Green Downstream Processing by Using Hydrothermal Extraction for Recovering Flavonoid, Phenolic, and Antioxidant Activity from Moringa

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ABSTRACT

Well known as rich in phenolic compounds, *Moringa oleifera* mature leaf extract shows significant antioxidant activity both in vitro and in vivo. It has been widely used in the cosmetic, medicine, and functional food sector. Hydrothermal extraction is one of the green environmentally friendly methods that fit *M. oleifera* feedstock for recovering natural antioxidants, namely flavonoid, phenolic, and antioxidant activity. In this study, three parameters were used: sample-solvent ratio, running a temperature, and extraction time. Flavonoids were evaluated by colorimetry of hydrazine 2,4-dinitrophenyl, whereas phenolics were measured by using tannic acid reacting with Folin ciocalteu reagent. DPPH measured antioxidant activity. The optimum result was obtained at 15 minutes with 140 °C for total flavonoid content (TFC) 18.98 mg/g whereas 170 °C for total phenolic content (TPC) and antioxidant activity 48.73 mg tanat acid/dried extract; 44.76 mg/L respectively.

KEYWORDS

Biomass
Extraction
Environmentally friendly
Natural antioxidant

INTRODUCTION

Moringa oleifera is widely used as a nutrition supplement to help malnutrition and its harmful effects ¹. Moreover, *M. oleifera* is claimed as a new source of food in China. High phenol and flavonoid content making *M. oleifera* as a source of antioxidant activity both in vivo and in vitro ^{2,3}. *M. oleifera*, exclusively at the leaf, having the highest phenol and antioxidant activity content than root, stem, bark, and seed ⁴. Nowadays, *M. oleifera* extract is widely applicable in medicine, nutrition supplement, cosmetics ^{5,6}. Several extraction methods have been studied for recovering phenolic and flavonoid compounds from *M. oleifera*. Many liters of volatile organic solvent, including methanol, ethanol, chloroform, and acetone, were utilized to extract these compounds. However, those extractions still in debate about using a toxic solvent that making harmful to the environment. The sophisticated method such as ultrasonic-assisted extraction (UEA), supercritical fluid extraction, microwave-assisted extraction (MAE). The supercritical extraction offered high yield and clean extract, but at this temperature, the phenolic and flavonoid compounds may be destroyed. This extraction method is not suitable for polar compounds. The UEA also provides high yield, clean extract, and short time extraction, but too many undesired

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compounds carried at extract that making hard and long steps in separation process ^{2 7}. The hydrothermal extraction in the subcritical situation, the most effective method to extract flavonoids and phenolics from *M. oleifera*, has been proved. This method was claimed as natural, eco-friendly among others ^{8 9 10}. Water could extract polar compounds or decompose lignin cellulose at this state, becoming valuable compounds such as organic acid, aromatic, protein ¹¹ and applicable for extract phenolic compounds ¹². This method to be an option due to only utilizing water as a solvent. Hence making the yield product more fit to edible food.

In this study, the extraction of flavonoid, phenolic compound, and antioxidant activity from *M. oleifera* mature leaves using hydrothermal extraction at a subcritical state was carried at a mini stainless steel reactor batch would be discussed.

MATERIALS AND METHODS

Materials

Pretreatment *M. oleifera* leaves were separated from ranting. Then chopped and dried in the open air under the sun. The dried leaves were grinding into powder for maximizing surface area. Powder kept at vacuum storage at freeze temperature. Reagent Cioicalteu and any chemical were purchased from a commercial source.

Maceration

Maceration, the classical extraction method used, is comparable to hydrothermal extraction. This method using ethanol as a solvent for 72 hours followed earlier study ¹³. Ratio 1:6 dried powder / solvent. Filtration with vacuum pump was done after maceration ended. The wet extract was collected and dried in the open air.

Hydrothermal extraction

The hydrothermal extraction was carried at 110 °C – 230 °C, ranging from reaction time 5-20 minutes. Ratio dried powder: water 1/6 (gr/ml) was poured in a mini tube stainless steel reactor and heated into a furnace. The pressure was kept at 7 MPa. After the end reaction time, the reactor was shocked into the cold water of a sudden to stop the reaction. The extract was collected with a vacuum pump and kept in a 50 mL bottle. The bottles are kept in the freezer and equipped with a black seal to protect the sample from light to prevent degradation when analyzed ².

Flavonoid Analysis

Flavonoid analysis utilized 2,4-Dinitrophenylhydrazine Colorimetric. The principal basis of this method is that 2,4-dinitrophenylhydrazine reacts with keton and aldehyd, becoming 2,4-dinitrophenylhydrazones. Hence, flavones were obtained, whereas flavonols and isoflavone with double-bound C2-C3 could not react with 2,4-dinitrophenylhydrazine. Meanwhile, hydrazone that is having a maximum of 495 nm was chosen as reaction wavelength. Sample 20 mg was

soluble in methanol to make variant concentrations. 1 mL of each concentration react with 2 mL reagent 2,4-dinitrophenylhydrazine 1% and methanol at 50 °C for 50 minutes. After cooling down at room temperature, the solution was added by 5 mL potassium hydroxide in 70 % methanol and incubated for 2 minutes at room temperature. About 1 mL was taken from the solution and react with 5 mL methanol. Then centrifuge at 1000 rpm to extinguish the suspended solid. When supernatant was obtained, then diluted becomes 25 mL. Analyzed at 495 nm to find out the quantity of flavonoid content¹⁴.

Phenolic Analysis

Initially make tannat acid standard curve with various concentrations, ranging from 10-100%. Each concentration was added with 0.5 mL Folin ciocalteu reagent, adding 2 mL Na₂CO₃ 7.5%, and then kept in the dark place at room temperature. UV-Vis was utilized to measure phenolic compounds' content with absorbance 779 wavelength¹⁵. Phenol analysis was carried with 0.1 mL extract diluted in methanol and demin water until 2 mL. Then, followed the steps standard curve. Results were shown in mg tanat acid/ mg extract.

Determine antioxidant activity

The standard solution was made in 10 mg vitamin C/100 mL and diluted into 2, 4, 6, 8 mg/100 mL. Six reaction tubes were filled with 0.1 mL mixture and diluted into 2 mL with methanol. Each reaction tube was added 2 mL of 35 µg/mL DPPH (1,1-diphenyl-2-picrylhydrazil) and kept at room temperature in a dark place for 30 minutes. UV-vis was set at 517 wavelengths, then measured absorbance of each solution, and the standard curve was obtained. The same steps were carried out for evaluating sample extract. The principle of evaluating antioxidant activity utilizing DPPH method when DPPH solution is mixed with compounds that donate hydrogen atoms, DPPH is reduced and loses its purple color.

RESULTS AND DISCUSSION

Hydrothermal process

M. oleifera were extracted at hydrothermal extraction under subcritical state 110 °C – 230 °C. Combination temperature and reaction time precisely bring out optimum yield and bioactive compounds (flavonoid, phenolic). The obtaining yields were described in Figure 1.

Figure 1. Fluid Temperature and Reaction Time on Yield Effect

When temperature and pressure are set, the yield always increases first with extended reaction time, then decreases slowly. However, different reaction times are corresponding to the highest yield at different reaction time temperatures. With the extension reaction time, the more prolonged contact between sample with water, and more ions H_3O^+ dan OH^- are released at that subcritical state^{16 17}. The highest yield was obtained at 170 °C for 15 minutes at the hydrothermal extraction method.

Total Flavonoid Compounds

In the present study, total flavonoid compounds (TFC) of the hydrothermal extraction were expressed as mg/ g sample. Figure 2 show that there were no significant differences about temperature 140°C and 170°C. However, the lower temperature is more acceptable due to flavonoids not resistant to high temperatures.

Figure 2. Temperature and Reaction Time Effect on Flavonoid Compounds

It was proofed that flavonoid content at 1700C; 17.8 mg/g sample little much lower even though highest yield obtained. On the other hand, at 1400C, the 18.98 mg/g sample was the highest flavonoid content, although the yield content not high enough. Several reports demonstrated the different results of total flavonoid content *M. oleifera* leaves from other extraction methods. Sreelatha and Padma reported that mature leaves have potent flavonoids was 27 mg/ g sample than those of tender leaves. As reported [Rodriguez Perez] before, the predominant group of phenolics compounds in *M. oleifera* leaves was the flavonoid group with the most predominant kaempferol and quercetin derivatives.

Total Phenolic Compounds

In this study, total phenolic compounds (TPC) of *M. oleifera* extracts were expressed as mg tannat acid/ mg dried sample. Phenolic compounds are extracted by hydrothermal extraction using subcritical water temperatures consisting of phenolic compounds and other non-phenolic compounds. Due to this study not characterizing particular phenolics, therefore, they called total phenolic compounds. The highest total phenolic compound was found 48.733 mg tannat acid/ mg dried sample at 1700C for 15 minutes. Temperature higher than 1700C is not allowed due to phenolic compounds gradually degrade. The data were shown in Figure 3.

Figure 3. Temperature and Reaction Time Effect on Phenolic Compounds

Total Phenolic Compounds

Antioxidant activity of *M. oleifera* leaves extracts were described as mg Vitamin C/L. Several studies reported that exert antioxidant activity by ascorbic acid at hot water tea extract^{18, 19}.

Figure 4. Temperature and Reaction Time Effect on Antioxidant Activity

The result described in Figure 4 shows increasing temperature and reaction time followed by increasing DPPH content. However, increasing too much temperature impact decreasing DPPH content. It could be inferred that free radical scavenging activity through proton donating ability in DPPH assay and the *M. oleifera* antioxidant activity leaves extract could be an excellent

reductone. The optimum conditions in 1700C for 15 minutes also, with 44.76 mg vitamin C/L. Extract with high TFC, and TPC content also promoted high DPPH scavenging activity.

Maceration

Maceration with various ethanol concentrations was utilized compared to the new method, hydrothermal extraction, by using water at subcritical temperature to substitute organic solvent. Maceration carried for 72 hours followed previous research¹³. However, water is still used as the mixture. Utilization water as a mixture of ethanol because of its polarity. Hence, reduce the amount of organic solvent. Effect of water mixture on yield, TFC, TPC, and antioxidant activity can see in Figure 5.

From Figure 5 could be seen that the higher the ethanol concentration, the higher yield obtained. On the other hand, pure water collected tiny yield, flavonoid, phenolic and antioxidant activity. This is perfectly natural, considering there is no physical treatment nor chemical addition. The highest yield results, TPC, TFC, and antioxidant activity, were obtained at 35.92%; 54.5 mg tannat acid/g dried extract; 15.9 mg/g; 53.5 mg vit C/L successively. Those results were higher than hydrothermal extraction. However, hydrothermal extraction gave more benefits such as escalated penetration from solvent into powder particles, reduced extraction time, and environmentally friendly.

Figure 5. Effect of Ethanol-Water Mixture on Yield, TPC, TFC, and Antioxidant Activity

CONCLUSION

Hydrothermal extraction with water at subcritical temperature and pressure as a natural solvent to substitute organic solvent was proofed as green downstream processing to obtain high yield extract that corresponds to high flavonoid, phenolic and antioxidant activity in a quick way. However, because flavonoids and phenolics are valuable compounds susceptible to temperature, it is necessary to characterize them.

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