



Kinetic Extraction of *Moringa oleifera* Leaves using the Microwave Assisted-Extraction (MAE) Method

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Abstract. *Moringa oleifera* leaves are known for their distinctive leaf shape and offer numerous health and nutritional benefits. They are rich in vitamins, minerals, proteins, and bioactive compounds and possess antioxidant and anti-inflammatory properties. This study aimed to explore the extraction process, identify the presence of tannin compounds in *Moringa* leaves, and determine the appropriate kinetic model for the extraction of *Moringa* leaf extract using the Microwave-Assisted Extraction (MAE) method. The extraction was performed using 96% ethanol as the solvent and the MAE technique, which is known to enhance extraction efficiency. Variables such as power (150 watts), a material-to-solvent ratio of 1:15 (b/v), and extraction times of 2, 4, 6, 8, and 10 minutes were tested. The results indicated that the highest yield was achieved after 10 minutes, with a value of 10.25%. The extraction time was extended at most 10 minutes due to time limitations and diminishing returns as extraction time increased. The study concluded that the second-order kinetic model ($R^2 = 0.9897$) was the most suitable for describing the extraction of tannin compounds from *Moringa* leaves, outperforming the first-order model with a value closer to 1.

Keywords: *extraction, Moringa oleifera, flavonoids, and microwave-assisted extraction*

1. Introduction

Moringa leaves, or *Moringa oleifera*, are tropical plants from South Asia known for their high nutritional content, especially in the leaves used in traditional medicine [1]. *Moringa* leaves are rich in essential nutrients such as protein, calcium, iron, and vitamins. *Moringa* leaves also contain bioactive compounds, including flavonoids, tannins, and saponins, which have antioxidant and anti-inflammatory benefits [2]. In Indonesia, *Moringa* leaves are used as a vegetable to accompany rice. *Moringa* leaves have many benefits, including being anti-diabetic, anti-hepatitis, a medicine for heart problems, and reducing cholesterol levels in the body.

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According to research by Rohyani *et al.* (2015), the results of phytochemical screening from Moringa leaves contain flavonoids, alkaloids, steroids, tannins, saponins, anthraquinones, and terpenoids [3]. The content per 100 grams of dried Moringa leaves contains 10% flavonoids, 0.075% water, 2.05% calories, 0.382% carbohydrates, 0.271% protein, 0.023% fat, 0.192% calcium, 3.68% magnesium, 2.04% phosphorus, 0.006% copper, 0.282% iron, and 8.7% sulfur [4].

Extraction is a key step in utilizing bioactive compounds from natural materials, such as plants, for various industrial and medical applications [5]. Optimal extraction allows maximum retrieval of valuable compounds with high efficiency, optimizing the final product yield. Factors such as temperature, time, solvent type, and extraction method affect the results. Extraction methods generally used are maceration, percolation, soxletation, and ultrasonic. Factors that need to be considered in selecting an extraction method are the nature of the material, the type of solvent, and the purpose of the extraction [6]. The duration of tannin extraction is often due to the chemical nature of tannin, which is easily soluble in water or other solvents, as well as the speed of diffusion of plant tissue into the solvent. Tannin is a polyphenolic compound with a high affinity for water, so the transfer process from the plant matrix to the solution can occur efficiently and quickly. In addition, modern extraction methods such as microwave-assisted extraction (sonication) speed up this process by increasing contact between the solvent and plant material, reducing diffusion barriers, and accelerating the breakdown of plant cell walls [7].

Extracting bioactive compounds from plants is a key step in developing vegetable products, including from Moringa leaves [8]. Microwave-assisted extraction (MAE) utilizes microwave energy to speed the extraction process. It allows the extraction of active compounds more efficiently and in a shorter time than conventional methods. In this research, we will explore the influence of three key parameters in the extraction method, solvent type, extraction temperature, and extraction time, on the tannin content in Moringa leaves extract using the MAE method [9]. MAE is an innovative technique that utilizes microwaves to speed up extracting active compounds from plant materials. This method has demonstrated significant advantages in extraction efficiency compared to conventional methods, which often require longer extraction times and more amounts of solvent [10].

This research aims to study extraction, determine the presence of tannin content in Moringa leaves, and determine the kinetic model of extraction of Moringa leaves extract yield using the MAE method. Tannin has antioxidant properties that can be beneficial for humans.

By knowing and understanding the variations in extraction, we can optimize the process to maximize the potential of bioactive compounds from Moringa leaves, providing benefits for the food industry, pharmaceuticals, and human health.

Based on research conducted by Handayani et al. 2020. The maceration method was used with 96% ethanol solvent, and qualitative testing was done using Alkaloid, Flavonoid, Saponin, and Tannin tests using reagents appropriate to the test parameters. Qualitative test results show that Moringa seeds contain positive alkaloids, indicated by an orange precipitate, flavonoids, marked by the formation of an orange-yellow color, saponins characterized by a stable foam, and tannins, indicated by a black color [17]. Research by EA Koesnadi et al. 2021 used rambusa leaves extract, which was extracted using the MAE method with an extraction time ratio of 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, and 6 minutes with 300 watts microwave irradiation power. The results showed that an extraction time of 4 minutes was able to produce rambusa leaves extract which had the highest antioxidant activity based on the percentage of free radical inhibition, namely 25.29% with an IC50 value of 196.17 mg/L, yield of 17.40%, total phenol 75.07 mg GAE/g extract, total flavonoids 33.05 mg QE/g extract and total tannins 2.76 mg TAE/g [18].

The problems in obtaining Moringa leaf extract using the MAE method are rarely solved. The exact ratio of ingredients to solvent and extraction time on total tannin content is unknown. Previous research on the extraction of Moringa leaves regarding total flavonoids and antioxidant activity can be seen in Table 1.

Table 1. Research on tannin extraction

| Material | Method | Result | Reference |
|---|--|--|------------------|
| Garlic Peel (<i>Allium sativum</i> L.) | Microwave Assisted Extraction (MAE) | In this study, variable raw material ratios of 1:10, 1:15, 1:20, 1:25, 1:30 were used, power of 10% and 50% of the maximum power of the tool (399) watts, and time of 5, 10, 15 minutes. The research results showed that the total yield value obtained was 2.04% from a solvent raw material ratio of 1:15, extraction time of 10 minutes, and 50% power. | [9] |
| <i>Psidium guajava</i> leaves | Soxhlet | This research uses ethanol solvent with a solid-liquid ratio of 1/20 and 1/60 (w/v). The extraction temperature was 80°C and extraction times were 30, 45, 60, 75, and 90 minutes. The highest tannin content was obtained at 60 minutes and a solid-liquid ratio of 1/20 (w/v), approximately 17% and 12%, respectively. The highest percentage of tannin content was for a solid-liquid ratio of 1/60 (w/v) at an extraction time of 60 minutes. If compared, the tannin content at a solid-liquid ratio of 1/20 (w/v) is greater than 1/60 (w/v). These results indicate that increasing the amount of solvent has no significant | [12] |

| Material | Method | Result | Reference |
|--|-------------------------------------|--|-----------|
| | | effect on the tannin diffusion rate of <i>Psidium guajava</i> leaves. | |
| Acacia Wood | Microwave Assisted Extraction (MAE) | This research used distilled water and ethanol solvents with microwave power of 100, 180, 300, 450 watts and extraction times of 1, 3, 5 minutes. The extraction results were analyzed using a UV-Vis spectrophotometer. This research obtained the highest tannin yield of 26,606 mg/g at 100 watts of power and 3 minutes with ethanol solvent. | [15] |
| Moringa leaves (<i>Moringa oleifera</i>) | Microwave Assisted Extraction (MAE) | Ethanol solvent, solvent concentration (60, 70, and 80%), raw material mass (25 and 50 grams), extraction time 10 minutes with a solvent volume of 500 mL and a power of 380 watts. The best yield value produced by the Microwave Assisted Extraction (MAE) method produces the largest yield, namely 9% for a material mass of 50 grams, time of 10 minutes and ethanol solvent concentration of 80%. | [16] |
| Moringa Seeds (<i>Moringa oleifera</i> Lam.) | Maceration | This research used the maceration method with 96% ethanol solvent, qualitative testing using Alkaloid, Flavonoid, Saponin and Tannin tests using reagents in accordance with the test parameters. Qualitative test results showed that Moringa seeds were positive for containing alkaloids, indicated by the presence of an orange precipitate, flavonoids indicated by the formation of an orange yellow color, saponins indicated by a stable foam, and tannins indicated by a black color. | [17] |
| leaves (<i>Passiflora foetida</i> L.) | Microwave Assisted Extraction (MAE) | % ethanol solvent with material ratio (1:10), extraction time ratio of 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes and 6 minutes with 300 watts microwave irradiation power. The results showed that an extraction time of 4 minutes was able to produce rambusa leaves extract which had the highest antioxidant activity based on the percentage of free radical inhibition, namely 25.29% with an IC50 value of 196.17 mg/L, yield of 17.40%, total phenol 75.07 mg GAE/g extract, total flavonoids 33.05 mg QE/g extract and total tannins 2.76 mg TAE/g. | [18] |

2. RESEARCH METHODS

2.1 Tools and materials

This research needs a series of MAE extraction tools, a 60 mesh sieve, analytical scales/balance, filter paper, a glassware set, a test tube rack, and a rotary evaporator. The ingredients used are Moringa leaves, distilled water, 96% ethanol, concentrated hydrochloric acid (HCl), anhydrous acetic acid, and 1% FeCl₃.

2.2 Sample preparation

2.2.1 Making Simplisia Powder

Raw materials are made from Moringa leaves from Kaliwates District, Jember Regency. The Moringa leaves are sorted and washed clean, then dried at an oven temperature of 60°C until the Moringa leaves are dry. The dried Moringa leaves are then blended and then sieved using a 60 mesh sieve to obtain Moringa leaves simplicia powder [23].

2.2.2 Simplicia and Water Content Test

Determination of simplicia water content was carried out using the gravimetric method. A total of 2 grams of simplicia powder was used to determine water content using an oven at 105°C for 3 hours. The water content requirement for simplicia, according to the applicable standard parameters, is no more than 10%. Determining the water content of simplicia is very important to provide a maximum limit on the water content in simplicia, because the amount of water that tends to be high can become a medium for the growth of bacteria and fungi which can damage the compounds contained in simplicia [24]. The calculation of % water content can be seen in the equation below [23]:

$$\% \text{ Water content} = \frac{(\text{mass of empty cup} + \text{ingredients before drying}) - (\text{mass of cup} + \text{ingredients after drying})}{\text{mass of simplicia}} \times 100\%$$

2.2.3 Moringa Leaves Extraction

The third stage of the procedure is extracting Moringa leaves. The variable ratio of material to solvent is 1:15 b/v [22], using the lowest microwave power, 150 watts. The solvent used in the extraction process is ethanol, with a concentration of 96%. The extraction process will take 2, 4, 6, 8, and 10 minutes [19]. The raw materials are put into the Erlenmeyer according to the predetermined ratio variations and then into the microwave with power according to the variables. The solution is irradiated in the microwave periodically to maintain a temperature of at least 80°C. The solution is then left to cool to room temperature and filtered, and the filtrate is concentrated using a rotary evaporator until it becomes a thick extract.

2.2.4 Yield Analysis

The resulting extract is then weighed in a container, and the weight of the concentrated extract is divided by the initial weight using the following formula [25]:

$$\% \text{ Yield} = \frac{\text{thick extract mass}}{\text{mass of simplicia powder}} \times 100\%$$

2.3 Tannin Phytochemical Analysis

The extract that has been put into the test tube, then 1-2 drops of 1% FeCl₃ solution are added as a reagent. If the extract contains tannin compounds, there will be a color change in the filtrate, indicating the presence of tannins with a color change to green or bluish-black. This color change occurs due to the interaction between the Fe³⁺ ion from FeCl₃ with the phenolic group in tannin, forming a complex that produces the color [25].



Figure 1. Color Change of Tannin Compound Extract

2.4 Extraction Kinetics

To determine the optimal operating conditions in the Moringa leaves extraction process, it is essential to calculate the extraction kinetics using the Lagergren equation, a widely used model for describing the rate of adsorption or extraction processes. The Lagergren equation, often applied to first-order kinetic reactions, provides insights into how quickly the active compounds in the Moringa leaves are extracted under varying conditions, such as time, temperature, and solvent concentration. By applying this equation, researchers can evaluate how different factors affect the extraction rate, including solvent type, extraction time,

temperature, and the ratio of solvent to plant material. The results help to identify the reaction rate constant (k_1), which quantifies the speed of the extraction process, and the equilibrium concentration (C_s), which indicates the maximum extractable concentration under specific conditions.

Using this model, we can minimize the number of variables by focusing on the most influential factors, such as temperature and extraction time, which are likely to have the most significant impact on the efficiency and yield of the extraction process. This allows for a more streamlined and controlled approach to optimize the extraction, ensuring the process is efficient and cost-effective. Moreover, understanding the kinetics can aid in determining the exact point at which the extraction process reaches equilibrium, ensuring that no resources are wasted and maximum yields are achieved without over-extraction.

2.4.1 First Order Kinetic Model

The first-order kinetic equation, as proposed by Lagergren, can be expressed in its differential form as follows:

$$\frac{dC_t}{dt} = k_1(C_s - C_t) \quad (1)$$

In this equation, t (min) represents the extraction time, and k_1 (min^{-1}) is the extraction rate constant for the first-order reaction. To integrate equation (1), the boundary conditions are applied: $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$:

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = k_1 t \quad (2)$$

The equation (1) that has been obtained can be converted into linear form as follows:

$$\log(C_s - C_t) = \log(C_s) - \frac{k_1}{2.303} t \quad (3)$$

Then, a plot is made between $(C_s - C_t)t$ and t are used to get the slope and intercept, which can be used to determine the value of the first-order extraction rate constant k_1 and the value of extraction capacity C_s .

2.4.2 First Order Kinetic Model

The second-order kinetic equation that describes the extraction rate can be expressed as follows:

$$\frac{dC_t}{dt} = k_2(C_s - C_t)^2 \quad (4)$$

Where k_2 ($\text{Lg}^{-1} \text{min}^{-1}$) is the extraction rate constant for the second order. By grouping the variables in equation (4), we obtain:

$$\frac{dC_t}{(C_s - C_t)^2} = k_2 dt \quad (5)$$

A further equation in (8) can be obtained by integrating equation (5) using boundary conditions $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$ and by rearranging as follows:

$$\frac{1}{(C_s - C_t)} - \frac{1}{C_s} = k_2 t \quad (6)$$

$$C_t = C_s - \frac{C_s}{1 + C_s k_2 t} \quad (7)$$

$$C_t = \frac{C_s^2 k_2 t}{1 + C_s k_2 t} \quad (8)$$

Equation (8) is an integrated extraction rate law for the second order and can be converted back into linear form as follows:

$$\frac{T}{C_t} = \frac{1}{k_2 C_s^2} + \frac{t}{C_s} \quad (9)$$

The extraction rate C_t/t can be obtained from equation (9), as follows

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{k_2 C_s^2}\right) + \left(\frac{t}{C_s}\right)} \quad (10)$$

And the initial extraction rate h , as $C_t = t$ approaches 0, can be defined as follows:

$$H = k_2 C_s^2 \quad (11)$$

Equation (8) can be changed again so that finally, it can be found:

$$\frac{t}{C_t} = \frac{t}{C_s} + \frac{1}{h} \quad (12)$$

Given the initial extraction rate h , extraction capacity C_s and extraction rate constant k_2 , the second order can be determined experimentally from the slope and intercept by plotting between t/C_t with t . [19].

3. RESULT AND DISCUSSION

The Moringa leaves extraction process requires an efficient approach to ensure optimal results. Several process stages are carried out to obtain the yield of Moringa leaf extract.

3.1 Sample preparation

The samples used in this research were *Moringa oleifera* L. plants in the Kaliwates sub-district, Jember Regency. The sample used was fresh Moringa leaves picked from the leaf's stalks that were still green. A total of 1 kg of Moringa leaves was washed with running water to remove impurities that could affect the extraction process results. If the sample has been cleaned, the drying process is then carried out using an oven at 60°C to remove the water content in the sample so that it avoids microbial growth and can be stored for a long time. The dried samples were then ground using a blender and then sifted using a 60 mesh sieve, which aims to limit variations in sample size so that a fine powder that is relatively the same size and uniform is obtained.

3.2 Water Content Analysis

Analysis of the water content in dry samples of Moringa leaves aims to determine the water content in the sample. The high water content in the sample can affect the extract concentration process. Low water content will inhibit the growth of microorganisms so that samples can be stored longer.

Water content analysis was carried out by heating an oven at 105°C for 3 hours, using a temperature higher than the water boiling point to maximize evaporation of the water in the sample. The results of the analysis of water content in dry samples of Moringa leaves were

4.58%, the results of the water content test were almost the same as in research conducted by Febriani *et al.* [24] namely 4.6% and were following the requirements for simplicia water content according to standard parameters. What applies is no more than 10%.

3.3 Extraction of Moringa Leaves

MAE is a modern method that utilizes microwave radiation to heat solvents and materials quickly and evenly. This technique increases solvent diffusion into plant cells and accelerates the release of active compounds, including tannins, from the cellular matrix. The advantages of MAE compared to conventional methods include shorter extraction time, less solvent use, and higher extraction efficiency.

The extract yield obtained was derived from the Moringa leaves extraction treatment using 96% ethanol solvent with a ratio of 1:15 w/v and with time variations of 2, 4, 6, 8, and 10 minutes using the MAE method. The yield results are obtained in the table below based on the treatment that the specified variations have carried out.

Table 2. Effect of Extraction Time with the MAE Method

| w/v (g/ml) | Extraction Time (minutes) | Yield (%) |
|------------|---------------------------|-----------|
| 1:15 | 2 | 4.1 |
| 1:15 | 4 | 6.34 |
| 1:15 | 6 | 8.52 |
| 1:15 | 8 | 9.66 |
| 1:15 | 10 | 10.25 |

Evaluation of the effect on the extraction time variable was carried out for 2 minutes to 10 minutes with a mass and solvent ratio and using a constant power of 1:15 b/v, respectively, using 96% ethanol solvent, and the power used was 150 watts. The experimental results can be seen in Table 2. Based on the table above, along with increasing extraction time, there is a significant difference in yield. It can be seen in the table that the 2nd minute is the lowest yield when compared to the yield at other time variations. It is suspected that the ability of the ethanol solvent to extract the material has not been maximized, so only a small number of components are taken from the material.

Whether or not the extraction process takes a long time significantly influences the resulting extract yield. From the table above, it can be seen that the yield of the extract produced has different results over various changes in time. An increase in the duration of the extraction time used will increase the yield value. Likewise, the length of the extraction time will improve

the ability of the solvent to penetrate a material. The solubility of components in the material progresses slowly as time increases; however, if the optimal time has been reached, the number of components taken from the raw material will experience a stable value or decrease. This is because the components contained in the material have a limited amount, and the solvent used has a limited ability to dissolve the existing material, so even if the extraction time is extended, the dissolved substances in the material are no longer there [22].

3.4 Identification of Tannins in Moringa Leaves Extract

The tannin content in *Moringa oleifera* leaves has significant potential in various industrial applications, from food to pharmaceuticals. Tannin is a phenolic compound known for its antioxidant and antimicrobial properties. Identifying the tannin content in Moringa leaves is an important first step in understanding the potential benefits of the extract.

The extract obtained was subjected to qualitative tests on tannins. The aim of conducting qualitative tests on tannins is to determine the presence of tannins in Moringa leaves. The phytochemical test carried out in this research was by adding tannin extract with 1% FeCl_3 . This can be seen in Figure 1, which shows the qualitative test results of the extract solution with 96% ethanol solvent.

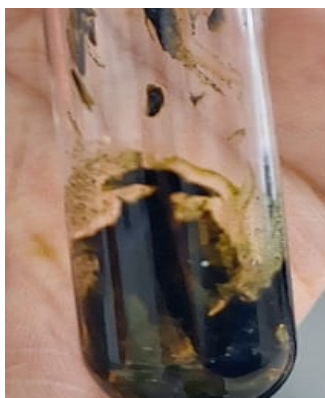


Figure 2. Results of Phytochemical Identification of Tannin Content.

Moringa oleifera L. leaves extract showed positive results. To determine whether the sample contained a phenol group, the researchers tested it by placing Moringa leaf extract into a test tube and adding 1% FeCl_3 . Then, observe the color change in the sample; the color of the sample will change to blackish green or blackish blue; this indicates that the sample is positive for containing phenol groups, and it is possible that one is tannin. A blackish-green or blackish-

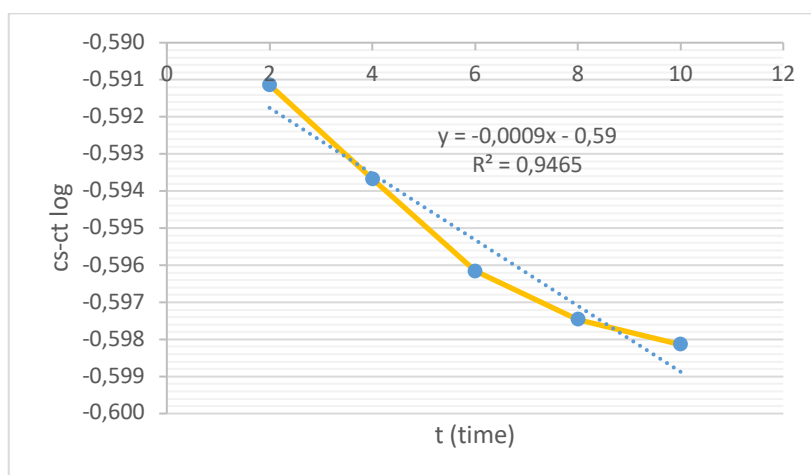
blue color is formed in the extract after adding FeCl_3 because tannins will form complex compounds with Fe ions [20].

Tannins are included in the phenolic group of active plant compounds, meaning they have an astringent taste. Tannin compounds are distributed in almost all plant species and have a role in protection from predators. They may also act as natural pesticides and regulate plant growth. Tannin compounds also function as antioxidants and inhibitors of tumor growth, and tannin compounds are also polyphenolic compounds found in plants [21].

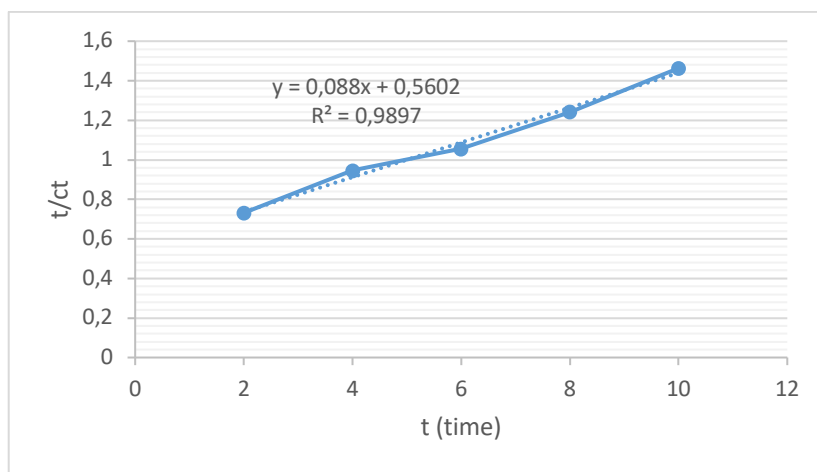
3.5 Extraction Kinetics

In studying extraction kinetics, it is important to understand how changes in extraction conditions affect the rate of tannin release from Moringa leaves. Parameters such as temperature, extraction time, and microwave power are significant variables. Kinetics studies are often carried out by monitoring tannin concentrations in extracts at different intervals, allowing researchers to identify appropriate kinetic models, such as first- or second-order models. These results help in optimizing extraction conditions to obtain maximum results.

The results of Moringa leaf extract are greatly influenced by the operational conditions used in the MAE process. For example, increasing the extraction temperature usually increases yields, but too high a temperature can cause the degradation of tannin compounds. Likewise, excessively long extraction durations can reduce process efficiency due to thermal or oxidative degradation. Therefore, finding the optimal balance between temperature, time, and microwave power is a step in obtaining high-quality extract yields.



(a)



(b)



(c)

Figure 3. Reaction kinetics in the extraction of Moringa leaves tannin compounds using the MAE method: (a) Zero order model, (b) First order model, and (c) Comparison between extraction kinetic models

The results of this study follow research carried out previously using different materials [26]. The extraction process using 96% ethanol as a solvent is partly controlled by desorption kinetics because the desorption force from the matrix into the fluid is the concentration gradient from the soil to the extraction fluid, which can influence the kinetic rate of extraction, which creates two regions in the kinetic rate curve, namely the "fast region" (k_1) and the "slow region" (k_2). This curve agrees with the second-order kinetic model [27]. The extraction rate constants for order 1 and order 2 modeling can be seen in Table 3.

Table 3. Rate constants of 1st-order and 2nd-order extraction kinetic models

| Material | Kinetic Model | | | |
|---|---------------|---------|-----------|--------|
| | Order 1 | | Order 2 | |
| Moringa leaves (<i>Moringa oleifera</i> L.) | Slopes | -0.0009 | Slopes | 0.088 |
| | k_1 | 0.0009 | k_2 | 0.088 |
| | Intercept | 0.59 | Intercept | 0.5602 |
| | R^2 | 0.9465 | h | 0.0059 |

| Material | Kinetic Model | |
|----------|----------------|---------|
| | Order 1 | Order 2 |
| | R ² | 0.9897 |

Based on Table 3, the kinetic model is in order 1 and 2. This table shows the value of the reaction rate constant and the value of Cs. The 2nd-order extraction kinetics model for the extraction of Moringa leaves with 96% ethanol solvent, and the MAE method has a coefficient of determination ($R^2 = 0.9897$), which is higher than the 1st-order extraction kinetics model ($R^2 = 0, 9465$). The R^2 value or coefficient of determination is a value that shows the level of conformity of the equation to the research conducted. The value of R^2 is getting closer to 1, meaning that the equation obtained is closer to the actual research results, so it can be said that modeling the kinetics of Moringa leaves extraction is more suitable using 2nd-order kinetics compared to 1st-order kinetics. The kinetic rate constant value of the 2nd order extraction model is 0.088 mL/g.min, and the Cs value in the 2nd order is 0.2591 mg/mL.

4. CONCLUSION

Moringa oleifera L. leaves using the MAE method have shown that the extraction process using this method is efficient and effective in optimizing extraction results. Identification of tannins using 1% FeCl₃ showed positive results by showing the presence of tannin content in Moringa leaves undergo a characteristic color change, namely, the sample changes color to blackish blue. The yield results show that the extraction time variable can influence the extract results. The lowest yield was obtained in the 2nd minute of extraction, namely 4.1%, and the highest yield was obtained in the 10th minute of the extraction process, 10.25%. The extraction kinetic model suitable for extracting tannin compounds from Moringa leaves is order 2, where the R² 0.9897 value is higher than order 1, and the value is almost close to 1.

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