blank. For the radical scavenging activity determination, 2 mg of the methanol extract was used to prepare test solutions at six concentrations ranging from 3.125 to 100 ppm. A quercetin standard was prepared at the same concentrations. 1 mL of each test solution was mixed with 2 mL of the 40 ppm DPPH solution and incubated for 30 minutes at room temperature. Absorbance was subsequently measured at 517 nm using a UV-Vis spectrophotometer. The experiment included two measurements and three replicates for each sample, after which the IC50 value—the concentration required to inhibit 50% of DPPH activity—was calculated using the regression equation, where a smaller IC50 value indicates higher antioxidant activity (Salazar-Aranda et al., 2011).

Research Location and Time

The determination test was conducted at the Biology Laboratory, Andalas University, while phytochemical testing was carried out at the Pharmacy Laboratory, Kader Bangsa University. Subsequently, the antioxidant activity analysis was performed at the Biology Laboratory, Sriwijaya University. The sample was collected from swamp located within Banyuasin regency. This research was completed in 2022.

Experimental Design

The research design employed an experimental design, and the sampling method used was simple random sampling.

Research Methods and Parameters

The parameter for phytochemicals testing following the previous method and describe in table 1.

Table 1. Phytochemical parameters for positive result

Ingredients of Phytochemical Compounds	Interpretation	Reference
Alkaloids	Formed a brown precipitate	Formed a brown precipitate with Dragendroff reagent (Kopon et al., 2020)
Flavonoids	A red color is formed on the layer	Red, yellow or orange color is formed (Kopon et al., 2020)
Saponins	Formed froth that persisted even though HCl 2 N was added (foam did not disappear)	Stable froth formed even though 2N HCl was added (foam did not disappear) (Kopon et al., 2020)
Tannins	Formed a black green color	Formed a black-green color (Kopon et al., 2020)
Steroids	Blackish green formed	Blackish green color is formed (Putra et al., 2016)
Triterpenoids	Blackish purple formed	Black purple color is formed (Putra et al., 2016)

Antioxidant capacity is quantified by measuring the decrease in absorbance of the DPPH solution after the sample is added. This change is calculated as the percentage of inhibition (% Inhibition) by comparing the absorbance before and after adding the extract. The resulting inhibition percentages are then plotted against the corresponding extract concentrations (ppm) to generate a linear regression equation in

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the form of Y=a+bX, where X is the concentration (X-axis) and Y is the percentage of inhibition (Y-axis). The crucial IC50 value is determined from this equation by calculating the extract concentration (X) required to achieve a 50% inhibition (Y=50), serving as the standard metric for expressing antioxidant strength (Salazar-Aranda et al., 2011).

Data Analysis

The phytochemical analysis was interpreted qualitatively, whereas antioxidant activity results were subjected to linear regression analysis to determine the IC50 value.

Results and Discussion

Results

The leaves of Gelam wood (*Melaleuca leucadendra* (L.) L.) are typically oblong or lanceolate (4.5 cm to 15 cm long), possess a hairy texture ranging in color from green to brownish, and emit a distinct cajeput oil fragrance when crushed. The plant produces compound spike inflorescences with bell-shaped, white flowers that emerge at the branch tips, leading to small, light-to-dark brown fruit (2.5 mm to 4 mm wide, as shown in Figure 1)

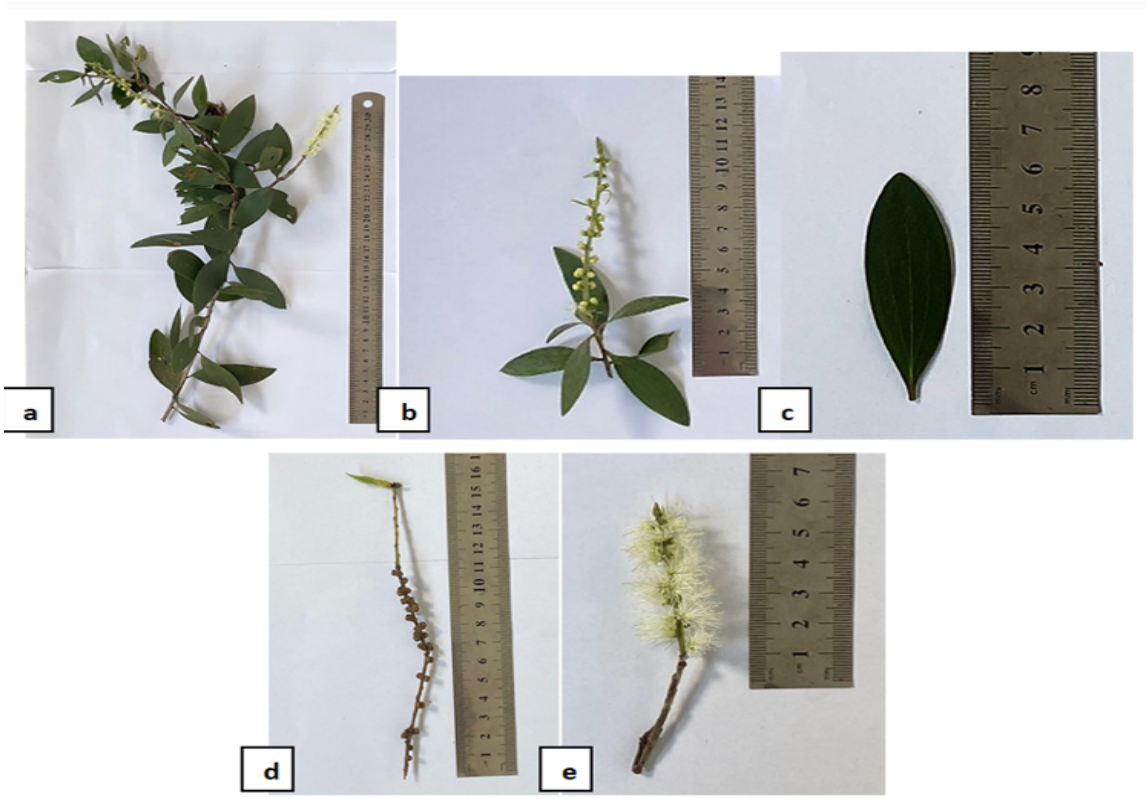

Figure 1. Morphology of Gelam Wood (*Melaleuca leucadendra* (L.) a) Leaf Petiole and Inflorescence (Flower Spike) (b) Immature Fruit/Seed Capsule (c) Leaf(d) Mature Fruit/Seed Capsule (e) Flower

The identification of Gelam wood's content began with extraction. The leaves and seeds were extracted using the maceration method with methanol as the solvent. The maceration was performed at room temperature in the dark for a total period of 3×24 hours (72 hours). The resulting crude extract from the leaves and seeds was a

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viscous material with a blackish-green color and a distinct cajeput oil-like fragrance. From an initial sample of 400 g of dried leaf and seed powder macerated in 12 L of methanol, the final evaporation yielded 154 g of concentrated extract.

Table 2. Results of Phytochemical Compound Identification in Gelam Wood Leaf and Seed Extracts

Bioactive compound	Reagent	Inference
Alkaloid	Wagner	+
Flavonoid	Shinoda's test	+
Saponin	Foam test	+
Tanin	FeCl3	+
Steroid	Liebermann-Burchard's test	+
Triterpenoid	Liebermann-Burchard's test	+

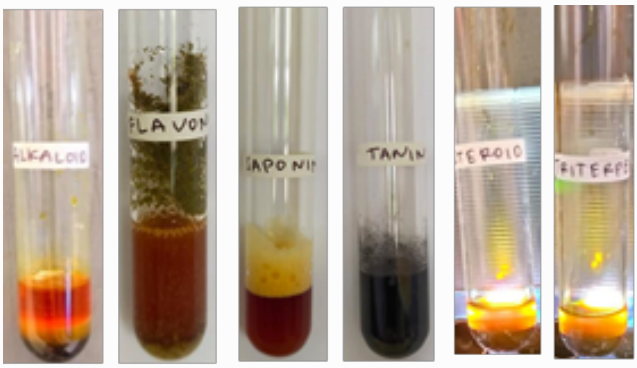

Figure 2: Phytochemical Screening Results. Showing positive identification for Alkaloids (A), Flavonoids (B), Saponins (C), Tannins (D), Steroids (E), and Triterpenoids (F).

The antioxidant activity of the Gelam wood leaf and seed extracts was measured using the widely favored DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which is simple, fast, and requires minimal sample (Safitri et al., 2020). The method's principle is based on the DPPH radical's ability to accept a hydrogen atom donated by an antioxidant compound. This reaction is monitored spectrophotometrically at 517 nm (the maximum absorption wavelength of DPPH) by observing the decrease in absorbance as the solution changes color from violet to the more stable, yellow DPPH-Hydrazine form; consequently, the intensity of the color change is directly proportional to the extract's antioxidant capacity (Molyneux, 2003).

Table 3. Percentage Inhibition of Quercetin

	Concentration (µg/mL)	Ln Concentration	% Inhibition		
			1	2	3
A	100	4.6052	86.269	86.008	86.269
B	50	3.9120	85.601	85.630	85.630
C	25	3.2189	85.281	85.281	85.223
D	12.5	2.5257	84.176	84.496	84.002
E	6.25	1.8326	82.461	82.955	83.014
F	3.125	1.1394	82.112	81.415	82.171

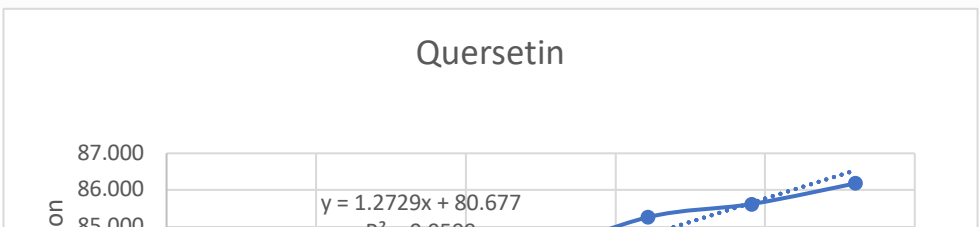

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Figure 3: Calibration Curve Showing DPPH Absorbance vs. Quercetin Concentration
The IC50 calculation results are exponential -24.10009 is 3.42 ppm

Tabel 4. Percentage of inhibition of antioxidant activity of Gelam Wood (leaves-seeds extract)

	Concentration (µg/mL)	Ln Concentration	% Inhibition		
			1	2	3
A	1000	6.9078	87.694	75.581	78.779
B	500	6.2146	78.682	61.047	65.698
C	250	5.5215	65.310	55.814	57.849
D	125	4.8283	57.461	47.384	44.186
E	62.5	4.1352	54.554	40.116	35.756
F	31.25	3.4420	48.740	37.791	37.151

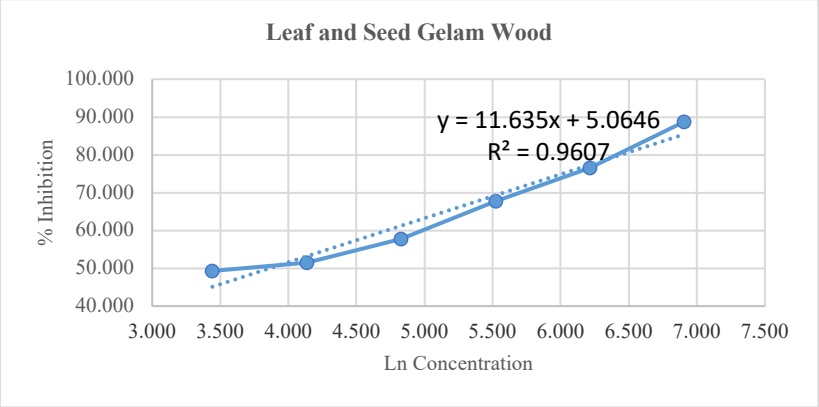

Figure 4: DPPH Absorbance vs. Concentration Graph for Gelam Wood Leaf and Seed ExtractsThe IC50 calculation results are as x = 3.862 exponential 3.862 is 47.565 ppm

Discussion

This resilient species thrives extensively in peat swamp forests, particularly well in eastern Indonesia and northern Australia, though it is also cultivated elsewhere with clear dry seasons. It is commonly found in barren soils with a pH of 5.6, is heat-tolerant, and can resprout after fire, growing from sea level up to 400 meters above sea level near beaches, behind mangrove forests, or in various wet or dry swampy lands (Supriyati et al., 2014). The comprehensive results of the phytochemical identification are presented

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in Table 2. The findings from the preliminary tests demonstrate that the methanol extract of the Gelam wood leaves and seeds contains alkaloid compounds. Alkaloids are significant secondary metabolites widely distributed across nature, found not only in plants but also in fungi and animals (Gwaltney-Brant et al., 2012; Maisarah et al., 2023). In plants, these compounds primarily function as toxic agents to deter insects that might otherwise harm the organism (Matsuura & Fett-Neto, 2015). In the field of pharmacology, alkaloids are highly valued for their therapeutic properties, serving as potent agents for anesthesia, cardioprotection, and anti-inflammation (Kurek, 2019). Furthermore, alkaloids are frequently utilized in pharmaceutical applications, including the development of various medicines and even some toxins (Roy, 2017).

The flavonoid phytochemical test on the Gelam wood leaf and seed extract yielded a positive result, evidenced by the formation of a red color. Flavonoids, known for their antibiotic properties, are functional in treating liver disorders and can act as hepatoprotectors by inhibiting prostaglandin synthesis (Dwika et al., 2016). Similarly, the phytochemical test for saponins was also positive, demonstrated by the presence of stable foam during the experiment. Saponins are essential antimicrobial agents whose concentration in plants is affected by variety and growth stage, with the highest levels typically found in the parenchymal cells of vegetative parts (Faizal & Geelen, 2013). aponins promote regeneration and re-epithelialization by supporting the host's immune response. In pharmaceutical applications, they are valued as anticarcinogenic and immunostimulant agents, with further uses spanning agriculture, cosmetics, and food production(Moghimipour & Handali, 2015).

The tannin phytochemical test yielded a positive result, evidenced by the formation of a blackish-green color resulting from the reaction between the phenolic hydroxyl groups in tannins and $FeCl_3$ (Iron(III) Chloride) to form an Fe^{3+} complex; tannins are recognized as pharmacologically active components (Oyetayo, 2007). The steroid phytochemical test was also positive, showing a green color that confirms the presence of steroid compounds; these compounds are vital for controlling plant growth and are used in medicine as detoxifying agents (Dwika et al., 2016). Finally, the triterpenoid phytochemical test was confirmed by a color change to purple-black, indicating the presence of triterpenoids, which primarily function in the plant as a defense mechanism against disruptive insects.

Antioxidant activity in this study is quantified by the IC50 value, which represents the concentration of the test compound required to scavenge 50% of free radicals; a smaller IC50 value indicates higher antiradical activity (Das, 2020). The IC50 for the Gelam wood leaf and seed extract was determined from the linear regression equation shown in Figure 4: $y=11.635x+5.0646$ ($r=0.9607$). By substituting 50 for the y variable, the concentration (x) was calculated to be 47.56 ppm, meaning the extract scavenges 50% of free radicals at this concentration. Although this value is higher than the quercetin control (3.42 ppm), its IC50 remains below 50 ppm, which is the recognized threshold for classifying a substance as a strong antioxidant, thereby confirming the potent antioxidant content of the Gelam wood leaf and seed extracts.
The antioxidant capacity of natural materials can vary significantly due to several factors, including the structure of the polyphenols present and the inherent, often weak, stability of those compounds. This stability is highly susceptible to chemical and physical factors such as pH, temperature, light, oxygen, enzymes,

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ascorbic acid, sugars, and metal ions (Lang et al., 2024). Additionally, research on plants like *Hyssopus officinalis*, *Lamium album*, and *Leonurus cardiaca* has shown that the highest antioxidant content is often concentrated in the upper parts of the plant or tissues close to the flowers (Skrovankova & Mlcek, 2025). Consistent with these findings, the strong antioxidant content identified in this study was derived from the Gelam wood leaves and seeds, without dismissing the vital role that chemical and physical factors play in maintaining this antioxidant potency.

Conclusions

Based on the determination test, the species of the Gelam wood plant was confirmed as *Melaleuca leucadendra*. The subsequent phytochemical screening revealed that the plant contains a diverse profile of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. Furthermore, the leaf and seed extracts demonstrated strong antioxidant activity, evidenced by an IC50 value of 47.56 ppm, confirming their ability to scavenge 50% of free radicals at this concentration. This study contributes new knowledge regarding the potential of Gelam wood. Future research should focus on a more in-depth quantitative analysis of its phytochemical content and further investigate its potential as an antifungal or antibacterial agent.

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