Extraction of Bioactive Compounds in Moringa Leaves (*Moringa oleifera Lam.*) using Modified Sonication Technique

Wafa Nida Faida Azra[1], *Bambang Susilo* [1*], *Ni'matul* Izza[1], *Abd. Rohim*[1], *Rizki Putra* Samudra[1]

[1]Department of Biosystems Engineering, Faculty of Agricultural Technology, Universitas Brawijaya, 65145 Malang, East Java, Indonesia

Abstract. *Moringa oleifera Lam.* is well-known for its health benefits due to its rich antioxidants, anti-hypertensive, and anti-inflammatory properties. Extraction methods are crucial in unlocking the medicinal potential of Moringa leaves. While traditional maceration methods are common, they are inefficient, requiring significant time and solvent usage. This study explores sonication as an alternative extraction method, utilizing specific wave frequencies to disrupt cell walls and release bioactive compounds without compromising integrity. Sonication offers a faster, more efficient process with reduced solvent usage, enhancing overall effectiveness. Results showed that fresh moringa leaf extraction without grinding using the maceration method yieled 99 metabolic compounds. In contrast, powdered moringa leaf extraction using the maceration method produced 117 compounds and sonication extraction resulted in 143 metabolic compounds. Dominant bioactive compounds include trigonelline, acetophenone, kaempferol, etc. Sonication is recommended for identifying bioactive compounds in Moringa leaf extract.

1 Introduction

Moringa leaves (*Moringa oleifera Lam*) contain many bioactive components that have high potential for treatment. Unfortunately, its utilization has not yet reached its maximum point. Even though it offers various benefits and minimal side effects compared to chemical drugs, inappropriate use of Moringa leaves as medicine can be dangerous and cause an overdose. Moringa, a widely used tropical plant, has a long history as a vegetable and traditional medicine. The natural chemical content in Moringa leaves is produced from primary metabolism, such as carbohydrates, proteins, lipids and nucleic acids and secondary metabolism, for example alkaloids, flavonoids, steroids, terpenoids and tannins. This content has extraordinary benefits and medicinal properties. Previous research has proven that Moringa leaves have anti-inflammatory properties [1] and antioxidants [2]. Therefore, there is a need for an appropriate process to obtain optimal bioactive components from Moringa leaves without breaking the compounds contained, this process is extraction. In extraction, the preferred solvent is very important, such as water or ethanol. The ethanol solvent is also considered a suitable solvent because it has selectivity, safety and ease of evaporation [3]. Extraction aims to isolate compounds that are soluble in solvents. There are various extraction methods used, such as cold methods (maceration, percolation) and hot methods (reflux, Soxhlet, *microwave assisted extraction* or MAE). Various extraction methods have

* Corresponding author: susilo@ub.ac.id

advantages and disadvantages in producing extract. Macerated extraction offers simplicity, does not require complicated equipment and preserves the bioactive content of the material. However, this method takes a long time and requires a lot of solvent. Another alternative is to use hot extraction such as ultrasonic extraction which offers fast extraction times and small energy consumption. This cost method is relatively cheap. The weakness this method is that inappropriate use of heat will cause bioactive degradation of the extracted material [3]

In this research, Moringa leaves (*Moringa oleifera Lam*) are included in the family of medicinal plants which have abundant nutritional content and medicinal properties. Various studies have proven the benefits of the Moringa plant from each part, such as Moringa leaves which can increase hemoglobin levels and overcome anemia [4], Moringa stems help to reduce high blood pressure or hypertension [5], Moringa leaves are useful for treating diabetes [5] and Moringa tree bark is efficacious for treating colitis [6]. Another advantage of Moringa leaves is that they contain essential amino acids, antioxidants such as vitamin C, vitamin E, flavonoids, tanning and are rich in minerals. Moringa is a herbaceous plant that contains antioxidants that are beneficial for the body. The human immune system can naturally neutralize free radicals to prevent damage. However, external factors such as unhealthy lifestyles, smoking, pollution and ultraviolet radiation can increase the production of free radicals. Free radicals can cause degenerative diseases such as cancer, rheumatism, coronary heart disease and Parkinson's disease. Antioxidants play a role in warding off or preventing these diseases. Many natural food ingredients have antioxidants, especially those that contain phenolics. One group of phenolics that have the ability to reduce free radicals are flavonoids. Therefore, Moringa leaves are known as "*The Miracle Tree*" because parts of the Moringa plant are a source of nutritious nutrition [6].

Currently, medicinal plants are widely used as the main ingredients for making herbal medicine. Traditional medicines is a drug that have been used for generations, where the process steps are carried out simply in several parts of the plant through plant extracts. It has been used by ancient people as a medicine for various diseases. Plants that are widely used for medicine or other functions such as papaya, cucumber, spinach function as a nutritional supplement for the family, while turmeric, galangal, ginger, lemongrass and bay leaves function as herbs or spices in cooking [7]. Along with advances in technology, traditional medicines are increasingly being used in the pharmaceutical industry. However, standards in the form of rules that have been set to meet the criteria for the content of efficacious active substances in herbal plants must be met so that these properties can be utilized optimally as medicines or cures for various diseases. Apart from that, recommended doses that are safe for consumption are an important requirement so that the benefits of the bioactive compounds contained in herbal plants, especially Moringa leaves, can be right on target. This research aims to compare the conventional extraction method with the ultrasonic extraction method using a modified sonication machine based on the results of its effectiveness in extracting the compounds contained in the material. In research [8] Moringa leaves are a medicinal plant that contains 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 25 times more iron than spinach. Apart from that, there are many compounds that are beneficial for body health.

2 Materials and Methods

The extraction method using maceration and modified sonication with the same solvent (50% ethanol). The results of the processed extraction were analyzed using LC-HMRS for metabolites identification.

2.1 Materials

The instruments used in this research were a prototipe sonication extractor machine which is equipped with double jacket system designed by author and LC-HMRS (*Liquid chromatography-mass spectrometry*) Q-exactive type (Thermo Fisher Scientific) to detect compound content and qualitatively identify extracts in Moringa leaves. The modified sonication extractor machine is an extraction machine that uses sonication waves with a frequency of >20kHz. This instument has a capacity of 2 liters and is equipped with a double jacket to stabilize the temperature during the process. Moringa leaf samples were obtained from Malang, East Java (7.97°S 112.12°E) and 1,440 feet above sea level which can be seen at **Fig 1.**

Fig 1. *Moringa oleifera Lam.*

2.2 Extraction

The fresh Moringa leaves was dried in the cabinet dryer until the water content 10% [9]. The dried leaves are powdered using a grinding machine and sifted with an 80 mesh sieve. The extraction technique using a modified extractor machine begins by inserting a sample of Moringa leaf powder, distilled water and solvent into the chamber (50 g/2L (w/v) for 15 minutes at a temperature of 30°C. The ratio of distilled water to solvent is 2:1. After extracting, the Moringa leaf extract was analyzed using LC-HMRS to obtain qualitative data regarding the bioactive components in the Moringa extract. LC-HMRS analysis (*Liquid chromatography-mass spectrometry*).

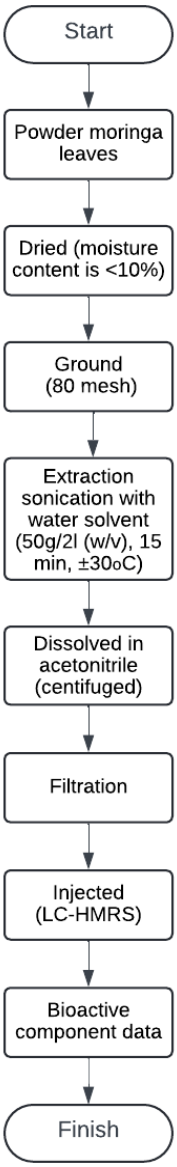

Fig 2. Research procedure

Qualification analysis of bioactive compounds in Moringa leaves is divided into several procedure, namely:

2.2.1 Sample preparation

Before testing begins, First, the sample dissolution stage is carried out. This dissolution was carried out using acetonitrile (LC-MS grade) in an appropriate volume. Furthermore, The sample was stirred using a vortex speed of 2500 rpm for 3 minutes. This vortex process ensures that the solution is mixed homogeneously. After stirring, The sample solution was centrifuged for 3 minutes to separate the solid and supernatant. Final, The supernatant was filtered using a PTFE ministart filter with a pore size of 0.22 micrometers. The filtration process aims to remove fine particles that may be dissolved.

2.2.2 Analisis LC-HRMS

After the sample preparation process is complete, Next, the sample was injected into the Hypersil Gold aQ pool High Performance Liquid Chromatography (HPLC) column. This column has a particle size of 50 x 1 mm x 1.9 micrometers, which is ideal for efficient separation of analytes. The mobile phase used consists of two solutions: A is 0.1% formic acid in water and B is 0.1% formic acid in acetonitrile. To separate bioactive components gradually, gradient elution method was used. Gradient elution started with 5% B for 2 minutes, then increased to 60%-95% B within 15-20 minutes. To clean the column, composition B was increased to 95% B for 3 minutes (20-23 minutes) and then reduced again to 5% B for 7 minutes (23-30 minutes). During the analysis process, some operating parameters are kept constant. HPLC operates in positive ion mode with a flow rate of 40 microliters per minute. The column oven temperature was maintained at 30 degrees Celsius and the total analysis time was 30 minutes.

2.2.3 Chromatogram Analysis

The resulting total ion chromatogram will be analyzed using Compound Discoverer 3 software. Identification of bioactive compounds is carried out by comparing mass spectrum data obtained with the mzCloud online library. Compound Discoverer 3 is software used to analyze LC-HMRS chromatograms while mzCloud online library is a chemical compound mass spectrum database used to identify bioactive compounds.

3 Results

Based on the results of research that has been carried out, it shows that the extraction process using maceration using a modified sonication extractor has a significant difference. The test results using LC-HMRS relate to the compounds detected. In the identification results, LC-HMRS results show that these two methods are successful in extracting bioactive compounds found in Moringa leaves, but the number of compounds detected or extracted is different, in the extraction results using maceration, fewer compounds are extracted compared to the extraction results using a modified sonication extractor. The number of compounds extracted as a result of extraction using a modified sonication extractor was 143 compounds, while the number of compounds extracted as a result of extraction using maceration was 117 compounds. This is in line with research [10] succeeded in extracting good ingredients in samples of seaweed material used sonication methode. The difference amount in compounds extracted by sonication and compounds extracted by maceration, there are three dominant compounds with the highest content which can be seen **Fig 3**.

Enhanced Bioactive Compound using Sonication:

Fig 3 demonstrates the superior efficacy of sonication over maceration in extracting bioactive compounds from Moringa leaves. The sonication technique yielded a wider range of bioactive compounds, including trigonelline, acetophenone, and kaempferol, compared to the maceration method. This enhanced extraction efficiency is attributed to the increased mass transfer facilitated by sonication waves, which promote agitation and cavitation within the solution, effectively transferring target compounds from the sample to the solvent [11]. Additionally, sonication-induced cavitation breaks down complex compounds into simpler, more soluble forms, further boosting the yield of extracted material [12]. Research on anthocyanin extraction from purple sweet potatoes also supports this finding, demonstrating that sonication waves can accelerate the movement of target compounds and expedite the extraction process [13].

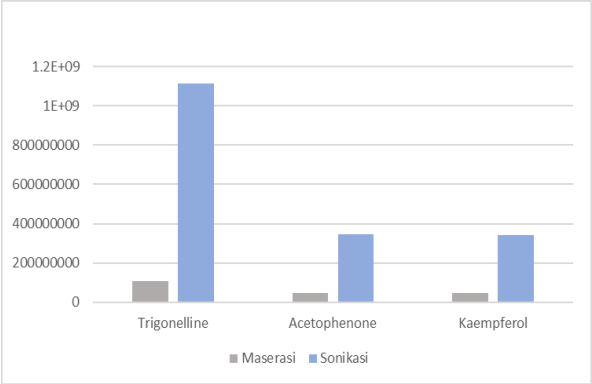

Fig. 3 Bioactive Compound Content Graph

4 Discussion

Powdered Moringa Leaves: A Richer Source of Bioactive Compounds

The study also revealed a higher concentration of bioactive compounds in powdered Moringa leaf extract compared to fresh leaf extract. The powdered form yielded 99 compounds, surpassing the number of bioactive compounds identified in fresh leaf extract. This finding aligns with previous research indicating that powdered marine seagrass (Syringodium isoetifolium) exhibits enhanced antioxidant activity [14]. Similarly, powdered seaweed (Sargassum cristaefolium) demonstrates superior antibacterial activity and a richer bioactive compound profile [15].

Unlocking Moringa's Potential through Powdering

Powdering Moringa leaves is not merely a processing step; it serves as a crucial gateway to unlocking the vast potential benefits of this remarkable plant [15]. Powdered Moringa leaves hold immense value as a functional food, offering enhanced nutritional value and significant health benefits [15]. Its bioactive properties position it as a promising anti-inflammatory and antioxidant agent [9], while its herbal simplicia form facilitates storage and utilization in traditional medicine [15]. Moreover, powdering Moringa leaves extends its shelf life and preserves its antioxidant content more effectively than fresh ingredients [10].

LC-HMRS: Unveiling Moringa's Bioactive Riches

Liquid chromatography-mass spectrometry (LC-HMRS) has emerged as a sophisticated tool for analyzing natural compounds. Its exceptional mass resolution and accuracy enable precise identification of bioactive compounds. LC-HMRS offers the ability to determine mass values with high accuracy (up to four to six decimal places), effectively eliminating interference and establishing it as an ideal tool for analyzing complex natural compounds. In this study, the Q-exactive LC-HMRS system was employed for non-targeted screening of bioactive compounds in the samples, eliminating the need for injecting authentic or standard compounds. Previous research employing LC-HMRS analysis of herbal oil, seaweed, and seagrass samples successfully identified the bioactive compound profiles of these materials.

The study's findings underscore the remarkable potential of Moringa leaves as a source of bioactive compounds, particularly when processed into a powdered form [15]. LC-HMRS analysis proved invaluable in elucidating the intricate composition of these bioactive compounds, paving the way for further exploration of their health-promoting properties and potential applications [15]

5 Conclusion

This research compares extraction methods using sonication and maceration to extract the bioactive compound content in Moringa leaves. The results showed that sonication was more effective in extracting bioactive compounds compared to maceration. This is indicated by the number of bioactive compounds extracted using sonication amounting to 143 while with maceration there are 117 compounds. The advantages of sonication are based on several factors, namely increasing mass transfer, breaking down complex compounds into simpler ones and accelerating the extraction process time. Furthermore, this research found that extraction of Moringa leaf powder resulted in more bioactive compounds being extracted compared to fresh Moringa leaves. This shows that the process of powdering Moringa leaves is able to increase the accessibility of bioactive compounds to solvents, thereby increasing extraction results. This research provides important insights into the extraction method using a modified sonication technique proven to be a superior method in terms of extracting the number of compounds from the extracted material, time efficiency and its effect on the raw material.

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