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ANALYSIS BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF EXTRACT BEKUL FRUIT (*Ziziphus jujuba* Mill.) AS A POTENTIAL NUTRACEUTICAL

Analisis Senyawa Bioaktif dan Aktivitas Antioksidan Ekstrak Buah Bekul (Ziziphus jujuba Mill.) Sebagai Potensi Nutraceutical

Komang Windayani[1], Paramasari Dirgahayu[1,2,4], Ratih Puspita Febrinasari[1,3]

[1]Master Program of Nutritional Sciences, Postgraduate School, Universitas Sebelas Maret, Surakarta, Indonesia
[2]Department Parasitology and Mycology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
[3]Department of Pharmacologi, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
[4]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
*Email: windayani534@gmail.com

ABSTRAK

Pemanfaatan bahan alami sebagai terapi diet atau nutrisi dalam mengobati atau mencegah suatu penyakit berkembang sangat pesat. Salah satu bahan alami yang memiliki potensi sebagai agen *nutraceutical* adalah buah bekul Bali (*Ziziphus jujuba* Mill.). Saat ini belum banyak bukti empiris terkait potensi *nutraceutical* yang terdapat pada buah bekul (*Ziziphus jujuba* Mill.) varietas Bali. Penelitian ini bertujuan untuk mengetahui kandungan senyawa bioaktif dan aktivitas antioksidan yang terdapat dalam ekstrak etanol buah bekul (*Ziziphus jujuba* Mill.) varietas Bali. Metode yang digunakan pada penelitian ini yaitu *eksploratorik desain* dengan sampel menggunakan buah bekul Bali (*Ziziphus jujuba* Mill.). Penelitian ini dilaksanakan di Laboratorium Penelitian dan Pengujian Terpadu (LPPT) Universitas Gajah Mada pada bulan April 2024. Proses ekstrasi menggunakan metode maserasi dan uji aktivitas antioksidan dilakukan dengan metode DPPH. Penentuan kadar total flavonoid, fenolik dan saponin menggunakan metode spektrofotometer UV-Vis, sedangkan penentuan kadar quercetin menggunakan metode HPLC. Hasil uji aktivitas antioksidan berdasarkan nilai IC_{50} tergolong kuat yaitu sebesar 78,57%, kandungan senyawa total flavonoid (114,85 mg QE/g), quercetin (60,00 µg/g), fenolik (109,34 mg GAE/g) dan saponin (9,90 mg/g). Dapat disimpulkan bahwa ekstrak etanol buah bekul (*Ziziphus jujuba* Mill.) varietas Bali diketahui memiliki kandungan senyawa bioaktif yaitu fenolik, flavonoid dan turunannya seperti quercetin, dan saponin serta memiliki aktivitas antioksidan tergolong kuat yang dapat dimanfaatkan sebagai agen nutraceutical.

Kata kunci: Buah bekul, *Ziziphus jujuba* Mill., nutraceutical, senyawa bioaktif, aktivitas antioksidan

ABSTRACT

The utilization of natural materials as dietary or nutritional therapy in treating or preventing disease is growing very rapidly. One of the natural ingredients that have potential as a nutraceutical agent is Balinese bekul fruit (Ziziphus jujuba Mill.). Currently, there is not much empirical evidence related to the nutraceutical potential contained in the of bekul fruit (Ziziphus jujuba Mill.) Bali variety. This study aims to determine the content of bioactive compounds and antioxidant activity in the ethanol extract of the bekul fruit (Ziziphus jujuba Mill.) Bali variety. The method used in this research is exploratory design with samples using bekul fruit (Ziziphus jujuba Mill.) Bali variety. This research was conducted at the Integrated Research and Testing Laboratory (LPPT) of Gadjah

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Mada University in April 2024. The maceration method was used in the extraction process. The DPPH method was employed to conduct the antioxidant activity test; the UV-Vis spectrophotometer method was employed to determine the total flavonoid, phenolic, and saponin content; and the HPLC method was used to determine the quercetin content. The results of the antioxidant activity test based on the IC_{50}, classified as strong at 78.57%, showed the content of total flavonoid compounds (114.85 mg QE/g), quercetin (60.00 µg/g), phenolics (109.34 mg GAE/g), and saponins (9.90 mg/g). It can be concluded that the ethanol extract of bekul fruit (Ziziphus jujuba Mill.) variety of Bali is recognized for its bioactive compounds, such as phenolics, flavonoids, and their derivatives, including quercetin and saponins. It possesses potent antioxidant properties that can be utilized as a nutraceutical agent.

Keyword: *antioxidant activity, bekul fruit, bioactive compounds, nutraceutical, Ziziphus jujuba Mill*

INTRODUCTION

Treatment using natural ingredients, also known as complementary and alternative medicine (CAM), is growing very rapidly [1]. Increasing public interest has encouraged esearchers to explore the potential of natural constituents as nutraceutical agents for disease treatment [2]. Nutraceutical is a term that combines the terms “nutrition” and “pharmaceutical.” It is defined as a food or a component of food that offers health benefits, such as the prevention and/or treatment of diseases [3]. Nutraceuticals have garnered significant attention because of their demonstrated impact on health and potential therapeutic actions in the treatment of numerous chronic diseases [4]. Research about nutraceutical components has the potential to improve the public’s comprehension of food sources in the health sector [5].

One fruit that has the potential to serve as a nutraceutical agent but is not widely recognized by the general public is the bekul or bidara fruit. The bekul fruit, scientifically identified as *Ziziphus jujuba* Mill., is a plant species that belongs to the *Rhamnaceae* family. Various regions of the world including Iran, India, and China, have been extensively cultivated [6]. *Ziziphus jujuba* Mill. or bekul fruit is capable of flourishing in regions with relatively severe temperatures and droughts [7]. Each region refers to the bekul fruit (*Ziziphus jujuba* Mill.) by a variety of names. For example, in the Java/Sundanese region, it is known as Bidara, in the Bima area as Rangga, in the Sumba area as Kalangga, in the Kupang area as Kom, and in the Bali region as Bekul [8]. The Bekul fruit (*Ziziphus jujuba* Mill.) is a thorny bush tree with a trunk diameter of 40 cm or greater and a height ranging from 4 to 16 meters. The Bekul fruit is characterized by a smooth, shiny, yellowish-green to reddish exterior and a white, crunchy, mildly sour to sweet flesh [6].

Bekul fruit (*Ziziphus jujuba* Mill.) is a fruit that is rich nutrients and phytochemicals, contributing to its high nutritional value. The nutritional value of 100 grams of fresh fruit is as follows: 20.23 g of carbohydrates, 1.2 g of protein, 0.2 g of fat, 7.3 g of fiber, 69 mg of vitamin C, 40 IU of vitamin A, and 0.9 mg of vitamin B3. It also includes minerals like magnesium (10 mg), phosphorus (23 mg), and potassium (250 mg) [9]. The water content of fresh bekul fruit (*Ziziphus jujuba* Mill.) typically ranges from 77% to 83%. Bekul fruit (*Ziziphus jujuba* Mill.) is a plant that is a source of bioactive components, including flavonoids, phenolics, saponins, triterpenic acids, and polysaccharides [10]. The known phytochemical content of Bekul fruit (*Ziziphus jujuba* Mill.) indicates its potential use as a source of antioxidants, antidiabetes, hepatoprotection, anti-inflammation, epilepsy, anti-cancer, and anti-dyslipidemia [11] [12].

The health sector's utilization of bekul fruit (*Ziziphus jujuba* Mill.) is suboptimal due to the dearth of public awareness regarding its nutraceutical potential. Further research is required to ascertain the bioactive compounds and antioxidant activity of the ethanol

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extract of the Bali variety of bekul fruit (*Ziziphus jujuba* Mill.). This is due to the potential presence of bioactive compounds that are recognized as beneficial to health. This research has the potential to provide empirical evidence regarding the content of bioactive compounds and antioxidant activity in Balinese bekul fruit (*Ziziphus jujuba* Mill.) varieties, as there has been limited research on the ethanol extract in the prevention and/or treatment of a disease.

METHODS

This study uses a exploratory design. The research sample was in the ethanol extract of the bekul fruit (*Ziziphus jujuba* Mill,) with the variables studied: antioxidant activity, total flavonoids, quercetin content, total phenolics, and saponins. *This research was conducted at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University in April 2024.* The research protocol was approved by the Health Research Ethics Committee of Sebelas Maret University (No. 46/UN27.06.11/KEP/EC/2024).

A. Research Materials and Equipment

The materials used are bekul fruit (*Ziziphus jujuba* Mill.), distilled water, 80% ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), $AlCl_3$, galic acid, sulfuric acid, acetic acid, methanol, dragendroff reagent, and vitamin C (ascorbic acid) Merck. The equipment used are a flouring machine, oven, rotary evaporator, incubator, UV-Vis spectrophotometer, balance sheet, test tube, dropper pipette, measuring cup, glass cuvette, filter paper, jar, and water bath.

B. Procedure

1. Sample Preparation

Bekul fruit (*Ziziphus jujuba* Mill.) was procured from cultivators in the Banjar Area of Buleleng Regency, Bali. The process of preparing simplysia involves the separation of bekul fruit between the pulp and seeds, followed by the cutting of the fruit into small pieces with a thickness of ± 1 cm. The pieces are then desiccated in an oven at 40°C. The sample is subsequently pulverized using a flouring machine to produce a fine powder or simplified form after it has dried [13].

2. Extraction

The maceration procedure was employed to extract the bekul fruit (*Ziziphus jujuba* Mill.) variety Bali by soaking the sample in an ethanol solvent. The results of the simplysia were subsequently weighed and dissolved in 70% ethanol for two 24-hour periods. The sediment and filtrate were separated by repeating the extraction and filtering it with whattman paper. The filtrate was subsequently concentrated using a rotary evaporator at 40°C and a speed of 100 rpm until the solvent evaporated, resulting in a viscous extract [14].

3. Antioxidant activity test

The antioxidant activity was assessed using the DPPH method. The sample extract was diluted into five distinct concentrations to evaluate its efficacy at varying concentrations. Subsequently, a DPPH solution was introduced and the mixture was incubated at 37°C for 30 minutes. The absorbance of the solution was determined at a wavelength of approximately 517 nm using a spectrophotometer following incubation. The inhibitory concentration (IC_{50}) value is the concentration of the sample that can reduce the DPPH free radical by 50%. This value is used to express the percentage of DPPH radical inhibition [15].

4. Total flavonoid

The determination of flavonoid levels was carried out using the UV-Vis spectrophotometer method. Samples of bekul fruit extract were taken at a dosage of 50 mg, and then 0.3 ml of 5% sodium nitrite was added to the sample. After 5 minutes, add 0.6 mL of 10% aluminum chloride and 2 mL of 1 M sodium hydroxide. Then add distilled

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water up to 10 ml with a volumetric flask, then transfer it into a cuvette. The absorbance was read with a spectrophotometer at a wavelength of 510 nm [16].

5. Quercetin content

Quercetin content was assessed through the utilization of the HPLC (High Performance Liquid Chromatography) technique. Samples weighing up to 500 mg were weighed and subsequently transferred to a 15 ml test tube. The addition of 1 ml of 50% ethanol. This was followed by sonication for 60 minutes and a vortex for 30 seconds. The sample was disentfused and subsequently transferred to a 5 ml volumetric vial. Add 50% ethanol and repeat the process 2-3 times. Dilute the solution twice and subsequently filter it through a 0.45 µm hypodermic filter. Add 25 µL of the sample to the HPLC. Conduct a 20 minute analysis of the sample at a wavelength of 370 nm [17].

6. Total phenolic

Determination of total phenolics using the UV-Vis spectrophotometer method by weighing 50 mg of sample material dissolved with 0.5 ml of folin-ciocalteu reagent and 5 ml of distilled water. The mixture was left for 10 minutes at room temperature, then 1.5 ml of 20% sodium carbonate was added. Adding distilled water to a volume of 10 ml with a volumetric flask, then transfer into a cuvette. The absorbance was read with a spectrophotometer at a wavelength of 760 nm [18].

7. Saponin content

The total saponins were determined using the UV-Vis spectrophotometer method. The sample was weighed to a maximum of 50 milligrams, and 2 ml of H2SO4 2N was subsequently added. The sample was autoclaved at 110°C for 120 minutes. Subsequently, it was extracted with ether, and the filtrate was desiccated. Added 1 ml of water and then used a vortex to extract it for 5 minutes. Homogenized the mixture after adding 50 µl of anisaldehyde. Allow the mixture to remain for 10 minutes, and then add 2 ml of 50% sulfuric acid. Heat on a water immersion at 60°C for a duration of 10 minutes. Using a volumetric flask, add 10 ml of distilled water and then transfer the mixture to a cuvette. The absorbance was measured at a wavelength of 435 nm using a spectrophotometer [19].

RESULT

1. Extraction of Bekul Fruit (*Ziziphus jujuba* Mill.) variety of Bali

The maceration procedure was employed to extract the bekul fruit with 70% ethanol. The weight extract was 409 grams, with a total of 4,242 grams of simplysia utilized. The weight of the extract is compared to the ultimate weight of the simplysia bekul fruit (*Ziziphus jujuba* Mill.) to determine the yield in percent. The resulting value is 9.6% (Table 1).

Tabel 1. Yield of Bekul Fruit (Ziziphus jujuba Mill.) Variety Bali

Fruit Type	Weight of simplysia (gram)	Weight of Extract (gram)	Yield (%)
Buah Bekul (*Ziziphus jujuba* Mill.)	4.242	409	9,6%

2. Antioxidant activity

The antioxidant activity of the Bekul fruit (*Ziziphus jujuba* Mill.) variety of Bali was evaluated using the DPPH method, which was based on the IC_{50} value and the equation y=bx+a. The ethanol extract of bekul fruit contains an IC_{50} of 78.57 µg/ml, as illustrated in Table 2. The antioxidant activity of the ethanol extract of bekul fruit is classified as strong, with an IC_{50} value ranging from 50 to 100 µg/mL.

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Table 2. Antioxidant Activity of Ethanol Extract of Bekul Fruit (*Ziziphus jujuba* Mill.) Variety Bali

Sample	Concentration (ppm) (x)	Mean Absorbance	Mean % Inhibition (y)	IC_{50} (µg/mL)
Ethanol extract of Bekul fruit (*Ziziphus jujuba* Mill.) variety Bali	1265	0,009	97,13	78,57
	632	0,059	81,21	
	316	0,093	70,38	
	158	0,143	54,46	
	79	0,196	37,58	

The antioxidant activity curve of the ethanol extract of bekul fruit (*Ziziphus jujuba* Mill.) is illustrated in Figure 1.

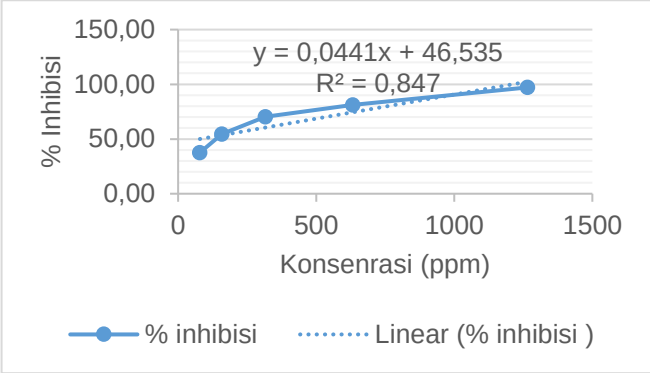

Figure 1. Linear Regression Curve the Antioxidant Activity of Bekul Fruit (*Ziziphus jujuba* Mill.) variety of Bali

3. Bioactive Compound Content (total flavonoids, phenolics, quercetin and saponins)

The ethanol extract of the bekul fruit (*Ziziphus jujuba* Mill.) contains a variety of bioactive compounds, such as total flavonoids, phenolics, quercetin, and saponins, as illustrated in Table 3. The test results were repeated up to two to three times, resulting in the average value being as follows:

Table 3. Results of Bioactive Compound Content of Ethanol Extract of Bekul Fruit (*Ziziphus jujuba* Mill.) Bali variety

Parameters	Unit	Analysis Result			Mean	Methods
		I	II	III		
Flavonoid Total	% (b/b)	11,21	11,29	11,96	11,49	Spektrofotometri UV-vis
Quercetin	µg/g	64,05	55,96	-	60,00	HPLC
Phenolic total	% (b/b)	11,16	10,52	11,12	10,93	Spektrofotometri UV-vis
Saponin	% (b/b)	0,99	0,99	-	0,99	Spektrofotometri UV-vis

DISCUSSION

In this study, the process of making bekul fruit extract (*Ziziphus jujuba* Mill.) using the maceration method with 70% ethanol solvent. Previous studies often used 70% ethanol extraction to obtain bioactive compounds from natural materials [20]. Table 1 shows the weight of the bekul fruit extract (*Ziziphus jujuba* Mill.) at 409 grams, with a yield

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calculation result of 9.6%. The percentage of yield calculation results shows the maximization of a solvent used in extraction [21].

Antioxidant activity testing of ethanol extract of bekul fruit (*Ziziphus jujuba* Mill.) variety of Bali was conducted using DPPH method. Antioxidant activity is the capacity of a compound or bioactive substance to protect body cells from the damage caused by free radicals [22]. Inhibitor Concentration 50 (IC_{50}) is the unit of measurement used to express antioxidant activity testing. The IC_{50} value is the concentration of an antioxidant compound that is necessary to reduce free radical activity by 50%. Antioxidant activity is inversely proportional to the IC50 value, with smaller IC50 values indicating greater antioxidant activity and conversely [23]. The antioxidant activity testing results in Table 2 indicate a value of 78.57%. Classified the antioxidant activity as strong due to its IC_{50}, which falls within the 50 – 100% range [24]. This is consistent with the research findings [25] which indicate that the antioxidant activity of bekul fruit (*Ziziphus jujuba* Mill.) is classified as strong, with a value of 77.40%.

Bioactive compounds are substances with biological activity that have the potential to prevent and treat a wide range of diseases [26]. Researchers have extensively investigated bioactive compounds, including flavonoid compounds and their derivatives, such as quercetin, phenolic compounds, and saponins, and found that they have beneficial effects on the body [27].

The analysis of bioactive compounds revealed that the ethanol extract from the Bali variety of bekul fruit (*Ziziphus jujuba* Mill.) contains a total of 11.49% (b/b) flavonoids, equivalent to 114.85 mg QE/g. The total flavonoid content is greater than that which was only 92 mg QE/g [28]. This may be attributed to variations in the type of solvent used and distinctions in geographical location. *Ziziphus jujuba* Mill. fruit from China was employed in the research conducted by [28], which employed an 80% methanol solvent. In contrast, the *Ziziphus jujuba* Mill. fruit used in this study was sourced from Bali and was dissolved in a 70% ethanol solvent. The optimal quantity of bioactive compounds is more effectively produced by utilizing 70% ethanol solvent, as it maintains the stability of active ingredients during the extraction process, resulting in a higher content of bioactive compounds. This is due to the solvent's appropriate polarity balance [29].

Quercetin is one of the flavonoid derivatives present in extract bekul fruit (*Ziziphus jujuba* Mill.) variety of Bali. Quercetin is a potent antioxidant that aids in the protection of cells from the harmful effects of free radicals [30]. The HPLC method was employed in this study to test for quercetin. The total quantity of quercetin detected was 60.00 µg/g. The test results of quercetin content in the study conducted by [31] were 54.63 µg/g, which is not significantly different from the current result.

Other bioactive compounds found in bekul fruit contain total phenolic compounds, which are 10.93% (b/b) or equivalent to 109.34 mg/g gallic acid equivalent (GAE). Phenolic compounds are secondary metabolite compounds that are found in all plants [32]. The total phenolic content of bekul fruit extract (Ziziphus jujuba Mill.) is greater than [9] which was 85 mg/100 g GAE, and [25] which was 29.49 mg/100 g GAE. Other studies have also reported lower results, specifically 16.33 mg GAE/g [28].

The extract of bekul fruit (*Ziziphus jujuba* Mill.) contained 9.90 mg/g of saponins. Saponins are bioactive compounds found in various types of plants [33]. The saponin content contained in the extract bekul fruit (Ziziphus jujuba Mill.) variety of Bali is lower than the saponin content in the study of [28], which amounted to 17 mg/g. Other studies also mention that the saponin content in Ziziphus jujuba Mill. amounted to 32 mg/g [34] and 9.5 mg/g [35].

The genetic factors of specific fruit varieties and the environmental conditions in which the fruit grows can contribute to variations in the yield of bioactive compounds. Plants that are cultivated in soil that is abundant in specific minerals may generate an increased quantity of bioactive compounds [36]. In addition, the bioactive substance

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content of certain fruit varieties may be influenced by their natural capacity to synthesize and retain their content, which is higher than that of others [37]. During the maturation process of fruit, the concentration of bioactive compounds may fluctuate [38]. This study employs fresh fruit that is still yellowish green and has a fruit age of approximately 70–75 days, in contrast to research conducted in Iran or India, which typically employs fruits that are extremely mature until they attain a reddish appearance. The content of bioactive compounds in the bekul fruit (*Ziziphus jujuba* Mill.) variety Bali can also be influenced by the difference in the level of fruit maturity. This study's limitations include not testing several bioactive compounds such as catechins, rutin, inulin, ferulic acid, and vitamin C due to limited laboratory testing.

CONCLUSIONS

The ethanol extract of bekul fruit (Ziziphus jujuba Mill.) Bali Variety is recognized for its bioactive compounds, including phenolics, saponins, and flavonoids including quercetin. Bekul fruit (Ziziphus jujuba Mill.) Bali Variety also possesses potent antioxidant properties that may be employed to prevent or treat diseases. Future studies should test additional bioactive compounds in the bekul fruit (*Ziziphus jujuba* Mill.) to learn more about its potential as a nutraceutical.

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