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Enhanced Extraction of Phenolic Compounds from *Moringa Oleifera* Leaves Using Subcritical Water Ethanol Mixture

S Zullaikah[1,*], R Y Naulina[1], P Meinawati[1], K Fauziyah[1], M Rachimoellah[1], O Rachmaniah[1], S Nurkhamidah[1], N M I P Suari[1] and E N Prasetyo[2]

[1]Department of chemical engineering, Institut Teknologi Sepuluh November (ITS), Kampus ITS Keputih Sukolilo, Surabaya 60111, Indonesia

[2]Department of Biology, Institut Teknologi Sepuluh Nopember (ITS) Kampus ITS Keputih Sukolilo, Surabaya 60111 Indonesia

Email: szulle@chem-eng.its.ac.id, szullehakim@gmail.com

Abstract. A *Moringa Oleifera* leaves are rich of phenolic compounds that have the ability to serve as antioxidants. Phenolic compounds as an antioxidant can stabilize free radicals with complementing the electron deficiency of free radicals. Conventional method (maceration) can extract phenolic compounds from *Moringa Oleifera* leaves. However, this method is known as time consuming, inefficient and using solvent that is non-environmentally friendly. In this study, the extraction of phenolic compounds from *Moringa Oleifera* leaves was enhanced using a subcritical water-ethanol mixture. Fresh and dried *Moringa Oleifera* leaves were extracted using ethanol solution with different ratio and ratio of dried leaves and ethanol solution at 200 ^{o}C for 15 min. The highest amount of phenolic compounds of 87.11 ± 0.81 mg GAE/g of dried leaves with flavonoid contents of 75.79 ± 0.73 mg/g of dried leaves, antioxidant activity of 88.75 ± 0.93 mg vitamin C/L and yield of extracts of 32.73 % ± 0.08 were obtained under following operation conditions: ratio of dried leaves 0.13:20 (g/mL) and ethanol solution 96 %. The utilization of ethanol solution as a solvent on the subcritical condition was able to enhance the amount of total phenolic compounds, flavonoid content, antioxidant activity and yield of extract.

1. Introduction

Worldwide consumption of herbal medicines has markedly increased. According to the Secretariat of the Convention on Biological Diversity, global sales of herbal products were estimated to be the US $60 billion in 2000. In 2008, the global market for herbal remedies was about the US $83 billion with a steady growth rate ranging between 3 % - 12 % per year [1]. A Moringa leaf is one of the plants that can be used as herbal medicine. High nutrients and antioxidants cause this plant is called the miracle of a tree. This magic tree is not used only as medicine but can also be used as a nutritional supplement. Based on [2], Moringa leaves can be an alternative food to reduce malnutrition, especially in children and toddlers, because it has a high concentration in protein, potassium, and iron. It has benefits in the health field and good source of natural antioxidants because of the content of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids[3],[4]. Moringa Oleifera is a plant that thrives and wild in Indonesia. These plants include in the Moringaceae family of the single genus Moringa. The genus Moringa is thought to include 13 species of which 11 are from

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Africa [5]. The growth of herbal plants as drugs triggers to increase the production of extracts of natural materials. It has developed several methods in extracting natural materials ranging from conventional to modern. Has been described in the literature [6], there are methods of extracting natural materials like maceration, decoction, percolation and Soxhlet extraction. However, in the application they need a lot of solvent and long time for extraction. An alternative method to increase the extraction of phenolic compounds from Moringa Oleifera leaves by using subcritical water extraction. This method is time efficient and water is using as solvent, therefore is environmentally friendly. Besides that, issue about design and economic potential for the extraction of active components from herbs to be another reason to enhance the product [7]. Subcritical water extracts rapidly with the use of low-pressure high temperatures thus avoiding the degradation of volatile and thermo labile compounds. Subcritical water is water at a temperature of 100 – 374 °C under mild pressure to keep water in the liquid condition, and the dielectric constant of water also changed under this condition. It changes the nature of water that was originally polar to non-polar [8]. In our previous work on extracting phenolic compounds using subcritical water extraction resulted a total yield of 30.70 % at T = 200 °C for 15 min. with total phenolic compounds of 48.73 mg tannic acid/g extract and antioxidant activity of 45.86 mg vitamin C/L. Since phenolic Compounds have low polarity, solvent extractions are the most typically procedures used for extraction of plant materials due to their ease of use, efficiency, and wide applicability [9]. In this work, a mixture of water and ethanol was used to enhance extraction of phenolic compounds from Moringa oleivera leaves. The objective of this work was to study the effect of sample to solvent ratio using dry Moringa leaves and fresh Moringa leaves and ethanol solution concentration on the yield of extract, total phenolic compounds, antioxidant activity and flavonoid content using subcritical water-ethanol mixture.

2. Experimental section

2.1 Materials

Fresh moringa leaves with water content of 74.55 % was obtained from yard around campus. The dry moringa leaves was obtained by drying in a oven at 60 °C for 24 h. The dry moringa leaves were reduced in size used a blender and fresh moringa leaves used a mortar. Aquades, ethanol 96 %, methanol, DPPH (2, 2-diphenyl-1-picryl-hydrazyl), vitamin C (ascorbic acid), Na_2CO_3, Follin-Ciocalteu reagent, quercetin, $AlCl_3$, CH_3COONa, HCl were obtained from commercial sources.

2.2 Subcritical Extraction

Extraction of phenolic compounds from moringa oleifera leaves using subcritical water ethanol mixture was carried out in a batch hydrothermal reactor (V = 45 mL) made by stainless steel tube (SUS 316) with a Swagelok fitting. Generally, a certain ratio of fresh/dried leaves of moringa oleifera and solvent (ethanol solution) were charged into reactor. The reactor was heated in a furnace at temperature of 200 ^oC (time to reach from ambient temperature to T = 200 ^oC was 20 min.) and extraction was carried for 15 min. Increasing temperature in the reactor was followed with increasing of vapor pressure. The ratio of fresh leaves to ethanol solution (0, 35, 70, 80 and 96 %, (v/v)) is 3 :18; 0.5 : 20 and 1 :20 (g/mL), while the ratio of dried leaves to ethanol solution is 0.7635 : 18; 0.1273 : 20 and 0.2545 : 20 (g/mL). The reactor is then quenched by direct insertion into cold water; the extract (liquid phase) is collected by filtered with filter paper. The extract is separated from the solvent by a rotary evaporator. To remove a residual solvent, the extract is placed in the oven at 60 °C to obtain constant weight.

2.3 Total Phenolic Compounds

Total phenolic compounds was analyzed using Folin-Ciocalteau reagents according to [6].

2.4 Antioxidant Activity

Free radical scavenging activity was measured by using DPPH (2, 2-diphenyl-1-picryl-hydrazyl) according to[10], [11] and [12]

2.5 Flavonoid Content

Total flavonoid content was analyzed according to [13].

3. Results and discussions

3.1 Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the yield of extract

Moringa leaves contain phenolic compounds that are useful as natural antioxidants. In this work, to enhance extraction of phenolic compounds was carried out by using subcritical water-ethanol mixture. The ratio used was maintained to the volume reactor of 21 ml in order to maintain the vapor space since increasing temperature in the reactor was followed with increasing of vapor pressure. For concentration of ethanol solution of 0 % showed that the solvent used was water and this condition is called subcritical water. Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the yield of extract was shown in figure 1.

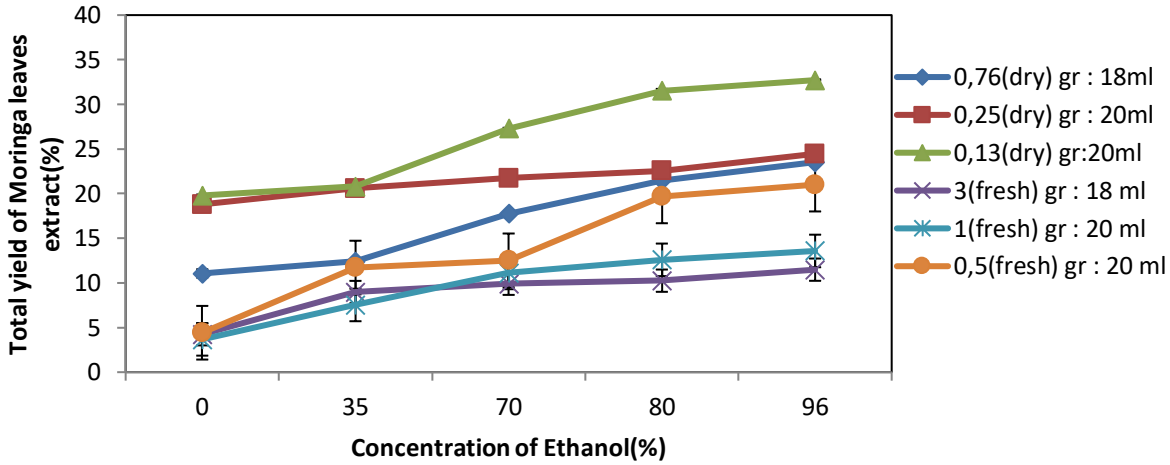


Figure 1. Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the yield of extract

From figure 1, it can be seen that the highest yield of extract was obtained using concentration of ethanol solution of 96 % for both fresh and dried leaves. Highest yield of extract from fresh leaves is 21.01 ± 0.75 at a ratio of 0.5 g: 20 mL and dried leaves of 32.73 ± 0.08 at ratio 0.13 g: 20 mL. The extract resulted from dried leaves is higher than that of fresh leaves. This shows that the ratio and concentration of ethanol solution used in the extraction has affected the yield of extract. This is consistent with literature [14] which states that the amount of the extract decreases with decreasing ratio of solid to solvent. The greater of the concentration of ethanol solution will increase the yield of extract. This is due to higher concentration of ethanol solution lowering solvent polarity, and lowering solvent polarity increasing extractable ability of total extract that is composed of phenolic and flavonoid compounds [9]. In accordance with the principle of mass transfer, the orientation of the thrust force during mass occurs gradient compilation of greater solids concentration [15]. The yield of extract from fresh leaves enhanced by 37.71 % by increasing concentration of ethanol solution from 0

% (solvent is water) to concentration of ethanol solution of 96 %, while using dried leaves the yield of extract enhanced by 60.50 %.

3.2 Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the total phenolic compounds (TPC)

The influence of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the TPC (figure 2) shows the same trend as the yield of extract, where the greater the concentration of ethanol solution, the higher the TPC concentration. TPC composed of flavonoid, where it belongs to flavonol such as quercetin and kaempferol. From figure 2, the highest TPC was obtained using concentration of ethanol solution 96 % at a ratio of dried leaves and solvent of 0.1273 g: 20 mL, with TPC concentration of 87.11 ± 0.81 mg GAE / g dried leaves. Phenolic compounds are less polar compounds; therefore organic solvents such as water and ethanol under subcritical conditions are particularly suitable for extracting. Extraction of moringa leaves using water (concentration of ethanol solution = 0 %) show that the content of phenolic compounds extracted is lower compared using concentration of ethanol solution 96 %. This is because the water polarity index is larger than ethanol 96 %, the lower polarity index (nonpolar solvent) is an effective solvent to extract TPC.

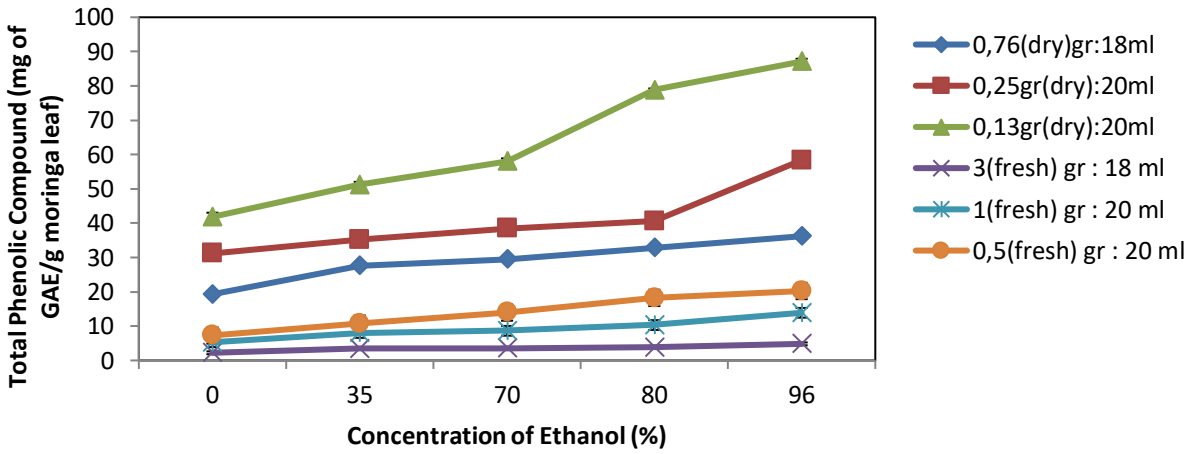


Figure 2. Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the TPC (Total Phenolic Compound)

3.3 Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the antioxidant activity

Antioxidants are any substances that when present at low concentrations versus oxidized substrate significantly delay/prevent substrate oxidation. Activity is measured by counting the amount of DPPH(2,2-diphenyl-1-picryl-hydrazyl) light intensity reduction equivalent to DPPH concentration reduction. Dyeing is produced by reacting the DPPH(2,2-diphenyl-1-picryl-hydrazyl) molecule with the hydrogen atom released by the component molecule of the sample to form the DPPH (2,2-diphenyl-1-picryl-hydrazyl) compound and causing the DPPH (2,2-diphenyl-1-picryl-hydrazyl) color decay from purple to yellow [16]. Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the antioxidant activity was shown in figure 3.

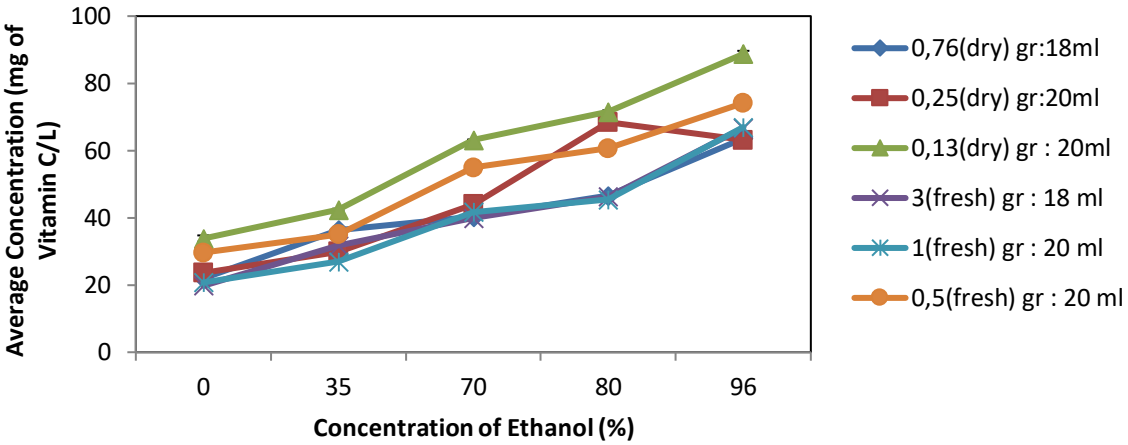


Figure 3. Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the antioxidant activity

Increased concentration of ethanol solution and ratio of leaves to solvent affect the antioxidant activity. The highest antioxidant activity (88.75 ± 0.93 mg vitamin C/L) obtained using concentration of ethanol solution of 96 % and ratio of dried leaves and solvent of 0.1273 g: 20 mL. The antioxidant activity tends to increase in proportion to the number of ratio of leaves to solvent and concentration of ethanol solution. This is because the yield of the extract is directly proportional to the antioxidant activity. The antioxidant activity obtained from dried leaves is higher than that of fresh leaves. Antioxidant activity analyzed by DPPH(2,2-diphenyl-1-picryl-hydrazyl) method is expressed by IC_{50} (Inhibitory Concentration). IC_{50} is a number that shows the concentration of extract that can inhibit DPPH(2,2-diphenyl-1-picryl-hydrazyl) activity by 50 %. The smaller the IC_{50} value means the higher the antioxidant activity. It can be concluded that the higher ratio of leaves to solvent and increasing concentration of ethanol solution enhanced the antioxidant activity, and resulting in less inhibition percentage. IC_{50} for dried leaves extracted using subcritical water-ethanol mixture (96 %) at 200 ^{o}C for 15 min are 181.45, 119.34, and 106.48 for ratio of dried leaves of 0.7635 g: 18 mL, 0.2545 g: 20 mL, and 0.1273 g: 20 mL, respectively. While IC_{50} for fresh leaves extracted using subcritical water-ethanol mixture (96 %) at 200 ^{o}C for 15 min are 166.91, 165.40, and 136.09 87 for ratio of fresh leaves of 03 g: 18 mL, 1 g: 20 mL, and 0.5 g: 20 mL, respectively.

3.4 Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the flavonoid content

Flavonoids are defenilpropane which is widely found in plants. The immediate family members of flavonoids include flavones, isoflavones, and the 2,3-dihydroderivatives of flavones, which are flavanones, it interconvertible with the isomeric chalcones. Flavanones undergo a series of transformations affecting the heterocyclic C ring to give rise to other family members of flavonoids, including anthocyanins and catechins. Some flavonoids have several fungi as antilipoperoxidant, antitumoral, antiplatelet, antiischemic, anti-allergic, and anti-inflammatory activities. There are also useful enzymes, such as lipoxygenase, cyclooxygenase, monooxygenase, xanthine oxidase, mitochondrial succinoxidase and NADH-oxidase, phospholipase A, and protein kinases. This biological function is a form of antioxidant property [17]. The principle method used for the determination of flavonoid content is when aluminum chloride forms stable acid complex with

carbonyl group at C4 and hydroxyl at C3 (flavonol) and C5 in flavonols and flavones, in addition to forming a labile acid complex with hydroxyl at ortho position in ring A or B flavonoids [4]. Total flavonoids extracted with subcritical water had lower levels and shown by figure 4.

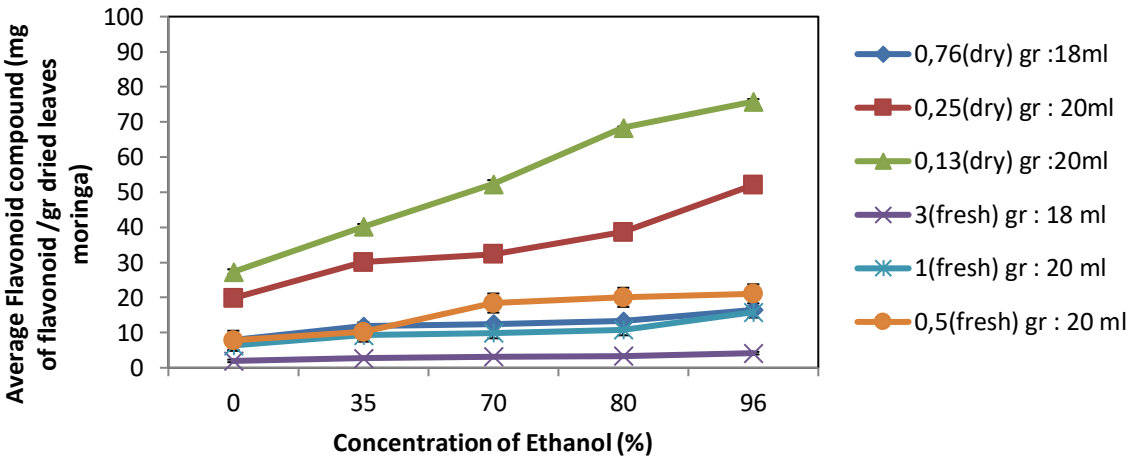


Figure 4. Effect of concentration of ethanol solution at different ratio of fresh/dried leaves and solvent on the flavonoid content

Figure 4 shows that by using higher concentration of ethanol solution and higher ratio of leaves to solvent extracted higher flavonoid content. The highest flavonoid content (75.79 ± 0.73 mg quercetin/ g of dried leaves) was found using dried leaves with ratio of leaves to solvent of 0.1273 g: 20 mL.

4. Conclusions

In this work the yield of extract, total phenolic compounds, antioxidant activity and flavonoid content of Moringa oleifera leaves was extracted using subcritical water-ethanol mixture. The highest yield of extract, total phenolic compounds, antioxidant activity and flavonoid content of 32.73 ± 0.08 %, 87.11 ± 0.81 mg GAE / g of dried leaves, 88.75 ± 0.93 mg of vitamin C/L with IC_{50} value of 119.34 µg / mL, and 75.79 ± 0.73 mg quercetin / g of dried leaves, respectively was obtained using concentration of ethanol solution 96 % and ratio of dried leaves to solvent of 0.1273 g: 20 mL. The yield of extract from fresh leaves enhanced by 37.71 % by increasing concentration of ethanol solution from 0 % (solvent is water) to concentration of ethanol solution of 96 %, while using dried leaves the yield of extract enhanced by 60.50 %. From this data we can conclude that the utilization of ethanol solution as a solvent on the subcritical condition was able to enhance the amount of the yield of extract, total phenolic compounds, antioxidant activity as well as flavonoid content.

Acknowledgments

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