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THE TEST OF ANTIOXIDANT ACTIVITIES WITH COMPARISON OF EXTRACTION METHODS FROM ROBUSTA COFFEE SEEDS (*Coffea canephora*)

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Abstract. The content of robusta coffee include alkaloids, flavonoids, saponin, tannins, caffeine, and phenol. Differences in the method of extraction of a plant can cause differences in the number of chemical compounds. The purpose of this study was to determine the phytochemical characteristics and antioxidant activity in robusta coffee beans extracted by maceration and sonication method. Antioxidant activity was performed by the method of DPPH (2,2-diphenyl-1-picrylhydrazyl) by spectrophotometry. The results showed that phytochemical characteristics of robusta coffee extract of maceration and sonication contain the same chemical compounds, namely alkaloids, flavonoids, tannins, and saponins. The antioxidant activity of robusta coffee seed extract of sonication result has IC50 of 54,14 ppm which is better than the extract of robusta coffee beans from maceration.

Keywords: coffea canephora, maceration, sonication, antioxidant

I. INTRODUCTION

Indonesia is one of the third largest coffee producing countries in the world. Coffee is one of the results of plantation commodities that have high economic value among other plantation crops such as cocoa and tea. It becomes an important role as a source of foreign exchange. It is also a good source of income for no less than one and a half million coffee farmers in Indonesia (Rahardjo [1]). In Indonesia, there are 2 types of coffee produced, namely arabica and robusta.

Based on research by Bettina Cämmerer and Lothar W. Kroh [2] in 2006 stated that robusta coffee beans contain high polyphenols which has a function as antioxidants.

Antioxidant activity can inactivate the development of oxidation reactions by forming free radicals. To extract polyphenols in plants, the extraction needs to be done. It is the process of withdrawing chemical compounds found in a plant or animal by using suitable solvents. There are various types of extraction methods, namely: conventional and modern extraction methods.

Conventional extraction methods include maceration, detox, soxhlet, reflux, percolation, and infusion. Modern extraction methods include microwave extraction, high-pressure extraction, super-critical fluid extraction, sonication extraction, enzymatic extraction and hydrotropy extraction (Hanani, [3]). The extraction methods such as maceration require less time, making it more efficient. Sonication extraction is also relatively efficient because it utilizes ultrasonic waves. The effectiveness of extraction depends on the experimental conditions used such as extraction time, solvents, and solvent types. Therefore it is necessary to optimize the best antioxidant activity against the effect of differences in maceration and sonication extraction methods.

II. RESEARCH METHODS

The research was conducted from January to May 2018 at the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor.

Tools and Materials

The tools used are dark bottles (chocolate bottles), sonicators, 40 mesh sieves, digital scales (AND G-120®), moisture balance (AND MX 50®), ovens, furnaces (Ney®), Vaccum Dryer, a set of spectrometer devices UV-Vis (Optizen®) and glassware.

Materials

The materials needed are robusta coffee beans, 96% ethanol, concentrated petroleum ether, methanol pa, concentrated ethyl acetate, Zn P powder, 2N HCl, concentrated HCl, Mg P powder, distilled water, Bouchardat LP, Mayer LP, concentrated ammonia P , ether P, 80% ethanol, gelatin, NaCl, $FeCl_3$.

Organoleptic Test

Description of organoleptic powder and Robusta coffee extract are observations of the shape, color, smell and taste of coffee [4].

Making Extract

Maceration Extraction

Put 200 grams of simplicial powder in a dark bottle. Put 2 L ethanol extraction liquid as much as 2 L into a dark bottle, soak it for the first 6 hours while stirring occasionally, then left for 18 hours. Separate it by

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filtration. Repeat the process three times with the same type and solvent. The filtrate was collected and then dried using a Vaccum Dryer to obtain a dry extract [5]. Repeated 2 times.

Sonication Extraction
Simplicia powder weighed 100 grams and then placed into a container/vessel and added 96% ethanol technically as much as 1 L then covered with aluminum foil and then put in a sonicator for 15 minutes. Then let stand for 30 minutes and filtered with batis cloth. The filtrate is dried using a Vaccum Dryer to obtain a dry extract [5]. Repeated twice.

Characteristics of Extracts
Water content
Determination of water content is ready by using moisture balance. The sample was weighed as much as 2 grams into the prepared instrument.

Ash content
The test material weighed carefully 1 gram, then inserted into the silicate crucible which has been incandescent and cast, flattened. Spread at ± 600 ° C slowly until the charcoal exhausted, then cooled and weighed until the constant weight is ± 0.25%. If this way the charcoal cannot be removed, hot water is added, stirred and filtered. The filtrate inserted into the crucible evaporated and spilled until the weight is constant. The total ash content is calculated against the material that has been dried in the air. Repeated twice (Duplo) [4].

Determination of Extract Renderman
Extracts were calculated by comparing the weight of the simplicia and the weight of the extract produced.
Extract yield = (Extract weight) / (Simplicia weight) x 100%

Phytochemical Test Extract
Identification of Alkaloids
Put 500 mg of the extract then put into Erlenmeyer. Added 1 ml of 2N HCl and 9 mL of water and heated over a water bath for 15 minutes, cooled and filtered. The solution was dripped on a watch glass and each was added to with alkaloid reagents (Dragendroff, Mayer, and Bouchard). The filtrate is used for the following experiment:
1. The filtrate was dripped on the watch glass then added 2 drops of Bouchardat reagent. The positive results formed brown to black deposits.
2. The filtrate was dropped on the watch glass and then added 2 drops of Mayer reagent. The positive results formed white deposits.
3. The filtrate was dropped on the watch glass then added 2 drops of Dragendroff reagent. The positive results formed brown orange deposits (Hanani, [3]).

Identification of saponins
A total of 0.5 grams of the extract is weighed and then put into a test tube. Add 10 mL of hot water then cooled, shaken vigorously for 10 seconds will produce a stable foam with the addition of hydrochloric acid (Hanani, [3]).

Identification of Flavonoids
Weigh 2 grams of extract and shake with dichloromethane for 15 minutes, then filter it. The filtrate is dried until dry. The residue is dissolved in 50% methanol, if necessary by heating it over a water bath, then adding a little magnesium metal or zinc powder and 5-6 drops of concentrated hydrochloric acid, then heat a few minutes above the water bath. Emerging colors are observed (Hanani, [3]).

Identification of Tanin Compounds
A total of 2 grams compound was extracted with 80% ethanol (30 mL) for 15 minutes, then filter it. The filtrate obtained is evaporated over the bath. Add hot aquadest to the remaining evaporation and stir it. After that the solution was centrifuged. The top liquid is separated by decantation and it is used as the experimental solution in the following tests:
1. The filtrate is added with a solution of 10% gelatin, and there will be white sediment.
2. The filtrate is added with NaCl-gelatin (a solution of 1% gelatin in 10% NaCl with a ratio of 1: 1). There is sediment.
3. The filtrate is added with a solution of 3% iron (III) chloride, blue-green to blackish (Hanani, [3]).

Antioxidant Activity Test (DPPH (1,1 diphenyl-2-pikrihidrazil))

Making Solution
a. DPPH 1 mM solution
DPPH powder was weighed exactly 39.432 mg, then put into a 100 mL volumetric flask. Dissolved with methanol p.a to the limit (previously the volumetric flask was coated with aluminum foil).
b. Blank solution
Pipe 1 ml of DPPH 1 mM solution, add methanol p.a to 10 ml, then homogenized. Blank solution is incubated at a temperature of around 25-30 ° C (room temperature) for 30 minutes (volumetric flask wrapped in aluminum foil).
c. Standard solution of 100 ppm vitamin C
Weigh 100 mg ascorbic acid and then put into a 100 ml volumetric flask, then dissolve with p.a methanol to the limit (1000 ppm). To get a 100 ppm vitamin C mother solution, pipe 10 ml of vitamin C (1000 ppm) into a 100 ml volumetric flask and dissolve with methanol to the 100 ppm limit (Purnamasari [6]).

Determination of Maximum Wavelength
Pipe methanol p.an approximately 8 mL then add 1 mL DPPH 1 mM solution then dilute into the limit with methanol p.a in a 10 mL volumetric flask and incubate it in the room temperature for 30 minutes. Then the absorption is measured at a wavelength of 500-600 nm

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(previously the volumetric flask has been coated with aluminum foil) (Purnamasari [6]).

Determination of Optimum Incubation Time
Pipe as much as 1 ml of standard 100 Cm standard vitamin C solution then put it into a 10 ml volumetric flask. Approximately 4 mL of methanol p.a is added and 1 ml of DPPH 1 mM solution. Then dilute with methanol p.a until the boundary mark and homogenized. Uptake is measured at the maximum wavelength and measured at 10, 20, 30, 40, 50 and 60 minutes so that optimum absorption time is obtained (previously the volumetric flask is coated with aluminum foil) (Purnamasari [6]).

Making Standard Solution of Vitamin C
A standard series of the ascorbic acid mix with a concentration of 2; 4; 6; 8 and 10 ppm from the 100 ppm extract liquor is put into a 10 mL volumetric flask. 1 ml of DPPH 1 mM solution is added and diluted with methanol p.a to the boundary mark and incubate at optimum incubation time and its absorption is measured at the maximum wavelength using a UV-Vis spectrophotometer (Purnamasari [6]).

Making a variation solution test
First making a 1000 ppm extract solution by dissolving 50 mg of Robusta coffee. Each is put into a 50 mL volumetric flask, then dissolve with methanol p.a to the boundary mark. The standard series is made with concentrations of 5, 10, 20, 40, 80 and 100 ppm from the extract liquor into a 10 mL volumetric flask. 4 mL of methanol p.a and 1 mL of DPPH 1 mM solution is added and diluted using methanol p.a to the boundary mark and homogeneous. The series of test solutions are allowed to stand as long as the optimum time is at room temperature. Absorbance is measured at the maximum wavelength (previously the volumetric flask has been coated with aluminum foil).

Antioxidant Testing with DPPH Method
A series of test solutions, a series of positive control solutions of vitamin C and blanks are measured for absorption in the spectrophotometer with the maximum wavelength obtained. The resistance value of DPPH is calculated using the IC (Inhibitor Concentration) value of 50 obtained from a line cut between 50% inhibition and the concentration axis using a linear equation (y = box + a), where y = 50 and x indicates IC50 (Molyneux, [7]).

III. RESULTS AND DISCUSSION

Characteristics of roasted Robusta coffee powder has a rather coarse powder, brown color, bitter taste, and distinctive aromatic odor.

Robusta roasting coffee powder

a **b**

Figure 1. Robusta Coffee Extract macerated results (a) and sonication results (b)

After obtaining the dried extract of Robusta coffee beans then the yield is calculated which aims to determine the comparison between extracts obtained with initial Simplicia (DepKes [8]). The yield obtained in Robusta coffee bean extract maceration of 14.80%, and sonication of 20.50%. The difference in yield is due to differences in extraction time, solvents and solvent types (Oktavia [3]).

Measurement of extract water content using moisture balance obtained robusta coffee beans macerated by 1.395% and sonication results of 2.685%. The results of the water content are in accordance with the requirements in traditional medicinal preparations, which are not allowed to exceed the limit of 10% (RI Ministry of Health, 1994). The ash content of the coffee bean extract obtained from the maceration was 9.80% and the sonication results were 7.30%. Requirements for extract ash content should not be more than 10.2% (RI Ministry of Health [9]).

Phytochemical Test Results
Robusta coffee bean extract results from maceration and sonication containing alkaloid compounds, flavonoids, tannins, and saponins.

Results of Antioxidant Activity
Determination of the results of antioxidant activity was carried out first with the determination of the maximum wavelength measured at 500-600nm obtained by 715nm. Then the optimum incubation time was carried out for 60 minutes and obtained in the 30th minute. Furthermore, the inhibition test of the DPPH was carried out with concentrations of 2,4,6,8 and 10 ppm so that the linear equation y = 6,1850x + 24,620 with the value obtained R2 = 0.9996. The antioxidant activity of Robusta coffee bean extract from maceration results has an IC50 of 56.48 ppm and the results of sonication have an IC50 of 54.14 ppm. This is due to differences in extraction time and extraction method.

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IV. CONCLUSION

Phytochemical characteristics of robusta coffee bean extract from maceration and sonication results contain the same chemical compounds, namely alkaloids, flavonoids, tannins, and saponins. The antioxidant activity of Robusta coffee bean extract from sonication has an IC50 of 54.14 ppm which is better than the Robusta coffee bean extract from maceration.

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