Optimization and characterization of n-hexane extracts of arabica coffee ground (Coffea arabica L.) from Gayo plateau as source of natural antioxidant

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Abstract. Arabica coffee is a major commodity crop in the Gayo Plateau, Aceh Province. Utilization of coffee in the area only as a raw material for making coffee drinks and produce waste that is not utilized. The aim of this study is optimization and characterization of n-hexane extract of Arabica Gayo coffee ground (Coffea arabica L.) as a source of natural antioxidant. The extraction process used soxhlet method with n-hexane solvent. Characterization of arabica coffee ground oil consist of analysis of functional group using FTIR, component analysis using GC-MS and antioxidant analysis using DPPH method. The optimum time of the soxhlet process of Arabica coffee is 180 minutes with rendement of 9.54%, analysis of functional group shows CH₂ asymmetric stretch at 2922 cm⁻¹, CH₂ symmetric stretch at 2853 cm⁻¹, C=O asymmetric stretch at 1741 cm⁻¹, CH₂ bending at 1458 cm⁻¹, C-CH₃ vibration at 1159 cm⁻¹ and CH vibration at 718 cm⁻¹. The main component of Arabica Gayo coffee ground oil is 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester with an area of 18.09%, antioxidant activity of Arabica Gayo coffee ground oil is very weak with IC₅₀ value = 1222,31 ppm.

1. Introduction

The Gayo Plateau is an area in Aceh that includes three regencies i.e. Bener Meriah, Central Aceh and Gayo Lues. The main commodity from these regencies is coffee. The coffee cultivated massively in the three regencies is Arabica Coffee (Coffea arabica L.) due to its best taste and high price. In Aceh, Arabica Coffee is only used as raw material to make coffee and has not been treated in industry yet. In making a cup of coffee, it will be obtained something called coffee ground. The coffee ground usually become waste product that not being used.

Coffee ground contains polyphenol 18,180 mg/g, but polar extract of coffee ground contains polyphenols 1,746 mg/g and has an antioxidant activity of 29.04% [1]. The coffee ground extracted using subcritical water extraction method contains phenol compound of 86,23 mgGAE/g with ABTS antioxidant activity (81,38 mmolTE/100 g) and DPPH (42,12 mmolTE/100 g) [2]. The concentration of polyphenols in water extracts of coffee grounds reaches 5.66 mgGAE/g and has an antioxidant activity of 1222,31 ppm.
activity of 80.5% [3]. Varying extraction methods (soxhlet, ultrasonic and supercritical fluids) and solvents (hexane, dichloromethane, ethylacetate, ethanol and CO₂) used to observe the antioxidant activities of coffee grounds, all result indicate the presence of antioxidant activities of coffee ground with different levels [4]. Other studies have shown that coffee grounds used as fertilizers can increase the antioxidants activities and bioactive compounds from plants [5].

The aim of this study was optimization of time extraction and characterization of Arabica Gayo Coffee ground oil (Coffea arabica L.) grown in Gayo Plateau, Aceh, Indonesia as source of natural antioxidant. Extraction process was performed using soxhlet method with nonpolar solvent i.e. n-hexane, analysis of functional groups was performed using FTIR, analysis of oil component was performed using GC-MS and activity test of antioxidant was performed using DPPH method.

2. Methodology
2.1. Tools and materials
Tools used in this study were glasses, analytical scale, soxhlet, vortex mixer, rotary evaporator, Fourier transform infrared (FTIR) (Agilent resolution Cary 630 FTIR spectrometer), gas chromatography – mass spectrometry (GC-MS) (Shimadzu qp ultra 2010, with stationary phase colom db5ms and mobile phase helium gas), spectrophotometry UV-Vis (UV mini-1240 Shimadzu). Materials used in this study were sample of Arabica Coffee ground from Bandar, Bener Meriah Regency, n-hexane solvent, methanol solvent, and DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) from sigma-aldrich.

2.2. Extraction of coffee ground oil
Extraction of coffee ground oil was performed using soxhlet method. Firstly, sample of coffee ground was measured of 30 grams and wrapped using a filter paper. Then, it was put in extraction tube. The solvent used was 200 mL n-hexane. The extraction process was performed at temperature 80°C with varying time of 90, 120, 150, 180 and 210 minutes. Rotary evaporator was used to separate coffee ground oil and the solvent at temperature 60°C for 30 minutes [6].

2.3. Analysis of functional groups
Analysis of coffee ground oil functional groups was performed using Fourier transform infrared (FTIR) Agilent resolution Cary 630 FTIR spectrometer at instrumentation laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh.

2.4. Analysis of Component
Analysis of coffee ground oil component was performed using gas chromatography–mass spectrometry (GC-MS) Shimadzu qp ultra 2010 with stationary phase colom db5ms and mobile phase helium gas, performed at chemical instrumentation laboratory, Department of Chemistry Education, Faculty of Mathematics and Science Education, Indonesia University of Education, Bandung.

2.5. Activity test of antioxidant
Analysis of antioxidant was performed using DPPH method (1,1-diphenyl-2-picrylhydrazyl) [7,8,9].

2.5.1. Preparation of DPPH solution
DPPH was measured of 7.9 mg, dissolved in methanol until 50 mL then homogenized, and obtained 0.4 mM DPPH solution. The DPPH solution must be stored in a dark bottle. The new solution was always made when needed.

2.5.2. Preparation of extract solution variation
The 500 ppm main solution was made by dissolving 5 mg extract of coffee ground oil into 10 mL flask using methanol. The main solution was diluted and obtained varying solution concentration of 25, 50 and 100 ppm. Each solution was homogenized using a vortex mixer and incubated at 37°C for 30 minutes.

2.5.3. Measurement of blanko absorbance
1 mL of 0.4 mM DPPH solution was set its volume to 5 mL using methanol, homogenized using a vortex mixer and incubated at 37°C for 30 minutes. Furthermore, its absorbance was measured using UV-Vis spectroscopy at 517 nm wavelength.

2.5.4. Measurement of sample absorbance
Arabica coffee ground oil was set at 25 ppm concentration of 250 μL, 50 ppm of 500 μL and 100 ppm of 1000 μL, each of them was added 1 mL of 0.4 mM DPPH solution and converted the volume to 5 mL using methanol, homogenized using a vortex mixer and incubated at 37°C for 30 minutes. Its absorbance was measured using UV-Vis spectroscopy at 517 nm wavelength.

2.5.5. Calculation IC<sub>50</sub>

The IC<sub>50</sub> (inhibition concentration 50) is an antioxidant concentration in ppm (μg/mL) that can inhibit 50% free radical. The determination of IC<sub>50</sub> value is obtained from the linear equation of 50% of inhibition and concentration. By using the equation y=a+bx where y=50 and x=IC<sub>50</sub> value. The percentage of inhibitory power is calculated using the equation (1):

\[
\text{inhibition} \% = \left( \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \right) \times 100
\]

3. Results and Discussion

3.1. Rendement of coffee ground oil

Rendement of coffee ground oil was calculated using equation (2), rendement obtained from mean soxhlet process was 9.03%. Time soxhlet affected rendement that was obtained. Figure 1 shows the correlation of rendement result with time soxhlet. As can be seen in Figure 1 that the optimum time used in the soxhlet process was 180 minutes with rendement obtained value of 9.54%. It can be seen that the longer time spent the amount of rendement increasing. This is due to more frequent contact between the solvent and the sample. The density of arabica coffee ground oil produced is 0.9227 g/cm<sup>3</sup>.

3.2. Functional groups of coffee ground oil

The result of analysis of functional groups of Arabica Coffee ground oil (Coffea arabica L.) is presented in Figure 2. The result of spectrum shows there are some specific functional groups for coffee oil as can be seen in Figure 1. There are some functional groups have sharp peaks (% transmit) like \( \text{CH}_2 \text{asymmetric stretch at 2922 cm}^{-1} \), \( \text{CH}_2 \text{symmetric stretch at 2853 cm}^{-1} \), C=O\text{asymmetric stretch at 1741 cm}^{-1} \), \( \text{CH}_2 \text{bending at 1458 cm}^{-1} \), C-\text{CH}_3\text{vibration at 1159 cm}^{-1} \) and C-H\text{vibration at 718 cm}^{-1} [10,11,12]. These show there are many functional groups in the sample.
Figure 2. FTIR spectrum of Arabica Coffee ground oil

Table 1. Characterization of FTIR peak of Arabica Coffee ground oil

| Peak position (cm⁻¹) | Characterization of Absorbance          |
|---------------------|----------------------------------------|
| 3008                | C-H alkene                              |
| 2922                | CH₂ asymmetric stretch                  |
| 2853                | CH₂ symmetric stretch                   |
| 1741                | C=O asymmetric stretch                  |
| 1458                | CH₂ bending                             |
| 1377                | C-H symmetric bending                   |
| 1269                | C-O stretch                             |
| 1159                | C-CH₃ vibration                         |
| 1042                | C-O-HDeformasi                          |
| 718                 | C-H vibration                           |

3.3. Component of Coffee Ground Oil

The result of component analysis of Arabica Coffee ground oil using GC is presented in Figure 3 and Table 2. The result shows there are some active compounds contained in coffee oil. As can be seen in Figure 3 that there are seven compounds with area above 1% i.e.: methylcyclopentane (14.93%), cyclohexane (1.36%), pentadecylic acid (8.81%), linoleic acid (9.00%), ethyl linoleate (6.36%), 2,3-dimethylbenzofuran (1.61%) and 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (18.09%), with time retention successively of 1.599; 1.697; 19.148; 21.193; 21.321; 25.078 and 25.724 minutes. The MS fragmentation form of the compounds are presented in Figure 4. The peak 1 with time retention of 1,532 minute is n-hexane solvent (34.45%).

The result of GC-MS shows that there is a main compound, 1,2-benzenedicarboxylic acid bis (2-ethylhexyl) ester (Figure 5) which has the largest area (18.09%) than others. The compound contains some bioactivities like anti-cancer, antimicrobial, antifungal dan antioxidant[13,14,15,16,17].
Figure 3. Chromatogram GC of Arabica Coffee ground oil

Table 2. Characterization of Arabica Coffee ground oil GC peak

| Peak | R.Time | Area | Area% | Height | A/H | Name |
|------|--------|------|-------|--------|-----|------|
| 1    | 1.523  | 27537902 | 34.45 | 10351345 | 2.66 | Hexane |
| 2    | 1.599  | 11934777  | 14.93  | 6498872  | 1.84 | Methylcyclopentane |
| 3    | 1.697  | 1086488   | 1.36  | 684829   | 1.59 | Cyclohexane |
| 4    | 1.795  | 48846     | 0.06  | 18680    | 2.61 | Heptane |
| 5    | 1.923  | 26983     | 0.03  | 21298    | 1.27 | Methylcyclohexane |
| 6    | 2.162  | 164556    | 0.21  | 122034   | 1.35 | Toluene |
| 7    | 4.479  | 80751     | 0.10  | 37044    | 2.18 | Decane |
| 8    | 4.811  | 25838     | 0.03  | 16858    | 1.53 | Benzene,1,4-dichloro- |
| 9    | 6.034  | 47378     | 0.06  | 31891    | 1.49 | Undecane |
| 10   | 9.853  | 35674     | 0.04  | 19625    | 1.82 | Decanoic acid, methyl ester |
| 11   | 11.032 | 37623     | 0.05  | 24545    | 1.53 | Tetradecane |
| 12   | 13.020 | 360953    | 0.45  | 171031   | 2.11 | Dodecanoic acid, methyl ester |
| 13   | 13.985 | 28151     | 0.04  | 12047    | 2.34 | Cyclohexadecane |
| 14   | 14.089 | 41180     | 0.05  | 26038    | 1.58 | Iron, tricarbonyl[N-(phenyl-2-pyridinylmethylene)benzenamine-] |
| 15   | 15.901 | 104645    | 0.13  | 59618    | 1.76 | Tetradecanoic acid, methyl ester |
| 16   | 16.858 | 26168     | 0.03  | 18121    | 1.44 | Eicosane |
| 17   | 18.518 | 509379    | 0.64  | 273230   | 1.86 | Hexadecanoic acid, methyl ester |
| 18   | 19.148 | 7042720   | 8.81  | 1635011  | 4.31 | Pentadecanoic acid |
| 19   | 19.345 | 787716    | 0.99  | 209359   | 3.76 | Hexadecanoic acid, ethyl ester |
| 20   | 20.561 | 88723     | 0.11  | 49981    | 1.78 | 9,12-Octadecadienoic acid(Z,Z), methyl ester |
| 21   | 20.621 | 597383    | 0.75  | 336751   | 1.77 | 9-Octadecenoic acid, methyl ester |
| 22   | 20.904 | 220964    | 0.28  | 118434   | 1.87 | Octadecanoic acid, methyl ester |
| 23   | 21.193 | 7193004   | 9.00  | 1644113  | 4.38 | Linoleic acid |
| 24   | 21.321 | 5084017   | 6.36  | 555188   | 9.16 | Ethyllinoleate |
| 25   | 21.617 | 287097    | 0.36  | 98682    | 2.91 | 1-Eicosanol |
| 26   | 21.655 | 134680    | 0.17  | 68347    | 1.97 | Pentacosanoic acid, ethyl ester |
| 27   | 23.747 | 47889     | 0.06  | 21518    | 2.23 | 1-Eicosanol |
|    |    |    |    |    | 2.29 Hexanedioic acid, bis(2-ethylhexyl) ester |
|----|----|----|----|----|-----------------------------------------------|
| 28 | 23.853 | 94535 | 0.12 | 41268 | 2.29 Hexanedioic acid, bis(2-ethylhexyl) ester |
| 29 | 25.078 | 1285779 | 1.61 | 412086 | 3.12 2,3-Dimethylbenzofuran |
| 30 | 25.455 | 312343 | 0.39 | 101688 | 3.07 2,3-Dimethylbenzofuran |
| 31 | 25.724 | 14460503 | 18.09 | 3848697 | 3.76 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester |
| 32 | 26.042 | 194991 | 0.24 | 58262 | 3.35 Pregnenoloneacetate |

**Figure 4.** MS fragmentation form of Arabica Coffee ground oil compound (a) methylcyclopentane (m/z = 84), (b) cyclohexane (m/z = 84), (c) pentadecylic acid (m/z = 242), (d) linoleic acid (m/z = 280), (e) ethyl linoleate (m/z = 308), (f) 2,3-dimethylbenzofuran (m/z = 146) and (g) 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (m/z = 279).
3.4. Antioxidant activities

Antioxidant activities of coffee ground oil extracted using soxhlet method with n-hexane was tested using DPPH method. Testing antioxidant activities of coffee ground oil was performed by varying extract concentration of 25, 50 and 100 ppm. The result of testing antioxidant activities of coffee ground oil is presented in Table 3. It can be seen that inhibition percentage and IC$_{50}$ value of coffee ground oil sample. Determination of IC$_{50}$ value of coffee ground oil sample is obtained from linear equation/interpolation presented in Figure 6. $y = 0.0413x - 0.4813$ with $y = 50$ as it was obtained $x = 1222.31$ ppm that is IC$_{50}$ value. It indicates that antioxidant activities of coffee ground oil is very weak, another study showed the same value of 1421.53 ppm [4].

| Sample           | Concentration (ppm) | Sample Absorbance | Blanko Absorbance (DPPH) | Inhibition (%) | IC$_{50}$ (ppm) |
|------------------|---------------------|-------------------|--------------------------|----------------|-----------------|
| Coffee ground oil| 25                  | 0.825             | 0.831                    | 0.722          | 1222.31         |
|                  | 50                  | 0.820             | 0.831                    | 1.324          |                 |
|                  | 100                 | 0.800             | 0.831                    | 3.730          |                 |

Figure 6. Correlation between concentration with Arabica Coffee ground oil inhibition curve

4. Conclusions

Extract of n-hexane Arabica Gayo Coffee ground (*Coffea arabica* L.) contains some active compounds, the main component is 1,2-benzenedicarboxylic acid, bis(2-ethylhexil) ester. Arabica Gayo Coffee ground oil also contains antioxidant activities of 1222.31 ppm IC$_{50}$ value.
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