Subcritical Water Extraction of Phenolic Compounds from Moringa Oleifera Leaf

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Abstract—Moringa oleifera leaf is a good source of phenolic compounds that are reported to exhibit antioxidant activity both in vitro and in vivo. This study investigated the extraction of phenolic compounds from Moringa oleifera leaf using subcritical water. Experiments were performed in a batch stainless steel reactor at temperature ranging from 100 to 300°C at residence time of 5 to 20 min. Subcritical water extraction resulted in the highest yield of product, total phenolic compounds and antioxidant activity at temperature of 200°C at residence time of 15 minutes. The yield of product, total phenolic compounds and antioxidant activity obtained were 30.661%, 48.733 mg tannat acids/g dry powder of extract and 45.863 mg ascorbic acid/L, respectively. Subcritical water at 200°C and 15 min might be a good substitute to organic solvents such as ethanol to obtain phenolic compounds from Moringa oleifera leaf.

Keywords—antioxidant, hydrothermal, Moringa oleifera leaf, phenolic, subcritical water.

I. INTRODUCTION

Antioxidant plays an important role in life. Natural antioxidant has more benefits, such as no toxic and more easily degradable by bodies than artificial antioxidant. Moringa oleifera leaf rich with natural antioxidant, it has many constituents such as phenolic, flavonoid, proanthocyanidin, vitamin C, vitamin E, β-carotene, zinc and selenium where it has very potent to antioksidant, it has many constituents such as phenolic, antioksidant. Moringa oleifera leaf rich with natural more easily degradable by bodies than artificial

As we known, conventional extraction methods have several drawbacks; e.g. they are time-consuming, low of selectivity, give low extraction yield and use large amount of expensive, explosive and sometimes toxic organic solvents [20]. Solubility of phenolic compounds in common organic solvent is low, so Moringa oleifera leaf are treated at high temperature and/or under acidic conditions [21]. Mostly of extraction methods used conventional method, such as squeezing extraction, decoction, maceration, percolation and Soxhlet extraction. The extraction method of Moringa oleifera leaf based on yield in which maceration got the best. Maceration with 70 % ethanol, for 72 hours, is the most suitable extraction method of the dried leaf of Moringa oleifera. It promoted high yield of the crude extract, the highest contents of total phenolics, total flavonoid, major activity compounds and the most potent antioxidant activity [19]. As we known, conventional extraction methods have several drawbacks; e.g. they are time-consuming, low of selectivity, give low extraction yield and use large amount of expensive, explosive and sometimes toxic organic solvents [20]. Solubility of phenolic compounds in common organic solvent is low, so Moringa oleifera leaf are treated at high temperature and/or under acidic and basic conditions [21].

In facts, its applications are due to the manipulation of its dielectric constant, and variable concentration of hydrogen hydroxide with temperature. For instance, its dielectric constant decreases from 80 (at room temperature) to 27 (at 250°C) almost equaling of ethanol at ambient temperature [22]. The increase/decrease in hydrogen is a hydroxide ions in subcritical water [23] along with decreasing of its dielectric constant, make it very suitable medium for the extraction and hydrolysis of natural matrices. In other word, Subcritical water...
II. MATERIAL AND METHOD

A. Materials

Folin-Ciocalteu reagent, sodium bicarbonate (Na2CO3) and methanol were purchased from Merck. Ethanol, Methanol, Natrium carbonate and ascorbic acid are purchased from Brataco, Surabaya, and supplier of chemical compounds. Tanat acid and 1,1-diphenyl-2-picrilhydrazyl (DPPH) purchased from WAKO, Japan.

B. Plant Materials

The dried powdered leaf were provided to particular ratio (1 grams: 6 cm3). The extract was filtered and the marc was re-extracted by the same process and solvent until the extraction was exhausted [24].

C. Hydrothermal Extraction

A batch reactor used for subcritical water treatment was stainless steel tube (SUS316, i.d. 16.5 mm × 150.4 mm) with a Swagelok fitting. In typical experiment, an accurately weighed amount of dried leaves of Moringa oleifera (about 3 grams) and about 18.0 cm3 of water were charged into reactor. The reactor was heated in furnace dependent on temperature (100, 150,200,250 and 300oC) and time (5, 10, 15, and 20 minutes). Increasing temperature of reactor was followed with increasing pressure which was depended of ratio [34].

D. Determine of Total Phenolic Compounds Content

The content of total phenolic compounds was determined using Folin-Ciocalteu procedure [25]. Each sample (1 mg/mL), 0.1 mL was mixed with 0.5 mL of the Folin-Ciocalteu reagent and 2 mL of sodium bicarbonate solution (7.5 %, w/v). The mixture was allowed to stand at room temperature for 60 minutes with intermittent shaking. The absorbance was measured at 779 nm using a UV-Visible (UV-VIS) Spectrophotometer (Pothitirat et al., 2009). Total phenolic compounds was expressed as mg of Tanat acid equivalents in 1 g of the extract and dried powder, using the following equation based on the calibration curve: Y = 378,99x, R² = 0.9796. Where x is the absorbance and Y is the Tanat acid equivalent (mg/g).

E. Determination of Antioksidant Activity

The free radical scavenging activity of the extracts and of standard solutions (Tanat acid and quercetin) were investigated using 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging method [25]. A total of 2 mL of the extract or standard (vitamin C) was added to 2 mL of DPPH in methanol solution. The mixture was allowed to stand at room temperature for 30 minutes with intermittent shaking. The absorbance of each solution was determined at 517 nm using UV-VIS Spectrophotometer (Pothitirat et al., 2009). Antioxidant activity was expressed as mg of vitamin C equivalents in 1 L of the solution, using the following equation based on the calibration curve: Y = 244,6x, R² = 0.9358. Where x is the absorbance and Y is the vitamin C equivalent (mg/g).

III. RESULT AND DISCUSSION

A. Effect of Time and Temperature on Yields, Total Phenolic Compounds and Antioxidant Activities at Subcritical Water Extraction.

Under subcritical conditions, the intermolecular hydrogen bonds of water break down and the dielectric constant of water decreases. The dielectric constant of ethanol and of pure water at ambient temperature and pressure are 27 and 79, respectively. As temperature increases to 250°C, the water dielectric constant is reduced to 27, which is similar to the dielectric constant of ethanol [26]. The subcritical water extraction was evaluated by the extraction yield (%) of crude extract. That process provided the highest yield of 30.661% at temperature of 200°C and time of 15 minutes. Figure 1 shown sharply effect of temperature and time on yield (%).

The effect of water temperatures in the range of 100 – 300°C on the efficiency of subcritical water extraction was investigated. The extraction rate of yield increased as the extraction temperature increased to 200°C, and then the extracted quercetin was gradually degraded as the temperature increased above 200°C. These results were similar to another author [27] although the extraction rate increases with temperature, some desired organics show substantial degradation at temperatures >150°C with subcritical water extraction that higher temperatures may lead to degradation and loss of desirable compounds because of thermal instability. These results indicate that the efficiency of subcritical water extraction is greatly affected by the extraction temperature. A higher temperature increases the solubility of phenolic compounds (Figure 2). Moreover, the temperature at which the highest amount of phenolic compounds (48.733 ± 0.01 mg Tanat acid/g of extracts of dried powder) was extracted (200°C) affects. The obtained results indicate that temperatures between 200 – 250°C are the most suitable for subcritical water extraction of phenolic compounds from Moringa oleifera leaf. Obviously, the production of phenolic compounds was also a function of residence time. Both yield, total phenolic compounds and antioxidant activity showed peak at around 15 min, and then decreased at around 20 minutes. After 15 min, produced total phenolic compounds may be decomposed by subcritical water.

Generally, phenolic compounds have antioxidant activity; however, it was probable that besides phenolic compounds, other nonphenolic compounds with antioxidant activity were also produced and/or extracted from Moringa oleifera leaf in subcritical water medium. Therefore, antioxidant activity as a criterion of total produced antioxidants was also investigated. Fig. 3 shown effect of temperature and time at the antioxidant activity.

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Decomposition and conversion of Moringa oleifera leaf into valuable chemical compounds were successfully conducted using subcritical water. Degradation of the phenolics complexes of Moringa oleifera leaf were achieved (up to 30.65 % of Moringa oleifera leaf) in the water without using organic solvent, acid, base, and/or enzyme. Decomposition of Moringa oleifera leaf have resulted almost the same amount of phenolic compounds; it was understood that phenolic compounds were mainly produced. Some of phenolic compounds and antioxidant activities were identified and quantified in this study.

Subritical water extraction resulted the highest yield of product, total phenolic compounds and antioxidant activity at temperature of 200oC at residence time of 15 minutes. The yield of product, total phenolic compounds and antioxidant activity obtained were 30.661%, 48.733 mg tannat acids/ g dry powder of extract and 45.863 mg ascorbic acid/L, respectively. Subritical water temperature and residence time were two studied parameters which influenced the decomposition of Moringa oleifera leaf and production of phenolic compounds. It was found that phenolic compounds could be selectively produced by temperature variations. From residence time point of view, production of phenolic compounds could be efficiently achieved in a very short time which was much less than those reported in conventional methods that increases economic feasibility of this method.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Rer. Nat Fredy Karniawan, M.Si, head of Instrumentation and Science Analytical Laboratorium, Chemistry, Natural of Science, Institute Technology of Sepuluh Nopemter. Any opinion expressed and conclusions arrived at, are those of the authors and not necessary to be attributed to Institute Technology of Sepuluh Nopemter.

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Figure 1. Effect of temperature and time on yield (%) at subcritical water extraction

Figure 2. Effect of temperature and time on total phenolic compounds (mg Tannat acid/g extracts of dried powder) at subcritical water extraction

Figure 3. Effect of temperature and time on antioxidant activities (mg ascorbic acid/L) at subcritical water extraction