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ANTIOXIDANTS ACTIVITIES OF SECONDARY METABOLITE COMPOUNDS FROM BUNI FRUIT (*ANTIDESMA BUNIVUS L.*) SEED EXTRACT

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ABSTRACT

In this study, the seeds of Buni fruit were extracted with methanol solvent using Ultrasound-Assisted Extraction at a temperature of 50°C for 45 minutes. The methanol extract of Buni fruit seeds produced two phenolic compounds as a result of characterization using GCMS, namely Eugenol and Alpha-Tocopherol-beta-D-mannoside. The resulting IR spectrum showed the presence of a hydroxyl group (-OH) and aromatic compounds in the methanol extract of buni fruit seeds. Total Phenolic Content of Buni fruit seed methanol extract was 38.667 ± 0.764 mg/g in GAE (Gallic Acid Equivalent) and Antioxidant Activity IC_{50} was 1.632 ± 0.280 mg/L. The methanol extract of Buni fruit seeds is classified as a very strong antioxidant so that it has the potential to be developed as a functional food for health.

Keywords: Buni Fruit Seed, Antioxidant, Total Phenolic Content, Extraction, Methanol.

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INTRODUCTION

Plants that are included in the *Antidesma* genus-group are known to have health properties such as antimicrobials and antioxidants^{1,2,3}. Buni fruit (*Antidesma bunivus L.*) is one of the plants belonging to the *Antidesma* genus. Buni fruit is one of the plants used as a traditional medicinal plant in the Bugis tribe in Indonesia. Exploiting every part of the plant has the potential to be used as a source of natural medicine. Several studies have only developed Buni fruit pulp and Buni fruit water as a potential part of being used as a source of treatment^{2,4,5}.

Buni plants consist of several parts such as leaves, stems, meat, and seeds. Buni fruit seeds are part of the buni fruit whose utilization has not been found much, even only used as a byproduct of the buni fruit, even though every part of a plant is believed to have properties because it contains secondary metabolites. Secondary metabolite compounds in the form of phenolic compounds in plants have the potential to be used as antioxidants because of their ability as electron donors that are in their hydroxyl groups so that they can ward off free radicals^{6,7}.

Optimization of the potential utilization of the seeds from the Buni fruit which really needs to be developed as an antioxidant because, in addition to the abundant amount, the seeds of the Buni fruit must contain secondary metabolite compounds. For this reason, this study aims to determine the antioxidant activity and to determine the characteristics of secondary metabolite compounds contained in the methanol extract of buni fruit seeds. Buni fruit seeds are extracted using Ultrasound-Assisted Extraction because the process is easy and fast but does not affect the quality of the extract produced^{8,9}.

EXPERIMENTAL

Reagent

All chemicals used to come from the Merck company such as methanol (C₂H₅OH), Folin-Ciocalteu Phenol, Sodium Carbonate (Na₂CO₃), Gallic Acid, while the chemicals that come from the Sigma Aldrich company are 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Extraction of Buni fruit seeds

The purplish-red color of the Buni fruit plant material from inside and around the Moncongloe-Maros area, Indonesia is collected, washed, separated between the meat and seeds then dried, and made into powder for further extraction.

The extraction of buni fruit seeds was carried out using the Ultrasound-Assisted Extraction method using methanol as a solvent. 300 g of Buni fruit seed powder that has been dissolved with methanol is then put into the sonic power Ultrasound of 40 W on a wave of 40 kHz at 50°C for 45 minutes. The resulting filtrate is then evaporated at low pressure and a temperature not exceeding 45°C to obtain a thick extract⁶.

Chemical Analysis of The Extract

The extract was characterized by Prestige-21 Shimadzu Infrared spectroscopy at the range of 400-4000 cm⁻¹ using KBr pellets and Characterization with GCMS using Ultra Shimadzu QP2010 Gas Chromatograph Mass Spectrometer^{2,10,11}.

Determination of Total Phenolic Content

1 mL of methanol extract of Buni fruit seeds is added with 1 mL of Folin Ciocalteu solution and 5 mL of 10% Na₂CO₃. The solution was incubated at room temperature for 1 hour. Furthermore, the absorbance was determined at a wavelength of 765 nm by using Orion Aquamate 8000 UV-Vis Spectroscopy. Total Phenolic Content expressed as mg gallic acid equivalent (GAE) / g, obtained from the calibration curve of the standard solution of gallic acid (range from 5 to 65 mg/L)^{1,12,13}.

Antioxidant Activity Test

The methanol extract of the seeds of the Buni fruit was measured using the DPPH method^{2,14,15}. 2 mL of methanol extract of buni fruit seeds (10, 20, 30, 40 and 50 mg/L) was added with 2 mL of 0.1 M DPPH into each of various concentrations of the methanol extract of Buni fruit seeds. The absorbance of the solution was measured by Orion Aquamate 8000 UV-Vis Spectroscopy at the wavelength of 517 nm.

RESULTS AND DISCUSSION

The methanol extract of Buni fruit seeds was analyzed qualitatively using GCMS and Infrared Spectroscopy to determine the chemical compounds contained in the extract. The use of GCMS to determine the amount and chemical structure of the compounds contained in the sample while Infrared Spectroscopy is used to determine the functional groups contained in the sample. The results of measurements using GCMS (Fig.-1 and Table 1), obtained secondary metabolite compounds included in the phenolic compound group, namely Eugenol (Fig.-4) and Alpha-Tocopherol-beta-D-mannoside (Fig.-5).

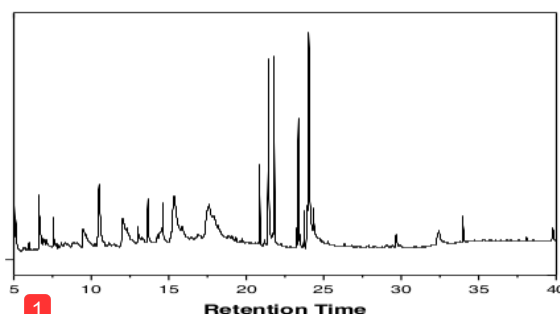


Fig.-1: GCMS Spectrum of Methanol Extract from Buni Fruits Seed

Table-1: Measurement results with GCMS from methanol extract of Buni Fruits Seed

| No | Retention Time | Name of Compound |
|----|----------------|---|
| 1 | 6.627 | 2-Cyclopentanedione |
| 2 | 7.552 | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one |
| 3 | 10.503 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- |
| 4 | 12.012 | 2-Furancarboxaldehyde, 5-(Hydroxymethyl)- |
| 5 | 12.294 | 1-Propanamine, 3-propoxy- |
| 6 | 13.008 | Eugenol |
| 7 | 13.654 | Eugenol |
| 8 | 14.408 | 2-Butanone, 4-(3-Ethoxyiranyl)-, Trans- |
| 9 | 14.636 | Caryophyllene |
| 10 | 15.362 | Ethanol, 2-(2-butoxyethoxy)-, acetate |
| 11 | 17.618 | 10-Hydroxy-d-mannonic lactone |
| 12 | 20.877 | Hexadecanoic Acid, Methyl Ester |
| 13 | 21.441 | l-(+)-Ascorbic acid 2,6-dihexadecanoate |
| 14 | 21.725 | 5-Tetradecen-1-ol, acetate, (Z)- |
| 15 | 21.807 | Hexadecanoic Acid, Ethyl Ester |
| 16 | 23.360 | 8,11,14-Docosatrienoic acid, methyl ester |
| 17 | 23.739 | Octadecanoic acid, methyl ester |
| 18 | 23.921 | 9,12-Octadecadienoic acid (Z,Z)- |
| 19 | 24.040 | 9,12,15-Octadecatrien-1-OL |
| 20 | 24.342 | Octadecanoic acid |
| 21 | 29.668 | Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester |
| 22 | 32.358 | 9-Octadecenoic Acid (Z)-, Octadecyl Ester |
| 23 | 32.432 | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- |
| 24 | 33.988 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- |
| 25 | 39.780 | alpha.-Tocopherol-beta.-D-mannoside |

The content of phenolic compounds contained in the methanol extract of Buni fruit seeds was also strengthened from the results of qualitative analysis using IR spectroscopy which indicated the presence of a typical spectrum (Figure 2) for aromatic groups at wave number $1454,38 \text{ cm}^{-1}$ as C=C-C (aromatic) bonds and the hydroxyl group -OH at wave number $3387,11 \text{ cm}^{-1}$ ^{16,17,18}.

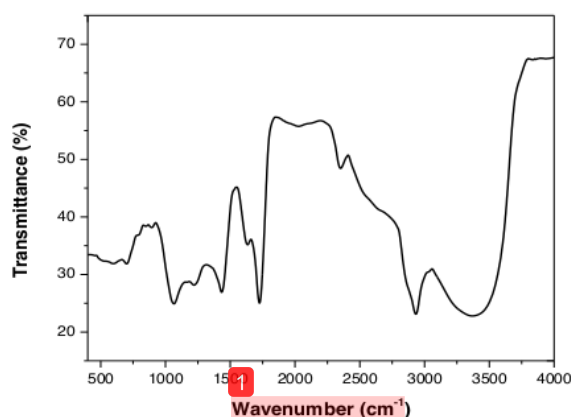


Fig.-2: IR Spectrum of Methanol Extract from Buni Fruits Seed

The content of Eugenol and Alpha-Tocopherol-beta-D-mannoside compounds in the methanol extract of Buni fruit seeds greatly impacts the total phenolic content and antioxidant activity. The results of Total Phenolic Content (Fig.-3 and Table 2) in the methanol extract of Buni fruit seeds were $38.667 \pm 0.764 \text{ mg/g}$ in GAE (Gallic Acid Equivalent) with IC_{50} Antioxidant Activity of $11.632 \pm 0.280 \text{ mg/L}$.

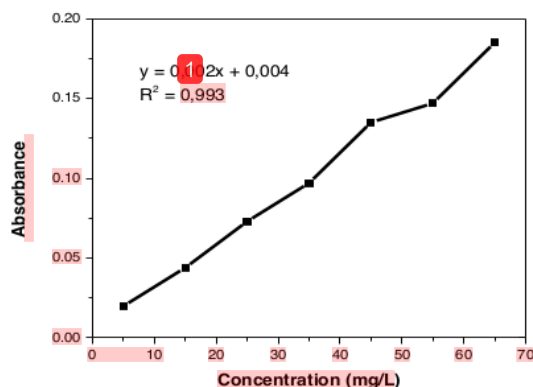


Fig.-3: Gallic Acid Standard Curves in the Concentration Range of 5 ppm - 65 ppm

Table-2 : Total Phenolic Content Of Ekstrak Metanol Biji Buah Buni

| Samples* | Absorbance | Total Phenol Content (mg/g in GAE*) |
|----------|------------|-------------------------------------|
| S1 | 0,083 | 38,667 ± 0,764 |
| S2 | 0,080 | |
| S3 | 0,081 | |

Table-3 : Antioxidant Activities of Ekstrak Metanol Biji Buah Buni

| Samples* | Linear Equations | R ² | IC ₅₀ (mg/L) | Average of IC ₅₀ (mg/L) |
|----------|--------------------|----------------|-------------------------|------------------------------------|
| S1 | y = 0,097x + 48,88 | 0,994 | 11,5464 | 11,6320 ± 0,280 |
| S2 | y = 0,108x + 48,71 | 0,991 | 11,9444 | |
| S3 | y = 0,121x + 48,62 | 0,996 | 11,4050 | |

According to Bezzera, et al. (2017)¹⁹ stated that Eugenol has many benefits such as being able to act as an antioxidant or prooxidant agent. In addition, it has anti-carcinogenic, cytotoxic and anti-tumor properties. While the Alpha-Tocopherol compound is known apart from being an antioxidant, Alpha-Tocopherol is also useful as a treatment in Alzheimer's²⁰ disease and anti-inflammatory disease²¹. The ability of eugenol^{22,23,24,25} and Alpha-Tocopherol as an antioxidant is due to the fact that eugenol is included in a group of phenolic compounds with characteristic aromatic rings²⁴. The presence of a hydroxyl group attached to the aromatic ring of phenol makes eugenol and Alpha-Tocopherol able to act as hydrogen donors so as to increase oxidation inhibition²⁶. The methanol extract of Buni fruit seeds is very strong in inhibiting 50% free radicals because it is included in the very strong antioxidant group because it has an IC₅₀ of < 50 mg/L^{2,27,28,29}.

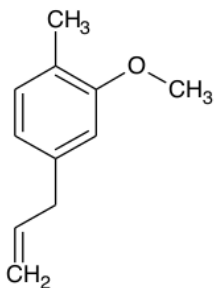


Fig.-4: Eugenol

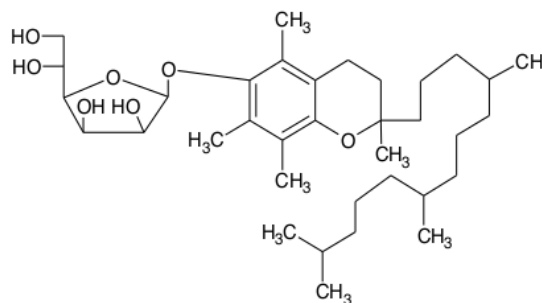


Fig.-5: Alpha-Tocopherol-beta-D-mannoside

CONCLUSION

Methanol extract of Buni fruit seeds is classified as a very strong antioxidant with an IC_{50} of 11.632 ± 0.280 mg/L and a Total Phenolic Content of 38.667 ± 0.764 mg/g in GAE (Gallic Acid Equivalent). Methanol extract of Buni fruit seeds contains phenolic compounds in the form of Eugenol and Alpha-Tocopherol-beta-D-mannoside.

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