Optimization of Encapsulation of *Moringa Oleifera Antioxidant Activity* with Ionic Gelation Method Using Sodium Alginate and $CaCl_2$

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Abstract

The diversity of medicinal plant benefits in Timor Leste. One of them is the Moringa plant which has not been utilized properly such as roots, bark and leaves. Unhealthy living habits can trigger poor human health. Namely malnutrition and exposure to free radicals cause cell damage in the body and ultimately have a major impact on health. Antioxidants as the right effort to be able to ward off and capture free radicals and replace damaged body cells, help in the regeneration of new cells. Antioxidants can be obtained from leaves, bark and roots of Moringa which are rich in high nutrition, encapsulated with Sodium Alginate and CaCl2. The purpose of the study was to determine the physical and chemical characteristics of Moringa leaf, bark and root extracts. Benefits for innovating functional Moringa food products with the highest antioxidant content, namely Moringa leaves. Using the ionic gelation method with nano synthesis testing with variations of Sodium Alginate and CaCl2 with variations in the ratio between (3: 1, 4: 1, and 5: 1). Testing of physical and chemical characteristics of superior results at a ratio of 5: 1. The results of the antioxidant test showed that the best Moringa leaves had an IC50 value of <50 ppm, its effectiveness in inhibiting free radicals was higher than extracts from other parts of Moringa. After encapsulation, the leaf extract yield was 29.81262851 ppm, the bark 40.69652802 ppm, and the roots 43.56171361 ppm.

Keywords: *Moringa, Free Radicals, Antioxidants, Encapsulation.*

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A. INTRODUCTION

The population conditions in Timor Leste, especially in the city of Dili, are getting worse along with the exponential increase in population from population growth in this region which is increasingly threatening the food security margin. In addition, the Covid-19 pandemic, the impact of which is still being felt today, has triggered a sharp increase in the prevalence of stunted toddlers (UNICEF, 2020). According to existing survey data on Food and Nutrition in 2020, the presentation rate was 47% of children suffering from stunting and 8.8 % of children under 5 years of age who were underweight. This condition is reinforced by data released by WHO (World *Health Organization)*, in 2021 which reported that around 47.1% of toddlers in Timor Leste suffered from malnutrition problems due to unsafe food (*Tatoli news,* 2021). Likewise, data from the Ministry of Health of Timor Leste from January to July 2022, in the first semester conducting a review and identifying the growth and development of around 44,238 children also found similar facts. From the available data, it was found that around 1,500 children suffered from malnutrition, including

1,327 cases of moderate malnutrition and 442 cases of acute malnutrition (*Tatoli news*, 2022).

Malnutrition is one of the serious health problems for toddlers in developing countries, including Timor Leste. Efforts that need to be made are to utilize biological resources to provide antioxidant compound supplements to toddlers, adolescents and adults who are at risk of health. Antioxidant compounds can repair, neutralize and stabilize free radicals that can damage body cells. Antioxidant compounds can also improve the immune system and protect the body from infection. Moringa plants are also widely known by the community with the name in the local language *Marungi* which has another advantage, namely that it grows a lot in tropical areas such as Timor Leste [16]. Malnutrition occurs due to protein imbalance which will cause a state of antioxidant deficiency and increase oxidative stress in the brain [14].

Antioxidants are substances that can inhibit and protect the body from free radical compounds. Antioxidants have the ability to neutralize free radicals by combining free electrons, thereby preventing damage to cells in the body. Free radicals that are abundant in the human body can cause many types of diseases such as heart attacks, cancer, stroke, kidney failure, premature aging and other chronic diseases. There are several commonly used antioxidants such as vitamin C, vitamin E, carotenoids, flavonoids, lipoic acid, and antioxidants from spices. Antioxidants obtained from plants are usually in the form of phenol compounds and their derivatives [5].

Many studies have been conducted by researchers to utilize the efficacy of the Moringa plant as a vegetable and also a medicine with excellent nutrition for stimulants in blood circulation, antiepileptic, anti-inflammatory, antitumor, antipyretic, antibacterial, antifungal and antioxidant. According to Nurulita et al. (2019), the ethanol extract of Moringa leaves has a total phenolic of 16.13 mg GAE / g with an antioxidant activity of 97.48 ppm [13]. Stating that the methanol extract of Moringa leaves has a chalcone compound [18]. Based on the results of this study, the results of the extraction of Moringa leaves have the potential as a source of natural antioxidants.

B. METHODS

The materials used in this study were leaves, bark and roots of Moringa taken from Comoro Village, Dom Aleixo District, Dili-Timor Leste. Samples were taken from 1 tree that was more than 1 year old. Each sample was washed in running water until clean, must be free from dust and soil. Dried by airing on a 40 x 40 cm baking sheet for 5 to 10 minutes, with the aim of ensuring that no water sticks to the sample. Then continue by drying each *Moringa oleifera sample* (leaves, bark and roots) in a digital oven at a constant temperature of 45 ^{o}C for 6 hours for each sample. After that, weigh each grinder at maximum speed for 45 seconds to obtain sample powder with

a 100-mesh sieve. Each powder for each sample is 100 grams for maceration preparation.

C. RESULTS AND DISCUSSION

1. Extraction Results from Maceration and Evaporation

Extraction of leaves, bark and roots *of Moringa oleifera* each 100-gr soaked in methanol pa 500 mL covered with *plastic wrap* protected from light or direct sunlight for 2 days (48 hours). Filtering the soaking of each sample, obtaining the filtrate which was then continued with evaporation in a *Buchi R-300 rotation evaporator* with an optimum temperature of 50 °C. With the following results of stages I and II:

Table 1. Maceration and Evaporation Extraction Results

Sample & Weight (gr)		Solvent Volume (mL)	Sample Maceration Time (O'clock)	Extract volume (mL)	Evaporation Time (Minutes)	Evaporation Result (mL)
Leaf	100	350	48	330	26	13.8
Bark	100	350	48	290	21	10.0
Root	100	350	48	300	23	10.2

2. Results of PSA measurements and zeta potential of encapsulation results

Measurement of encapsulated particles using *Particle Size Analyzer* (PSA) which has a sensitivity between 3-10,000 nm and is able to measure particles in the range between 0.15 -10 μm. Particle size can affect the stability of the powder due to the influence on the surface area, so smaller particles have a larger surface area per unit mass. This can increase interaction with the surrounding environment. The results of the particle size analysis are shown in table 2 below:

Table 2. Particle Measurement Analysis Results

Type	PSA Results (nm)			Average size (nm)	PDI
	D10	D50	D90		
Leaf	180	245	310	245	0.28
Stem	160	215	280	218	0.22
Root	200	275	350	275	0.32

The results of PSA testing on three parts of the Moringa plant showed interesting differences in particle size characteristics. Moringa roots have the largest average particle size of 275 nm, followed by Moringa leaves with 245 nm, and Moringa stems with the smallest size of 218 nm. From the results of this particle size, it has met the nanoparticle size standard according to Jonassen (2014), which states that a particle with a size range between 10-1000 nm can be called a nanoparticle [8,12]. For nano particles with an average size of 50 to 500 nm, they can be used as drugs (nanocarriers) for drug delivery purposes [3,12]. In addition, the test results show PDI or often referred to as particle distribution uniformity. The distribution of

particles in the tail has the widest range of 0.32, while in the stem it has the narrowest distribution range of 0.22. The lower the PDI value indicates a more even distribution. *Particle Size* Distribution is an important property that affects drug loading, drug release, and stability [2,11]. The value of PDI (Polydispersity *Index)* approaching zero indicates a uniform distribution of nanocapsule particle size [20]. However, a PDI value exceeding 0.5 indicates a non-uniform distribution of nanocapsule particle size or high heterogeneity [7]. So, the conclusion from the observation of particle size obtained the best results in the rod sample.

Table 3. Results of Zeta Potential Determination

Sample Type	Zeta potential (mV)
Leaf	-28.5 ± 2.3
Stem	-24.7 ± 1.9
Root	-31.2 ± 2.7

In addition to observing the particle size to determine the nature of the nanocapsule results, the determination of zeta potential was also carried out. The results of the zeta potential measurement of the nanocapsules on the sample can be seen in table 2, which shows the surface value of the particles on the negatively charged sample. Determination of this zeta potential was carried out to determine the surface charge of the nanocapsules of the leaf, stem and root samples of moringa. The results of the expected zeta potential have a value greater than +25 mV or -25 mV because if it has a high zeta potential it will be electrically stable. Whereas if the zeta potential is low it will tend to clump or fluctuate which can then cause poor physical stability [2,6].

However, zeta potential is not the main parameter in determining the stability of a nanoparticle because it can be influenced by several factors including; pH of the solution, electrolyte concentration, and the type of coating material used in the encapsulation process [2,19]. Based on the results of the three samples, the values above ± 25 mV mean that the three samples have good stability. Moringa root extract shows the most negative zeta potential value, which may indicate better colloidal stability compared to leaf and stem extracts.

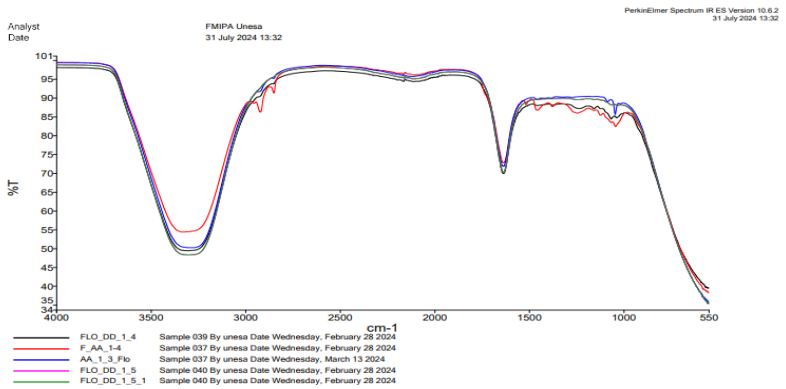

Figure 1. FTIR Test Result Graph

FTIR spectroscopy analysis was performed on five samples. Measurements were made in the wave number range of 4000-550 cm $^{-1}$ using the *PerkinElmer Spectrum IR ES Version* 10.6.2 instrument. The results of the analysis are displayed in the form of a transmittance spectrum as shown in Figure 1. The FTIR spectra of the five samples show similar patterns, indicating similar chemical structures. However, there are variations in the intensity of several peaks indicating differences in concentration or minor structural modifications between samples. Significant absorption peaks were observed in several regions of the spectrum:

a. Region 3300-3400 cm^{-1}: The broad peak in this region indicates the presence of OH or NH stretching vibrations. The presence of this peak indicates the possibility of hydroxyl or amine groups in the compound structure. This is in line with research [17] which identified similar peaks in the analysis of organic compounds.
b. Region 2900-3000 cm^{-1}: Peaks in this range are characteristic for CH stretching vibrations of alkyl groups. The fairly strong peak intensity indicates the presence of hydrocarbon chains in the molecular structure.
c. Region 1500-1700 cm^{-1}: Peaks in this range can be attributed to stretching vibrations with C=C of the aromatic ring or stretching vibrations with C=O of the carbonyl group. The presence of these peaks indicates the presence of aromatic elements or carbonyl groups in the compound structure. This interpretation is supported by research [15] which used FTIR for the analysis of aromatic and carbonyl compounds.
d. Area below 1500 cm^{-1}: (*fingerprint region*): The peaks in this region provide specific information about the various types of bonds and functional groups in the molecule. The complex patterns in this region can be used for specific identification of compounds. The importance of this fingerprint region is emphasized in a comprehensive study by [22] on the application of FTIR in molecular structure analysis.

Comparison between samples shows general similarity of spectral patterns, but there are variations in intensity of some peaks. This difference can be caused by concentration variations or minor structural modifications between samples. Sample AA_1_3_Flo shows consistency with other samples, indicating the stability of the compound over the time period.

The quality of the obtained spectra shows good resolution with minimal noise, allowing accurate interpretation. However, for definitive identification of the compound, further analysis is required with other spectroscopic techniques such as NMR or mass spectrometry, as well as comparison with reference spectra. This multi-technique approach is in line with the recommendations [10] for comprehensive molecular structure characterization.

Based on the results of the FTIR analysis, it shows that the five samples have similar chemical structures with some minor variations. The presence of functional

groups such as OH or NH, CH, and possibly C=C or C=O can be an initial clue in determining the molecular structure of the compound being analyzed.

3. Antioxidant Test Before and After Encapsulation

In the antioxidant activity test using the *DPPH method* with a concentration of 100 ppm and methanol solvent pa Then the next measurement was carried out using *a UV-Vis spectrophotometer* at a wavelength of 400-700 nm. For testing antioxidant activity was carried out before and after the encapsulation process can be seen in table 4 and table 5 below:

Table 4. Antioxidant Activity Test Before Encapsulation Process

Moringa Leaves						
Concentration (ppm)	**Absorbance**			**Average**	**Inhibition %**	**IC $_{50}$ (ppm)**
	1	**2**	**3**			
1000	0.047	0.047	0.048	0.04733	90.66968	39.1082
100	0.162	0.163	0.163	0.16267	64.63506	
10	0.319	0.319	0.32	0.31933	29.27013	
1	0.428	0.429	0.429	0.42867	4.589917	
Moringa Root						
1000	0.053	0.054	0.054	0.05367	89.24003	42.52075
100	0.198	0.198	0.198	0.19800	56.65914	
10	0.333	0.334	0.334	0.33367	26.03461	
1	0.447	0.448	0.448	0.44767	0.300978	
Moringa Bark						
1000	0.029	0.029	0.029	0.02900	86.70077	45.61974
100	0.079	0.079	0.079	0.07900	61.12532	
10	0.141	0.141	0.142	0.14133	29.24126	
1	0.187	0.188	0.189	0.18800	5.370844	

Table 5. Antioxidant Activity Test After Encapsulation Process

Moringa Leaves						
Concentration (ppm)	**Absorbance**			**Average**	**% Inhibition**	**IC $_{50}$ (ppm)**
	1	**2**	**3**			
1000	0.025	0.025	0.026	0.0253333	95.63582	29,8126
100	0.142	0.143	0.143	0.1426667	69.14974	
10	0.299	0.299	0.300	0.2993333	33.78480	
1	0.428	0.429	0.429	0.4286667	4.589917	
Moringa Root						
1000	0.038	0.039	0.039	0.0386667	92.62603	40,6965
100	0.183	0.184	0.184	0.1836667	59.89466	
10	0.318	0.319	0.318	0.3183333	29.49586	
1	0.447	0.448	0.448	0.4476667	0.300978	

Moringa Bark						
1000	0.027	0.027	0.028	0.0273333	87.55330	43,5617
100	0.077	0.077	0.078	0.0773333	61.97780	
10	0.14	0.141	0.141	0.1406667	29,58230	
1	0.187	0.188	0.189	0.188	5,370844	

Calculation of Antioxidant test with *Inhibition Concentration* (IC50) is the sample concentration that can reduce DPPH radicals by 50%. The IC50 value is obtained from the x value after replacing y = 50. Then the inhibition percentage of each concentration is obtained, the equation is y = ax + b which can be determined by linear regression calculation where x is the concentration (ppm) and y is the inhibition percentage [9].

Each of the moringa extract parts was tested for its best activity as antioxidant activity data before encapsulation. This also aims to determine the reduction or increase in antioxidant activity during the encapsulation process. Several studies have examined the potential for free radical reduction from moringa leaf extract (*Moringa oleifera)*. One of the most widely used methods is 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis 3-ethylbenzothiazoline-6-sul- fonic acid (ABTS) [4]. As in the study of Fombang et al revealed that the alcohol extract of moringa leaves had an IC50 value of 1.28 using the DPPH test. In this study, an evaluation was carried out on various parts of the moringa plant, namely the leaves, bark, and roots of moringa to determine the most potential part as an independent antioxidant utilization.

In the context of this research, antioxidant activity was obtained based on the IC50 value of DPPH testing from the leaves, roots, and bark of Moringa oleifera, respectively, which were 39.1082; 42.52057; and 45.61974. The highest activity was shown by the leaves, which is in accordance with [21] which revealed high antioxidant activity in isoquercetin, crypto-chlorogenic acid, and astragalin compounds from Moringa oleifera leaves. To assess the antioxidant activity of the extracts and isolated phenolic compounds in cell lines, the same researchers examined the possibility of inhibiting the production of reactive oxygen species (ROS) induced by H2O2 and the potential impact on the expression of mRNA encoding for antioxidant enzymes [1]. In general, both extracts and isolated phenolic compounds showed a suppressive effect on ROS production and increased the expression of antioxidant enzymes such as catalase, heme oxygenase, and superoxide dismutase. Based on this study, it can be concluded that Moringa leaves can function as a valuable source of natural antioxidants, having direct and indirect antioxidant abilities.

Based on the calculation results, the IC 50 value of the extract of leaves, roots, and bark of the Moringa plant after the encapsulation process respectively is 29.81262851 ppm; 40.69652802 ppm; 43.56171361 ppm. This IC 50 value shows that Moringa leaves have the best antioxidant activity, namely having an IC 50 value <50 ppm. It can be stated that its effectiveness in inhibiting free radicals is higher than extracts from other parts.

The encapsulation method can help in increasing the stability and bioavailability of antioxidant compounds, thereby strengthening their antioxidant potential.

D. CONCLUSION

The results showed that all samples were successfully encapsulated into nanoparticles with sizes ranging from 10-1000 nm. Moringa stems had the smallest particle size and the most uniform distribution. Zeta potential measurements indicated good stability in all samples. Antioxidant activity tests using the DPPH method showed that Moringa leaves had the best antioxidant activity, both before and after encapsulation. The encapsulation process successfully increased antioxidant activity in all samples, with the greatest increase in Moringa leaf extract.

FTIR analysis confirmed the presence of functional groups commonly associated with antioxidant activity in the studied samples. In conclusion, this study successfully optimized the encapsulation process of Moringa oleifera extract, resulting in nanoparticles with good physicochemical characteristics and enhanced antioxidant activity. Among the plant parts studied, Moringa leaves showed the greatest potential as a source of natural antioxidants. The results of this study open up opportunities for researchers to further develop this research in the utilization of *Moringa oleifera* as an effective and stable source of natural antioxidants.

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