

Research Article



## Phenolic, Flavonoid, and Antioxidant Profiles of Sago (*Metroxylon sagu* Rottb.) Leaf Ethanol Extract

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### Abstract

Oxidative stress drives many degenerative conditions, motivating the search for safe, plant-based antioxidants. Sago (*Metroxylon sagu* Rottb.) leaf is comparatively underexplored. This study evaluated its antioxidant activity and related it to phenolic and flavonoid contents. A 96% ethanolic leaf extract was assayed for radical-scavenging activity by DPPH using ascorbic acid as the positive control; IC<sub>50</sub> values were obtained from linear regression of % inhibition versus concentration (2.5–10 ppm). Composition was profiled by total phenolic content (TPC; Folin–Ciocâlteu, expressed as mg gallic acid equivalents per g, mg GAE/g) and total flavonoid content (TFC; AlCl<sub>3</sub> colorimetry, expressed as mg quercetin equivalents per g, mg QE/g). The extract showed very strong DPPH activity (IC<sub>50</sub> = 11.873 ± 0.025 µg/mL), while ascorbic acid yielded 3.166 ± 0.025 µg/mL; both fall within the <50 µg/mL category. The extract contained TPC = 1.61% w/w (~16.1 mg GAE/g) and TFC = 3.05% w/w (reported on the quercetin-equivalent scale). These indices are consistent with the low IC<sub>50</sub> and support a phenolic-driven antioxidant profile. *Metroxylon sagu* leaf extract exhibits very strong radical-scavenging activity, supported by appreciable levels of phenolics and flavonoids, which identify *M. sagu* leaves as a promising natural antioxidant source. The findings provide a concise quantitative basis for follow-up work focused on targeted phenolic/flavonoid profiling, as well as broader antioxidant evaluations.

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## INTRODUCTION

Oxidative stress, driven by the accumulation of free radicals, highly reactive atoms or molecules characterized by unpaired electrons, poses a significant threat to biological systems. These unstable entities destabilize healthy cells by sequestering electrons from vital macromolecules, including proteins, lipids, carbohydrates, and DNA, leading to structural and functional cellular damage. The unchecked accumulation of this damage contributes directly to various chronic and degenerative diseases, necessitating the body's defense mechanisms<sup>1</sup>. To mitigate these detrimental effects, antioxidants are essential, defined as compounds capable of delaying, slowing, or preventing processes like lipid oxidation<sup>2</sup>.

Antioxidant compounds function through diverse, complementary mechanisms that extend far beyond simply intercepting lipid peroxidation. These mechanisms include chain-breaking radical scavenging via hydrogen-atom transfer, transition-metal chelation (which suppresses Fenton-type radical generation), and the quenching of reactive species, such as singlet

oxygen. Furthermore, they support the upregulation of endogenous defenses through pathways such as the Nrf2-ARE (which enhances enzymes like superoxide dismutase, catalase, and glutathione peroxidase) and facilitate antioxidant regeneration networks<sup>3,5</sup>. While synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are highly effective, their use at high doses has been associated with adverse health effects, including hepatic toxicity<sup>6</sup>. This safety concern has driven a critical need for research into natural antioxidants, such as flavonoids, ascorbic acid, and  $\beta$ -carotene, found within natural materials<sup>7</sup>.

Indonesia's vast biodiversity offers immense potential for the discovery of novel antioxidant compounds. Plants produce flavonoids, hydroxylated phenolic compounds, often in response to microbial infections. These compounds are distributed throughout various plant parts and possess antioxidant properties primarily due to the hydroxyl groups bound to their aromatic rings, allowing them to effectively capture free radicals<sup>8,9</sup>. Furthermore, both flavonoids and other phenolic compounds are generally recognized as non-toxic and exhibit a broad range of biological activities, including anti-inflammatory, antiproliferative, antimutagenic, and anti-allergic effects, which help prevent or ameliorate degenerative conditions<sup>10,11</sup>.

Among the promising natural resources is the sago palm (*Metroxylon sagu* Rottb.), which has been documented to have medicinal uses. The leaves of *M. sagu* contain a rich profile of secondary metabolites, including alkaloids, saponins, tannins, steroids, phenols, and flavonoids<sup>12</sup>. Specifically, the pith and log matrix of *M. sagu* yields identifiable small molecules, including (+)-catechin, (-)-epicatechin, and 4-hydroxybenzoic acid, with various plant parts demonstrating antioxidant, antibacterial, and anti-acne effects<sup>13</sup>. Given the known medicinal potential and the abundance of polyphenolic compounds in *M. sagu*, research on its specific antioxidant activity remains limited. Therefore, this study aims to determine the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of *M. sagu* leaf ethanol extract. This quantitative assessment provides a necessary foundation for characterizing the chemical constituents responsible for its antioxidant capacity, supporting the further development of this local resource as a therapeutic agent.

## MATERIALS AND METHODS

### Materials

The chemical reagents and solvents utilized in this study were of analytical grade. These materials included: distilled water (Sigma-Aldrich, Singapore), methanol (Merck, Indonesia), 96% ethanol, citric acid, chloroform, and phosphate saline (all from Sigma-Aldrich, Singapore). Key assay reagents comprised ascorbic acid (Merck, Indonesia), quercetin (Merck, Indonesia), gallic acid (Merck, Indonesia), sodium acetate (Merck, Indonesia), aluminum chloride ( $\text{AlCl}_3$ ; Merck, Indonesia), Folin-Ciocalteu reagent (F-C; Sigma-Aldrich, Singapore), and 2,2-diphenyl-1-picrylhydrazyl (DPPH; Merck, Indonesia). The plant material, *M. sagu* leaves, was collected from Kendari City, Southeast Sulawesi, Indonesia. Botanical authentication was rigorously performed at the Pharmacognosy-Phytochemistry Laboratory, Universitas Mandala Waluya, under the determination letter No. 132/09.03.01/VII/2023, and a voucher specimen was subsequently deposited.

### Methods

#### Sample collection and preparation

Only mature, fully expanded, green, and disease-free leaves from the mid-canopy of *M. sagu* were collected; young/unfolded and senescent/yellowing leaves were rigorously excluded. Harvesting was performed during dry weather conditions between 8:00 and 10:00 local time to minimize surface moisture and prevent enzymatic degradation. The collected leaves were first rinsed under running water, gently blotted with lint-free paper, and allowed to air-dry for approximately 30 minutes. Subsequently, the material was oven-dried at a controlled temperature of  $40 \pm 2^\circ\text{C}$  for 48 hours, or until a constant weight was achieved (defined as two consecutive weighings differing by less than 1%). Drying was conducted with the leaves arranged in a single layer under forced airflow. The dried leaves were then cooled to room temperature in a desiccator before being pulverized using a stainless-steel mill. Milling was performed using intermittent bursts to ensure the powder temperature remained below  $40^\circ\text{C}$ . The resulting powder was sieved to pass through a 40-mesh sieve, with any retained coarse fractions being remilled until they also passed through the sieve. The final powdered material was stored in amber, airtight containers over silica-gel desiccant at  $4^\circ\text{C}$  until required for extraction.

### Extraction

A total of 500 g of dried *M. sagu* leaf material was subjected to maceration using 96% ethanol as the solvent. A solid-to-solvent ratio of 1:10 (w/v) was employed (500 g sample to 5.0 L solvent). The sample was placed in a sealed, amber glass vessel and kept in the dark at room temperature ( $25 \pm 2^\circ\text{C}$ ) for 24 hours, with intermittent stirring to ensure optimal solvent penetration. The solvent was refreshed every 24 hours for three consecutive cycles, resulting in a total maceration time of 72 hours. Each 24-hour macerate was first filtered through muslin cloth and then through Whatman No. 1 filter paper. The combined filtrates were concentrated under reduced pressure using a rotary evaporator, maintaining a water bath temperature of  $40 \pm 2^\circ\text{C}$  and a vacuum of 200–250 mbar, until a syrupy consistency was achieved. Residual solvent was removed in a vacuum oven at  $40^\circ\text{C}$  until a constant weight was reached, yielding a thick ethanolic extract. The final extract was weighed to calculate the extraction yield (% w/w) and subsequently stored in amber, airtight vials at  $4^\circ\text{C}$  until the analysis.

### DPPH radical-scavenging assay

The antioxidant capacity of the extract was determined using the DPPH radical-scavenging assay. A DPPH working solution was freshly prepared by dissolving 5 mg of DPPH in methanol and adjusting the volume to 50 mL (100 mg/L), ensuring it was protected from light<sup>14</sup>. The maximum wavelength ( $\lambda_{\text{max}}$ ) for the DPPH solution was confirmed to be 528 nm using a UV-Vis spectrophotometer after scanning the 400–600 nm range, and all subsequent measurements were taken at this wavelength. A stock solution of *M. sagu* leaf ethanol extract (100 mg/L) was prepared in methanol and serially diluted to working concentrations of 10, 7.5, 5, and 2.5 mg/L. Ascorbic acid served as the standard and was prepared in parallel at the same concentrations.

For each determination, 2.0 mL of sample (or standard) was mixed with 1.0 mL of the DPPH solution (total volume, 3.0 mL), and the mixture was incubated in the dark at  $37^\circ\text{C}$  for 30 minutes. A control (blank) consisting of 2.0 mL of methanol and 1.0 mL of the DPPH solution was also prepared. The absorbances of the control and sample were measured at 528 nm.

The antioxidant activity, expressed as the percentage inhibition of DPPH absorption, was calculated using the following Equation 1. The Inhibition Concentration 50% ( $\text{IC}_{50}$ ) value, which represents the extract concentration required to reduce 50% of DPPH radicals, was determined by plotting the inhibition percentage ( $y$ -axis) against the extract concentration ( $x$ -axis). Linear regression analysis yielded the equation  $y = bx + a$ , and the  $\text{IC}_{50}$  value was calculated by setting  $y$  to 50<sup>15</sup>.

$$\% \text{inhibition of DPPH absorption} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100\% \quad [1]$$

### Data analysis

#### Determination of total phenolic content

The TPC of the extract was quantified using the standard F-C method and measured with a UV-Vis spectrophotometer. Initially, the F-C reagent was diluted 1:10 (v/v) with distilled water, and a 7.5% (w/v) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was prepared. A stock solution of gallic acid (500  $\mu\text{g}/\text{mL}$ ) in methanol was used to create working standards ranging from 20 to 60  $\mu\text{g}/\text{mL}$ . For the assay, 0.50 mL of either the standard solution or the appropriately diluted extract solution (stock extract concentration, 1.0 mg/mL in methanol) was mixed with 2.50 mL of the diluted F-C reagent. After a 5-minute pre-incubation period, 2.00 mL of the 7.5%  $\text{Na}_2\text{CO}_3$  solution was added, bringing the final volume to 5.00 mL. The mixtures were then incubated in the dark at room temperature ( $25 \pm 2^\circ\text{C}$ ) for 30 minutes. Absorbance was subsequently measured at  $\lambda_{\text{max}} = 765 \text{ nm}$ , which was previously determined by scanning the 400–800 nm range after full color development, using a reagent blank (methanol instead of the sample) for background correction. All measurements were conducted in triplicate. A calibration curve was generated by plotting the absorbance against the gallic acid concentration ( $y = ax + b$ ). The concentration of phenolics in the sample ( $c$ , expressed in mg/mL GAE) was calculated using the equation  $c = (a-b)/a$ . The final TPC was expressed as mg GAE/g using Equation 2. This calculation incorporates the concentration  $c$  obtained from the calibration curve (mg/mL), the dilution factor (DF) applied to the extract stock before the assay, and the concentration of the extract stock ( $S$ , mg/mL)<sup>16</sup>.

$$\text{TPC} = \frac{(1000 \times c \times \text{DF})}{S} \quad [2]$$

### Determination of total flavonoid content

The TFC was quantified using the  $\text{AlCl}_3$  colorimetric method employing a UV-Vis spectrophotometer. Quercetin was utilized as the standard for the calibration curve. A stock solution of quercetin (1,000 mg/L) was prepared by dissolving 10 mg of quercetin in ethanol and adjusting the volume to 10 mL. Working standards ranging from 20 to 60 mg/L were then freshly prepared by appropriate serial dilution of the stock solution in ethanol. The  $\lambda_{\text{max}}$  for measuring the quercetin- $\text{AlCl}_3$  complex was determined by scanning the spectrum from 400 to 600 nm after full color development, which was established at 415 nm<sup>17</sup>. All subsequent absorbance measurements were recorded at this wavelength.

For color development, 1.0 mL of the standard or appropriately diluted extract solution was sequentially mixed in a 10-mL volumetric flask with 4.0 mL of ethanol, 1.0 mL of a 10% (w/v)  $\text{AlCl}_3$  solution, and 1.0 mL of 1 M sodium acetate. The mixture was then brought to the final volume of 10.0 mL with distilled water. The solutions were incubated in the dark at room temperature ( $25 \pm 2^\circ\text{C}$ ) for 30 minutes. The reagent blank was prepared identically, but the sample/standard aliquot was replaced with 1.0 mL of ethanol. Absorbance readings were taken at 415 nm in 1-cm quartz cuvettes. The extract stock solution was prepared at a concentration of 2.0 mg/mL and diluted as necessary to ensure readings fell within the established calibration range. All measurements were conducted in triplicate.

A calibration curve (absorbance versus quercetin concentration,  $y = bx + a$ ) was constructed from the standard values. The quercetin-equivalent concentration of the sample solution in the reaction mixture ( $c$ , mg/L) was calculated using the formula  $c = (a-b)/a$ . The final TFC was expressed as mg QE/g using the following Equation 3<sup>18</sup>, in which  $V$  is the reaction volume (0.010 L),  $DF$  is the dilution factor applied to the extract before color development, and  $m$  is the mass of extract (g) contained in the aliquot used for the reaction ( $m = 0.002$  g for a 1.0 mL aliquot of the 2.0 mg/mL extract solution).

$$\text{TFC} = \frac{(c \times V \times DF)}{m} \quad [3]$$

## RESULTS AND DISCUSSION

As demonstrated in Table I, spectral scanning of the DPPH solution across the range of 400 nm to 600 nm yielded a broad absorption band. The highest absorbance was recorded at 528 nm (0.651), confirming this wavelength as  $\lambda_{\text{max}}$  under the assay conditions. The spectral profile showed a steady increase in absorbance starting from 400 nm (0.215), peaking sharply at 528 nm, and then decreasing toward 600 nm (0.357). Notably, a minor shoulder was observed near 520 nm (0.513). This established  $\lambda_{\text{max}}$  of 528 nm was subsequently used for all DPPH readings and the calculation of  $\text{IC}_{50}$  values, ensuring maximal sensitivity and comparability across all experimental measurements.

**Table I.**  $\lambda_{\text{max}}$  measurement of DPPH at 450-600 nm.

Wavelength (nm)	Absorbance (Å)
400	0.215
420	0.379
440	0.352
460	0.462
480	0.431
500	0.427
520	0.513
<b>528</b>	<b>0.651</b>
540	0.509
560	0.432
580	0.385
600	0.357

Note: Numbers in **bold** indicate  $\lambda_{\text{max}}$ .

The antioxidant capacity of *M. sagu* leaf ethanol extract was determined using the DPPH radical scavenging assay at concentrations of 2.5, 5.0, 7.5, and 10.0 ppm; the detailed concentration-dependent results are presented in Table II. We observed a clear dose-response relationship: as the extract concentration increased, the solution's absorbance decreased, corresponding to a proportional increase in the percentage of DPPH radical inhibition. Linear regression analysis of the inhibition percentage versus concentration yielded an  $\text{IC}_{50}$  value of  $11.873 \pm 0.025$  ppm for *M. sagu* leaf extract. For the positive

control, Ascorbic acid,  $IC_{50}$  was determined to be  $3.166 \pm 0.025$  ppm. Both *M. sagu* extract and ascorbic acid demonstrate very strong antioxidant activity, falling well below the standard threshold of  $<50$   $\mu\text{g/mL}$ . This categorization allows us to interpret the findings based on their biological effect size, rather than focusing strictly on statistical significance. Ascorbic acid was selected as the control due to its high solubility in polar media, its established redox behavior, and its widespread use as a standard benchmark for comparing DPPH results across various studies<sup>19</sup>.

**Table II.** Antioxidant test of *M. sagu* leaf extract.

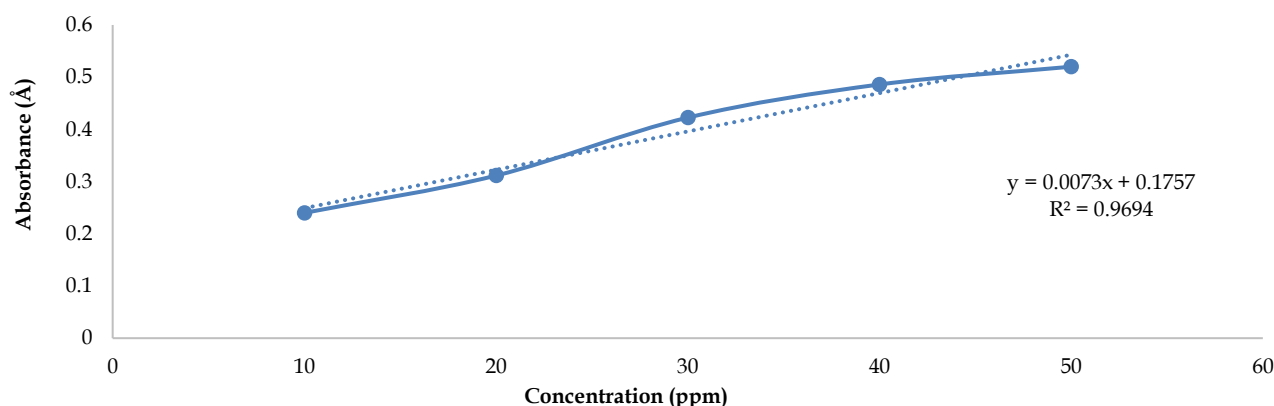
Sample	Concentration (ppm)	Blanko absorbance (Å)	Sample absorbance (Å)	Inhibition (%)	$IC_{50} \pm SD$ (ppm)
<i>Metroxylon sagu</i> leaf extract	2.5	0.722	0.534	26.039	11.9 $\pm$ 0.025
	5		0.517	28.440	
	7.5		0.497	31.117	
	10		0.475	34.211	
Ascorbic acid	2.5	0.724	0.364	49.724	3.2 $\pm$ 0.025
	5		0.347	52.026	
	7.5		0.326	55.018	
	10		0.305	57.873	

The antioxidant efficacy of *M. sagu* leaf extract, as evidenced by an  $IC_{50}$  value of 11.9  $\mu\text{g/mL}$  in the DPPH assay, compares favorably with that of several widely recognized medicinal plants. For instance, this potency is substantially higher than the  $IC_{50}$  of approximately 49.3  $\mu\text{g/mL}$  reported for the methanolic extract of *Moringa oleifera* leaves<sup>20</sup>. Furthermore, the activity is competitive with the strong antioxidant capacity documented for *Syzygium aqueum*, where multiple studies report DPPH  $IC_{50}$  values below 50  $\mu\text{g/mL}$ , with some nearing the potency of synthetic ascorbic acid<sup>21,22</sup>. Providing another frame of reference, reports on *Curcuma longa* extracts show DPPH  $IC_{50}$  values ranging from 8 to 21  $\mu\text{g/mL}$ , depending on the specific cultivar and extraction methodology. The observed  $IC_{50}$  of *M. sagu* leaf extract, therefore, overlaps with the upper range of potency exhibited by *C. longa*<sup>23</sup>, collectively underlining the significant antioxidant potential of *M. sagu* leaves.

The TPC of *M. sagu* leaf extract (96% ethanol) was quantitatively determined using the established F-C assay and expressed in GAE<sup>24</sup>. The gallic acid calibration curve, generated across the concentration range of 10–50 ppm, demonstrated strong linearity ( $R^2 = 0.9694$ ), as detailed in Table III and visually represented in Figure 1. Based on replicate absorbance measurements (Table IV), the TPC of the extract was calculated to be 1.61 % (w/w), or equivalently, 16.1 mg GAE/g of dry extract. Reporting the TPC in mg GAE/g is aligned with standard pharmacological reporting practices, allowing for direct comparison with the published literature<sup>25</sup>. Significantly, these compositional data are consistent with the very strong DPPH radical-scavenging activity previously observed for this specific extract (Table II), suggesting that its high phenolic load underpins its strong antioxidant capacity.

**Table III.** Concentration and absorbance data of gallic acid standard curve.

Sample	Concentration (ppm)	Absorbance I (Å)	Absorbance II (Å)	Mean $\pm$ SD (Å)
Gallic acid	10	0.248	0.232	0.24 $\pm$ 0.011
	20	0.309	0.314	0.3115 $\pm$ 0.004
	30	0.435	0.410	0.4225 $\pm$ 0.018
	40	0.491	0.481	0.486 $\pm$ 0.007
	50	0.523	0.517	0.52 $\pm$ 0.004



**Figure 1.** Gallic acid standard curve.



**Table IV.** TPC of *M. sagu* leaf extract.

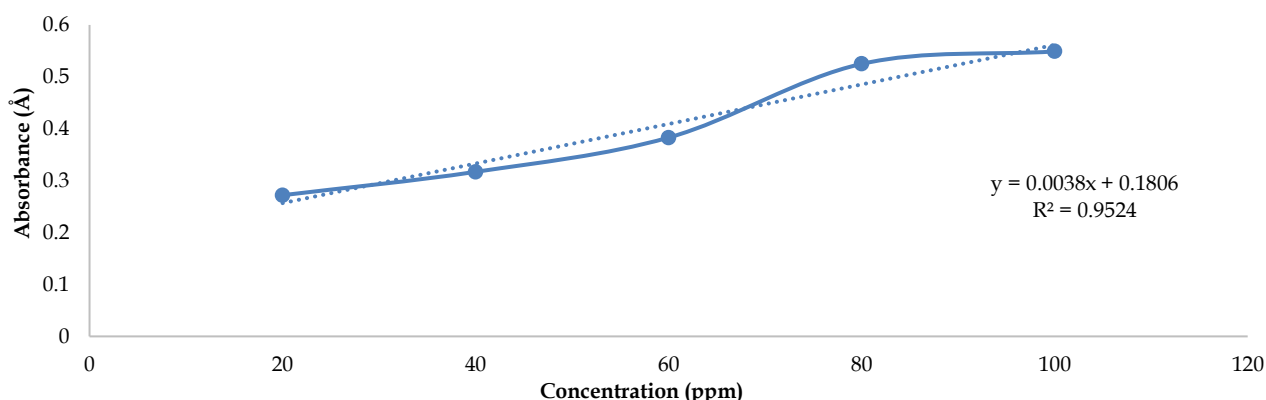
Sample	Absorbance I (Å)	Absorbance II (Å)	Absorbance III (Å)	Phenolic content (%)
<i>Metroxylon sagu</i> leaf extract	0.382	0.425	0.427	1.61±0.009

The utilization of GA as the calibration standard for F-C analysis aligns with consensus practice in phenolic quantification. This choice is predicated on GA's high chemical stability within the typical analytical range, its ready commercial availability, and its capacity to produce a linear and reproducible spectrophotometric response under standard conditions. While other standards, such as catechin or tannic acid, are sometimes employed, their use introduces unique calibration characteristics that ultimately hinder the direct, cross-study interpretation of results. Consequently, expressing the outcome as mg GAE/g provides the most broadly accepted and interpretable metric for standardized comparisons across diverse botanical matrices<sup>26</sup>.

The TFC of *M. sagu* leaf extract (96% ethanol) was accurately quantified using AlCl<sub>3</sub> colorimetric assay, with quercetin serving as the calibration standard. The quercetin standard curve, prepared across a range of 10–50 ppm and measured at λ<sub>max</sub> of 424 nm, demonstrated excellent linearity, yielding the equation  $y = 0.0038x + 0.1806$  with a strong correlation coefficient ( $R^2 = 0.9524$ ) (Table V; Figure 2). Replicate measurements of the extract (Table VI) determined the TFC to be 3.05% (w/w). This result confirms that the *M. sagu* leaf extract is a considerable source of flavonoids. For standardized comparison across different studies, this value can also be expressed in terms of mg QE/g of extract.

**Table V.** Concentration and absorbance data of quercetin standard curve.

Sample	Concentration (ppm)	Absorbance I (Å)	Absorbance II (Å)	Mean±SD (Å)
Quercetin	10	0.293	0.251	0.272±0.0297
	20	0.323	0.311	0.317±0.0085
	30	0.386	0.381	0.383±0.0035
	40	0.506	0.544	0.525±0.0269
	50	0.544	0.554	0.549±0.0071

**Figure 2.** Quercetin standard curve.**Table VI.** TFC of *M. sagu* leaf extract.

Sample	Absorbance I (Å)	Absorbance II (Å)	Absorbance III (Å)	Flavonoid content (%)
<i>Metroxylon sagu</i> leaf extract	0.445	0.406	0.389	3.05±0.029

The TFC of the extract was quantified using the AlCl<sub>3</sub> colorimetric assay. Quercetin is widely employed as the standard for this method because its distinct flavonol structure—specifically the 3-OH/4-keto conjugation and the O-dihydroxyl substitution—forms a strong, stable chromogenic complex with the aluminum ion (Al<sup>3+</sup>). This reaction yields high molar absorptivity and an excellent linear response in the 415–430 nm range, facilitating robust calibration and enabling comparison of TFC results when reported as mgQE/g<sup>27</sup>. While other standards, such as catechin or rutin, are available, quercetin remains the most commonly accepted reference for this analytical technique<sup>28</sup>. The same extract demonstrated a TPC of 1.61% (w/w), as determined by the F-C method.

Furthermore, the extract exhibited very strong DPPH radical-scavenging activity, characterized by an IC<sub>50</sub> value of 11.9 µg/mL. These combined results are highly coherent with the established pharmacological roles of polyphenols. Phenolic

compounds, including flavonoids, are the major drivers of antioxidant behavior, primarily functioning via hydrogen-atom transfer (HAT) or single-electron transfer (SET) mechanisms to neutralize radicals and form highly resonance-stabilized phenoxyl radicals. This activity is structurally enhanced by the presence of catechol motifs and extended  $\pi$ -conjugation, which effectively lowers the O–H bond dissociation enthalpy. Complementary to direct radical scavenging, many polyphenols also chelate transition metal ions ( $\text{Fe}^{2+}/\text{Cu}^{2+}$ ), thereby attenuating Fenton chemistry and suppressing the formation of secondary reactive oxygen species (ROS)<sup>29</sup>.

## CONCLUSION

The ethanol extract derived from *M. sagu* leaves demonstrated very strong antioxidant activity in the DPPH assay, yielding an  $\text{IC}_{50}$  value of approximately 11.9  $\mu\text{g/mL}$ . Complementary compositional analysis corroborated this potent activity, revealing a significant TPC of 1.61 % and a high TFC of 3.05%, which collectively indicate a potent phenolic-driven radical-scavenging profile. These findings strongly suggest that *M. sagu* leaves are a promising and readily available natural source of antioxidants with substantial value for further therapeutic and nutritional development. Future research should prioritize the targeted characterization of specific phenolic and flavonoid compounds, as well as broader *in vitro* and *in vivo* bioactivity evaluations, to translate these results into practical applications.

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## AUTHORS' CONTRIBUTION

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**Software:** -

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## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declared no conflict of interest related to this research.

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