

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

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ABSTRACT

In this study, methanol solvent was used to extract the seeds of Buni fruit with Ultrasound-Assisted Extraction at a temperature of 50°C for 45 minutes. The methanol extract of Buni fruit seeds produced two phenolic compounds because 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 11.632 ± 0.280 mg/L. The high antioxidant content of the methanol extract of buni fruit seeds has the potential to be used as a healthy food ingredient for the body.

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

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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.¹⁻³ Buni fruit (*Antidesmabunius 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 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 characterize the secondary metabolite compounds contained in the methanol extract from the seeds of Buni fruit and to determine their potential antioxidant activity. 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 grams of Buni seed powder that has been dissolved with methanol are then put into Ultrasound-Assisted Extraction at a temperature of 50°C with a soaking time of 45 minutes with a wave of 40 KHz. The resulting filtrate is then evaporated at low pressure and a temperature of less than 45°C.⁶

Chemical Analysis of The Extract

To determine the content of the bound functional groups, the methanol extract from the seeds of Buni fruit was characterized using Infrared Spectroscopy (Prestige-21 Shimadzu Infrared spectroscopy), while to determine the composition of the compounds contained in the methanol extract from the seeds of Buni fruit, measurements were carried out using GCMS (Ultra Shimadzu QP2010 Gas Chromatograph Mass Spectrometer).^{2,10,11}

Determination of Total Phenolic Content

The solution containing a mixture of 1 mL of methanol extract from the seeds of Buni fruit was added with 1 mL of Folin Ciocalteu and 5 mL of 10% Na₂CO₃ and then incubated at room temperature for 1 hour. Furthermore, the absorbance measurement of the mixed solution was carried out at a wavelength of 765 nm using UV-Vis Spectroscopy (Orion Aquamate 8000). Total Phenolic Content is obtained from plotting the absorbance of the sample in the calibration curve of the standard solution of gallic acid (range from 5 to 65 mg / L) expressed as Gallic Acid Equivalent per gram (GAE / g).^{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 at concentrations of 10, 20, 30, 40 and 50 mg/L were added each with 2 mL of 0.1 M DPPH into the test tube. The absorbance of the solution was measured by UV-Vis Spectroscopy at a 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 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).

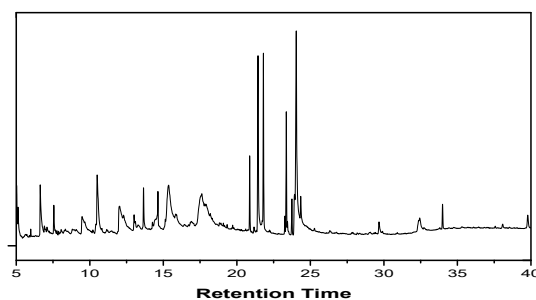


Fig.-1: Chromatogram of Measurement Results of Methanol Extract from Buni Fruit Seeds using GCMS

Table-1: Measurement Results with GCMS from Methanol Extract of Buni Fruits Seed

No	Retention Time	Name of Compound
1	6.627	1,2-Cyclopentanedione
2	7.552	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one

No	Retention Time	Name of Compound
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-Ethylxiranyl)-, Trans-
9	14.636	Caryophyllene
10	15.362	Ethanol, 2-(2-butoxyethoxy)-, acetate
11	17.618	3-Deoxy-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 (Fig.-2) for aromatic groups at wave number 1454.38 cm^{-1} as C=C-C (aromatic) bonds and the hydroxyl group -OH at wave number 3387.11 cm^{-1} .¹⁶⁻¹⁸

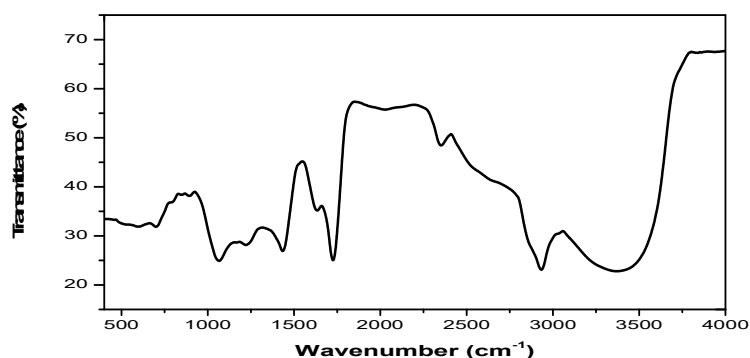


Fig.-2: Spectrum of Measurement Results of Methanol Extract from Buni Fruit Seeds using IR Spectroscopy

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 ± 0.764 mg/g in GAE (Gallic Acid Equivalent) with IC_{50} Antioxidant Activity of 11.632 ± 0.280 mg/L.

According to Bezzera, *et al.*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 disease.²¹ The ability of eugenol²²⁻²⁵ and Alpha-Tocopherol as an antioxidant is because eugenol is included in a group of phenolic compounds with characteristic aromatic rings.²⁴ The presence of a hydroxyl group attached to the aromatic ring of phenol makes eugenol and Alpha-Tocopherol able to act as hydrogen donors to increase

oxidation inhibition.²⁶ The methanol extract of Buni fruit seeds is very strong in inhibiting 50% free radicals. It is included in the very strong antioxidant group because it has an IC_{50} of < 50 mg/L.^{2,27-29}

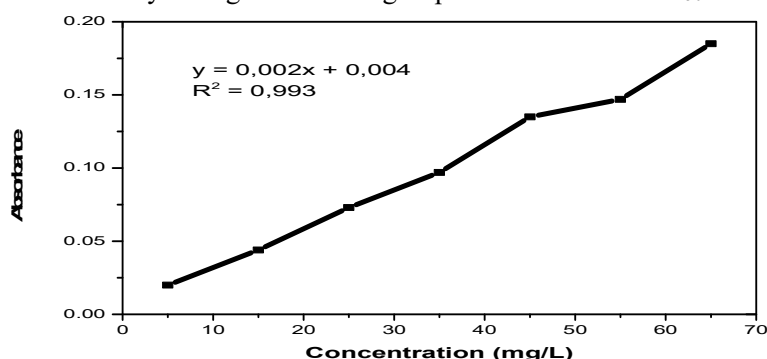


Fig.-3: The Standard Curve of the Measurement Results of Gallic Acid Solution at a Concentration of 5 ppm - 65 ppm

Table-2: Total Phenolic Content of Methanol Extract from Buni Fruits Seed

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 Methanol Extract from Buni Fruits Seed

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	

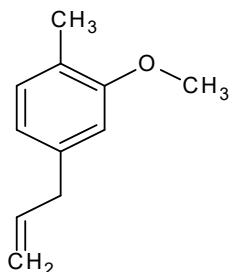


Fig.-4: Eugenol

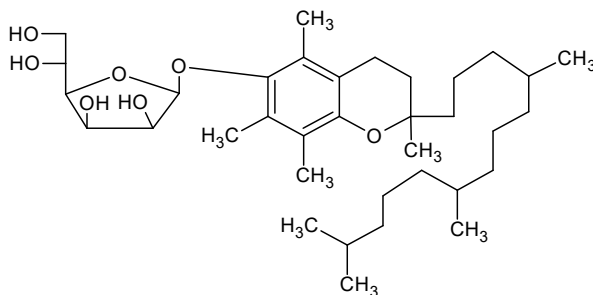


Fig.-5: Alpha-Tocopherol-beta-D-mannoside

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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