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By M. Yasser

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF BUNI FRUIT (*ANTIDESMA BUNIUS L.*) IN MONCONGLOE MAROS DISTRICT EXTRACTED USING ULTRASOUND ASSISTED EXTRACTION

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ABSTRACT

This study aims to extract Buni Fruit obtained from Moncongloe, Maros district, South Sulawesi, Indonesia using Ultrasound Assisted Extraction technology with ethanol as solvent. The effect of extraction temperature on the Total Phenolic Content and Antioxidant Activities of extract was also studied. The result shows that at the temperature of 50°C was obtained the best extract with the Total Phenolic Content of 6.7415 ± 0.0721 mg/g in GAE and the value of IC₅₀ of 29.9618 ± 1.9521 mg/L. This result shows that Buni fruit extract has potential to be developed as a healthy food.

Keywords: Buni Fruit, Ultrasound Assisted Extraction, Total Phenolic Content, Antioxidant activities, Ethanol

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INTRODUCTION

Buni fruit (*Antidesma bunius L.*) is a traditional plant spread all over South Sulawesi, this includes Moncongloe, Maros district. Buni fruit is used as a natural food coloring due to its a unique color¹. The red-purple color of Buni fruit indicates the content of flavonoid compound in Buni fruit. Flavonoid has various health benefits² such as antioxidant³, antimicrobial⁴ and anti-inflammatory agent^{5,6}.

Various methods have been to obtain the extract of flavonoid. The common method used in extraction process is maserasi⁷. Recently, ultrasound assisted technology has been developed as an extraction method⁸. The advantages of extraction using ultrasound technology are low cost, faster, simple process, save energy and more efficient. Ultrasound technology can also increase the yield of extraction due to acoustic cavitations effect of ultrasound wave generated in solvent. Mechanical effect of ultrasound technology can increase surface contact area between solid phase and liquid phase in addition, it can accelerate diffusion process of dissolved compound from solid phase to solvent⁹. Ultrasound Assisted Extraction technology did not generate significant change in chemical structures and raw material used¹⁰. Ultrasound Assisted Extraction technology can also facilitate effective mixing, faster energy transfer, reduce thermal gradient and extraction temperature, selective extraction, faster respond and higher yield¹¹⁻¹³.

Abundant to be Buni fruits in Moncongloe, Maros district, potentially developed to be extracted as an antioxidant. Ultrasound technology utilization in extraction process can increase extraction yield and efficiency, compared to conventional extraction method. For this reason, this study was aimed to was investigated Total phenolic content and antioxidant activities from extract of Buni fruit obtained from Moncongloe, Maros district. The results also were characterized by UV-Vis spectroscopy and IR spectroscopy.

EXPERIMENTAL

Material and Methods

Plant Material

Buni fruit (*Antidesma bunius L.*) was obtained from Moncongloe, Maros district, South Sulawesi, Indonesia in April, 2019. Buni fruit is ripe fruit with a deep red color (burgundy). Buni fruit's flesh and seeds were separated, and then its flesh was extracted.

Materials

All the chemicals i.e. Ethanol (C₂H₅OH), Folin-Ciocalteu phenol, Sodium Carbonate (Na₂CO₃), Gallic Acid were purchased from Merck, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich.

General procedure

Buni fruit Ultrasound Assisted Extraction

300 g of sample was added ethanol as a solvent in order to make the sample being soaked, after that it was extracted for 45 min by power sonic Ultrasound of 405 on wave of 40 kHz^{8,13-14}. The extraction was conducted by applying various temperature to find out the effect of temperature. The extract was then filtered using paper filter, then evaporated in a low pressure and temperature that was no more than 45°C until thick extract obtained.

Table-1: The Variables of Experiment

Samples	The Temperature of Ultrasound Extraction (°C)	The Time of Ultrasound Extraction (Minutes)
A	40	45
B	50	45
C	60	45

Characterization of extracts

UV-Vis Spectroscopy Analysis

Buni fruit extract was measured by Orion Aquamate 8000 UV Vis spectroscopy in wavelength of 200 to 700 nm¹⁵. Characteristic peaks were obtained for the different functional groups.

IR Spectroscopy Analysis

The IR spectrum of buni fruit extracted with ethanol solvent was measured by Prestige-21 Shimadzu Infrared spectroscopy at the range of 400-4000 cm⁻¹ using KBr pellet¹⁶ to identify its various functional group.

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Determination of the Total Phenolic Content

Total phenolic content of Buni fruit extracted was measured using reagent of Folin Ciocalteu¹⁷. 1 mL standard solution of Gallic Acid (3,6, 9, 12, 15, 18 and 21 mg/L) it was added 13.1 mL of Folin-Ciocalteu and 5 mL Na₂CO₃ 10%. The sample was then placed outside for 1 hour at room temperature. Absorbance of this solution was measured at the wave length of 765 nm by using Orion Aquamate 8000 UV-Vis Spectroscopy. The same procedures was conducted to sample of buni fruit extracted with ethanol solvent. 1 mL of sample was added with solvent in the same condition with gallic acid standard curve.

Antioxidant Activities

Antioxidant activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) method^{18,19,20}. 2 mL of each sample of buni fruit extracted with ethanol solvent in concentration of 10, 20, 30, 40 and 50 mg/L was added with 2 mL DPPH 0.1 M. This solution was placed outside for 1 hour at the room temperature before being measured by Orion Aquamate 8000 UV-Vis Spectroscopy at the wave length of 517 nm. IC₅₀ (efficient concentration of extract to decrease initial DPPH radical concentration of 50%) was obtained by interpolation in linear regression analysis. DPPH solution was used as the control.

RESULTS AND DISCUSSION

Characterization of Extract using UV-Vis Spectroscopy

Buni fruit extract analysis conducted by UV-Vis Spectroscopy was aimed to predict secondary metabolite compound contained in it. Each secondary metabolite compound had a unique absorbance that was measured by UV-Vis Spectroscopy. The result of sample analysis using UV-Vis Spectroscopy shows that there were two unique peaks (band) in each extract. Extract A represents two bands at absorbance of 279nm and 550 nm, extract B represents on absorbance at 277 nm and 548.5 nm and extract C represents on absorbance at 278.5 nm and 548.5 nm. Those three extract were identified as flavonoid indicated by formation of maximum absorbance at the range of 300 to 500 nm as band I and the maximum absorbance at the range of 240 to 290 nm as band II^{21,22}.

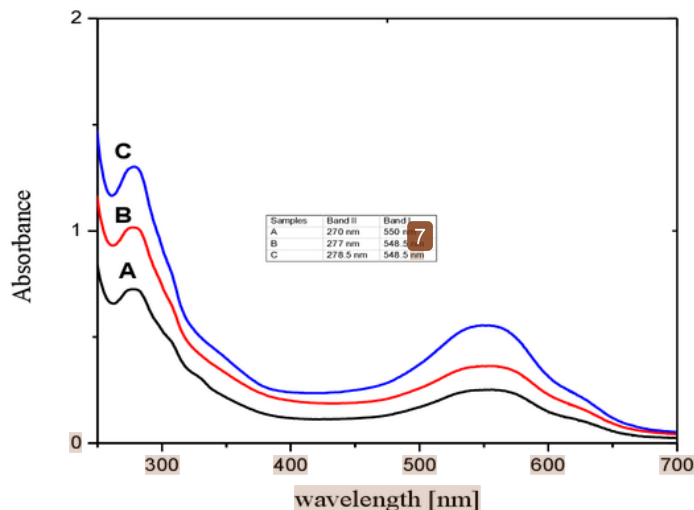


Fig.-1: UV-Vis Spectrum of Buni Fruits Extracted

Characterization of Extract using IR Spectroscopy

Buni fruit extract measurement was conducted by Infrared Spectroscopy to find out the functional groups contained in extract. The measurement result (Fig.-2 and Table-2) shows that buni fruit extract contained aromatic functional group shown in wave number of 1452.45 to 1454.38 cm^{-1} as C=C-C aromatic. In addition O-C-H bond was also identified in a form of phenolic on wave number of 1224.84 to 1238.34 cm^{-1} . There was also carbonyl functional group, C=O indicated by the absorbance of wave number of 1708.99 to 1734.06 cm^{-1} . Functional group of -OH was also identified with the unique wave number of 3421.83 to 3524.06 cm^{-1} . Functional group contained in Buni fruit extract strengthen the extract measurement by using UV-Vis Spectroscopy (Fig.-1) which identifies that buni fruit extract contained a flavonoid compound. Flavonoid compound was known contains three rings which two of those are aromatic. It was also known that flavonoid compound has carbonyl functional group. In addition, several derivatives of flavonoid compound contains -OH functional group.

Tab.15: Measurement peaks result of Buni fruit extract analyzed by IR Spectroscopy

A [cm^{-1}]	B [cm^{-1}]	C [cm^{-1}]	Functional Group Assignment
1224.84	1238.34	1224.84	O-C-H bending (phenolic) ²³⁻²⁵
1454.38	1452.45	1454.38	C=C-C Aromatic ²⁴⁻²⁵
1647.26	1643.41	1643.41	C=C stretching of phenyl ²⁴⁻²⁶
1734.06	1708.99	1722.49	Carbonyl compound, C=O ^{23-24,27}
3524.06	3421.83	3431.48	-OH ^{24-25,27}

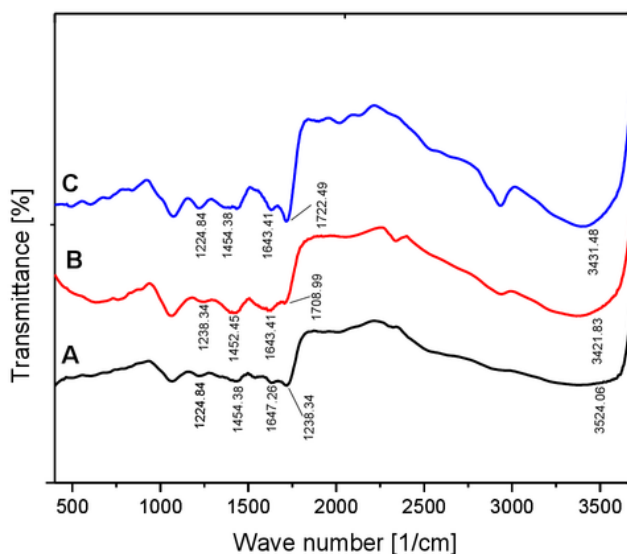


Fig.-2: IR Spectrum of Buni Fruits Extract

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Total Phenolic Content

Total phenolic content of buni fruit extract was measured by Folin-Ciocalteu method. The result of total phenolic measurement shows that the extraction at the temperature of 50°C using ultrasound had total phenolic content of 6.7415 ± 0.0721 mg/g in GAE, it was higher than a total phenolic content obtained extraction temperature of 40°C and 60°C. Temperature of 50°C was the best temperature range in extraction process to produce a high total phenol content and antioxidant activities²⁸. The result of buni fruit extract was analyzed by UV-Vis Spectroscopy (Fig.-1) and IR Spectroscopy (Fig.-2 and Table-2) shows that buni fruit extract contained phenolic compound especially flavonoid. The total difference of phenolic content on the three temperature variations was caused by phenolic compound content in buni fruit extract. Several phenolic compounds were vulnerable to pH and high temperature^{1,29,30}.

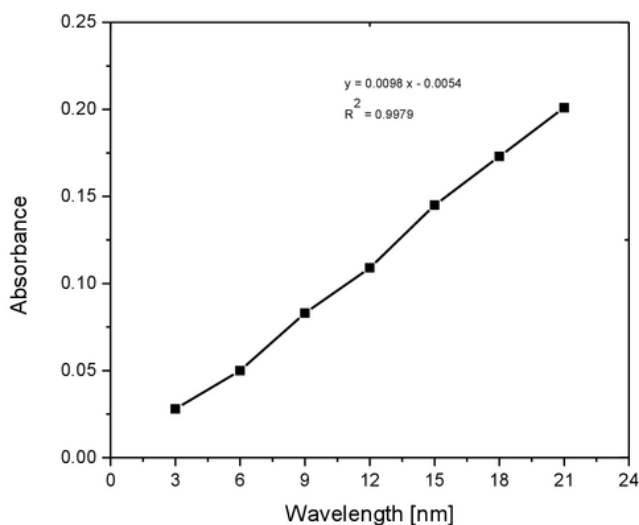


Fig.-3: Gallic Acid Calibration Curves at the various concentrations

Table-3 : Total Phenolic Content of Buni Fruits Extract

Samples	Absorbance	Total Phenol Content (mg/g in GAE*)
A1	0.0470	5.3571 ± 0.0267
A2	0.0469	
A3	0.0474	
B1	0.0500	6.7415 ± 0.0721
B2	0.0510	
B3	0.0500	
C1	0.0500	5.6871 ± 0.0589
C2	0.0510	
C3	0.0510	

*GAE is Gallic Acid Equivalent

Antioxidant Activities

Antioxidant activities of buni fruit extract was measured by DPPH method. The ability of buni fruit extract used as an antioxidant was based on phenolic compound content³⁰⁻³². The ability as an antioxidant can be seen by the value of IC₅₀³³.

The value of IC₅₀ in three ultrasound assisted extraction temperature shows insignificant difference. Extraction at the temperature of 50°C given better value of IC₅₀ compared to value obtained by extraction at the temperature of 40°C and 60°C. The value of IC₅₀ was affected by phenolic compound in the extract. The higher total phenolic content, the stronger antioxidant value of IC₅₀ would be. The value of IC₅₀ < 50 mg/L indicates a strong antioxidant³⁴. Phenolic compound has antioxidant property because of its own hydroxyl functional group leading to be able for hydrogen donor in radical compound which made it stable^{35,36}.

Table-4 : Antioxidant Activities of Buni Fruit Extract

Samples	Linear Equations	R ²	IC ₅₀ (mg/L)	Average of IC ₅₀ (mg/L)
A1	y = 1.1039x + 11.169	0.9983	35.1762	36.7707 ± 1.3815
A2	y = 1.1948x + 5.0649	0.9975	37.6089	
A3	y = 1.2597x + 2.7273	0.9948	37.5270	
B1	y = 1.2792x + 14.481	0.9923	27.7666	29.9618 ± 1.9521
B2	y = 1.3831x + 6.4286	0.9983	31.5027	
B3	y = 1.2662x + 11.234	0.9849	30.6160	
C1	y = 1.1558x + 13.636	0.9960	31.4622	32.8612 ± 1.3668
C2	y = 1.0649x + 14.935	0.9988	32.9280	
C3	y = 1.2078x + 8.7013	0.9877	34.1933	

CONCLUSION

Buni fruit by Ultrasound Assisted Extraction using ethanol solvent at the variation temperature of 40°C, 50°C and 60°C produced extract that was identified as a flavonoid and its derivatives. The extract formed by three variation generated a high total phenolic content and strong antioxidant activities.

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