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Research Article

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Analysis of Antioxidant Activity of Lontar Fruit (*Borassus flabellifer* Linn.) using Various Solvents

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ABSTRACT

Background: *Borassus flabellifer* Linn. (lontar) fruit contains essential nutrients and bioactive secondary metabolites, including flavonoids and phenolic compounds, which exhibit antioxidant activity. However, information on antioxidant properties based on different extraction solvents remains limited.

Aims: This study aimed to analyze the nutritional composition and evaluate the antioxidant activity of lontar fruit extracts obtained using solvents with different polarities.

Methods: Proximate analysis was conducted to determine the nutritional composition of lontar fruit. Extraction was performed using ethanol, methanol, and n-hexane as solvents. The antioxidant activity of each extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and inhibitory concentration (IC_{50}) values were calculated to compare antioxidant effectiveness.

Results: Proximate analysis revealed that lontar fruit contains 78.61% carbohydrates, indicating its potential as a high-energy food source with an energy value of 3762 cal/gram. All extracts demonstrated free radical scavenging activity. At a concentration of 0.40 mg/mL, the n-hexane extract exhibited the highest inhibition (64.27%), followed by ethanol (59.25%) and methanol (53.69%). The lowest IC_{50} value was observed in the n-hexane extract (0.23 ± 0.02 mg/mL).

Conclusion: The results indicate that lontar fruit possesses strong antioxidant activity, with nonpolar compounds contributing predominantly to this effect, suggesting its potential application as a source of antioxidant supplements or functional food ingredients.

INTRODUCTION

Indonesia, India, and Thailand have tropical palms like *Borassus flabellifer* Linn., or lontar. It has long been utilised in medicine and food. Lontar fruit contains carbs, proteins, vitamins (particularly B-complex and vitamin C), and minerals like calcium, magnesium, and potassium. Along with these nutrients, lontar fruit contains flavonoids, phenolics, tannins, saponins, and alkaloids. The plant benefits from these chemicals' antimicrobial, anti-inflammatory, and antioxidant properties. However, little is known about lontar fruit's chemical makeup and health impacts, especially how solvents alter antioxidant extraction (Le et al., 2020, 2021; Vengaiah et al., 2021). More research is needed to know how solvents extract antioxidant molecules.

Free radical accumulation causes oxidative stress, which is connected to cancer, diabetes, and brain illnesses (Hajam et al., 2022; Leyane et al., 2022). Antioxidants neutralise free radicals by scavenging or suppressing oxidative processes. Plant antioxidants such phenolics and flavonoids are safer and more sustainable than butylated hydroxyanisole (*BHA*) and butylated hydroxytoluene (BHT) (Chaudhary et al., 2023; Parcheta et al., 2021). These chemicals' extraction effectiveness depends on the solvent.

Methanol is selected as a standard polar solvent due to its low molecular weight and high efficiency in penetrating cell membranes to release hydrophilic phenolics and tannins (Alara et al., 2021). However, considering the potential application in functional foods, Ethanol is chosen as a safer, food-grade (GRAS) alternative that favors the extraction of flavonoid glycosides and supports green chemistry principles (Plaskova & Mlcek, 2021). Furthermore, the vibrant yellow-orange hue of lontar fruit indicates the presence of carotenoids and tocopherols. To extract these lipophilic substances, a non-polar solvent like n-hexane is essential to isolate the non-polar fraction which is often overlooked in aqueous extractions (Ramesh et al., 2024).

Previous studies support this polarity-based approach. Mohammed et al. (2022) found that methanol and ethanol effectively extract tuber plant phenolics, while Tripathi et al. (2025) reported that solvents with a specific polarity index (5.1–5.2) optimize polyphenol yield. Conversely, extraction of lipophilic substances requires nonpolar solvents. Thus, this study systematically tests n-hexane, ethanol, and methanol to establish a complete polarity gradient (0.1 to 5.1 polarity index), ensuring that both hydrophilic phenolics and lipophilic antioxidants in lontar fruit are comprehensively evaluated.

The most common antioxidant activity test is the DPPH test (2,2-diphenyl-1-picrylhydrazyl), which uses a chemical to quench DPPH radicals via a hydrogen-donor mechanism. This test was chosen because it is rapid, simple, and widely used in phytochemical studies. This study examined the effect of solvent on the phytochemical composition and antioxidant activity of lontar fruit extracts. This study provides scientific data for optimising the extraction of bioactive compounds from lontar fruit for pharmaceutical and food applications. Finding efficient, eco-friendly solvents supports green chemistry while enabling the exploration of lontar fruit bioactives for standardised herbal medicines and increasing this commercial value of this underutilized plant.

MATERIALS AND METHODS

Extraction Preparation

Lontar fruit was identified in the National Research and Innovation Agency (BRIN) Cibinong (No. BO-1997818). Jeneponto Regency, Makassar, yielded dark-purple mature fruits. Running water was used to wash, drain, and sun-dry the translucent white fruit flesh on a black cloth-lined tray for 3–5 days. After drying, fruit flesh was mixed with an electric blender. Extraction was done by macerating with 70% ethanol, methanol, and n-hexane (1:10) for 14–16 hours at 30–37 °C with a shaker at 150 rpm (method modified) (Anbessa et al., 2024; Frederick et al., 2021; Ishtiaq et al., 2020). The filtrate was filtered and evaporated at 50 °C in a rotary evaporator to make a thick extract kept at 4–8 °C.

Proximate Analysis

The Food Analysis Laboratory, Biotechnology IPB University, used modified AOAC 1980 for proximity analysis (AOAC, 2015). Moisture content was measured as the weight loss % of samples dried at 105 °C. The ash content was determined by burning the samples at 600 °C. Crude fat was quantified using Soxhlet extraction with hexane. Protein content was determined using the Kjeldahl method, which involves selenium digestion, H_2SO_4, NaOH distillation, and HCl titration. Crude fiber analysis involved acid-alkali digestion and incineration. Total carbohydrates were calculated by subtracting water, ash, protein, and fat from 100%. The gross energy was measured using a bomb calorimeter under oxygen pressure

Antioxidant Activity Test

BRIN Cibinong used DPPH (2,2-diphenyl-1-picrylhydrazyl) to assess antioxidants. 0.007 g of DPPH powder was dissolved in 50 mL of ethanol to make a 0.15 mM solution. One mL of this solution was combined with five mL of ethanol and left for 30 min. After mixing, 5 mL of ethanol and 1 mL of the solution were pipetted in and left in the dark for 30 min (Ariandi et al., 2024). The extract was then produced at 0.05, 0.10, 0.15, 0.25, 0.30, 0.35, and 0.40 mg/mL (Banu et al., n.d.; Renuka et al., 2018). A UV-Vis spectrophotometer assessed absorbance at 517 nm (Sudiono, 2021). The results were compared to vitamin C solution under the identical conditions (Ariandi et al., 2024). A formula calculated antioxidant activity as DPPH inhibition percentage.

$$\% \text{ inhibition} = \frac{A0 - A1}{A0} \times 100$$

A_0 = blank absorbance
A1 = sample absorbance (lontar extract)
Next, the IC_{50} is calculated, obtained from the linear regression y = ax + b using the formula:

$$IC_{50} = \frac{50 - a}{b}$$

Data Analysis

Data were analysed using a completely randomised design with three replicates. A 95% confidence level analysis of variance (ANOVA) was performed using SPSS 29.0. If substantial differences were observed, Duncan's test was used.

RESULTS AND DISCUSSION

Proximate Analysis

A study on lontar fruit identified and measured key nutritional components such moisture, ash, protein, fat, and fibre. Table 1 provides lontar fruit proximate analysis results.

Analysis shows 11.89% water content. The majority is carbohydrates (78.61%). Other components comprised 2.30% ash, 0.69% crude fat, 6.51% crude protein, and 3.17% crude fibre. Lontar fruit has 3762 cal/gram. Next, Lontar fruit was extracted using ethanol, methanol, and n-hexane. The antioxidant activity of these extracts was investigated. Results are shown in Figures 1-3. The antioxidant IC_{50} values for each solvent are in Table 2.

This comprehensive investigation evaluated the nutritional composition and antioxidant capacity of *Borassus flabellifer* Linn. fruit. Proximate analysis revealed the fruit as a high-energy source (3762 cal/gram) dominated by carbohydrates (78.61%). While proteins (6.51%) and fats (0.69%) are present in smaller quantities, their interaction with antioxidants is significant; for instance, high carbohydrate and fiber content has been linked to varying antioxidant release in wild carob pulp (El Chami et al., 2025). Other nutrients included ash (2.30%) and fibre (3.17%). These findings suggest that lontar fruit is a good source of carbs (Dubey et al., 2025; Mary & Jasmin, 2022). This information is crucial for using lontar fruit in meals or supplements. These findings support greater research on enhancing these nutrients. Protein decreases free phenolics, however protein-phenolic complexes are still active. High fat extracts compounds. Hydrolysis releases bound phenolics from carbohydrates and fibres. These findings show how macronutrients alter antioxidant molecule release and extraction.

Antioxidant Activity Test

Borassus flabellifer Linn was tested. removes free radicals. The fruit extract was compared to vitamin C, a powerful antioxidant. Stronger lontar fruit has

Table 1. Proximate Composition of *Borassus flabellifer Linn.* Fruit

Parameters	Results
Moisture content (%)	11,89
Ash content (%)	2,30
Crude fat content (%)	0,69
Crude protein content (%)	6,51
Crude fiber content (%)	3,17
Carbohydrate content (%)	78,61
Gross energy (cal/gram)	3762

Table 2. IC_{50} test results of antioxidant activity of lontar fruit using 3 solvents

Solvents	IC_{50} (mg/ml)
Ethanol	0.24 ± 0.03
Methanol	0.37 ± 0.27
N-hexane	0.23 ± 0.02

stronger antioxidant activity when tested with ethanol. The maximum concentration tested was 0.40 mg/mL, close to 0.35%. Inhibition was 59.25% for both. The negative control had 7.76% antioxidant activity, while lontar fruit had substantially higher activity. Vitamin C has the highest antioxidant activity (84.26%). Figure 1 shows the ethanol test results.

Lontar fruit antioxidant activity with methanol was not significantly varied between concentrations. Most antioxidant activity was at 0.40 mg/mL, with 53.69% inhibition. This was similar to 0.35, 0.30, and 0.25 mg/mL. The negative control had 7.76% inhibition and the positive control had 84.26% inhibition, however methanol-treated lontar fruit was different. Methanol antioxidant activity testing are shown in Figure 2.

The antioxidant activity of n-hexane treatments differed greatly. The most active concentration was 0.40 mg/mL, 64.27%. All treatments differed from the negative control (7.76%) and positive control (84.26%). Figure 3 illustrates the test findings.

This investigation showed which solvent treats lontar fruit better. At 64.27%, n-hexane exhibited the highest antioxidant activity. Methanol and ethanol had lower activity, 53.69% and 59.25%, respectively. Figure 4 shows findings for 0.40 mg/mL lontar fruit. Table 2 shows lontar fruit IC50s for ethanol, methanol, and n-hexane. The results of the IC50 test from the three solvents showed that n-hexane was the solvent with the lowest IC50, with a concentration of 0.23 mg/mL.

To assess health benefits, lontar fruit antioxidant activity was examined. Samples were extracted using ethanol, methanol, and n-hexane. These solvents were chosen to extract antioxidant chemicals from samples. The study revealed that solvent polarity significantly influences antioxidant activity (Mahayasa et al., 2024). Surprisingly, the non-polar n-Hexane extract exhibited the highest antioxidant activity (IC50 0.23 mg/mL; 64.27% inhibition at 0.40 mg/mL), outperforming both ethanol (IC50 0.24 mg/mL) and methanol (IC50 0.37 mg/mL). The IC_{50} value classifies antioxidant activity as <50 g/mL (very strong), 50-100 g/mL (strong), 101-150 g/mL (moderate), and >150 g/mL (weak) (Zongo et al., 2023) (Bageshwar & Desai, 2021; Muflihunna et al., 2019). This superior performance of n-Hexane suggests that the dominant antioxidants in lontar fruit are lipophilic (fat-soluble). This aligns with findings in other

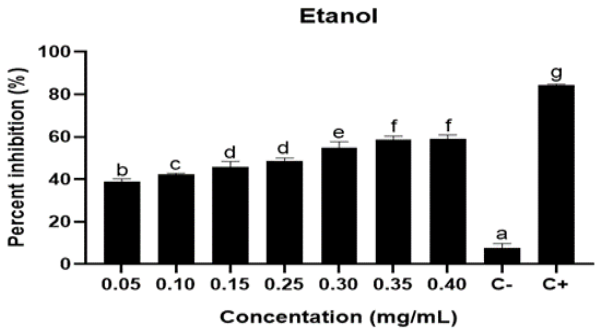

Figure 1. The results of antioxidant activity testing using ethanol solvent (C-: negative control; C+: positive control), different letters (a – g) show statistically significant differences ($p < 0.05$)

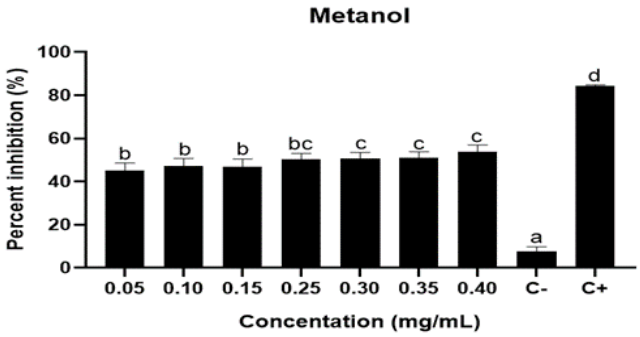

Figure 2. Results of antioxidant activity testing using methanol solvent (C-: negative control; C+: positive control), different letters (a – d) show statistically significant differences ($p < 0.05$)

palm species, such as *Elaeis guineensis* (oil palm), which is rich in tocopherols and tocotrienols—potent non-polar antioxidants. The n-hexane likely facilitated the extraction of these tocopherols, carotenoids, and other non-polar sterols that polar solvents like methanol failed to dissolve efficiently. Conversely, the lower activity in methanol extracts suggests that while polar phenolics are present, they may be less potent or less abundant than the lipophilic fraction in this specific fruit maturity stage. N-Hexane extraction showed that lontar fruit contains antioxidants that can be extracted with other solvents. Nonpolar n-hexane removes different molecules than polar solvents (Mahayasa et al., 2024; Srivijeindran et al., 2025).

Comparison with Vitamin C and Industry Implications Although the n-hexane extract showed strong potential, it is important to note that its activity (64.27% inhibition) is still lower than the positive control, Vitamin C (84.26%). From an industrial perspective, these results present a trade-off. While n-

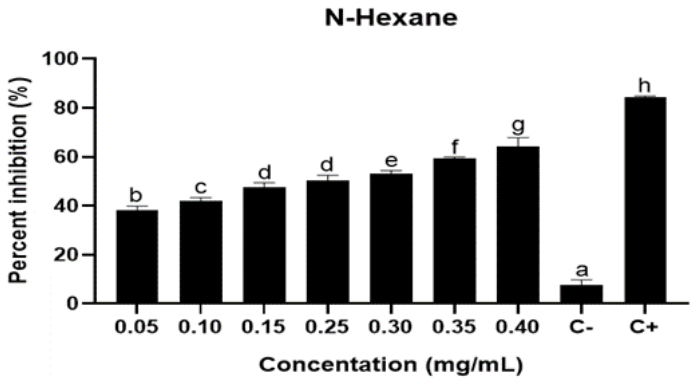

Figure 3. Results of antioxidant activity testing using n-hexane solvent (C-: negative control; C+: positive control), different letters (a – h) show statistically significant differences ($p < 0.05$)

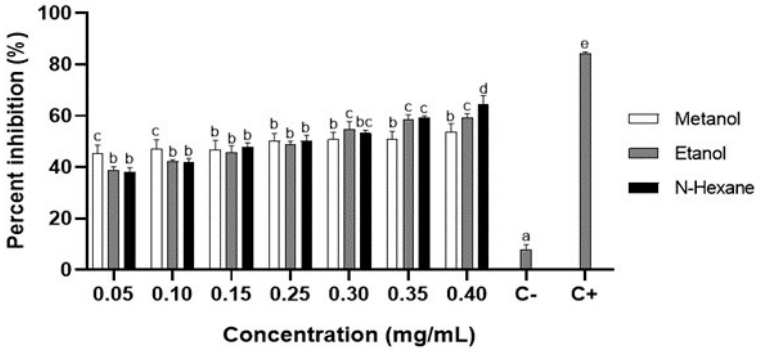

Figure 4. Comparison of lontar fruit antioxidant activity using ethanol, methanol, and n-hexane solvents (C-: negative control; C+: positive control), different letters (a – e) show statistically significant differences ($p < 0.05$)

hexane offers the highest potency, it is toxic and requires complete removal for food applications. Ethanol, with an IC50 (0.24 mg/mL) extremely close to n-hexane, offers a more strategic advantage for the food and pharmaceutical industries. Ethanol is Generally Recognized As Safe (GRAS) and eco-friendly, making it the preferred solvent for developing functional food additives or standardized herbal medicines from lontar fruit without the safety concerns associated with hexane residues.

CONCLUSION

Thus, it can be concluded that this study successfully profiled the proximate composition and antioxidant potential of *Borassus flabellifer* fruit. The fruit is confirmed as a carbohydrate-rich energy source with significant antioxidant capabilities. The extraction optimization revealed that the non-polar solvent n-hexane is the most effective, yielding the lowest IC50 value (0.23 ± 0.02 mg/mL), indicating that the fruit's most potent bioactive compounds are lipophilic in nature. However, this research relies solely on the DPPH assay, which primarily measures the reducing ability of antioxidants via hydrogen atom transfer. To fully validate the antioxidant mechanism, future research should incorporate complementary assays such as ABTS (for electron transfer capacity), FRAP (ferric reducing ability), and ORAC.

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AUTHORS CONTRIBUTION

H.D.S. contributed to the conceptualisation of the study, sample preparation, data collection, statistical analysis, and manuscript drafting. W.M. served as the principal investigator and corresponding author, contributing to the study design, research supervision, data interpretation, and critical revision of the manuscript. H.M. contributed to the research design, laboratory procedures related to antioxidant analysis, and validation of the experimental data. H.S.D. contributed to the study implementation, laboratory analysis, and data interpretation. All authors approved the final manuscript.

"The author declares that there is no conflict of interest with parties involved in this research".

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