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Comparative Study of Antioxidant Activity of Buni Fruit Extract (*Antidesma bunius* (L.) Spreng.) Using Various Extraction Techniques

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ABSTRACT

Buni fruit (*Antidesma bunius* L.) is a native Indonesian plant that contains anthocyanin, flavonoid, and phenolic compounds, so it has the potential as a source of natural antioxidants that are beneficial for health. This study aims to evaluate the antioxidant activity of extracts taken using various extraction methods because they can affect the results, composition of bioactive compounds, and the ability to scavenge free radicals. Buni fruit simplicia was extracted using 96% ethanol through three extraction techniques, namely maceration, soxhletation, and reflux. In vitro antioxidant activity testing was carried out using the DPPH method by determining the IC_{50} value. The reflux method gave the highest yield (13.99%) when compared to maceration (9.42%) and soxhletation (12.60%). However, the extract from the maceration method had an IC_{50} value (74.445 µg/mL) indicating the strongest antioxidant activity compared to soxhletation (155.104 µg/mL) and reflux (161.894 µg/mL). The results of one-way ANOVA analysis showed that the extraction method did not have a significant effect on the percentage of DPPH inhibition ($p>0.05$). This indicates that differences in extraction methods have not been able to produce statistically significant differences in antioxidant activity.

Key Messages:

- This study successfully demonstrated that the soaking method obtained from the reflux method gave a higher yield of 13.99%, this is likely due to the increased solubility and diffusion of compounds at high temperatures.
- However, extraction using high temperatures has the potential to cause degradation of thermolabile antioxidant compounds so that the maceration method carried out at room temperature is able to maintain higher stability of antioxidant compounds with an IC50 of 74.445 µg/mL.

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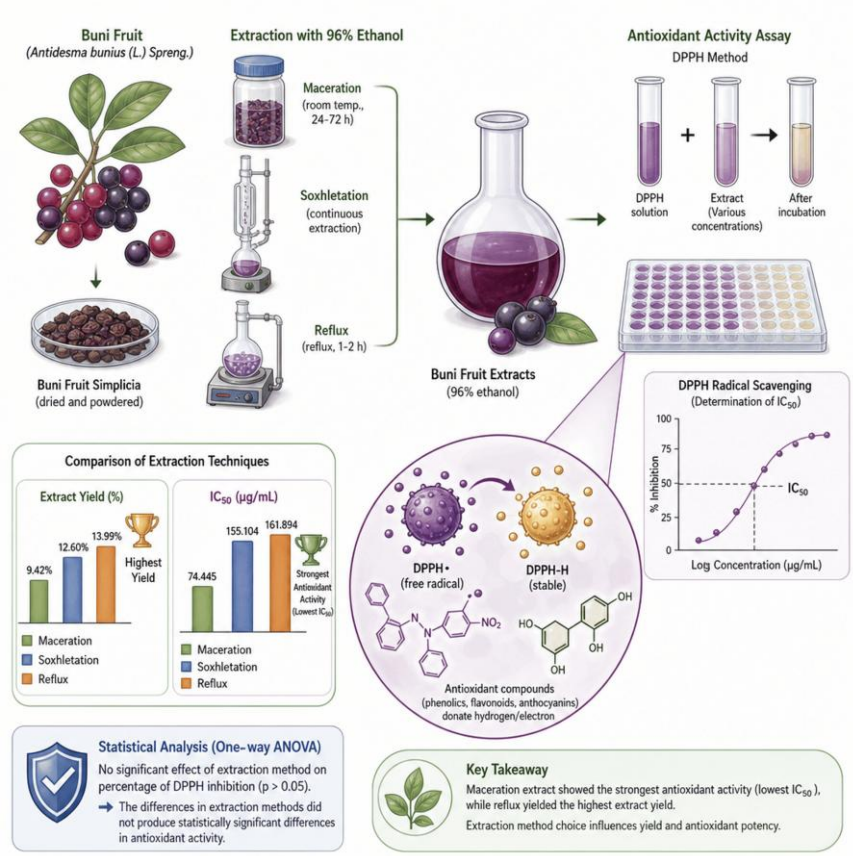
GRAPHICAL ABSTRACT



INTRODUCTION

Oxidative stress caused by excess free radicals plays a significant role in the development of various degenerative diseases, so the search for natural antioxidant sources remains a major research focus. Antioxidants derived from natural sources are considered safer and have the potential to be developed as functional foods and basic pharmaceutical ingredients (1).

Buni (*Antidesma bunius* (L.) Spreng), a member of the Phyllanthaceae family, has long been used as a traditional medicine in Southeast Asia. Buni plant extract contains compounds with potential α-glucosidase inhibitory activity. Buni fruit is also reported to contain various groups of secondary metabolite compounds, such as steroids, saponins, tannins, alkaloids, polyphenols, and triterpenoids, which have the potential to have pharmacological activity (2). *Antidesma bunius* (L.) Spreng. is rich in polyphenolic compounds with strong antioxidant properties. This study also showed that the total flavonoid content obtained from each extract of n-hexane, ethyl acetate, and methanol, through a multistage extraction method, was 10.72%, 7.9%, and 3.56%, respectively. The extract from red buni fruit showed the ability to inhibit the α-glucosidase enzyme with an IC_{50} value of 85.27 µg/mL. Most likely, this activity occurs due to the presence of polyphenolic compounds and strong antioxidants found in buni fruit, such as flavonoid compounds (3). Ethanol extract from buni leaves contains flavonoid, phenolic, tannin, alkaloid, saponin, terpenoid, and steroid compounds, and shows quite strong antioxidant activity with an IC_{50} value of 61.8 µg/mL (4).

Although there is evidence that berries contain high levels of polyphenols or anthocyanins and may act as antioxidants, several practical and scientific limitations exist. Some studies use only one extraction method or solvent, making it difficult to compare the effectiveness of different solvents in extracting antioxidant compounds. Some studies report information on phenolic or flavonoid content without directly comparing the various solvents n-hexane, ethyl acetate, and ethanol, which are often used in multistage extractions. Variations in antioxidant activity measurement methods using DPPH, ABTS, and FRAP make it difficult to assess the antioxidant potential of extracts (5).

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Sampling method and solvent type are key factors in how efficiently phenolics and flavonoids can be extracted from plant parts, as the solubility of bioactive compounds varies depending on the polarity of the solvent and the method used (6). The concentration of ethanol as a solvent affected the antioxidant activity of the ethanol extract. The 96% ethanol extract showed the highest value compared to the 70% ethanol extract. The antioxidant activity of the two extracts differed significantly, although both had very strong activity (7). Recent research has shown that the choice of solvent and extraction technique influences the total amount of phenolics or flavonoids that have antioxidant activity in the resulting extract. Therefore, a comparison of extraction methods and solvent selection provides practical guidance for improving the extraction process of natural ingredients (5).

Several studies on buni fruit have demonstrated strong antioxidant potential using DPPH, ABTS, and FRAP as well as other biological activities (8). Of the various methods, ethanol reflux produced the highest antioxidant activity (DPPH and ABTS scavenging), with a total phenolic content of 82.54 mg GAE/g and a flavonoid content of 31.90 mg QE/g. These findings indicate that ethanol reflux extraction is a highly effective method for producing extracts rich in bioactive compounds (9). The extraction method can affect the total phenol content and antioxidant activity of *S. polyanthum* leaf extract. Where the best antioxidant activity was obtained by the maceration method with an IC_{50} of 17.53±0.11 µg/mL, followed by soxhlet and infusion which were 18.73±0.31 and 40.26±0.18 µg/mL, respectively (10). Based on the variation of extraction methods which emphasize the importance of structured research on the influence of extraction methods on phytochemical content and antioxidant activity in berry fruit (11).

This study aims to evaluate the potential antioxidant activity of berry fruit extract obtained through three extraction techniques, namely maceration, soxhlet and reflux. Buni fruit was chosen because it contains anthocyanin pigments, flavonoids, and phenolic compounds, known to have potential as natural antioxidants. Differences in extraction methods are assumed to affect the amount of bioactive compounds, yield, and free radical scavenging capacity of the resulting extract.

METHODS

Research Design

The design used was an experimental study conducted in July 2022, at the Pharmaceutical Biology Laboratory (Sample Preparation and Extraction) and the Integrated Research Laboratory (Antioxidant Activity Measurement), Faculty of Health Sciences, Almarisah Madani University, Makassar, South Sulawesi. The sample used in this study is the buni fruit (*Antidesma bunius* (L.) Spreng), obtained from a plantation in Wattangpulu District, Sidenreng Rappang Regency, South Sulawesi. Located at coordinates -3.922588 South Latitude, 119.698108 East Longitude. The buni fruit was first wet sorted to separate dirt and other unwanted materials. After that, the fruit was washed with running water and drained before being dried in a simplicia oven at a temperature of 40°C for 72 hours. After the drying process was complete, the simplicia from the buni fruit was ground using a blender before entering the extraction stage.

Extraction of Buni Fruit Using the Maceration Method

175 g of dried buni fruit simplicia was macerated with 96% ethanol extract. The maceration process was carried out for 3x24 hours with occasional stirring, the maceration vessel must be tightly closed to avoid external contamination. After that, the residue and filtrate were separated using filter paper. Then the residue was re-macerated with 96% ethanol extract for 3x24 hours, then filtered again. Each filtrate from the maceration results was combined and evaporated using a rotary vacuum evaporator to obtain a thick buni fruit extract **(12)**.

Extraction of Buni Fruit Using the Soxhlet extraction Method

175 g of dried berry fruit simplicia, wrapped in filter paper, tied both ends with thread, then put into a soxhletation tube, added with 96% ethanol solvent. The berry fruit simplicia divided into 25 g for 1x extraction, with 250 mL of 96% ethanol solvent put into the soxhletation tube to wet the sample. The extraction process was carried out at a temperature of 70°C (the temperature setting of 70°C on the device aims to ensure a stable extraction process, without excessive boiling that can cause compound degradation

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or solvent loss). Extraction was carried out until it reached 20 cycles. The extracts were combined and evaporated using a rotary vacuum evaporator until a thick berry fruit extract was obtained.

Extraction of Buni Fruit Using the Reflux Method

175 g of dried buni fruit was placed in a round-bottom flask, then 96% ethanol was added and heated at 60°C for 4 hours. The extracts were combined and evaporated using a rotary vacuum evaporator to obtain a thick buni fruit extract.

Antioxidant Activity

Preparation of Control Solution

Weighed 19.71 mg of DPPH powder was dissolved in ethanol p.a and diluted to a volume of 100 mL in a volumetric flask to obtain a 0.5 mM DPPH solution. The solution was homogenized and stored in the dark to prevent degradation due to light. The maximum wavelength was determined after incubation for 30 minutes and the maximum absorbance of the DPPH solution was obtained at a wavelength of 517 nm using a UV–Vis spectrophotometer.

Making Buni Fruit Sample Solution

25 mg of 96% ethanol extract of buni fruit was dissolved in 25 mL of pro-analysis ethanol. The sample solution was varied at 80, 160, 240, 320, and 400 ppm. It was made by taking 400; 800; 1,200; 1,600 and 2,000 µL of the stock solution from 1,000 ppm, then put into a 5 mL volumetric flask and made up with pro-analysis ethanol.

Antioxidant Activity Test of Buni Fruit Extract Using DPPH Method

Each concentration of buni fruit extract was pipetted. 1 mL of DPPH reagent solution was added and the volume was made up to 5 mL with analytical grade ethanol in each volumetric flask. The solution was homogenized and allowed to stand for 30 minutes, then measured at a wavelength of 517 nm using UV-Vis spectrophotometry.

Determination of IC_{50} Value

Antioxidant activity in a sample is determined by the magnitude of the inhibition of DPPH radical absorption by calculating the percentage (%) of DPPH absorption inhibition using the formula:

$$\%\ Inhibition = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}}\ x\ 100\%$$

The IC_{50} value indicates the concentration of the sample solution that can reduce DPPH free radicals by 50%. From the equation y=a+bx, the IC_{50} value can be calculated using the formula.

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

y = % Inhibition (50)

x = Concentration

a = Intercept (line intersection on the y-axis)

b = Slope

RESULTS

The extraction process is crucial for maintaining the quality of natural extracts. This study evaluated three extraction methods: maceration, reflux and soxhlet extraction for buni fruit (Antidesma bunius). The aim was to determine the effect of extraction methods on the antioxidant activity of buni fruit extract. Maceration is performed without heating, soxhlet extraction involves continuous extraction, and reflux involves extraction with controlled heating. A temperature of 60°C was used for the reflux method and 70°C for the soxhlet extraction method with 96% ethanol to optimize the extraction process and protect heat-sensitive bioactive compounds. This temperature was chosen because it is below the boiling point of ethanol, thus accelerating diffusion and dissolution without damaging the chemical structure of the compounds.

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Comparative of Yield Values Based on Extraction Methods

The extract obtained from the maceration method was 16.49 g, the reflux method was 24.49 g and the soxhlet extraction method was 22.05 g. The percentage yield obtained from the maceration method was 9.42%, the reflux method was 13.99% and the soxhlet extraction method was 12.60% (table 1).

Table 1. Ethanol Extract Yield Value of 96% Buni Fruit

Method	Simplicia Weight	Extract Weight	Type of Extract	Yield Value (%)
Maceration	175 g	16.49 g	Thick	9.42
Soxhlet	175 g	22.05 g	Thick	12.60
Reflux	175 g	24.49 g	Thick	13.99

Comparative Antioxidant Activity Assessment

Buni fruit extract (*Antidesma bunius* L.) showed increased antioxidant capacity with increasing concentration. The maceration method had the highest antioxidant activity with an IC_{50} of 74.445 µg/mL, superior to reflux and soxhlet extraction. This result is influenced by the extraction method without heating in maceration, maintaining the stability of the bioactive compounds (Table 2). Absorbance measurements in the antioxidant activity test were performed once at each concentration. Therefore, data are presented as a single value without standard deviation. The IC_{50} value was determined based on a curve plotting the relationship between concentration and percent inhibition. The results of a one-way ANOVA analysis showed that the extraction method did not significantly affect the DPPH inhibition percentage ($p>0.05$). This indicates that different extraction methods have not been able to produce statistically significant differences in antioxidant activity.

Table 2. Results of Comparison of Antioxidant Activity of Buni Fruit Extracts

Method Extraction	Concentration (ppm)	Absorbance	Blank	% Inhibition	IC_{50} value (µg/mL)
Maceration	80	0.429	0.828	48.188	74.445
	160	0.335		59.541	
	240	0.242		70.773	
	320	0.168		79.710	
	400	0.154		81.400	
Soxhlet extraction	80	0.516	0.828	37.681	155.104
	160	0.418		49.517	
	240	0.277		66.546	
	320	0.211		74.517	
	400	0.158		80.918	
Reflux	80	0.529	0.828	36.111	161.894
	160	0.410		50.483	
	240	0.298		64.010	
	320	0.219		73.551	
	400	0.175		78.865	

Figures 1, 2, and 3 show a positive linear relationship between the increase in the activity of 96% ethanol extract of buni fruit and the percentage of DPPH free radical inhibition. This indicates that as the concentration of the extract increases, its antioxidant capacity also increases. In Figure 1, the maceration method obtained a regression equation of $y = 0.1082x + 41.945$ with an R^2 value of 0.9511, which indicates a strong linear relationship and the effectiveness of the extract in capturing free radicals at relatively low concentrations. The reflux method (in Figure 2) produces an equation of $y = 0.1357x + 28.031$ with an R^2 value of 0.9702, while the soxhlet extraction method (in Figure 3) shows an equation of $y = 0.1393x + 28.394$ and $R^2 = 0.969$, both of which also indicate a very strong linear correlation between concentration and percentage of inhibition.

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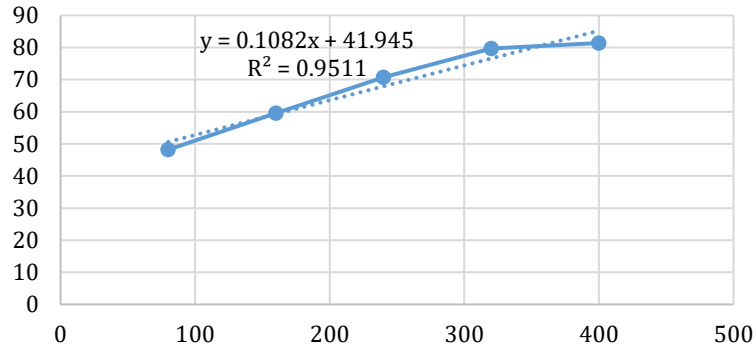

Figure 1. 96% Ethanol Extract Curve from Maceration Method

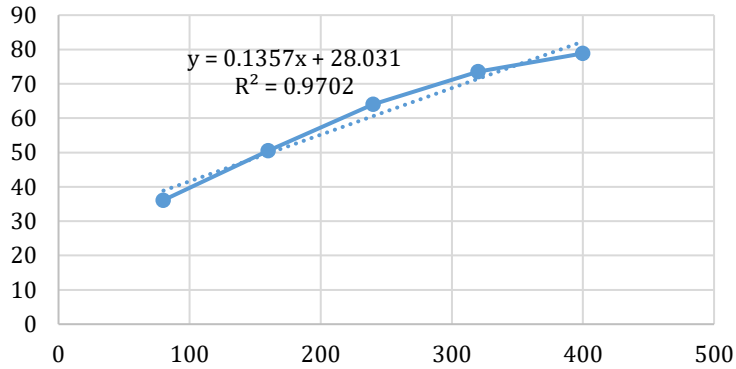

Figure 2. 96% Ethanol Extraction Curve from Reflux Method

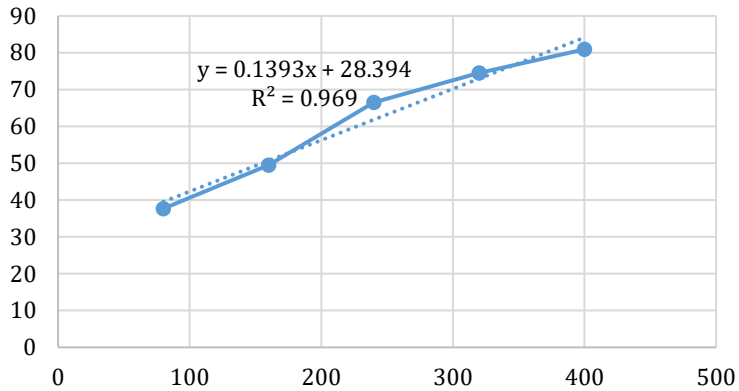

Figure 3. 96% Ethanol Extraction Curve from Soxhlet extraction Method

DISCUSSION

Comparative of Yield Values Based on Extraction Methods

The initial stage of the research was the extraction of berry fruit using maceration, reflux, and soxhlet techniques. The solvent used was 96% ethanol in each extraction method. From this process, extracts were obtained with different yields: 9.42% from the maceration method, 13.99% from the reflux method, and 12.60% from the soxhlet method (Table 1). Heat extraction methods such as reflux and Soxhlet produce higher yields due to increased solubility and diffusion of compounds at high temperatures. However, high temperatures have the potential to cause degradation of thermolabile antioxidant compounds, such as phenolics and flavonoids. Conversely, the maceration method carried out at room temperature is able to maintain the stability of antioxidant compounds, resulting in higher antioxidant activity despite lower yields.

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Yield is the ratio of the weight of the resulting extract to the weight of the medicinal plant used. The yield value is related to the number of active compounds contained in medicinal plants, where compounds have various benefits for human life. Determining the percent yield serves to determine the level of secondary metabolites attracted **(13)**. The higher yield from reflux is due to the use of high temperatures that increase the permeability of cell walls and accelerate the process of diffusion of dissolved compounds in the solvent. The soxhlet process also produces high yields, but the cyclic extraction mechanism causes the efficiency of the relationship between the solvent and the medicinal plant to be lower than that of reflux. Meanwhile, maceration produces a moderate yield because it is carried out without heating, so the compound diffusion process is slower. Overall, the resulting yield value still meets acceptable standards for natural ingredient extracts in the pharmaceutical field **(14)**. Research results obtained from the cold and hot extraction methods show that the hot method, namely reflux and soxhlet, produces more extract. The presence of a temperature factor or heating of the solvent at the extraction stage can increase the transfer of metabolites into the solvent more quickly.

Comparative Antioxidant Activity Assessment

Antioxidant properties testing using the DPPH method is based on the potential of antioxidant compounds to neutralize DPPH free radicals, which is seen from the reduction in purple color. This color reduction was measured quantitatively using a UV-Vis spectrophotometer. Each sample concentration (80, 160, 240, 320, and 400 ppm) was measured using a UV-Vis spectrophotometer with a wavelength of 517 nm. The processing or mixing process was carried out in dark conditions to prevent contact with light, because DPPH is very sensitive to light **(15)**. The results of the antioxidant activity test using the DPPH method showed that all berry extracts, based on their extraction techniques, had the ability to capture free radicals depending on their concentration. When the extract concentration was increased from 80 to 400 ppm, there was an increase in the percentage of inhibition and a decrease in absorbance values in all extraction methods used. This indicates that the higher the extract concentration, the more antioxidant compounds are ready to donate electrons or hydrogen atoms to reduce DPPH radicals **(16)**.

The extract obtained through the maceration technique showed the highest antioxidant activity, as seen from the IC_{50} value (74.445 µg/mL), when compared to the Soxhlet (155.104 µg/mL) and reflux (161.894 µg/mL). A lower IC_{50} value indicates a stronger antioxidant capacity. The high antioxidant activity in the maceration method is thought to be due to the extraction process being carried out without heating, thus maintaining the stability of heat-sensitive phenolic and flavonoid compounds. Although the Soxhlet and reflux methods produced a higher percentage of inhibition at high concentrations, the IC_{50} values of both methods were higher. The use of high temperatures in these two methods can cause some antioxidant compounds to degrade or extract inactive compounds, which can reduce the relative effectiveness of the extract. This finding underscores the importance of the extraction method in determining the quality of antioxidant activity, where yield results or increased concentration do not always align with the resulting antioxidant strength.

CONCLUSION

This study proves that the extraction technique with heating can produce a greater yield, such as 13.99% in reflux, this occurs because of the increased solubility and diffusion of compounds at high temperatures. Meanwhile, the extraction technique without heating can increase the antioxidant activity of the extract, such as in maceration, which obtained an IC50 of 74.445 µg/mL, this occurs because the antioxidant compound is stable at room temperature.

DECLARATION OF THE USE OF AI

The authors declare that no artificial intelligence (AI), AI-assisted technologies, or large language models (LLMs) were used in the conception of the study, data analysis, or the drafting, writing, and editing of this manuscript. The only exception is the graphical abstract, which was created using the design platform Illustrae (https://illustrae.co/). The authors take full responsibility for the content and accuracy of the graphical abstract and the entire manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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