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Antioxidant Effectiveness and Phenolic Content of Saga Seed (*Adenanthera pavonina)* Extracts In Vitro Using DPPH and ABTS Methods

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Abstract: Oxidative stress plays a crucial role in the development of various degenerative diseases, highlighting the need for safe and effective natural antioxidants. This study aimed to evaluate the antioxidant activity of saga seed (Adenanthera pavonina) extracts using DPPH and ABTS assays and to examine their relationship with total phenolic content (TPC). An in vitro experimental study with a quantitative approach was conducted. The extraction process was performed using maceration with 95% methanol and 95% ethanol as solvents. Antioxidant activity was determined based on IC_{50} values, while TPC was analyzed using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g extract. The results demonstrated that the methanolic extract exhibited stronger antioxidant activity (IC_{50} DPPH = 86.43 ppm; IC_{50} ABTS = 54.07 ppm) compared to the ethanolic extract (IC_{50} DPPH = 99.11 ppm; IC_{50} ABTS = 72.41 ppm). The methanolic extract also showed a higher TPC value (319.90 mg GAE/g) than the ethanolic extract (194.33 mg GAE/g).

Keywords: ABTS; Antioxidants; DPPH method; Phenolic content; Saga seeds

Introduction

Degenerative diseases have increasingly become a major topic of discussion in the field of public health. Commonly encountered degenerative diseases include diabetes mellitus, stroke, coronary heart disease, and various other cardiovascular disorders (Kurniawati et al., 2021). One of the key factors contributing to the rising prevalence of degenerative diseases is the modern lifestyle, which is closely associated with increased oxidative stress. Oxidative stress occurs when the production of free radicals exceeds the capacity of the body's antioxidant defense system, thereby triggering cellular and tissue damage. Fine particulate matter (PM2.5), which is widely present in urban environments and exhibits pro-oxidant properties, can further enhance the production of free radicals in the human body (World Health Organization, 2021).

In Indonesia, more than 272 million people are reported to live in areas with PM2.5 concentrations exceeding the safe threshold of 5 µg/m³ (EPIC, 2023), thereby increasing the risk of cellular damage induced by oxidative stress. Free radicals are molecules or atoms that possess one or more unpaired electrons, making them highly reactive and capable of damaging cellular structures through oxidative reactions (Widiasriani et al., 2024). To mitigate the adverse effects caused by free radicals, the human body requires antioxidant compounds that function to neutralize these reactive species. Consequently, the development of natural antioxidant sources that are safe, readily accessible, and sustainable has become an increasing concern, particularly in Indonesia, which is recognized as one of the countries with the greatest biodiversity in the world.

One local plant with considerable potential as a natural antioxidant source is saga seeds (Adenanthera pavonina). The saga tree is a medicinal plant that has

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been traditionally used to treat various health conditions (Dwitanti et al., 2020; García-Cervantes et al., 2025; Susilowati et al., 2021). A. pavonina exhibits a wide range of biological activities, including astringent, antihemorrhagic, antidiarrheal, antihematuric, anti-inflammatory, antirheumatic, antigout, and antioxidant properties (Ramzan, 2025; Sundarasamy et al., 2022). Saga is regarded as a rich source of bioactive compounds due to its ability to produce diverse secondary metabolites with strong anticancer and antioxidant activities (Asma et al., 2022; Domínguez-Arca et al., 2025; Wijesekara et al., 2024).

Several previous studies have demonstrated that the seeds, bark, and leaves of this plant contain phenolic compounds, flavonoids, and other bioactive constituents with high antioxidant potential. A study conducted by Yumita et al. (2023) reported that saga leaves and bark extracted using 70% ethanol exhibited high total phenolic contents, amounting to 81.379 mg GAE/g and 80.630 mg GAE/g, respectively, which significantly contributed to their antioxidant activity. Meanwhile, Owoeye et al. (2023) found that saga leaf extracts contain flavonoid and alkaloid compounds, which are well recognized for their effectiveness as natural antioxidants.

Most existing studies have primarily focused on the leaves and bark, whereas research on the antioxidant potential of saga seeds remains very limited. Furthermore, nearly all previous investigations employed only a single antioxidant assay, namely DPPH, without comparison to other methods such as ABTS. In fact, the DPPH assay is more sensitive to lipophilic compounds, whereas the ABTS method is capable of detecting antioxidant activity from both water- and lipid-soluble compounds (Mujahid et al., 2013). These data limitations indicate a clear research gap in the evaluation of the antioxidant activity of saga seeds using two complementary methods with differing detection characteristics.

Method

This study employed a quantitative approach with an in vitro experimental design to evaluate the antioxidant capacity of saga seed (*Adenanthera pavonina*) extracts. The research samples consisted of mature saga seeds collected from the BNI Urban Forest area, Banda Aceh. Sample selection was conducted purposively based on specific criteria, namely dark red seed color, absence of physical damage, and freedom from contaminants. The seeds were washed, dried, and ground into a fine powder prior to extraction through a maceration process for 3 × 24 hours using 95% ethanol and 95% methanol as solvents. The resulting filtrates were evaporated at low temperature using a rotary evaporator to obtain concentrated extracts for further analysis.

Considering the differing characteristics of antioxidant assay methods and the relevance of their relationship with phenolic content, this study was designed to address three main research questions: (1) What is the antioxidant activity of saga seed extracts based on the DPPH assay? (2) What is the antioxidant activity of saga seed extracts based on the ABTS assay? (3) Is there a relationship between total phenolic content and the free radical scavenging capacity of saga seed extracts? These questions form the analytical foundation of the study, as both aspects are essential for understanding the extent to which saga seeds can be considered a viable candidate as a natural antioxidant source for further development.

The objectives of this study were to analyze the in vitro antioxidant activity of saga seed extracts using the DPPH and ABTS methods and to evaluate their correlation with total phenolic content. The selection of both methods was intended to obtain more comprehensive comparative data that may serve as a reference in assessing the antioxidant characteristics of natural materials. In addition, examining the relationship between phenolic content and antioxidant effectiveness is expected to provide clearer insight into the contribution of major bioactive compounds present in saga seeds. Scientifically, this research contributes to the advancement of phytochemical studies on local Indonesian plants that remain underexplored.

The findings of this study are expected to enrich the scientific database regarding the potential of saga seeds as a source of antioxidants for applications in functional foods, health products, and natural-based cosmetics. Furthermore, the results may serve as a foundation for future studies, including the isolation of active compounds, investigation of antioxidant mechanisms, and safety evaluation through in vivo testing, thereby supporting broader practical applications.

Data collection was carried out by assessing antioxidant activity using two approaches, namely the DPPH and ABTS methods. Saga seed extracts were prepared at concentrations of 20, 40, 60, 80, and 100 ppm, and each concentration was reacted with DPPH or $ABTS^{+}$ radical solutions. Absorbance values were measured at specific wavelengths using a UV–Vis spectrophotometer to determine the percentage of free radical inhibition. Total phenolic content was analyzed using the Folin–Ciocalteu reagent, and the results were expressed as mg gallic acid equivalents (GAE) per gram of dry extract.

The collected data were subsequently analyzed quantitatively through the calculation of percentage inhibition and determination of IC_{50} values using linear regression equations. Total phenolic content (TPC) was

calculated based on a gallic acid standard calibration curve, while the relationship between phenolic content and antioxidant activity was evaluated using Spearman correlation analysis. All analytical procedures were designed to provide a comprehensive assessment of the effectiveness of saga seed extracts as a natural source of antioxidants.

Result and Discussion

Antioxidant Activity Assessed by the DPPH Method

The evaluation of antioxidant activity using the DPPH method demonstrated that all tested samples vitamin C, methanolic extract, and ethanolic extract were classified as strong antioxidants, with IC_{50} values ranging between 50 and 100 ppm. The IC_{50} value of vitamin C was recorded at 78.27 ppm, followed by the methanolic extract at 86.43 ppm and the ethanolic extract at 99.11 ppm.

These findings indicate that the bioactive compounds present in the methanolic extract exhibit higher effectiveness in reducing free radicals compared to those in the ethanolic extract. This difference in antioxidant efficacy is presumably related to solvent polarity, as methanol is more efficient in extracting phenolic and flavonoid compounds that possess higher reactivity toward free radicals. The regression curve depicting the percentage inhibition of saga seed extracts using the DPPH method is presented in Figure 1.

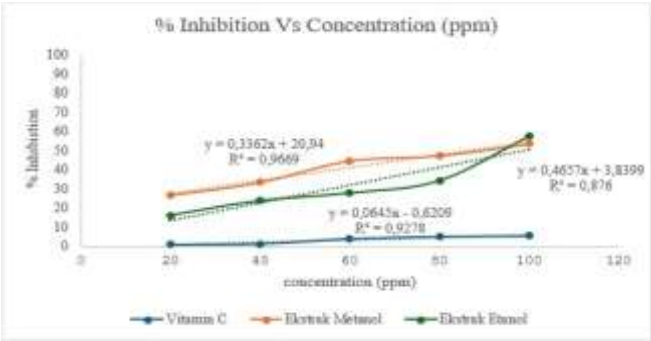

Figure 1. Linear regression curve of percentage inhibition of saga seed extracts using the DPPH method

The linear regression curves demonstrate a consistent increase in inhibition across all tested concentrations, with the methanolic extract exhibiting a steeper regression slope and better linearity than the ethanolic extract. This pattern indicates a more stable dose dependent response and further supports the conclusion that the bioactive components in the methanolic extract possess more effective electron- and hydrogen-donating abilities. These findings are consistent with recent studies by Ramadhani et al. (2022) and Putri et al. (2023), which reported that polar solvents exhibit higher efficiency in extracting phenolic compounds.

Antioxidant Activity Assessed by the ABTS Method

The results obtained using the ABTS method showed a pattern consistent with that observed in the DPPH assay. The IC_{50} values of the methanolic extract (54.07 ppm) and vitamin C (54.67 ppm) were classified as strong antioxidant activity, whereas the ethanolic extract exhibited an IC_{50} value of 72.41 ppm, which also falls within the strong category but with lower activity compared to the methanolic extract. These results further confirm that the methanolic extract contains phenolic compounds with a high radical-scavenging capacity. The difference in IC_{50} values between the two solvents once again emphasizes the critical role of solvent polarity in determining extract composition.

The regression curve illustrating the percentage inhibition of saga seed extracts using the ABTS method is presented in Figure 2.

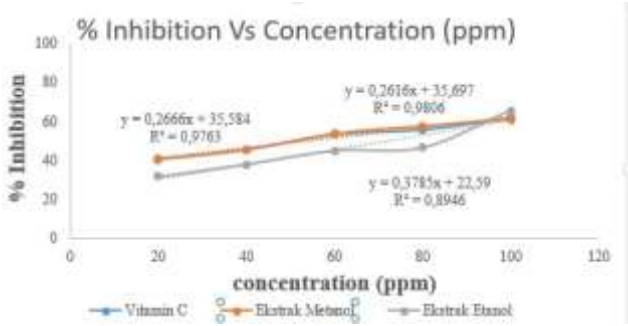

Figure 2. Linear regression curve of percentage inhibition of saga seed extracts using the ABTS method

The stable and steep regression slope observed for the methanolic extract in the ABTS assay reflects a more uniform increase in antioxidant activity compared to the ethanolic extract. The consistency of results between the DPPH and ABTS methods aligns with the findings of Muflihah et al. (2021), who reported that plant materials with high phenolic content typically exhibit a strong positive correlation between these two antioxidant assays.

Total Phenolic Content

The total phenolic content of each saga seed extract was determined using a regression equation derived from the Folin Ciocalteu assay. This reaction produces a greenish yellow coloration, which occurs when phenolic groups in gallic acid react with the Folin Ciocalteu reagent in the presence of sodium carbonate (Na_2CO_3). During the reaction, phenolic compounds reduce the molybdenum–tungsten complex in the reagent, forming a colored complex that is subsequently measured using a UV–Vis spectrophotometer.

The total phenolic content obtained was expressed as gallic acid equivalents (GAE) and is presented in Table 1.

Table 1. The Total Phenolic Content Obtained was Expressed as Gallic Acid Equivalents (GAE)

Extract	absorbance	mg GAE/g
methanol	3,774	319,8983
ethanol	2,292	194,3333

The methanolic extract yielded a total phenolic content of 319.8983 mg GAE/g, whereas the ethanolic extract exhibited a total phenolic content of 194.3333 mg GAE/g.

Correlation between Antioxidant Activity and Total Phenolic Content (TPC)

Spearman correlation analysis revealed a correlation coefficient between total phenolic content (TPC) and IC_{50} values of $r = -0.27$ with $p = 0.694$, indicating a very weak and statistically non-significant relationship. Therefore, an increase in total phenolic content cannot be considered a direct indicator of enhanced antioxidant activity in saga seed extracts. This finding highlights that total phenolic content does not necessarily reflect the specific characteristics or types of phenolic compounds present in the extract.

Each class of phenolic compounds, such as tannins, flavonoids, catechins, or phenolic acids, exhibits distinct antioxidant capacities. Consequently, two samples with comparable TPC values may demonstrate different antioxidant activities if their phenolic compositions differ. This observation is consistent with the report by Sari et al. (2025), which emphasized that antioxidant effectiveness is more strongly influenced by the molecular structure of phenolic compounds than by their total concentration.

In addition, differences in the sensitivity of the DPPH and ABTS assays toward specific types of antioxidants may also contribute to discrepancies between TPC and IC_{50} values. A study by Rahmawati et al. (2026) similarly reported that correlations between TPC and antioxidant activity are not always significant in natural product samples due to the diversity of secondary metabolites involved in antioxidant mechanisms.

Overall, this study demonstrates that saga seed extracts possess significant antioxidant potential, particularly when polar solvents such as methanol are employed during the extraction process. The concordance of results obtained from the DPPH and ABTS assays confirms the presence of bioactive compounds in saga seeds with high reactivity toward free radicals through multiple mechanisms. However, the non-significant correlation between total phenolic content and IC_{50} values provides an important academic contribution, indicating that the antioxidant activity of saga seed extracts is not solely dependent on the quantity of total phenolics, but also on their specific composition, molecular structures, and synergistic interactions among bioactive compounds. These findings open opportunities for further research focusing on the identification of dominant phenolic constituents using chromatographic techniques, as well as more comprehensive mechanistic analyses. Furthermore, the results reinforce the potential of saga seeds as a promising natural antioxidant source that can be optimized for the development of functional foods, cosmetics, and natural-based health products in accordance with modern industrial demands.

Conclusion

Saga seed (*Adenanthera pavonina*) extracts demonstrated strong antioxidant activity in both DPPH and ABTS assays, with the methanolic extract exhibiting superior performance. The methanolic extract also contained a higher total phenolic content than the ethanolic extract. However, the correlation between total phenolic content and antioxidant activity was weak and non-significant, indicating that antioxidant efficacy is influenced not only by the total amount of phenolic compounds but also by their structural characteristics and composition.

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