Volume 13 | Number 1 | 1-779| January-March|2020
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# RASĀYAN
## JOURNAL OF CHEMISTRY
(An International Quarterly Research Journal of Chemical Sciences)

*Rasayan J. Chem.*
ISSN (Print): 0974-1496
ISSN (Online): 0976-0083
CODEN: RJCABP

Abstracted in: Since, 2008
ELSEVIER
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RASĀYAN
JOURNAL OF CHEMISTRY
An International Quarterly Research Journal of Chemical Science



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RASĀYAN *J. Chem.*
Vol. 13 | No. 1 |684 – 689| January - March | 2020
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# 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 the 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 $IC_{50}$ of 29.9618 ± 1.9521 mg/L. This result shows that Buni fruit extract has the potential to be developed as a healthy food.

**Keywords:** Buni Fruit, Ultrasound-Assisted Extraction, Total Phenolic Content, Antioxidant Activities, Ethanol



## 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 unique color[1]. The red-purples color of Buni fruit indicates the content of the flavonoid compound in Buni fruit. Flavonoid has various health benefits[2]such as antioxidant[3], antimicrobial[4], and anti-inflammatory agent.[5,6]

Various methods have been to obtain the extract of flavonoids. The common method used in the extraction process is maserasi.[7] Recently, ultrasound-assisted technology has been developed as an extraction method.[8]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 the acoustic cavitations effect of ultrasound wave generated insolvent. The mechanical effect of ultrasound technology can increase the surface contact area between the solid phase and liquid phase also, it can accelerate the diffusion process of the dissolved compound from solid phase to solvent.[9] Ultrasound-Assisted Extraction technology did not generate significant change in chemical structures and raw material used.[10] Ultrasound-Assisted Extraction technology can also facilitate effective mixing, faster energy transfer, reduce thermal gradient and extraction temperature, selective extraction, faster response and higher yield.[11-13]

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

*Rasayan J. Chem.*, 13(1), 684-689(2020)
**http://dx.doi.org/10.31788/RJC.2020.1315584**

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## EXPERIMENTAL

### 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 ($C_2H_5OH$), Folin-Ciocalteau Phenol, Sodium Carbonate ($Na_2CO_3$), 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 to make the sample being soaked, after that it was extracted for 45 min by power sonic Ultrasound of 405 on a wave of 40 kHz.[8,13-14] The extraction was conducted by applying various temperatures to find out the effect of temperature. The extract was then filtered using a paper filter, then evaporated in low pressure and temperature that was no more than 45°C until thick extract obtained.

Table-1: The Variables of Experiment

| Samples | 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 nm.[15] 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 $cm^3$ using KBr pellet[16] to identify its various functional group.

### Determination of the Total Phenolic Content

The total phenolic content of Buni fruit extracted was measured using a reagent of Folin Ciocalteau.[17]1 mL standard solution of Gallic Acid (3,6, 9, 12, 15, 18 and 21 mg/L) it was added with I mL of Folin-Ciocalteu and 5 mL $Na_2CO_3$ 10%. The sample was then placed outside for 1 hour at room temperature. The absorbance of this solution was measured at the wavelength of 765 nm by using Orion Aquamate 8000 UV-Vis Spectroscopy. The same procedures were conducted to sample of buni fruit extracted with ethanol solvent. 1 mL of sample was added with a solvent in the same condition with a 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 wavelength of 517 nm. $IC_{50}$ (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 compounds 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 an absorbance of 279 nm 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 extracts were identified as flavonoid indicated by the formation of maximum absorbance at the range of 300 to 500 nm as a band I and the maximum absorbance at the range of 240 to 290 nm as band II.[21,22]

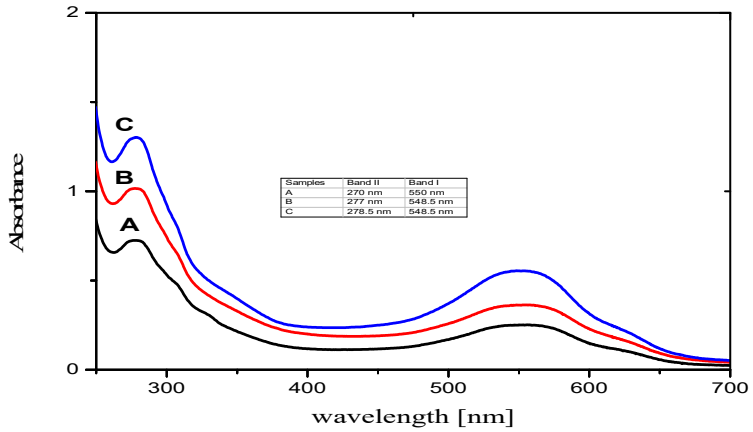


Fig.-1: UV-Vis Spectrum of Buni Fruits Extracted

### Characterization of Extract using IR Spectroscopy

Buni fruit extract measurement was conducted by Infrared Spectroscopy to find out the functional groups contained in the extract. The measurement result (Fig.-2 and Table-2) shows that buni fruit extract contained an aromatic functional group shown in wavenumber of 1452.45 to1454.38 $cm^{-1}$ as C=C-C aromatic. Also O-C-H bound was also identified in a form of phenolic on wave number of 1224.84 to 1238.34 $cm^{-1}$. There was also a carbonyl functional group, C=O indicated by the absorbance of wavenumber of 1708.99 to 1734.06 $cm^{-1}$. The functional group of -OH was also identified with the unique wave number of 3421.83 to 3524.06 $cm^{-1}$. The functional group contained in Buni fruit extract strengthen the extracted measurement by using UV-Vis Spectroscopy (Fig.-1) which identifies that buni fruit extract contained a flavonoid compound. Flavonoid compound was known to contain three rings which two of those are aromatic. It was also known that the flavonoid compound has a carbonyl functional group. Also, several derivatives of the flavonoid compound contain -OH functional group.

Table-2: 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] |

### Total Phenolic Content

The total phenolic content of buni fruit extract was measured by the Folin-Ciocalteau 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 the extraction process to produce a high total phenol content and antioxidant activities.[28]

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]

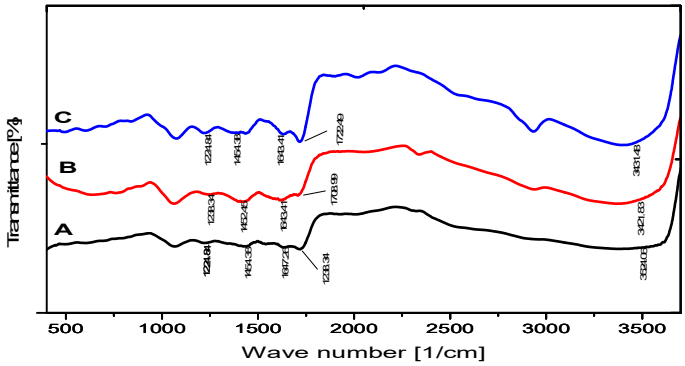


Fig.-2: IR Spectrum of Buni Fruits Extract

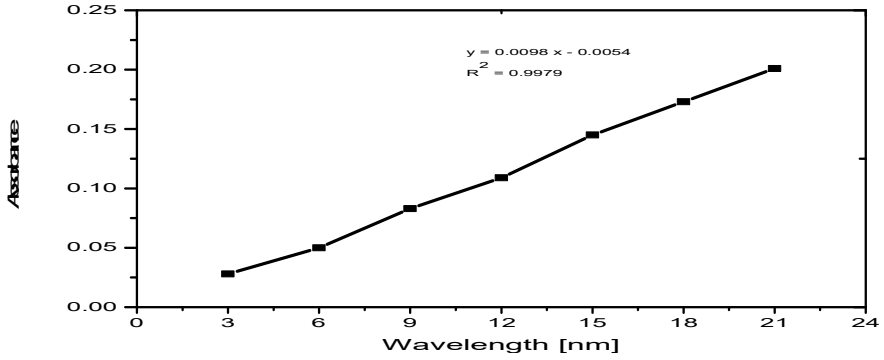


Fig.-3: Gallic Acid Calibration Curves at the Various Concentrations

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 were measured by DPPH method. The ability of buni fruit extract used as an antioxidant was based on phenolic compound content[30-,32]. The ability as an antioxidant can be seen by the value of $IC_{50}$.[33]

The value of $IC_{50}$ in three ultrasound-assisted extraction temperature shows an insignificant difference. Extraction at the temperature of 50°C given better value of $IC_{50}$ compared to the value obtained by extraction at the temperature of 40°Cand 60°C.The value of $IC_{50}$ was affected by the phenolic compound in the extract. The higher the total phenolic content, the stronger the antioxidant value of $IC_{50}$ would be. The value of $IC_{50}$< 50 mg/L indicates a strong antioxidant.[34] Phenolic compound has antioxidant property

because of its hydroxyl functional group leading to be able for hydrogen donor in the 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 varying 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 variations generated a high total phenolic content and strong antioxidant activities.

## ACKNOWLEDGMENT

Our gratitude to the Ministry of Science, Technology, and Higher Education of Indonesia for financial support and also to Bogor Agricultural University especially to Analytical Chemistry Division which has cooperated with authors in conducting a research study.

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