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Original Article

Comparison of Extraction Methods, Antioxidant Activities, Total Phenol in Seeds and Seed Shells of Kabau (*Archidendron bubalinum* (Jack) I.C. Nielsen) From Lampung and South Sumatra

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ABSTRACT

Background: Kabau is one of the famous plants in Lampung and South Sumatra. Kabau is usually used as an ingredient to increase the aroma of cooking. Kabau is a plant belongs to the same genus with *Archidendron pauciflorium* (Benth.) IC. Nielsen and in the same family with *Leucaena leucocephala* L. **Objective:** The purpose of this research was to compare the total phenolic content and the antioxidant activity between seed and fruit carp (shell) of kabau grown in Lampung and South Sumatra. **Method:** Kabau seeds and the shells were obtained from Lampung and South Sumatra. Extractions were conducted by maceration and a continuous extraction using Soxhlet apparatus with ethanol as the solvent. Phenolic content and the antioxidant activity of the extract were then measured. **Results:** The antioxidant activity and high phenol levels were found in kabau shell extract obtained by maceration method with IC-50 value of seed skin from Lampung 17.61 µg/mL and for those from South Sumatra. Phenol content of seed shell from Lampung 11.74 g GAE / 100 g and South Sumatra 5.88 g GAE / 100 g. **Conclusion:** Based on the research that has been done, it can be concluded that high antioxidant activity and high levels of total phenol were found in shell extract obtained by maceration of shell from Lampung and South Sumatra.

Key words: *Archidendron bubalinum* (Jack) Nielsen, Antioxidants, Phenols, Seeds, Seed shell.

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INTRODUCTION

Free radicals present in the body can affect health where oxidants have very reactive properties. Free radicals are compounds that have one or more unpaired electrons in their outer orbitals. These compounds always try to attack cellular components such as lipids, lipoproteins, proteins, carbohydrates, RNA and DNA.[1]

Oxidative stress is an imbalance between the number of free radical molecules and antioxidants in the body.[2] The substances needed by the body can help to protect the body from attacks by free radicals or radical compounds, namely antioxidants. Certain levels of antioxidants can inhibit or slow down the damage due to the oxidation process.[1]

Indonesian use plants as traditional medicines one of them is kabau (*Archidendron bubalinum* (Jack) I.C. Nielsen) which is empirically used as an antidiabetes. Diabetes mellitus can be induced by free radicals. Diabetes mellitus is a chronic disorder characterized by the increase of glucose in the blood. The link between diabetes mellitus and free radicals is the damage to ß-pancreatic cells.[3]

Kabau is one of the natural resources that has benefits as a food ingredient, but it is not widely known by people outside of the growing area. Kabau is also a close relative of "jengkol" (*Archidendron pauciflorium* (Benth.) IC. Nielsen) which is a member of the *Archidendron* that can be developed as an antidiabetic drug.[4] Jengkol fruit cortex contains alkaloid compounds, flavonoids, tannins, polyphenols, saponins, and triterpenoids or steroids.[5] Jengkol seeds contain carbohydrates, protein, vitamin A, B vitamins, phosphorus, calcium, alkaloids, essential oils, steroids, glycosides, tannins, and saponins.[6,7] Chinese petai seeds (*Leucaena leucocephala.* L) that is in the same family with kabau also have antioxidant activities by increasing SOD (Superdioxide Dismutase) of red blood cells.[8] According to has Antioxidant activity of Chinese petai seeds depends on the type of solvent used for extraction.[9-12] Therefore it was very interesting to study the comparison of extraction method on antioxidant activity and total phenolic content of seed and shell of kabau from Lampung and South Sumatra.

MATERIAL AND METHOD

Plant materials

The materials used in this study were kabau seeds and seed shell from Lampung and South Sumatra.

Phytochemical screening

Phytochemical screening was performed toward all extracts. Magnesium ribbon, HCl and amylalcohol for flavonoid compound. FeCl3 10% used for phenolic compound, gelatine for tannin, Dragendorf and Mayer reagents for alkaloid, Potassium hydroxide 5% for quinone, vanillin 10% in H_2SO_4 for monoterpene and sesquiterpene, Lieberman-

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Burchard for steroid and triterpenoid [10,11,12]. Saponins showed by a stable foam formation for 10 min after shaking of water extracts.[13]

Sample preparation

Archidendron bubalinum were collected from Lampung and Sumatera Selatan. The materials used in this study were the seed and the shell. The taxonomic confirmation of the plant was performed at the "Herbarium Bogoriense", Bogor Botanical Gardent the Biology Research Center-LIPI. The materials were washed under running water to remove soil and dirt, dried at 25^{0}C for one week to dryness. Ash content, water soluble material and ethanol soluble material were determined in accordance to WHO guidelines.[14] There were four samples, i.e. seeds from Lampung (SL), seed from South Sumatra (SS), Shell from Lampung (ShL), Shell from South Sumatra (ShS).

Extraction

Extraction was carried out using maceration and continuous extraction methods (Soxhlet apparatus). The test materials 100 g each was transferred into a macerator, soaked three times with ethanol for 24 hours respectively. Continuous extraction method was carried out by wrapping test material with a filter paper to form timble as that of Soxhlet extraction flask. The solvent was added into a round flask on the mantle heating. The extraction process takes place until the solvent in the timble becomes clear which indicates the extraction process has been completed. The results of the filtrate obtained were concentrated using a rotary evaporator at 50 °C. The remaining solvent in the extract was then removed by heating on a water bath and the yield calculated. There were eight extracts, seed maceration from Lampung (SLM), seeds maceration from South Sumatra (SSM), shell maceration from Lampung (ShLM), shell maceration from South Sumatra (ShSM), seed Soxhlet from Lampung (SLS), seed Soxhlet from South Sumatra (SSS), shell Soxhlet from Lampung (ShLS), shell Soxhlet from South Sumatra (ShSS).

Thin layer chromatography

The extracts that have been calculated for the yield were analyzed by thin layer chromatography (TLC) using a stationary phase. Silica Gel GF254 (Merck KGaA 1.05554.0007), mobile phase $CHCl_3$–EtOAc (8:2). The spot was visualized under UV 254 and 366 nm, 10% sulfuric acid in methanol, 1% iron (III) chloride, and 5% DPPH. Spraying 10% sulfuric acid will produce colors, 1% iron (III) chloride shows a blackish green color and DPPH yellow.

Antioxidant activities

Antioxidant activity in the kabau samples were assayed using the DPPH method. The use of this method is because DPPH is stable, simple and fast time analysis and can be observed with the occurrence of color changes from purple to yellow after the reaction.[15] Ascorbic acid which functions as a secondary antioxidant capable of counteracting various extracellular and intracellular free radicals was used as reference. Ascorbic acid is an antioxidant that works as an electron donor by moving one electron. This is because ascorbic acid has a free hydroxy group that can capture free radicals and if it has a polyhydroxy group it will increase the antioxidants activity.[16] For antioxidant activity, a standard ascorbic acid curve was made with various series of concentrations, then DPPH 50 µg/mL solution was measured at a wavelength of 517 nm. The extracts of kabau shell and seeds from Lampung and South Sumatra were made a series of concentrations, then each of the concentrations of extract was pipetted as much as 1.5 mL then 1.5 mL DPPH 50 µg/mL solution was added subsequently incubated for 30 minutes and the absorbance at 517 nm measured.

Determination of phenolic content

Phenolic content in the sample was measured as gallic acid equivalent. Gallic acid was weighed as much as 10 mg then added with methanol 20 mL then homogenized and transfer to a 100 mL volumetric flask, and the volume adjusted. The gallic acid is then made a series of concentrations of 10, 15,20,25,30 µg/mL. As much as 0.5 mL of each concentrations of gallic acid was added with 0.5 mL and then added 5 mL of Folin-Ciocalteu Reagent (which has been diluted with distilled water 1:10) and 4 mL added 1 M Na_2CO_3 mixed homogeneously, and left for 15 minutes at room temperature. Absorbance at 765 nm was measured, then make a calibration curve between the concentration of gallic acid (µg/mL) and absorbance was made.[16] The concentrated extract was treated in the same manner as that of standard solution. Measurements were repeated three times and the phenol content obtained was calculated using linear regression of the gallic acid calibration curve and expressed as gallic acid equivalent per 100 grams of extract (g GAE/100 g).

RESULTS

Taxonomic determination

The taxonomic determination of kabau plants was carried out in "Herbarium Bogoriense", the Botanical Garden of the Biology Research Center-LIPI Bogor, number of determination: 408/IPH.1.01/If.07/II/2018. The plants used was *Archidendron bubalinum* (Jack) I.C.Nielsen) Fabaceae. Figure 1 shows the picture of kabau seeds obtained from Lampung and South Sumatra.

Crude drug characterization

Characterization of crude drug that was carried out include water content, water soluble materials, total ash content, ethanol soluble materials and loss on drying. The results can be seen in Table 1.

Based on the results presented in Table 1, the highest moisture content was in Lampung shells and the lowest in Lampung seeds. Water content that meets the requirements, will prevent the growth of fungi and will last long during storage. Based on previous report[17] Lampung seeds have a water content of 0.23%, that is much lower than the result presented in Table 1. However, all samples tested fulfilled the requirement on the water content i.e less than 10%.[18]

Kabau shell of South Sumatra contained higher internal and external mineral content compared to Lampung shell, Lampung seeds and South Sumatra seeds. However, the characterization was still based on the value of the ash content in kabau which was 8.6%. Further analysis is needed to determine the content of inorganic and mineral originating from the internal or external sources. The highest water-soluble materials was found in South Sumatra seeds while the highest ethanol

Figure 1.1: *Archidendron bubalinum* (Jack) I.C.Nielsen from Lampung.

Figure 1.2: *Archidendron bubalinum* (Jack) I.C. Nielsen from South Sumatra.

Table 1: The characteristics of seeds and shells of kabau.

Characterization	Percentage			
	SL	SS	ShL	ShS
Water content	2	6	8	4
Total Ash Content	1,5	0,5	2,5	5,5
Water soluble materials	22	25	15	18
Ethanol soluble materials	7.0	3,9	8.0	7.0
Loss on drying	0,46	0,49	0,61	0,64

soluble materials was in Lampung shell. Analysis on the ethanol and water-soluble materials were carried out to determine the amount of material that will be extractable using those solvent. This will determine the extract yield during extraction process.[19] Loss on drying of the seeds of South Sumatra and Lampung was not much different. Likewise, on Lampung shell and South Sumatra shell the result was only 0.3% difference.

Extraction

The method used to obtain extracts was based on hot and cold methods. Hot method using Soxhlet apparatus while cold method maceration. The yields % obtained can be seen in Table 2.

Based on Table 2 the highest yield was obtained by the continuous extraction. From the data obtained that the seeds and shell of Lampung had lower yields compared to that of South Sumatra. This can be influenced by the limited solvent used by the maceration method where 1 L of the solvent used was divided into 3 part for three stages of maceration. As for the Soxhlet, the yield was higher due to the solvent used was 1L and recirculated as new solvent through evaporation and condensation. In addition, the temperature of extraction was also higher.

In addition, the geographical location may also affect the yield obtained where the altitude of the Lampung area from the Sea surface is 450-1700 m above sea level while South Sumatra area is 900-1200 m. Altitude was reported to be related to plant metabolic processes such as biochemical processes and the synthesis of secondary metabolites.[18] This will affect growth, morphological characters and the content of active compounds in a plant. The higher the altitude the higher the stress of the environment such as the temperature gets lower and the humidity gets higher, the intensity of the light gets smaller and the period of irradiation becomes shorter. The stress of temperature, light, humidity and others can affect the production of plant secondary metabolites. When plants experience stress, the production of secondary metabolites will increase. This is an effort to fight against environmental stress.

The variety of kabau from the Lampung and South Sumatra seems to be different and consequently their metabolic profile will be most likely to be different. Seed of kabau from Lampung region are smaller compared to those from South Sumatra. In addition, the length of the shell of kabau from the Lampung area was ± 5 cm while from the South Sumatra region ± 10 cm. Kabau pod from South Sumatra is larger, it has a bulkhead that separates seeds from one another. Insulation on the shell of about ± 1.5 cm in length. The kabau pod of Lampung does not have a bulkhead that separates seeds from one another. Seed coat of South Sumatra kabau is thicker compared to Lampung kabau.

Phytochemical screening

Phytochemical screening of kabau seeds and shells from Lampung and South Sumatra showed the presence of alkaloid, flavonoids, phenols, tannins, terpenoids/steroids, mono /sesquiterpenes and quinones. For the results of flavonoids, orange or yellow changes occur on the amyl alcohol layer. The addition of magnesium and hydrochloric powder causes the reduction of flavonoids to give red color. Flavonoid compounds are a group of the largest phenol compounds found in nature. Flavonoids can act as antioxidants as free radical catchers because their hydroxyl groups donate hydrogen to free radicals. In testing the saponin compound the results were positive, the froth as high as 1-1.5 cm was marked. This froth occurs because of the presence of a hydrophilic group which binds to water while hydrophobia binds to air.

Testing of positive results of tannin is indicated by the presence of white deposits and cloudy appearance when in 1% gelatin droplets which will precipitate protein in the gelain. Tannin compounds have activities as astringents, antidiarrhea, antibacteria and antioxidants.[20] The positive phenol test results are indicated by changes in blackish green when reacted with $FeCl_3$. The color change is due to the presence of hydroxyl phenolic groups in the compound.

Testing of terpenoids/steroids is indicated by formation of red or green colour when reacted with Liberman-Burchard which shows a positive steroid content. The results of the positive mono/sesquiterpenes test formed purple when reacted with vanillin sulfate. Tests for the positive quinone are marked with red color when reacted with 5% KOH.

Based on the results of phytochemical screening all extracts tested showed the same result.

Thin layer chromatography

Thin layer chromatography is carried out to determine the presence of compounds qualitatively. The mobile phase used is chloroform-ethyl acetate (8:2) and the stationary phase used was silica gel GF_{254}. Spraying the TLC plate using H_2SO_4, DPPH 5%, and $FeCl_3$.

TLC testing on 8 extracts showed that all extracts had antioxidant phenol compounds. This compound is semi-polar because it was eluted using chloroform and ethyl acetate. The use of $FeCl_3$ as a spray reagent appears to determine the presence of phenol compounds in the sample marked by the appearance of blackish green. All samples sprayed with DPPH 5% and $FeCl_3$ 1% had the same Rf value (0.1-0.375). TLC results that give a blackish green color change after spraying $FeCl_3$ and give a

Table 2: The yield from seed and seed shell.

Sample	Yield (%)	
	Maceration	Soxhlet
Seed from Lampung	5,63	8,13
Seed from South Sumatra	12,67	14,95
Shell from Lampung	6,52	9,35
Shell from South Sumatra	13,31	17,02

yellow color after DPPH spraying were demonstrated by spots with Rf values approximately 0.1 and 0.37. The TLC chromatogram that show phenol compounds and antioxidant activity can be seen in Figure 2.

Antioxidant activity

The parameters used to show the results of the DPPH method are IC_{50} (Inhibition Concentration 50), which is the concentration value of antioxidant compounds that can inhibit antioxidant activity by 50. The IC_{50} value produced is smaller, the stronger the antioxidant activity. The IC_{50} of ascorbic acid was 1,14 µg/mL. Ascorbic acid is a compound that is easily soluble in water, very sensitive to damage from outside, such as temperature, sugar, salt, pH, oxygen and metal catalysts.[1]

Figure 3 shows the results of IC_{50} of extracts obtained by hot extraction using Soxhlet and cold extraction by maceration. Based on this picture it can be seen that highest antioxidant activity was found in Lampung kabau shell extracted by maceration method. This may be due to lower level of decomposition of antioxidant compounds during extraction using maceration method compared to extraction using Soxhlet.

As shown in Figure 3, extract from seed and shell of kabau from Lampung has slightly stronger antioxidant activity compared to those obtained from South Sumatra. This can be influenced by the geographical location of plants, climate factors (temperature, air, humidity) and essential factors (light, water, and nutrient elements) and pest or disease and weed disorders.[21] The altitude of the Lampung region from is 450-1700 m, while South Sumatra area is 900-1200 m above sea level. In addition, the land in the South Sumatra is more acidic compared to Lampung, so that the land in the Lampung area does not need to be fertilized because there are elements (N, P and K). It is different in the South Sumatra area which must be given fertilizer. However, these two regions will be more fertile with rainfall.[22]

Higher antioxidant activity is found in the seed shell compared to the seeds. Visually, it can be seen that the extract of the shell was mach darker than the seeds. The chemical compounds present in the seeds and shell were alkaloid compounds, flavonoids, tannins, phenols, monoterpenes, steroids and quinones. Differences in the levels of compounds contained in seeds and shell may affect antioxidant activity. Compounds that have many hydroxyl phenolic will increase their antioxidant activity. Phenolic compounds and flavonoids have more than one hydroxyl group (polyhydroxyl) so it is very good at neutralizing a free radical.[21]

Determination of total phenol levels

Determination of total phenol levels in seed extract and kabau shell was carried out using Folin-Ciocalteau reagents. Phenol compounds will react with Folin-Ciocalteau reagents to form a blue complex. The phenolic content was measured as gallic acid equivalent that gave maximum absorption at a 780.5 nm.

Figure 4 showed the total phenol contents of kabau after extraction by Soxhlet and maceration. The highest level of phenol is in Lampung seed shell extracted maceration. The presence of high temperature treatment can damage the content of non-heat-resistant compounds including

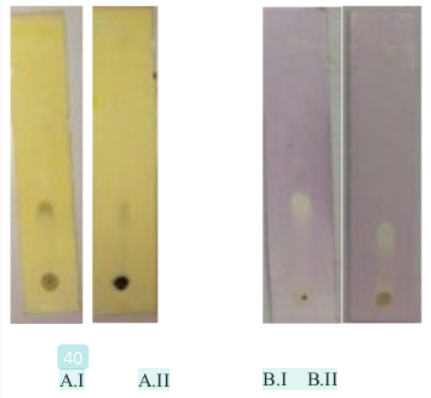

Figure 2: Chromatogram TLC of phenol compound with FeCl3 spray reagent on seed extract (A.I); shell (A.II) and Chromatogram TLC of antioxidant activity with DPPH spray reagent on seed extract (B.I); shell (B.II) from Kabau.

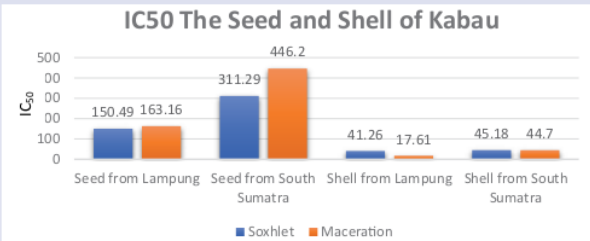

Figure 3: Testing of antioxidant activity based on extraction methods.

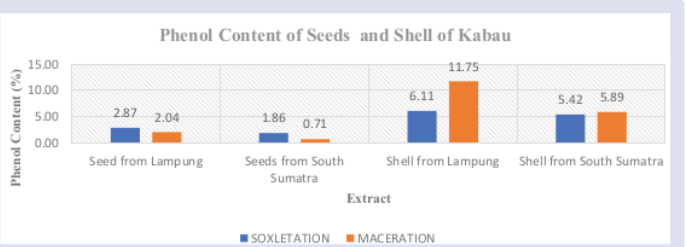

Figure 4: Total phenol total content based on extraction method.

phenol compounds. These results was line with those reported by Dewi, 2006 that showed the decrease of phenol content of *Aloe chinensis* juice upon extraction at high temperature.

The location of grow may also affect the total phenolic content of kabau. Comparison based on Lampung kabau had higher total phenol content whether in shells or seeds. This is cerrespond to the higer antioxidant activity of Lampung compared to those obtained from South Sumatra. However, this finding need to be further tested, since their morphology is slightly different indicating that they are different varieties.

CONCLUSION

Based on the research that has been done, it can be concluded that antioxidant activity and total phenolic content is higher upon extraction by cold method, the shell accumulated highest phenolic compounds. Lampung kabau demonstrated higher antioxidant activity and higher phenolic content compared to those obtained from South Sumatra.

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GRAPHICAL ABSTRACT

SUMMARY

Based on the research that has been done, it can be concluded that high antioxidant activity and high levels of total phenol were found in shell extract obtained by maceration of shell from Lampung and South Sumatra.

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