Artikel Penelitian

Instant Powder Formulation for Anti Anemia and Optimazation Exctraction Condition of *Moringa pterygosperma* Gaertn Leaves Using MAE

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ABSTRACT: *Iron supplement containing ferrous sulfate is commonly used for anaemia. Unfortunately, it has bad taste, can cause nausea, and made adverse effects if taken in large doses for long periods. It is necessary to find an alternative source of raw materials, including those from plants. Moringa pterigospera Gaertn leaves was selected because it contains iron and other nutritions. The purpose of this work was to make instant powder formula for anti anaemia using Moringa leaves extract as an alternative for ferous suphate iron suplementation. The extraction was performed by Microwave Assisted Extraction method. Optimization of extraction condition was performed by creating some variations in solvent composition (0-70% ethanol), microwave power (450 to 900 watts) and extraction time (3 to10 min). Iron content was determined by Atomic Absorption Spectrophotometer at wave length of 248 nm. Instant powder formula was made in 3 concentrations of sodium CMC as suspending agent. Results of the study showed that the most optimal extraction condition which resulted the highest iron content (2.4 mg/g extracts) achieved with 900 watts microwave power, 10 min extraction time and aqua demineralisata. According to 30 panelists of hedonic test, formula which used 5% w/w of sodium CMC got the highest scores. Its powder form had 2.31% of loss on drying and 7.74 g/sec of flow rate for powder form and pH of 5.78, viscosity of 15.98 cps for reconstituted form.*

Keyword: *anti anemia, instant powder, iron, microwave assisted extraction, Moringa pterygosperma Gaertn*

ABSTRAK: Suplemen besi yang mengandung ferrous sulfat umum digunakan untuk anti anemia. Sayangnya sediaan ini memiliki rasa tidak enak, menyebabkan mual dan jika digunakan dengan dosis besar dan jangka waktu lama dapat menyebabkan efek samping. Perlu dicari alternative sumber lain, termasuk dari tanaman. Daun *Moringa pterigospera* Gaertn dipilih karena mengandung besi dan suplemen lain. Penelitian ini bertujuan untuk membuat formulasi serbuk instan untuk anti anemia sebagai alternatif suplementasi zat besi selain dari ferous sulfat, dengan menggunakan ekstrak dari daun kelor (*Moringa pterygosperma* Gaertn). Ekstrak diperoleh dengan metode *Microwave Assisted Extraction*. Optimasi kondisi dibuat dengan memvariasikan pelarut etanol (0-70%), daya listrik microwave (450-900 watt) dan waktu ekstraksi 3-10 menit. Analisa kadar besi dilakukan menggunakan Spektrofotometer Serapan Atom pada panjang gelombang 248 nm. Formula serbuk instan dibuat dengan 3 konsentrasi natrium CMC sebagai bahan pensuspensi. Penelitian menunjukkan bahwa kondisi optimal ekstraksi yang menghasilkan kandungan besi paling besar (2,4 mg/g ekstrak) dicapai dengan daya listrik 900 watt, waktu ekstraksi 10 menit dan pelarut air suling. Berdasarkan uji hedonis dengan 30 panelis, formula dengan 5% natrium CMC paling disukai. Bentuk serbuk memiliki kadar air 2,31%, laju alir 7,74 g/detik dan bentuk rekonstitusinya memiliki pH 5,78, dan viskositas 15,98 cps.

Kata Kunci: anti anemia, serbuk instan, besi, *Microwave Assisted Extraction*, *Moringa pterygosperma* Gaertn

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INTRODUCTION

Iron supplements -currently available as anti-anemia- is commonly used raw materials ferrous sulfate which has a bad taste, causing nausea, and if taken in large doses for long periods can cause heart failure [1]. Therefore, it is necessary to find an alternative of raw materials including source from plants contain high iron, such as Moringa leaves. Moringa leaves is selected for antianemia therapy because it contains iron and nutrients, such as protein [2] and abundant in Indonesia. The formula of instant powder made from Moringa leaves should instantly soluble in water, practical, easy to carry, easy to use and "customer friendly". Anti-anemia preparations should contain sufficient iron content, therefore, it is necessary to find the optimal extraction condition in order to obtain high iron content that could be extracted from Moringa leaves. Several previous studies conducted for extracting the leaves of Moringa include decoction, maceration, percolation and soxhlet [3], Supercritical Fluid Extraction [4], reflux [5]. The method requires a complicated process, a long time and required a lot of solvents, therefor, today was developed a microwave extraction method. The advantages of using microwaves for extraction is the shorter extraction and cooling time and use less solvent [6].

To optimize the extraction process, some experimental design optimization of extraction conditions are made. The experiments are designed-by modifying multiple extraction conditions, such as electric power of microwave, extraction time and solvent composition in order to obtain the maximum iron content. Optimization of the instant powder formula of Moringa leaves are also conducted in order to obtain a formula that most consumers preferred. Some physical parameterswere conducted to evaluate the instant powders including loss on drying, flow rate and angle of repose and for the reconstituted form, the tests were pH, viscosity and hedonic test (colour, aroma and taste).

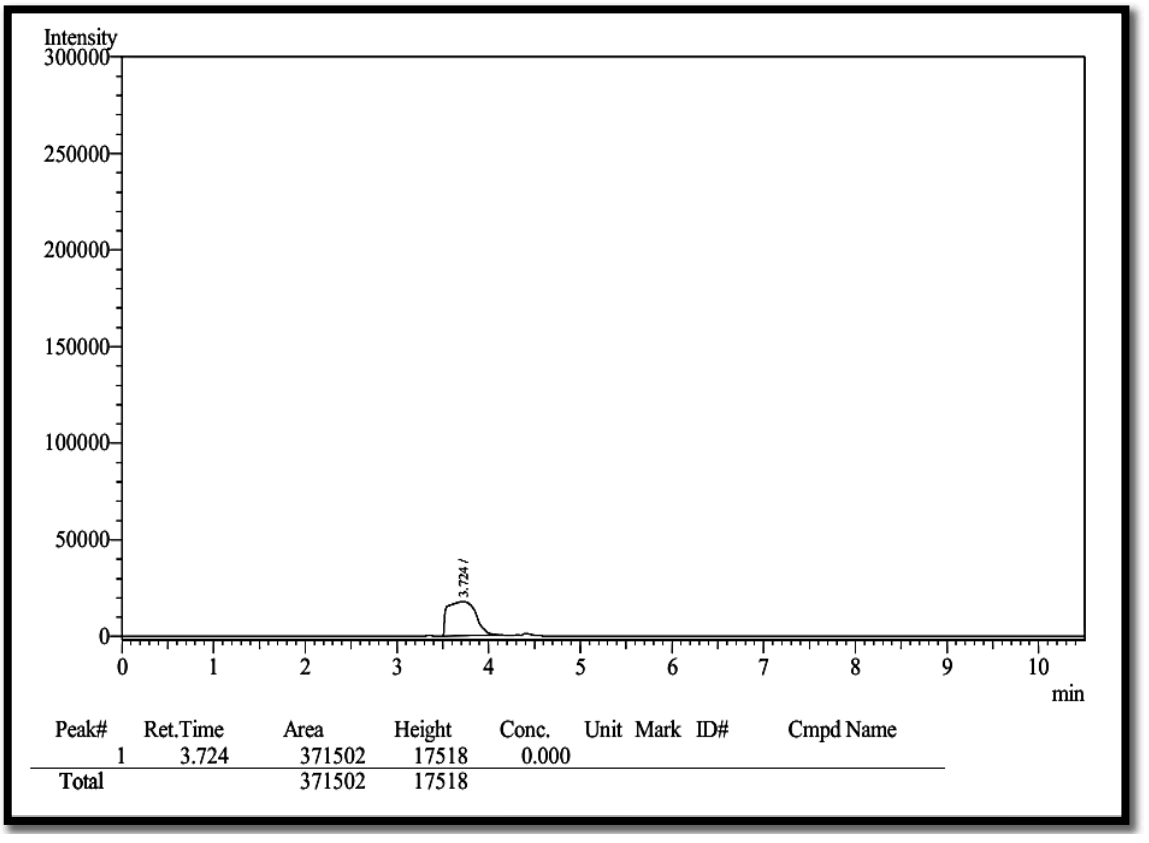
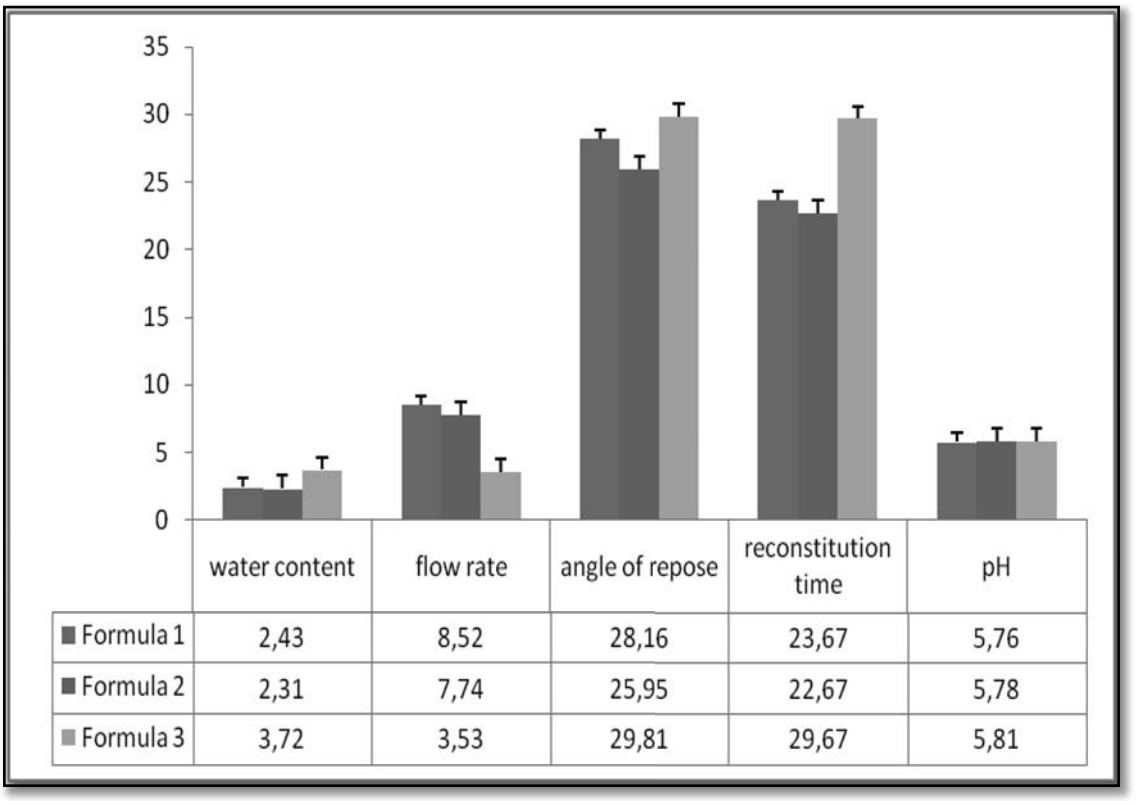


Figure 1 : Result of Residual test performed with GC-FID



	water content	flow rate	angle of repose	reconstitution time	pH
Formula 1	2,43	8,52	28,16	23,67	5,76
Formula 2	2,31	7,74	25,95	22,67	5,78
Formula 3	3,72	3,53	29,81	29,67	5,81

Figure 2 : Physicochemical test for instant powder

MATERIALS, INSTRUMENT AND METHOD

Materials:

Dried Moringa leaves obtained from Sukabumiand determined by the Indonesian Institute of Sciences, Bogor Botanical Gardens; n-hexane, ethanol, and aquademineralisata (Brataco, Indonesia), iron standard solution (Merck, Germany), nitric acid (HNO_3) (Merck, Germany), $HClO_4$ (Merck, Germany), manitol (Merck, Jerman), maltodextrin (Zhucheng Dongxiao Biotechnology Ltd, China), aerosil (Cabot Blue Star, China), D-mannitol (Merck, Jerman), CMC sodium (Dai-ichi, Kagyo), potassium sorbate (local, Indonesia), flavor and coloring Grape (local, Indonesia).

Instruments:

MAE - Microwave Assistance Extraction (Modena MV Series) which had been modified by installing additional double reverse condensor, Rotary Vacuum Evaporator (Buchi), destilation flask, Atomic Absorption Spectrofotometer (Shimadzu AA-6300 PC), GC-FID, Whatman # 42 filter paper, waterbath, magnetic stirer, thermometer, spatula, viscometer Brookfield, analytical balance and moisture balance.

Methods:

Preparation

Dried Moringa leaves were cleaned then blended to become a powder. The amount of 1,430 g of powdered Moringa leaves were then macerated with 4 liters of hexane for 24 hours. The process is repeated 7 times. The macerated powder was dried in drying cabinet.

Microwave Assistance Extraction Process

Before extraction process, the macerated powder was stirred using a magnetic stirrer for 10 minutes until obtained a homogeneous mixture, then extracted by MAE method. The optimization was applied by varying the type of solvent as follow: distilled water, ethanol 30% , 50% and 70% ; varying the electrical power; and varying the time of extraction. The parameters were analyzed using d–optimum design from Design Expert. As the result there were 29 types of extraction condition that will be made for the optimization with modification.

Testing for extract

Los on drying test using oven : The amount of 1

Table 1. Formula of Moringa Extract Granule

Ingredients	Formula (gram)	Function
Moringa leaves extract	1.375	Active Ingredient
Maltodextrin	3.321	Filler
Aerosil	0.704	Adsorbence
Total	**5.400**	

Table 2. Instant Powder Formula

Ingredients	Formula (gram)		
	I	II	III
Moringa's leaf extract dried granul	5.400	5.400	5.400
Mannitol	8.745	8.370	7.650
Sodium CMC	0.375	0.750	1.275
Grape's flavour and colouring paste	0.450	0.450	0.450
Potassium sorbate	0.030	0.030	0.030
Total	15.00	15.00	15.00

gram sample were weighed and placed in a platinum crucible, then heated in oven at 105 ° C for 3-5 hours.

Total ash: The amount of approximately 1 gram of sample was put in a platinum crucible and then ignite slowly until the charcoal is depleted then cooled and weighed .

Acid insoluble ash : After determination of the ash content, the sample was added 25 mL of H_2SO_4 10 % then heated 10 minutes to boil. the liquid is filtered with Whatman filter paper in a beaker glass. Whatman's paper was then placed into the porcelain plate and put into the oven for ± 2 hours at a temperature of ± 600°C. The plate was allowed to stand in a desiccator for ± 1 hour. The porcelain plate is weighed until obtain the fix weight .

Hexane residual test: using GC - FID instrument.

Organoleptic test: visual observed to the appearance, color , and taste.

Identification of iron (qualitative) : react the filtrate with 3 drops of NaOH, potassium hexacyanoferrate (II), potassium hexacyanoferrate (III)

Instant Powder Preparation

The recommended daily dose of iron is in the range of 10 mg which coud be divided into a 3.33 mg single dose. The amount of 1.375 g of Moringa extract was required to make a 3,33 mg single dose of iron (assuming that the content of Iron in the extract is 2.4 mg/g).

Dried granules was prepared by absorbing Moringa leaves extract with aerosil then mixed with maltodextrin until obtained a homogeneous mass. Then, dried granule in drying oven at 40°C for 6 hours, then crushed and sieved with mesh #40 .

Instant powder made by adding sodium CMC as suspending agent, pasta flavor and colorants, preservatives then stirred homogeneously. The mixture was then dried at a temperature of 40°C for 2x6 hours, then crushed and sieved with a mesh # 40. The powder was filled into alu-foil sachet and stored in a tight container

Evaluation of granules :

Moisture content measured with a moisture balance, flow rate and angle of repose, measured by flowmeter

Evaluation of Reconstituted powder :

Reconstitution time, pH and viscosity and organoleptic test aroma, color, taste).

Table 3. Iron Content Obtained Form The Optimization of Extraction Condition

No	Solvent composition	Eletrical Power (Watt)	Extraction Time (minutes)		Iron content (mg)
			Teoritic	Actual	
1	0	450	4.38	4.30	0.9865
2	0	450	5.97	6	0.9836
3	0	450	9.56	9.30	0.8824
4	0	630	3.55	3.30	0.8564
5	0	630	8.02	8	0.8733
6	0	630	10.00	10	0.9633
7	0	900	3.00	3	1.4406
8	0	900	6.23	6	0.9422
9	**0**	**900**	**9.78**	**10**	**2.4046**
10	30	450	5.34	5.30	2.0774
11	30	450	9.17	9	1.2672
12	30	630	3.99	4	0.9958
13	30	630	5.56	5.30	1.4650
14	30	630	9.56	9.30	2.3741
15	30	900	4.93	5	1.3211
16	30	900	6.48	6.30	1.5422
17	30	900	10.00	10	1.6070
18	50	450	5.21	5	1.3275
19	50	630	4.73	4.30	1.5342
20	50	630	4.73	4.30	1.3165
21	50	900	6.35	6.30	1.4952
22	50	900	7.26	7.30	1.2985
23	50	900	7.26	7.30	1.1058
24	70	450	7.05	7	0.8152
25	70	450	7.05	7	0.8227
26	70	450	7.05	7	0.8877
27	70	630	6.77	7	0.7403
28	70	630	6.77	7	0.7812
29	70	900	3.86	4	1.3160

RESULTS AND DISCUSSION

Dried Moringa leaves obtained from Sukabumi determined in the Indonesian Institute of Sciences, Bogor Botanical Gardens Conservation Center. The results of the determination states that the plant is *Moringa pterygosperma* Gaertn. The value of nutritional contents in Moringa leaf powder is reported not change even if it is not stored in the refrigerator until a few months [7]. Furthermore, the fine moringa leaves powder were macerated with hexane, to extract non-polar compounds that may interfere the assay of the active substance (Fe) which are polar and to eliminate bad odors of Moringa leaves. The essential oils contained in the leaves of Moringa were 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester, nonacosane, heptacosane,

Table 4. Total Score of Hedonic Test

Parameter	Formula 1	Formula 2	Formula 3
Texture	108	105	109
Colour	99	100	111
Aroma	103	112	107
Taste	93	107	68
Total	**403**	**424**	**395**

β-Amyrin [4]. Maceration process was repeated for 7 times, since the solvent of the 7th process began clear indicating that most of non-polar substances had been extracted. The rendemen of this hexane-maceration was 79.47 % .

The regression liniear equation obtained from several measurement of iron (Fe) reference standard solution at the concentration of 2, 4, 6 and 8 ppm was y = 0,043755x - 0.0318 with r = 0.9971. The visual test of the viscous extract showed a brown, hygroscopic with a distinctive aroma and flavor of Moringa. The viscous extract was then dried in a water bath.The physical tests were conducted to the dried extract and resulted loss on drying of 1.06 %, total ash of 12.76 %, acid insoluble ash of 1.69 %, the protein content of 0.18 % and the residual hexane of below 100 ppm using GC-FID, which was far below the limit 290 ppm.

Qualitative test identificatioin with NaOH produces a reddish brown color (positive Fe), using potassium hexacyanoferrate (III) produces a dark blue color (positive Fe^{3+}), and using potassium hexacyanoferrate (II) produced a light blue color (positive Fe^{2+}).The presence of iron in $Fe^{2+}$and or Fe^{3+} form did not affect the anti-anemia function, as both can be absorbed either in the body .

The data showed that the maximum iron content was obtained under the extraction condition as follow: Aqua demineralisata as solvent, 900 watt microwave power and 10 minutes extraction time which resulted of 2.4 mg iron/g extract or 25.4 mg iron/100 g of dry powder Moringa leaves. Comparing to the previous study, the iron content of 25.4 mg/100 g of dry powder Moringa leaves is higher than the amount of 16.42 mg/100 g dried Moringa leaves which was extracted used MAE method but without macerated with hexane [8]. However, it was lower than 28.2 mg/100 g of dry powder Moringa leaves obtained by Fuglie [2]. This is possibly due to the difference of extraction method as well as the difference of geographical location where the plants grow.

Visual observation of the powder preparation showed that the formula 1, 2 and 3 formed fine granules, colored purple with the aromas of grape. After dissolving in water, the dark purple-slight grape aroma solution was formed. Physical flow rate test of formula 1 and 2 showed a good flow ability between 4-10 g/sec, while the formula 3 showed a poor flow ability between 1.6 to 4 g/sec. The angle of repose test show that the formulas 1, 2 and 3 showed a good angle of repose which is between 25-300. The reconstitution time test (with 200 mL water) of all formulas are below 1 minute, but with a large standard deviation. It is probably influenced by the manual reconstitution method which vary in the stirring speed and strength.

The results of pH test of the reconstitution form, showed a pH of 5.76± 0.01 of formula 1; 5.78 ± 0.02 of formula 2 and 5.81 ± 0.02 of formula 3. This difference were mainly caused by the different amount of sodium CMC added into the formula. The higher the amount of sodium CMC, the higher value of pHwill increase. All formulas have a pH near to 6 so it did not irritate the stomach. Viscosity test was performed using Brookfield viscometer. The result is 9.61 cP of formula 1; 15.98 cP of formula 2 and 80.03 cP

of formula 3.

A hedonic test was performed to the preparations before and after reconstitution. The parameters tested were texture (before reconstituion), color, aroma and taste (after reconstitution). 30 panelists were randomly selected, which aimed to reduce variables that may interfere the results [9]. In this study, the total of 30 people with vary in gender and age as follow: female (80 %) and male (20 %), age of 16-20 years (36.7 %), 21-25 years (56.7 %) and 26-30 years (6.7 %). The collected data was analysed using a numerical scale (scoring) ranged from 1 to 5 The meaning of the value was as follows: 1 = not like, 2 = less like, 3 = normal, 4 = like and 5 = very like [10,11]. All data were analyzed using SPSS version 17.

CONCLUSION

The optimum condition for extraction of iron molecule from Moringa leaves using MAE is obtained by using aqua demineralisata solvent, 900 watt (P 100%) of microwave power and 10 minutes of extraction time. This condition resulted the maximum amount of Iron of 2.4 mg/gram of extract. The formula 2 wich applying the amount of 5% w/w of sodium CMC as suspending agentwas the most prefered formula selected by panelist with total score of 424. The formula has a physical properties of 2.31% loss on drying, 7.74 g/sec of flow rate, 5.78 of pH, and 15.98 cP of viscosity.

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