World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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The effect of hot extraction on total flavonoid content in *Moringa leaves aquous extract*

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World Journal of Advanced Research and Reviews, 2024, 23(03), 2245–2249

Publication history: Received on 03 August 2024; revised on 11 September 2024; accepted on 14 September 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.3.2786

Abstract

Moringa oleifera leaves extract is some natural compound rich in antioxidants such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins, and saponins. Until recently, this plant has been widely used as an antioxidant source and as a vegetable in the Indonesian community. Previous studies have demonstrated that the extraction with methanol and ethanol are effective because they have the ability to extract both the lower and higher molecular sizes of antioxidant compounds. However, the extraction of this compound using water has not yet been established. In this report, we investigated the total phenolic compound of *Moringa leaves extract.* Moreover, the flavonoid compound of *Moringa oleifera* leaves aquous extract has been characterized by using of several temperatures (40^0, 70^0, and 100^0 C) in order to mimic the real-life condition in the community. Here we showed that the phenolic compound in *Moringa oleifera* leaves aquous extract is 1.01%. Moreover, using quercetin standard, we evaluate the concentration of flavonoid in *Moringa oleifera* leaves aquous extract. We found that the flavonoid compound of *Moringa oleifera* leaves aquous extract with the temperature of 40^0, 70^0, and 100^0 C were for 5.245 µg/ml; 7.725 µg/ml; 5.728 µg/ml respectively. This report confirms the optimum temperature for Moringa leaves aquous extract is 70°C in order to obtain the maximum flavonoid compound.

Keywords: Hot extraction; *Moringa oleifera*; Leaves; Antioxidant

1. Introduction

Moringa oleifera (*Moringa oleifera* Lam.) leaves extract has been known for its antioxidant compound [1]. Over the past centuries, research has been carried out to explore the antioxidant compound of this plant [2, 3]. Leaves, stem, and root from this plant have been known for the antioxidant-rich compound [4, 5] such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins, and saponins and act as a potent antioxidant [3, 6, 7]. The antioxidant is the substance that can inhibit oxidation in our body and has the ability to defend many free oxidants thus has many benefits to our body [8]. That is why it is very crucial to measure the antioxidant compound in plants.

Previously, the extraction process for this antioxidant compound has been widely used by methanol and ethanol as their solvents [9]. These methods are very effective and efficient since these chemicals have the ability to extract the broader molecular weight of polyphenols from the smaller to the bigger size of molecular weight [10]. Unfortunately, this method could not be used as the reference to measure the antioxidant compound in houses-processed food.

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Moringa oleifera is a cultivated plant that grows well in almost all parts of Indonesia due to the rain due to the high annual rainfall [11]. Because of the widespread distribution of this plant, Indonesian usually use *Moringa oleifera* as a vegetable source on daily basis. To process the leaves, water is boiled with the leaves and spices, and serve the vegetables directly. Unfortunately, it was unknown whether the compound in *Moringa oleifera* was still intact in the water-based processed. The aqueous technique is very crucial to be explored since the large community applied the various part of this plant on aquous-based processed food.

In this study, we used a aquous-based extraction process to *Moringa oleifera* leaves extract to demonstrate the most effective temperature to process this compound.

2. Material and Methods

2.1. Chemicals and instrument

Quercetin were used as standard to measure flavonoid concentration. Rotary vacuum evaporator (BUCHI, Switzerland) was used for recovery of solvents under reduced pressure. UV/VIS spectrophotometer (Schimadzu, Japan) was used for taking absorbance of test samples

2.2. Plant materials

The *Moringa oleifera* plant we used was from 875 meters above the sea levels (mdpl) with an environment temperature was 20-25°C. The leaves of *Moringa oleifera* were collected from Batu city, Indonesia in 2017 were identified by the Materia Medica Batu Antanarivo, (Batu, Indonesia). The leaves then were dried frozen in the Physiology laboratory, Barawijaya University, Indonesia, where the samples were stored until used.

2.3. Methods for extracting phenolic compounds from *Moringa oleifera* leaves

One hundred and fifty grams of *Moringa oleifera* leaves were soaked in the 1,5 liters of water at different temperatures (40, 70, 100^0 C) for 1 h, stirred, and filtered with Whatman filter paper (number 3). The extract then was evaporated using a rotary evaporator with 45^0C then stored in 4^0C freeze until use.

3. Result

3.1. Total phenolic compound

The total phenolic compound in the *Moringa oleifera* leaves extract was assessed in 3 different parts of the samples. The sample showed the total phenolic compound of 0.66%, 1.38%, 0.99% respectively.

Table 1 The total phenolic compounds obtained by spectrophotometry expressed in % of dry freeze *Moringa oleifera* leaves

No	Code	Analysis result	
		Levels	Unit
1	A	0.66±0.01	%
2	B	1.38± 0.00	%
3	C	0.99±0.01	%
Mean		1.01	%

The mean percentage of phenolic compound in the *Moringa oleifera* leaves extract were 1.01% derived from 3 times of measurements.

3.2. Phenol and flavonoid identification in different temperature

The flavonoid was then identified in the *Moringa oleifera* leaves aquous extract using quercetin standard comparison.

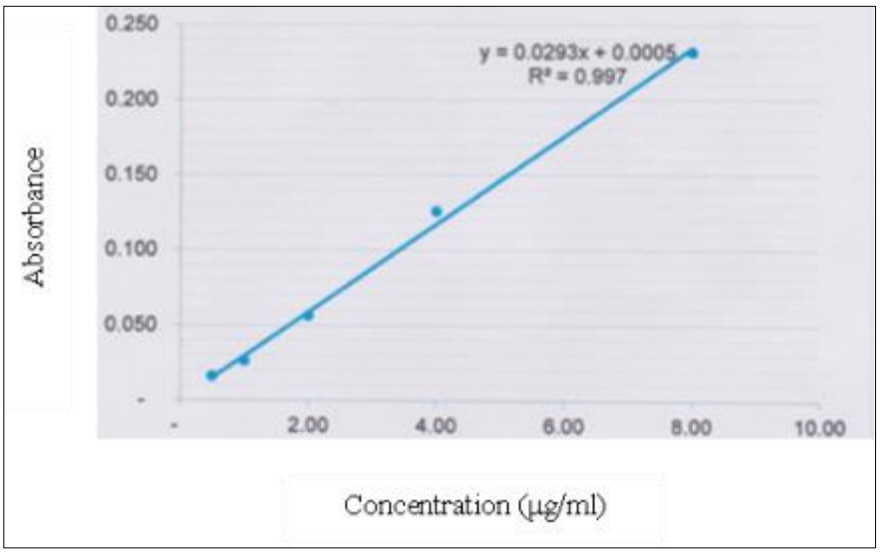

Figure 1 Standard curve of absorbance against quercetin concentration y= 0.0293x + 0.0005; R^2= 0.997

Our result showed a standard curve for quercetin concentration with formula y= 0.0293x + 0.0005; R^2= 0.997 and were used for flavonoid concentration measurement. Subsequently, the flavonoid concentration was assessed with water based extraction 3 times using 3 different temperatures (40^0, 70^0, 100^0 C).

Table 2 Flavonoid concentration from *Moringa oleifera* leaves aquous extract with different water temperature

	Temperature (^{0}C)		
Sample No	**40^0**	**70^0**	**100^0**
Sample 1	2.218	3.549	2.56
Sample 2	4.693	6.826	5.154
Sample 3	8.823	12.799	9.471
Mean concentration (µg/ml)	5.245	7.725	5.728

Our result showed that low temperature (40^0C) has the concentration of flavonoid of 5.245 µg/ml. Moreover, 70^0C resulted in flavonoid concentration of 7.725 µg/ml, while 100^0C temperature has the flavonoid concentration of 5.728 µg/ml.

4. Discussion

Moringa oleifera leaves extract was used in this experiment because of the high antioxidant compound such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins, and saponins and act as a potent antioxidant. It is of paramount importance to assess the amount of antioxidant compound in the plants in order to know the efficiency and using the correct amount of antioxidants for a specific application [12]. In addition, a study for the determination of antioxidant compounds could contribute to the observation of new antioxidant compounds [13].

In this study, the measurement of the antioxidant compound was started by the assessment of the total phenolic compound. Phenol is the main antioxidant component of the samples and their number reflects the total antioxidant activity [14]. Phenolic compounds are a group of molecules with a specific structure of at least one phenol unit [15]. In this study, we find that the *Moringa oleifera* leaves are rich in the phenolic compound for about 1% of total weight. It reflected that *Moringa oleifera* leaves extract has the ability as a defence response in our body such as such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities [15].

The estimation of flavonoid compounds of *Moringa oleifera* leaves aquous extract for this study was maximized using different temperatures. This method was used in order to observe the optimum temperature to obtain the highest flavonoid compound in *Moringa oleifera* leaves. Our observation displayed that the highest flavonoid was at a 70^0C. The mechanism of how this temperature affects the highest amount of flavonoid is unknown, but it possible that flavonoid is sensitive to temperature. A recent finding has been postulated by Sharma and colleagues where flavonoid was destroyed at higher temperature [16]. Moreover, in agreement with this study, the flavonoid component in garlic also displayed the sensitivity in temperature with the variation of cooking temperature and time [17].

5. Conclusion

Our result suggested that the most optimum temperature for Moringa leaves extraction using water is 70^0C and might have the highest antioxidant activity.

Compliance with ethical standards

Acknowledgments

The authors thank to Universitas Brawijaya and Universitas Airlangga for the support and help during conduct of the research.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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