

THE 8TH INTERNATIONAL SYMPOSIUM OF PUBLIC HEALTH 2024

DEVELOPMENT OF MORINGA OLEIFERA LEAF EXTRACT CAPSULES TO PREVENT ANEMIA AMONG WOMAN WITH POSTPARTUM ANEMIA

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ABSTRACT

Background: Anemia is the leading cause of high maternal mortality and it occurs due to postpartum hemorrhage. *Moringa oleifera* leaf can prevent anemia. The aim of this study was to determine the physical characteristics, stability, contamination, and the effect of *Moringa oleifera* leaf extract capsule among women with post-partum anemia. **Methods:** The first step of this study was physical properties test: the drying process of Moringa leaf, extraction process, and capsule formulation. Then we also conducted water moisture test, uniformity of weight and size test, disintegration time, and microbiological contamination tests using total plate count (TPC). The second step of this study was to determine the effect of the capsule among women with post-partum anemia using control and experiment groups. The experiment group was given Moringa leaf extract capsules and the control group used iron supplements, each twice a day. **Results and Discussions:** The results showed that the physical characteristics of capsules were water: 5.33%, a uniform weight of 500.44 mg, size of 2.25 cm, a disintegration time of 4 minutes 41 seconds, and had authentic powder fragments. The capsules were physically stable when stored at room temperature for three months and not contaminated with microbes. Also, Moringa leaf extract capsules increased hemoglobin and hematocrit levels, and it equivalent with iron supplements. **Conclusions:** Moringa leaf extract capsules meet the physical requirements for capsules, stable, safe from bacteria, and can increase hemoglobin and hematocrit among post-partum anemia.

Keywords: *Moringa oleifera*, Hemoglobin, Hematocrit, anemia.

1. Introduction

Moringa leaves have been used as complementary and alternative medicine (Meireles, Gomes, Lopes, Hinzmann, & Machado, 2020; Murti et al., 2024). Moringa leaves (*Moringa oleifera* Lam) are tropical plants originating from northern India and have spread to various tropical and subtropical regions in the world (Gupta, 2022). Moringa leaves have significant nutritional value and health benefits (Meireles et al., 2020). In recent years, scientific attention to Moringa leaves (*Moringa oleifera* Lam) has increased rapidly, especially because of its potential in improving postpartum maternal health and preventing anemia (Rotella, Soriano, Llopis-Gonzalez, & Morales-Suarez-Varela, 2023; Thenmozhi, Nirmala, & Subalakshmi, 2020).

Postpartum maternal health needs attention. The postpartum period begins immediately after delivery and lasts about six weeks. During this period, the body undergoes physical and hormonal changes and requires optimal nutritional support for recovery. Malnutrition during the postpartum period can cause various health problems, such as anemia, infection, and slow wound healing (Rotella et al., 2023). Moringa leaves (*Moringa oleifera* Lam) offer great potential to support postpartum maternal health due to their high nutritional content and broad pharmacological effects (Rotella et al., 2023; Thenmozhi et al., 2020).

Postpartum mothers who experience anemia have an impact on the breastfeeding process so that it is at risk for the child's health, especially related to stunting (Labbok, 2001; Nadhiroh, Micheala, Tung, & Kustiawan, 2023). Anemia strategies, namely providing iron supplements (Yanti, Ginting, & Susanti, 2024). Complementary and alternative medicine using natural ingredients can be another strategy to prevent anemia in postpartum mothers. The formulation of moringa leaves in capsule form makes it easier to consume compared to the fresh form of moringa leaves (Yanti et al., 2024). The capsule formulation with traditional medicine industry standards so that it becomes a product prototype to be patented and used by the traditional medicine industry. However, it was needed to test the quality of moringa leaf extract capsule.

2. Literature Review

Moringa leaves have been used traditionally to cure various health conditions. Moringa leaves contain 10 times more vitamins than carrots, 7 times more vitamin C than oranges, 17 times more calcium than milk, and 15 times more potassium than bananas (Rockwood, Anderson, & Casamatta, 2013). Moringa leaves also help increase antioxidant levels in the blood (Kushwaha, Chawla, & Kochhar, 2014) and reduce blood sugar levels (William, Lakshminarayanan, & Chegu, 1993) and inflammation (Libby, 2012). So, it can be concluded that Moringa leaves have many important nutrients for the body such as protein, vitamins, calcium, iron, ascorbic acid, and antioxidants (carotenoids, flavonoids, and phenols). Previous studies have mentioned that various developing countries in the world feed their children with Moringa. Moringa Oleifera has hematopoietic activity; it is especially beneficial to platelet count (when taken in low doses), and it is also beneficial to red blood cell formation in humans. Moringa leaves can also increase haemoglobin for postpartum mothers. This is in line with previous study of Zainab et.al (2023). The study found that moringa leaf extract capsules have the same ability as iron supplement, and can even increase hemoglobin and hematocrit levels by up to 28,84% and 22,99% respectively. Further, a study has proven that moringa leaf capsules can increase hemoglobin levels in postpartum mothers (Fitri, Y, 2022)

3. Research Method

The first step of this study was the physical properties of Moringa Leaf Extract: drying process of Moringa leaf, extraction process, and capsule formulation. Then we also conducted water content test, uniformity of weight and size test, disintegration time, and microbiological contamination tests using total plate count (TPC). The second step of this study was to determine the effect of the capsule among women with post-partum anemia using control and experiment groups. The experiment group was given Moringa leaf extract capsules and the control group used iron supplements, each twice a day. All of procedure was granted by ethical clearance number 411/UMB/KE/VI/2023 from Universitas Muhammadiyah Banjarmasin Indonesia.

3.1 Physical Properties of Moringa Leaf Extract Capsules

Moisture test of moringa leaf extract capsules

Moisture test is required for measuring the capsule quality. In accordance with Indonesian food and drug authority (BPOM) Regulation Number 29 of 2023 concerning the Safety and Quality Requirements for Natural Medicines, the water content in the capsule powder is <10% (BPOM, 2023). We used 10 g of Moringa leaves and dried it at a temperature of 105⁰ C for five hours and reweigh it. Then we calculated the water content

Microscopic identification of moringa leaves extract

The extract of Moringa leaf was added with a few drops of Chloral hydrate. Then it was placed on a glass object and covered with a cover glass. The preparation was viewed under a microscope with a magnification of 100 times. The aim of this test was to see fingerprints and prevent counterfeiting of moringa leaf extract.

Weight and content uniformity of capsule

The uniformity test of the capsules was tested by weighing 20 capsules. We weighed empty capsules and capsules containing Moringa leaf extract powder. Then the difference between capsule with powder and empty capsule was calculated as the weight of the powder, then the average was calculated. Standard: ≤ 2 capsule has weight deviate by more than 10% from the mean weight and no capsule should deviate by more than 25% (BPOM, 2023).

Disintegration Time Test

We put 900 mL of distilled water into a 1000 mL beaker glass. The disintegrator temperature was set at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. We put 6 capsules for testing in each basket tube. Then, we put 10 mesh gauze on the surface of the upper plate of the basket. We added 1-liter distilled water. Turn on the device for 30 minutes, then we lifted the basket and observed all capsules. All capsules must be destroyed, except for the part of the capsule shell. The standard for the disintegration time of herbal medicine in capsules is < 30 minutes (BPOM, 2023).

3.2 Stability Test of Moringa Leaf Extract Capsule Preparation

The stability test was carried out for three months by storing the capsules at room temperature. And it was carried out after testing the physical properties of the capsule preparation to see the starting point.

3.3 Microbial Contamination Test

Microbial contamination has been tested using the total plate count (TPC) according to the Indonesian Food and Drug Authority Guidelines (BPOM, 2023). We took a homogenization sample of 1 mL of sample and dilution was carried out. A total of 1 mL of sample from each dilution was poured into a sterile petri dish and added 10-20 mL of NA (Nutrient agar) for testing the number of bacteria and SDA (Sabouraud Dextrose Agar) for testing the number of yeast molds. We incubated it at 37°C for 24 hours for bacteria and at 25°C for 48 hours. Then we observed the number of colonies.

3.4 Clinical Trial of Moringa Leaf Extract Capsules among Women with Anemia

The Moringa leaf extract capsules were tested the effectiveness among postpartum women with anemia. A total of 40 postpartum mothers with anemia were selected. The agreed to join in this study and filled the informed consent. Furthermore, we divided it into two groups randomly. The hemoglobin and hematocrit levels were measured for pretest. Furthermore, the control group was given iron supplements, every day for 10 days with 2 tablets per day while the experiment group was given moringa leaf extract capsules every day for 10 days with 2 capsules per day. We conducted posttest on the 11th day (posttest) hemoglobin and hematocrit levels were checked. Clinical trial data were analyzed statistically using SPSS version 26. The results of the normality and homogeneity tests conducted on hemoglobin and hematocrit levels in respondents who received moringa leaf extract capsules and iron supplements showed normally distributed data with a Sig value > 0.05 . So, the data was analyzed using Annova.

4. Results and Discussion**4.1 Physical properties of Moringa leaf extract in capsule***Moisture content of capsule*

Table 1 showed the moisture content of moringa leaf extract in capsule. The result showed that the powder in capsule is safe. The mean of moisture content was 5.33% and range of moisture content between 4.1-6.2 %. According to Indonesian Food and Drug Authority, the moisture content of powder in a capsule is less than 10 (BPOM, 2023). The results of this study are appropriate with the requirement. Excess moisture in the tablet had

effect on microbial growth, such as bacteria and fungi. The low of moisture content can prevent the tablet from microbial growth (Organization, 2018).

Table 1. Moisture content of Moringa leaf extract

Replication	Initial weight (g)	Final weight (g)	Moisture content (g)	Water content (%)	BPOM standard
1	10.00	9.59	0.41	4.1%	≤ 10 %
2	10.00	9.43	0.57	5.7%	
3	10.00	9.38	0.62	6.2%	
			Mean	5.33%	

Microscopic identification of moringa leaves extract

Microscopic test was used for measuring the fragment contain in the powder of Moringa leaf extract. We used microscope with a magnification of 100x. The figure showed that there were leaf fragments in the powder. The figure showed that upper epidermal cell, hypodermis, parenchyma, palisade tissue, fibers, air cavities and vascular bundler. Spongy tissue, calcium oxalate, lower epidermis and papillae. This test can be used as fingerprint to prevent imitation of capsule. This study also similar with previous study (Bekoe, Jibira, & Agyei, 2020).

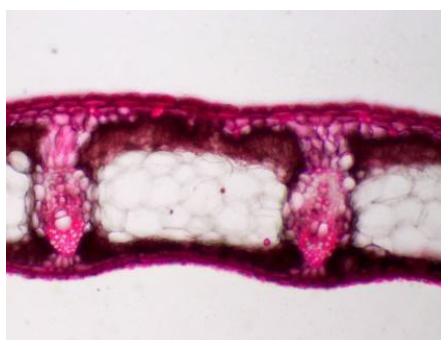


Figure 1. Powder characteristic of Moringa leaves extract

Weight and content uniformity of capsule

The weight and size of capsule must be uniform to avoid excess weight. Table 2 showed the weight uniformity of powder in capsule and table 3 showed the size uniformity of capsule

Table 2. Weight uniformity of Moringa leaf extract capsule

No	Weight (mg)	No	Weight (mg)	No	Weight (mg)	No	Weight (mg)
1	500.83	6	500.56	11	500.57	16	500.33
2	501.21	7	500.43	12	500.94	17	499.89
3	500.28	8	500.23	13	499.43	18	499.93
4	500.87	9	501.03	14	499.89	19	500.35
5	501.04	10	500.31	15	500.21	20	500.54
Mean 500.44 mg							
Standard: ≤ 2 capsule has weight deviate by more than 10% from the mean weight							

Tabel 3. Size uniformity of Moringa leaf extract capsule

No	Size (cm)	No	Size (cm)	No	Size (cm)	No	Size (cm)
1	2.25	6	2.25	11	2.25	16	2.26
2	2.26	7	2.25	12	2.25	17	2.26
3	2.25	8	2.26	13	2.26	18	2.26
4	2.24	9	2.26	14	2.25	19	2.26
5	2.25	10	2.27	15	2.25	20	2.25
Mean 2.254 cm							

Weight uniformity testing was conducted among 20 capsules randomly and according to the procedures of the Indonesia Food and Drug Authorities Agency. We weighed empty capsules and capsules containing Moringa leaf extract powder. Then the difference between capsule with powder and empty capsule was calculated as the weight of the powder, then the average was calculated. The results showed that an average of the powder weight in the capsule of 500.44 mg. Standard: ≤ 2 capsule has weight deviate by more than 10% from the mean weight. The result indicated that the capsule was fit. Testing of capsule size was also conducted among 20 capsules. Size is the length of the capsule. Measurements were made using calipers. The mean of capsule size was 2.254 cm. capsules that have a uniform size with no deviation exceeding 10% are of good quality. The results of the size test showed that the capsules have a relatively uniform size. This test is needed to guarantee the quality of capsule (Osorio & Muzzio, 2013).

Disintegration test

Disintegration time of capsule is one of parameter in capsule preparation. The disintegration time was shown in table 4.

Table 4. Disintegration time of moringa leaf capsule

Capsule	Disintegration time	Mean	Standard
1	4 minutes 43 second	4 minutes 41 second	≤ 30 minutes
2	4 minutes 54 second		
3	4 minutes 23 second		
4	4 minutes 44 second		
5	4 minutes 39 second		
6	4 minutes 43 second		

The results of the disintegration time test on 6 random capsules showed that the average disintegration time was 4 minutes 41 seconds. The disintegration time is in accordance with the standards of The Indonesian Food and Drug Authority, which is <30 minutes. The fast disintegration time of the capsule will be easy to digest, thus accelerating the absorption process of the active ingredient into the blood vessels. This condition is related to the efficacy of the capsule and the good quality of the capsule (Markl & Zeitler, 2017).

4.2 Stability test of Moringa leaf extract capsule

The stability of Moringa leaf extract capsule preparation was assessed based on the physical parameters, such as moisture content, weight uniformity, and disintegration time. The test was conducted on 1st month, 2nd month, and 3rd month. Table 5 showed the stability test of Moringa leaf extract capsule.

Table 5. Stability test of Moringa leaf extract capsule

Parameter	Replication	1 st month	2 nd month	3 rd month
Moisture content	1	5.45 %	5.42 %	5.42 %
	2	5.36 %	5.35 %	5.34 %
	3	5.28 %	5.25 %	5.23 %
Weight uniformity	1	500.54 mg	500.34 mg	500.31 mg
	2	500.74 mg	500.73 mg	500.69 mg
	3	500.62 mg	500.52 mg	500.51 mg
Disintegration time	1	4 minutes 52 second	4 minutes 51 second	4 minutes 48 second
	2	4 minutes 47 second	4 minutes 46 second	4 minutes 42 second
	3	4 minutes 32 second	4 minutes 30 second	4 minutes 30 second

The results of the three-month stability test showed that all three replications were physically stable. The parameter was moisture content at 5%. Weight uniformity shows the powder weight of around 500 mg, there is no significant change during storage. The disintegration time parameter is 4 minutes, there is no decrease to 3 minutes or increase to 5 minutes. The disintegration time also still meets the requirements of The Indonesian Food and Drug Authority, which is <30 minutes (BPOM, 2023). This process is to ensure the safety and quality of capsule (Bankoti, Rana, & Bharadwaj, 2012).

4.3 Microbial contamination test of Moringa leaf extract capsule

Microbial contamination has been tested using the total plate count parameter according to the Indonesian Food and Drug Authority Guidelines. The results of the microbial contamination test of powdered preparations in Moringa leaf capsules are shown in table 6.

Table 6. Microbial contamination test of Moringa Leaf extract capsule

Replication	Total plate count (Colonies/g)	Mean	BPOM standard
1	5.3×10^3	5.53×10^3 colony/g	$< 10^5$ colony/g
2	6.2×10^3		
3	5.1×10^3		

The results of the microbial contamination test using the total plate count (TPC) parameter. TPC showed mean of colonies was 5.53×10^3 colonies/g, and the Indonesian Food and Drug Authority standard is <105 colonies/g. TPC showed the number of aerobic bacterial colonies in the sample. These bacteria have a normal number. If the number was abnormal, so it can cause health problems if consumed. Microbial contamination can also be influenced by conditions during storage and the process of making extract powder in capsules (Okunlola, Adewoyin, & Odeku, 2007).

4.4 Clinical Trial of Moringa Leaf Extract Capsules among women with anemia

The capsule was clinically tested on women with anemia as an experimental group. Anemia is characterized by low hemoglobin levels. The control group was given iron tablets. Each group consisted of 20 respondents whose hemoglobin (HB) and hematocrit (HCT) levels were measured before (pretest) and after (posttest) treatment in the control and intervention groups (Table 7).

Table 7. The experiment and control group before and after intervention

Respondent	Experiment				Control			
	Haemoglobin		Haematocrit		Haemoglobin		Haematocrit	
	Pretest	Post test	Pretest	Post test	Pretest	Post test	Pretest	Post test
1	10.8	12.9	32	38	10.5	11.9	31	35
2	5.4	12.5	16	37	7.1	8.1	21	24
3	8.5	10.8	25	32	10.5	11.5	31	34
4	10.5	12.2	31	36	8.4	10.5	25	31
5	8.5	9.5	25	28	8.4	9.8	25	29
6	9.5	10.5	28	31	8.8	8.1	26	24
7	10.2	11.2	30	33	9.8	11.5	29	34
8	7.4	10.5	22	31	8.8	9.8	26	29
9	10.8	12.9	32	38	10.2	10.8	30	32
10	7.4	10.5	22	31	6.8	10.2	20	30
11	8.5	11.2	25	33	9.1	11.2	27	33
12	9.5	11.2	28	33	8.1	9.1	24	27
13	6.8	10.8	20	32	9.8	10.5	29	31
14	10.2	12.9	30	38	10.5	11.2	31	33

15	9.1	11.2	27	33	8.1	10.5	24	31
16	9.8	11.5	29	34	10.5	11.9	31	35
17	7.1	10.5	21	31	10.5	12.2	31	36
18	9.8	12.5	29	37	10.8	11.9	32	35
19	10.5	13.2	31	39	9.8	10.8	29	32
20	10.8	14.2	32	42	8.8	11.5	26	34
Mean	9.0	11.6	26.7	34.3	9.2	10.6	27.4	31.4
Percentage of increase	28.8%		28.4%		14.9%		14.7%	

Moringa leaf extract capsules were tested on the experimental group, among 20 women with anemia. The control group was given iron supplements among 20 people. The mean of hemoglobin levels before pretest showed that the intervention group had a hemoglobin: 9.0 and hematocrit level of 26.7%, in the control group the hemoglobin level was 9.2 and hematocrit level of 27.4%. These results indicated that both groups had anemia. Furthermore, the intervention group was given moringa leaf extract capsules, while the control group was given iron capsules. The intervention was carried out for 10 days with 2 capsules per day. On the 11th day, we measured hemoglobin and hematocrit levels. The post-test showed that the mean hemoglobin level in the experiment group was 11.6 and the hematocrit level was 34.3%. Meanwhile, the control group; the mean hemoglobin level was 10.6 and the mean hematocrit level was 31.4%. Based on the results, there was an increase in hemoglobin and hematocrit levels in the experiment and control group. In the group given moringa leaf extract capsules, there was an increase in hemoglobin levels of 28.8% and hematocrit levels of 28.4%. In the control group, there was an increase in hemoglobin levels of 14.9% and hematocrit levels of 14.7%. So, moringa leaf extract capsules can be used to increase hemoglobin and hematocrit levels in anemia women. The ability to increase hemoglobin and hematocrit levels in this study was greater than the use of iron capsules. Moringa leaf extract capsules can be used as an alternative for iron capsules in women with anemia.

Table 8. Comparison of the mean score in experiment group

Parameters	Experiment		Control		Sig
	Pretest	Post test	Pretest	Post intervention	
Hemoglobin	9	11.6	9.2	10.6	0.086
Hematocrit	26.7	34.3	27.4	31.4	0.085

Table 8 showed the results of hemoglobin after giving Moringa leaf extract capsules with iron capsules with p value = 0.086 ($p > 0.05$), it means that the difference was not significant. It can be concluded that the test results of moringa leaf extract capsules with iron capsules had similar ability to increase hemoglobin levels based on statistical analysis. The hematocrit results after giving extract capsules with iron capsules showed p value of 0.085 (p value. 0.05) which means that the difference that occurred was also not significant. Iron capsules had ability to increase hematocrit levels based on statistical analysis. However, the mean was increased. This study in line with previous study which mentioned that Moringa leaf increased Hb and hematocrit among pregnant woman (Iskandar, Hadju, As'ad, & Natsir, 2015; Nur et al., 2020; Nurdin, Hadju, Ansariadi, & Arundhana, 2018), however this study was conducted among post-partum women with anemia.

5. Conclusion and Implications

The physical characteristics of the moringa leaf extract capsules were water content: 5.33%, weight uniformity: 0.44 mg, size: 2.25 cm, disintegration time: 4 minutes 41 seconds, and distinctive powder fragments. These characteristics were appropriate with the standard. Moringa leaf extract capsules were physically stable when stored at room temperature for three months, and were not contaminated by microbes. Furthermore, moringa leaf

extract capsules increased hemoglobin and hematocrit levels, and this ability is equivalent to iron capsules based on statistical tests. This capsule can to be alternative for preventing anemia.

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