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Antioxidant potential and identification of active compounds on Kabau seed (*Archidendron bubalinum*) flesh and husk extract

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Abstract

Kabau (*Archidendron bubalinum*) is one of the plants that is often used as food ingredients and has the potential to be developed in the health sector. This study aims to determine the antioxidant potential of kabau seeds using 2,2-diphenyl-1-picril hidrazil (DPPH) and ferric reducing ability power (FRAP) methods and to identify active compounds of kabau seeds using GC-MS. Kabau seeds were macerated using 70% ethanol and 99.9% ethanol. The results showed that 99.9% ethanol extract of kabau seed coat had the highest antioxidant activity with IC_{50} values of 26.75 ppm for the DPPH method and 121.55 ppm for the FRAP method, respectively. Identification of active compounds using GC-MS showed that 99.9% ethanol extract of kabau seed coat contained the most 9,12-octadecadienoic acid compounds (linoleic acid) so that it could be developed as an antioxidant.

1. Introduction

One of the severe health problems in Indonesia is non-communicable diseases such as heart disease, stroke, cancer, and diabetes mellitus. The disease is a chronic disease that can be caused by metabolic degeneration. Based on the results of basic health research in 2018, the prevalence of non-communicable diseases has increased when compared with 2013 health research for cancer, stroke, chronic kidney disease, diabetes mellitus, and hypertension [1]. According to WHO that non-communicable diseases have killed 41 million people each year, equivalent to 71% of all deaths in the world [2].

One of the causes of metabolic degeneration is free radicals, which are molecules that contain one or more unpaired electrons in the outer orbitals, are highly reactive and unstable. The stability of these molecules can be achieved if they react with other stable molecules to obtain their electron pairs [3]. Excessive oxidation reactions in the body can trigger the formation of free radicals through metabolic processes from food or endogenous, but the body has an antidote to free radicals called antioxidants. When the amount of free radicals exceeds the body's capacity to neutralize these free radicals, oxidative stress is formed, which can cause damage to cells, tissues, and organs [4]. For that, we need antioxidant compounds from outside the body, known as secondary antioxidants. These antioxidants can be obtained from food ingredients [5].

Kabau seed (*Archidendron bubalinum*) is one of the food ingredients that is widely consumed by people in Darmasraya-West Sumatra because it is arousing appetite. Kabau seed is also used as a vegetable that is very popular with the people of Jambi but has not been cultivated much like its relatives, jengkol [6]. Kabau is one of the food plants that resemble jengkol but has a smaller size and a sharper aroma than jengkol seeds, the distinctive aroma of kabau seeds can improve appetite [7]. In Malaysia, kabau seed is known as kerdas and is often used as a salad but by the younger generation is not

preferred because of the firm and pungent odor. Mushrooms isolated from mature kabau seeds have the potential to be yeast [8].

Phytochemical analysis of methanol extract from *Archidendron bubalinum* seed coat containing secondary metabolites, i.e., flavonoids, tannin polyphenols, and terpenoids [9]. Other studies have shown that kabau seed extracts contain a lot of flavonoids, tannins, and saponins [10]. However, until now, there have not been many scientific reports on bioactivity and active compounds in kabau seed. The bioactivity of a natural material needs to be known because it can be a piece of scientific information for the development of medicines from natural materials so that the kabau seed, which has only been used as food, can also be used for medical treatment. Therefore, the research carried out aims to determine the antioxidant potential and content of active compounds in mature kabau seed extracts.

2. Research Methods

2.1. Equipment and Materials

The equipment used includes glassware, evaporators (Buchi, Switzerland), EpochTM microplate readers (Biotek, United States), Boeco m-24A centrifuges (Boeco, Germany), UV-VIS BMG Labtech nano spectrophotometers, pH meter 510 Thermal scientific (United States), GC-MS Agilent 5975C Inert XL (Canada), Toshiba refrigerators (Japan), microplate Starderc (Italy), Philips blenders, water baths, sieves, and scales.

The ingredients used include distilled water, absolute ethanol (99.9%), DPPH reagents (2,2-diphenyl-1-picryl hydrazyl), ascorbic acid (vitamin C), phosphate buffer, trichloroacetic acid, potassium hexacyanoferrate ($FeCl_3$) and kabau seed from Dharmasraya Regency-West Sumatra that has been determined at the Bogoriense Herbarium in the Botany Division, Biology Research Center, LIPI Bogor.

2.2. Kabau seed extraction

2.2.1. Sample preparation

A total of 10 kg of ripe and fresh kabau seed was dried under the sun. During the drying process, kabau seed was separated from the husk so that it was separated between flesh and kabau seed husk. Both were dried to obtain a dry weight of 10% of the wet weight, and a sample of kabau seed flesh and husk was obtained. Each sample was mashed using a blender, then filtered with a 100-mesh sieve to obtain a powder sample.

2.2.2. Extraction

Four Erlenmeyer flasks were each filled with 100 g of sample in which two Erlenmeyer were filled with kabau seed flesh powder, and the other two Erlenmeyer were filled with kabau seed husk powder. Each sample was macerated with 500 mL of 99.9% ethanol and 70% ethanol. Then four main samples were obtained i.e., kabau seed flesh - ethanol 99.9% called sample A, kabau seed flesh - 70% ethanol as sample B, kabau seed husk - 99.9% ethanol as sample C and kabau seed husk - 70% ethanol as sample D. Each mixture was stored in a refrigerator (10°C) for 4 x 24 hours and occasionally shaken to be homogeneous. The filtrate of each mixture was taken every 24 hours, and the supernatant was added again with new solvents. Each filtrate obtained was collected to be concentrated using a vacuum rotary evaporator, so that a kabau seed extract was obtained.

2.3. Measurement of Antioxidant Activity from Extracts

2.3.1. The modified 2,2-diphenyl-1-picryl hydrazyl (DPPH) method [11]

One hundred µL extracts from samples A, B, C, and D were dissolved in ethanol, each diluted to various concentrations, i.e., 3,125 ppm, 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm, 200 ppm, 500 ppm, and 1000 ppm. Then for each sample solution, 100 mL of DPPH 125 µmol/L was added, which was dissolved in ethanol, then put into the microplate, shaken until homogeneous, and incubated in a dark room at room temperature (27°C) for 30 minutes. Sample absorption was measured at a wavelength of 517 nm with a microplate reader. Ethanol was used for the negative control, while positive control used ascorbic acid (vitamin C). The antioxidant activity of kabau seed was expressed as an IC_{50} value. The IC_{50} value was obtained by calculating the amount (%) of inhibition against DPPH using the following formula:

$$\%\ \text{DPPH inhibition} = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}}$$

Notes:
A_{DPPH}: DPPH absorption
A_{sample}: Sample absorption

2.3.2. Modified ferric reducing ability power (FRAP) method [12]

1 mL of extracts of samples A, B, C, and D dissolved in ethanol were each diluted with various concentrations, i.e., 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm, 200 ppm, 500 ppm, and 1000 ppm. 1 mL of phosphate buffer 0.2 M (pH = 6.6) and potassium hexacyanoferrate were added to the sample. Then the mixture was shaken and incubated in a 50°C water bath for 20 minutes. 1 mL of trichloroacetic acid (TCA) 1% was added to the sample, then shaken and centrifuged at 3000 rpm for 10 minutes. A total of 1 mL of the supernatant sample was taken, and 0.2 mL of 0.1% $FeCl_3$ was added, then the mixture was incubated at 37°C for 30 minutes in a dark room. Adsorption of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 700 nm. As a blank, ethanol was used, and a standard curve was made using a solution of ascorbic acid (vitamin C). Antioxidant activity was compared with the reducing power of the sample where the value of %reducing power of the sample was obtained using the following equation:

$$\% \text{ reducing power} = \frac{A_{samples} - A_{blank}}{A_{samples}}$$

Notes:
A_{sample}: adsorption of sample
A_{blank}: adsorption of sample

2.4. Identification of Active Compounds

The kabau seed extract, which had the highest antioxidant activity of the two antioxidant analysis methods, was then identified using GC-MS. A total of 5 µL samples were inserted into the GC-MS tool, which had an HP Ultra 2 column with a column length of 30 m, a column diameter of 0.20 mm, and a stationary phase thickness of 0.11 µm. The initial temperature was 80°C and rose 3°C/minute until the temperature of 150°C was held for 1 minute then the temperature was raised 20°C/minute until the temperature of 280°C was held again for 26 minutes, while the injection temperature was carried out at 250°C. The carrier gas used was helium with a flow rate of 1.2 mL/min and a split ratio of 8: 1.

3. Results and Discussion

3.1. Antioxidant from kabau seed extract

3.1.1. Antioxidants obtained from the DPPH method.

DPPH is a free radical that is stable at room temperature. In DPPH solution can be reduced by antioxidant compounds so that the color changes from purple to yellow. The intensity of the color change that occurs can be measured at a wavelength of 517 nm to show antioxidant activity. One parameter commonly used to indicate that antioxidant activity is inhibition concentration (IC_{50}). IC_{50} values indicate the concentration of an antioxidant compound that can cause 50% DPPH to lose its free radical character [13]. However, the IC_{50} value is inversely proportional to antioxidant activity. The results of the IC_{50} values of vitamin C, kabau seed flesh, and husk are presented in Figure 1.

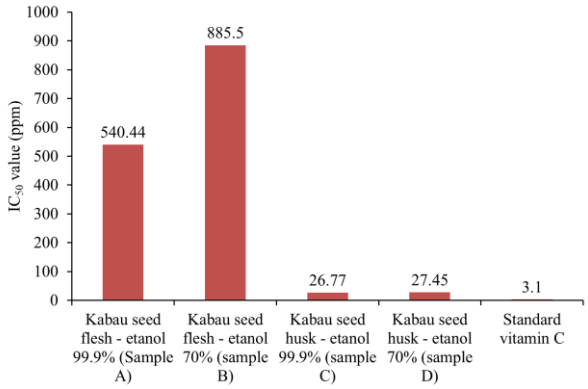

Figure 1. IC_{50} values of various extracts of kabau seed flesh and husk and vitamin C, which were analyzed using the DPPH antioxidant method.

Figure 1 shows that kabau seed extract has antioxidant activity with different IC_{50} values. IC_{50} values of kabau seed flesh - 99.9% ethanol, kabau seed flesh - 70% ethanol, kabau seed husk - 99.9% ethanol and kabau seed husk - 70% ethanol are 540.44 ppm, 885.50 ppm, 26.77 ppm, and 27.45 ppm respectively. Based on these values, it is known that 99.9% ethanol extract of kabau seed husk has the highest antioxidant activity, compared to other kabau seed extracts. However, it is still lower when compared with vitamin C. The antioxidant ability of kabau seed husk in the study was higher than that of the *Archidendron bubalinum* decoction extract with an IC_{50} value of 99.50 µg/ml reported in previous studies [14]. The results of this study are also higher than the results of other studies that report that the antioxidant activity of Kabau seed husk extract (*Archidendron bubalinum*) using the DPPH method with methanol, ethyl acetate, and hexane solvents has IC_{50} values of 324.913 ppm, 273.57 ppm and 735 ppm [15]. While the antioxidant activity found in the kabau seed husk extracts from Lampung and South Sumatra by the maceration method had IC_{50} values of 17.61 µg/mL and 44.7 µg/mL [16].

3.1.2. Antioxidant activity analyzed using the FRAP Method

The principle of the FRAP method is to measure the reduction reaction in the yellow Fe^{3+} complex compound (potassium hexacyanoferrate) that occurs in an acidic atmosphere, forming a Fe^{2+} complex that produces a bluish-green color due to obtaining electrons from antioxidant compounds. The ability to reduce Fe^{3+} determines the strength of antioxidants as a reducing agent [17, 18]. The FRAP method is an alternative method of antioxidant analysis for small samples and fast analysis time [19]. Measurement of antioxidant activity by the FRAP method can be used to determine the IC_{50} parameters by measuring the ability of an extract to reduce Fe^{3+} contained in a 1% potassium hexacyanoferrate solution. The results of the antioxidant analysis of kabau seed extract by the FRAP method are presented in Figure 2.

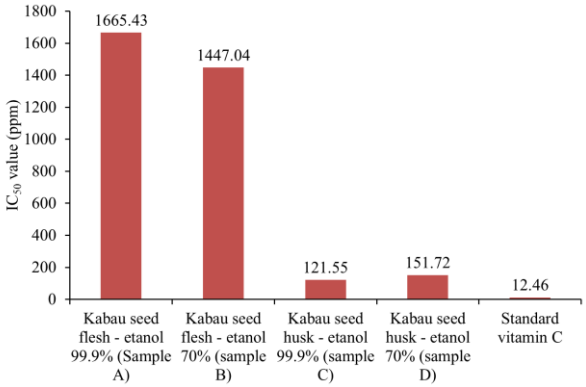

Figure 2. IC_{50} values of flesh and husk kabau seed extracts and vitamin C analyzed by the FRAP antioxidant method

Figure 2 shows that all kabau seed extracts, i.e., kabau seed flesh - 99.9% ethanol, kabau seed flesh - 70% ethanol, kabau seed husk - 99.9% ethanol and kabau seed husk - ethanol 70% have a reducing power to Fe^{3+}, indicated by IC_{50} produced by each extract of 1665.43 ppm, 1447.04 ppm, 121.55 ppm, and 151.72 ppm. Kabau

seed husk - 99.9% ethanol extract has the lowest IC_{50} value of all other extracts, which is 121.55 ppm, meaning that the kabau seed husk - 99.9% ethanol has the highest antioxidant activity compared to the other kabau seed extracts. The smaller the FRAP value of a sample, the higher the antioxidant activity [20]. Previously it was reported that the IC_{50} value of *Archidendron bubalinum* boiled water using the FRAP method was 2.19 mmol Fe^{2+}/g dry weight [14].

3.2. Identification of Active Compounds

Based on the data above, it is known that the kabau seed husk - 99.9% ethanol extract has the highest antioxidant activity compared to other kabau seed extracts. GC-MS results showed that there were five main compounds in the kabau seed husk - 99.9% ethanol extract (Figure 3). The chromatogram peaks of the kabau seed extract - 99.9% ethanol, which have similarities to the compounds in the GC-MC data bank are presented in Table 1.

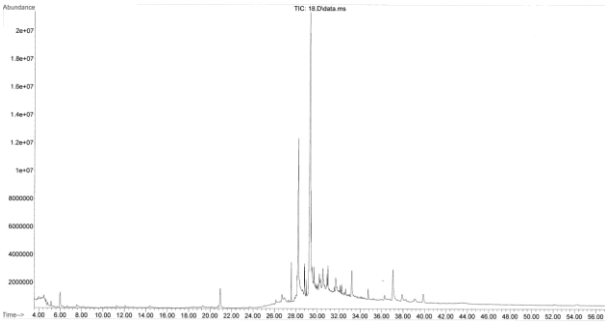

Figure 3. GC-MS chromatogram of kabau seed husk - 99.9% ethanol extract

Table 1. GC-MS analysis of kabau seed husk - 99.9% ethanol extracts

Peak	Retention Time (minutes)	*Quantity*	Chemical Components	percentage
1	5.993	95	2,3-hydro-3,5-dihydroxy-6-methyl-pyran-4-one	1.39
2	20.956	99	Lentionin	2.30
3	27.576	99	Methyl hexadecanoic	1.22
4	28.251	99	Hexadecanoic acid (palmitic acid)	19.63
5	28.831	99	Methyl Elaidate	1.34
6	29.341	99	9,12-octadecadienoic acid (linoleic acid)	36.31
7	29.417	99	Octadecanoic acid (stearic acid)	5.64
8	29.513	99	9,12-octadecadienoic acid (linoleic acid)	3.20
9	29.692	99	9,12-octadecadienoic acid (linoleic acid)	2.64
10	30.189	99	9,12-octadecadienoic acid (linoleic acid)	1.67
11	30.520	59	(+) - Beyeren-19-ol	2.60
12	31.720	95	9,12-octadecadienoic acid (linoleic acid)	1.32
13	33.216	47	m-phenetidine	2.44
14	37.070	87	Kondrilasterol	4.43
15	37.919	90	Stigmast-7-enol	1.04
16	39.898	97	Methyl beta-boswellanote	1.18

The bioactivity of some of the main compounds in the kabau seed husk - 99.9% ethanol extract can be seen in Table 2.

Table 2. Bioactivity of several active compound components of the kabau seed husk - 99.9% ethanol extract obtained from GC-MS

Chemical component	Bioactivity
9,12-octadecadienoic acid (linoleic acid)	anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotector, nematicide, insecticide, antihistamine, antiacne, antiandrogenic, antiarthritis, anticoroner and antimicrobial [20, 21]
Hexadecanoic acid (palmitic acid)	Antioxidants, antibacterial, nematicides, anti-inflammatory, hypocholesterolemic, pesticides, antiandrogenic, antitumor, immunostimulant, chemopreventive, hemolytic enzyme inhibitors 5-α reductase inhibitors of the lipoxygenase enzyme [20, 21]]
Octadecanoic acid (stearic acid)	Antibacterial Activity [22]
Kondrilasterol	Antimicrobial Activity [23]
Lentionin	Antimicrobial, reduces the production of tumor necrosis factor α and anti-inflammatory [24]

Table 1 shows that the most compound contained in the kabau seed husk - 99.9% ethanol extract is 9,12-octadecadienoic acid. The 9,12-octadecadienoic acid or linoleic acid is known as an antioxidant that protects cell membranes from damage [25]. Linoleic acid is a new compound identified in the kabau seed husk - 99.9% ethanol extract. According to Irawan *et al.* [15] that hexadecanoic acid (palmitic acid) is present in all methanol, ethyl acetate, and hexane extracts from kabau.

4. Conclusions

The results showed that kabau seed husk - 99.9% ethanol extract had the highest antioxidant activity compared to other kabau seed extracts, both using DPPH and FRAP antioxidant methods with IC_{50} values of 26.75 ppm and 121.55 ppm, respectively. In the process of identifying active compounds using GC-MC, it is known that the extract kabau seed husk - 99.9% ethanol contains 9,12-octadecadienoic acid (linoleic acid).

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