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The Extraction and Nano Particles Production of Moringa Leaf Bioactive Compounds and Their Identification

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ABSTRACT

Moringa leaves (Moringa oleifera) is a vegetable plant from the Brassica order and belongs to the Moringaceae family, known as the "Magic Plant" which has a wide variety of protein content contained in it.
Therefore, the bioactive compounds used are Moringa leaves (Moringa oleifera) which have such great potential that they can be developed as raw materials for medicine. However, because the secondary metabolite compounds contained by plants have hydrophobic properties. This property can be one of the obstacles for these secondary metabolites to be easily absorbed by the body because of their nature which is difficult to dissolve in body fluids so that they can be easily degraded by the body's enzymatic system, to overcome this problem Nanoparticles can be a solution in overcoming this problem. the manufacture of nanoparticles has been carried out.
To produce nanoparticles of Moringa leaf bioactive compounds, synthesized using tween 80 surfactants, has been carried out using the spontaneous emulsion method.
The bioactive compounds contained in Moringa leaves (Moringa oleifera) are: (1) 9-Octadecenoic acid (29.64%), (2) Hexadecanoic acid (18.42%), (3) Octadecanoic acid (7.07%), (4) Oleic Acid (5.88%), and (5) 14-Methyl-8-hexadesin-1-ol (5.47%). Synthesis using four different concentrate masses of these compounds, namely: 2 mg, 1.5 mg, 1 mg and 0.5 mg dissolved in 100 ml of ethanol, resulted in an average diameter of each nanoparticle of 15.9 nm; 15.5nm; 14.7nm; and 14.8 nm. The polydispersity index is 0.306; 0.258; 0.122; and 0.282.
From the results above, it has been shown that the process of synthesizing nanoparticles of secondary metabolites of ethanol extract using the spontaneous emulsion method has produced small-sized particles. This allows the development of site results to be used in applications in various fields such as in the pharmaceutical field in the drug development process.

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Introduction

Moringa leaves (Moringa oleifera) is one of the vegetable plants of the Brassica order and belongs to the Moringaceae family, known as "Miracle Plants" due to its high protein contents vitamin A ten times higher than carrots, vitamin C seven times higher than oranges, calcium seventeen times higher than milk, and fifteen times higher potassium concentration than bananas (Kumari.,D. J., 2017). It is traditionally used to treat various diseases and some chronic diseases (Amaglo et al., 2018; Jennifer Lutz,2018). The benefits of Moringa leaves include two nutritional sources, (Kushwaha, Chawla, & Kochhar, 2014) that are antioxidants, which can reduce blood glucose levels (Abdulrahman, F. I., & Onuoha, S. C 2020) and anti-inflammatory properties (Santos AR et al., 2018) to maintain heart health (Sacks FM et al ., 2017, Susan Zogheib. (2017) and increase immunity (Obi, Egwurugwu, , S. O. Ojefa, Ekweogu, & Ogunnaya, 2018) , as well as maintain healthy skin (Madhuri Parikh, 2020).

Several investigations have been conducted on bioactive compounds from Moringa leaves because they have extensive benefits and are still believed to be used for alternative medicine because of their affordable cost (Ali S et al., 2021 ; Sharmin F et al., 2021). A study on Moringa leaves conducted by (Yong-Bing, Gui-Lin, & Ming-Quan, 2019) showed that Moringa leaf extracts have high antioxidant activity on the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (*Ferric-Reducing Antioxidant Power*). According to (E. T. Akintoye et al., 2020), Moringa leaves have anti-aging compounds from the natural cytokinin group called zeatin and also its active compounds are effective in fighting skin cancer (Kim MK et al., 2018).
Moringa leaves can also strengthen the immune system. Vitamin C and antioxidant compounds contained in Moringa leaves can help strengthen the immune system and fight disease. Vitamin E and A contained in Moringa leaves can also help maintain healthy skin by preventing damage to the skin layer caused by free radicals (Padalia, 2017).

Experimental studies conducted on experimental animals on rats conducted in Pakistan showed that Moringa leaf extract can reduce the level of oxidative stress experienced by male rats who have reproductive problems (Shakya, 2017).

The life compounds of secondary metabolites contained by plants have hydrophobic characteristics. This is one of the obstacles for this secondary metabolite compound to be easily absorbed by the body because it is difficult to dissolve in body fluids so that it will easily be degraded by the body's enzymatic system (Siew, Chan, & Wong, 2022). Nanoparticles can be a solution in overcoming these problems because they can be active pharmacological agents that can help in the drug distribution system, improve drug stability, and control the release of drug active substances (Gupta,A., & Eral H.B. 2019; Kaur, R., Goyal, A. K., & Rath, G. 2018).

A nanoparticle is defined to be a very small particle, which less than 100 nanometers (0.1 microns) (Rajendra Kumar, Singh & Rohit Srivastava 2019). Nanoparticles consist of various materials, such as minerals, metals, polymers, and other organic materials. The behavior and properties of nanoparticles differ from larger particles due to their very small size. Nanoparticles have many applications in various industries, such as pharmaceuticals, electronics, and cosmetics (Ghotbi, Z., & Rezaei, M. 2019). There are two definitions of nanoparticles application in the pharmaceutical field, where the first is a drug compound synthesized into nanometers and the second is a drug compound encapsulated in a nanometer-sized carrier system (nanocarrier) (Raza.A et al., 2019).

However, the use of nanoparticles has an environmental impact and can also affect human health. So, further research is needed related to the impact of their use. The advantages of nanoparticles in the pharmaceutical field include the faster absorption process of drugs containing nanoparticle compounds, allowing the body to quickly respond to drugs and for the drugs to work faster (Briganti, 2016).

This study aims to identify the active compounds contained in Moringa leaves extract and synthesize nanoparticles using tween 80 surfactants as an ingredient for encapsulation. The choice of tween 80 surfactants is because this surfactant has non-toxic properties and is stable to the influence of stomach acid. Besides, it can reduce viscosity on the surface of particles to increase its bioavailability in the body (Singh et al ., 2020, Ghosh et al ., 2019).

Method

Materials and Tools

The equipment used in this study included a beaker, measuring cylinder, erlenmeyer flask, scales, stirring rods, funnels, Whatman no 1 filter paper, label paper, glass bottles, spatulas, shakers, vortex mixers, and syringes. The materials used in the study involved Moringa leaves, 96% ethanol, Tween 80, and distilled water/aquades. Moringa leaves came from Dodaek village, South Rote District, Rote Ndao Regency. Moringa trees and leaves can be seen in Figure 1. All tools to be used were washed first until clean and dried in a clean place and ready to be used.

Figure 1. Moringa Tree and Moringa Leaves

Producing Moringa Leaf Simplicia Powder

The process of making Moringa leaf simplicia powder begins with sorting Moringa leaf simplicia. Then, the simplicia was washed with clean running water and drained. The simplisia of Moringa leaves that have been clean was dried by aerating for seven days. Furthermore, dried simplicia leaves were pureed using a blender until they formed a fine dry powder. This grinding aims to increase the surface contact area with solvent molecules during extraction. After grinding with a blender, then it would be sifted using a 40-mesh sieve. Once sifted, it was ready to be extracted.

Extraction of Moringa Leaf Secondary Metabolite Compounds

Moringa leaves extraction process using maceration method with the absolute ethanol solvent for analysis 99.9% from Merck KGaA Germany. A total of 100 g Moringa leaves powder was soaked in 250 mL of ethanol solvent in the Erlenmeyer flask. Next, the mixture was stirred using a shaker at room temperature for one hour. After the stirring process, it was allowed to stand for four hours at room temperature. This stirring and silence process was carried out eight times. Furthermore, the maceration solution or macerate was separated from the residue by filtering it using Whatman No.1 filter paper. After filtration, a separation process was carried out from the solvent to produce a concentrated extract. Separation was done by evaporating the solvent using a rotary evaporator device operated at 80 rpm for three hours. The evaporation process was carried out at a pressure of 0.5 atm and a temperature of 40^{o}C. After the evaporation process completed, concentrated Moringa leaves extracts were obtained. The concentrate was then stored in a sealed glass bottle container in a refrigerated room at -10°C before

being identified using gas chromatography-mass spectroscopy or GC-MS techniques.

Identification of Moringa Leaves Secondary Metabolite Compounds

Identification of secondary metabolite compounds contained in Moringa leaves was carried out by gas chromatography-mass spectroscopy (GC-MS) technique using Shimadzu GC-MS QP2010 Ultra (Japan) tool. By measuring the parameters of retention time (minutes) and mass spectroscopy, the measured parameters of the ratio spectrum of mass (m) to charge (z) obtained were used to identify the types of metabolite compounds.

Synthesis of Nanopaticles Secondary Metabolite Compounds of Moringa Leaves

The synthesis process of Moringa leaves extracts nanoparticles was carried out by spontaneous emulsion method technique. The steps for making nanoparticles of Moringa leaves ethanol extract metabolite compounds with emulsion techniques was started with making four samples of Moringa leaves ethanol extracts diluted with concentration variations of 2mL, 1.5mL, 1mL, 0.5mL respectively in a beaker glass. Furthermore, in each sample, 10 mL of Tween 80 material was added at each sample concentration and then stirred it until homogeneous. Finally, add 30 mL distilled water which was slowly added using a 10 mL syringe. This process was repeated four times to ensure homogeneity. After the synthesis process of nanoparticles of secondary metabolite compounds of Moringa leaf extract was completed, the samples were placed in a tightly closed glass bottle container and stored in a room protected from the sun. Next, prepared each sample as much as 1 ml for particle analysis using the Horiba SZ-100 (USA) PSA (*Particle Size Analyzer*).

Results

Identification of Compounds Contained in Moringa Leaves

Procedure for examining compounds contained in Moringa leaves used Gas chromatography—mass spectrometry. This compound then was identified as the active substance found in Moringa leaves. A total chromatom ion (TIC) was obtained from the GC-MS measurements. It indicated the peaks of the secondary metabolite compounds of Moringa leaves extracts as seen in Figure 2 where there are 25 peaks of TIC. The magnitude values of each TIC peak are summarized in Table 1.

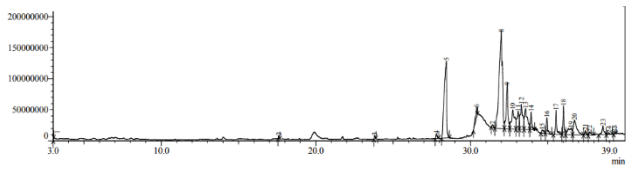


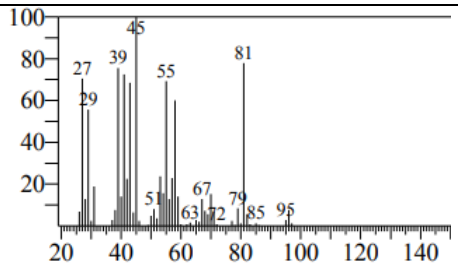
Figure 2. GC-MS chromatogram results of Moringa leaves secondary metabolite compounds using ethanol solvent

Figure 2 is the identification result of secondary metabolite compounds in Moringa leaves extracts concentrate. There are chromatogram peaks of total ion abundance (total ion chromatogram, TIC) of secondary metabolite compounds contained in Moringa leaves. Maximum wavelength absorption measurements were in the range of 300-500 nm and the maximum wavelength produced was ±435 nm. The area and percent of each peak can be seen in Table 1.

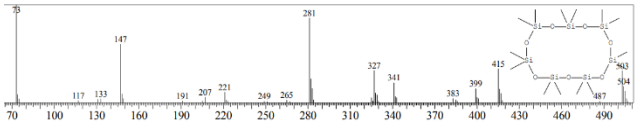
Table 1. The amount of retention time, peak area, and percentage peak area of each peak abundance of TIC secondary metabolites compunds of Moringa leaves.

Top #	Retention Time (Minutes)	Peak Area	% Peak Area
1	3.024	8850727	0.11
2	17.600	14418752	0.19
3	23.806	20170943	0.26
4	27.794	44962718	0.58
5	28.456	1432107338	18.42
6	30.400	116213502	1.49
7	31.430	22021429	0.28
8	31.997	2304772939	29.64
9	32.380	550043742	7.07
10	32.737	425178058	5.47
11	33.090	281501084	3.62
12	33.302	457337536	5.88
13	33.563	422143679	5.43
14	33.925	172284917	2.22
15	34.640	42976210	0.55
16	34.947	139731243	1.80
17	35.541	241743761	3.11
18	36.026	259502082	3.34
19	36.515	213051177	2.74
20	36.735	356699372	4.59
21	37.435	46993785	0.60
22	37.722	48661901	0.63
23	38.574	124912968	1.61
24	38.965	19288260	0.25
25	39.340	10716884	0.14
	Total	7776285007	100.00

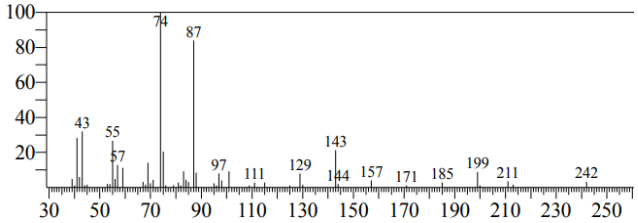
In Table.1, it can be seen that there are 25 peaks of TIC representing 25 secondary metabolite compounds contained in Moringa leaves ethanol extracts concentrate. There are five dominant secondary metabolite compounds seen in Table 1, namely at peaks 5, 8, 9, 10, 12, When the molecules of secondary metabolite compounds have ionized and fragmented, their mass ratio (m) and their charge ratio (z) are measured. The measured m/z spectra of the secondary metabolite compounds that produce the peaks in Figure 2 are shown in Figure 3.



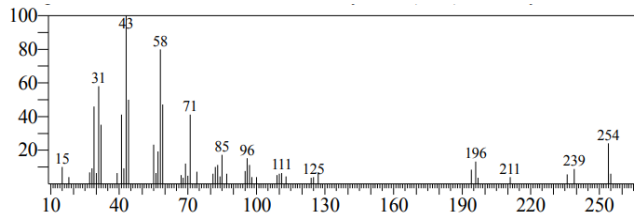
#1 the ratio spectrum of molecular m/z of the compounds producing peak



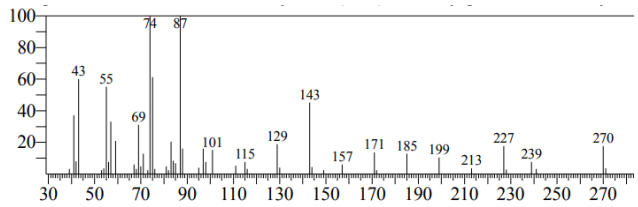
#2 the ratio spectrum of the molecular m/z of the compounds producing peak



#3 The ratio spectrum of the molecular m/z of the compounds producing peak



#4 The ratio spectrum of the molecular m/z of the compounds producing peak



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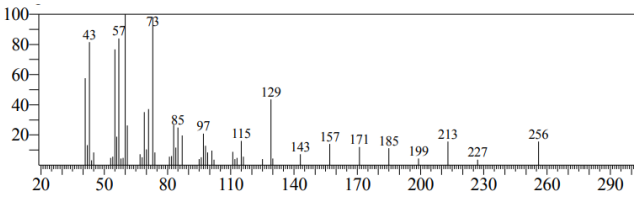
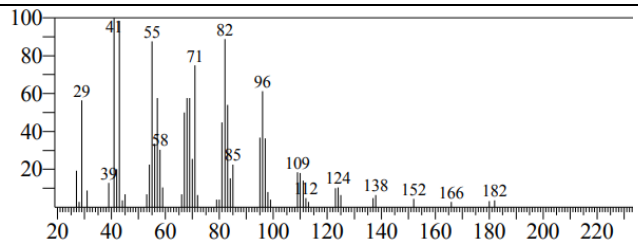
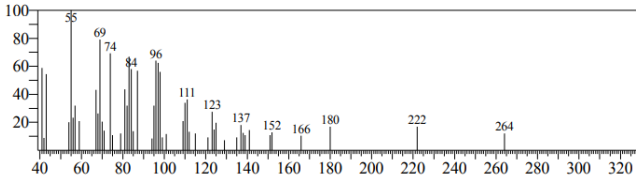


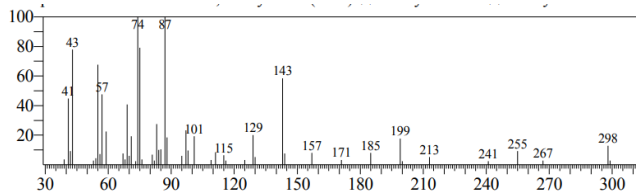
#6 The ratio spectrum of the molecular m/z of the compounds producing peak



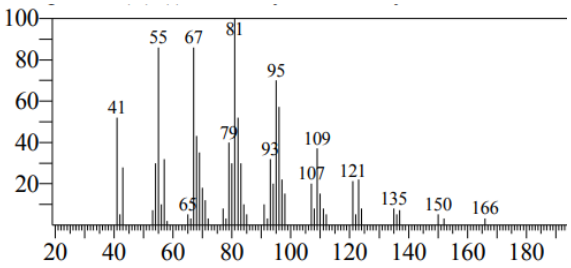
#7 The ratio spectrum of the molecular m/z of the compounds producing peak



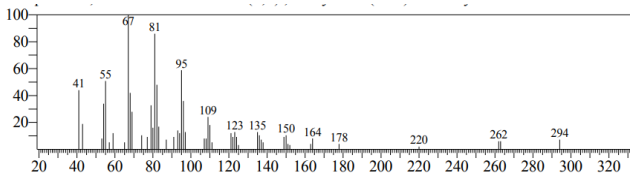
#8 The ratio spectrum of the molecular m/z of the compounds producing peak



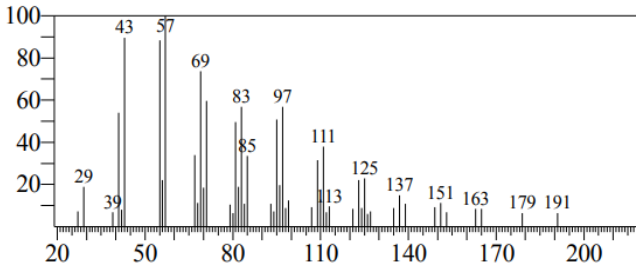
#9 The ratio spectrum of the molecular m/z of the compounds producing peak



#10 The ratio spectrum of the molecular m/z of the compounds producing peak



#11 The ratio spectrum of the molecular m/z of the compounds producing peak



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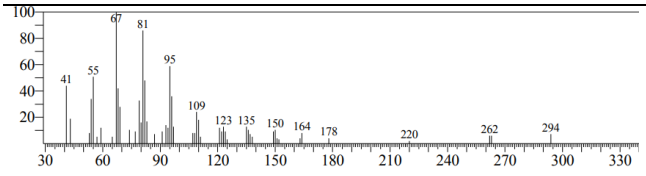
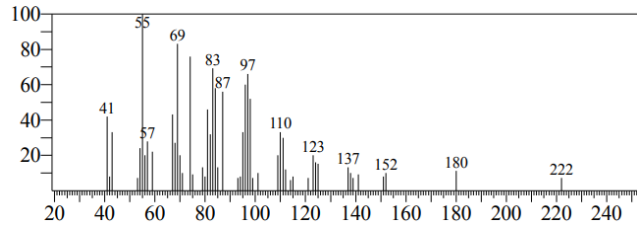


#13 The ratio spectrum of the molecular m/z of the compounds producing peak



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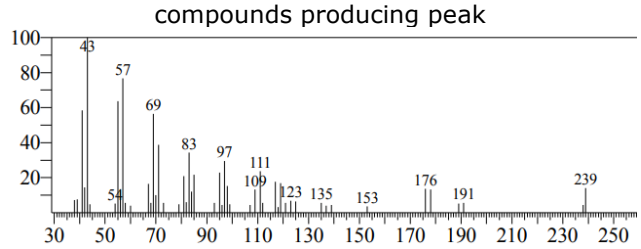

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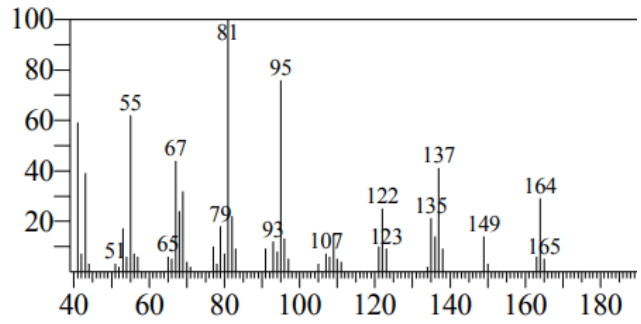
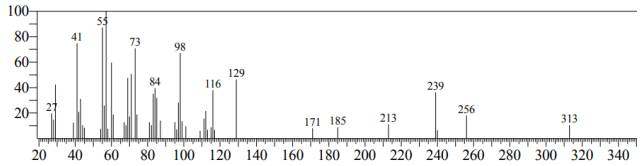
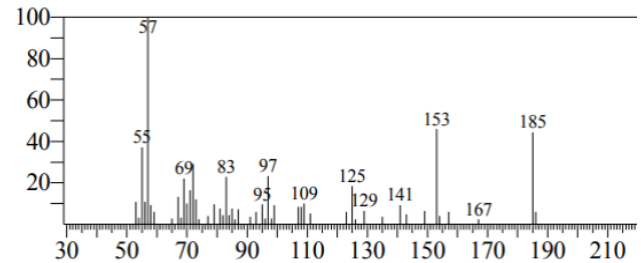


#16 The ratio spectrum of the molecular m/z of the compounds producing peak



#17 The ratio spectrum of the molecular m/z of the compounds producing peak



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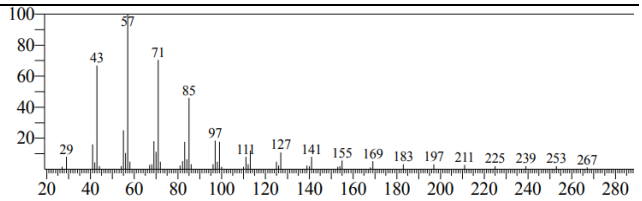

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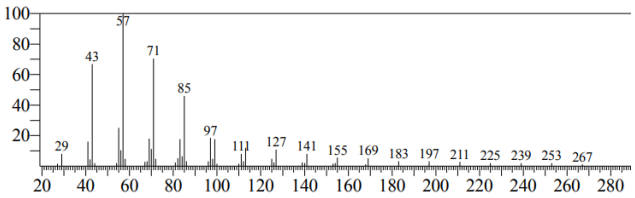
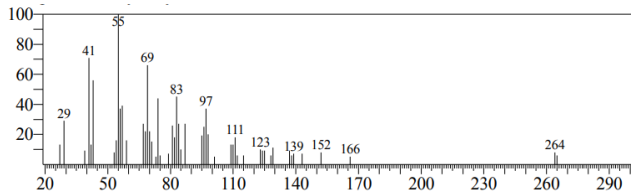
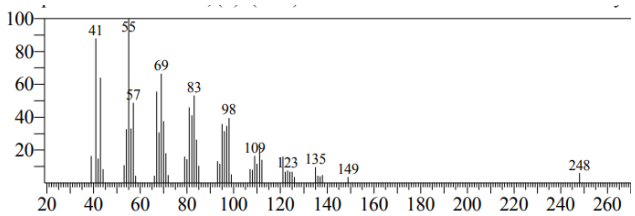


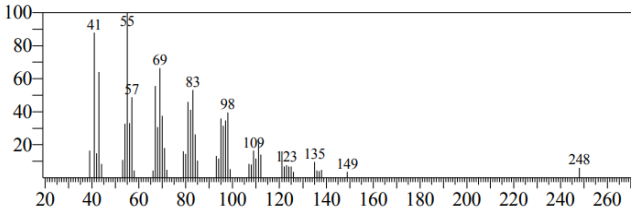
#20 The ratio spectrum of the molecular m/z of the compounds producing peak



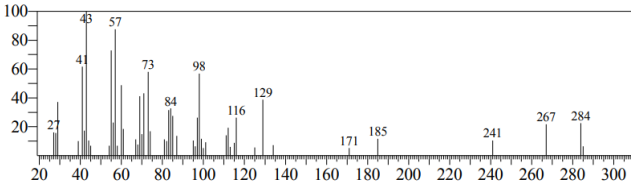
#21 The ratio spectrum of the molecular m/z of the compounds producing peak



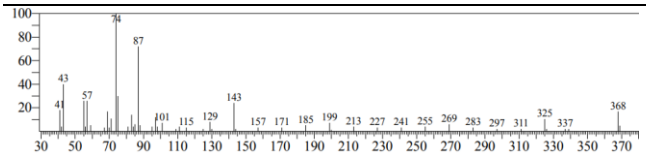
#22 The ratio spectrum of the molecular m/z of the compounds producing peak



Molecular m/z ratio spectrum of peak producing compound #23



#24 The ratio spectrum of the molecular m/z of the compounds producing peak



#25 The ratio spectrum of the molecular m/z of the compounds producing peak

Figure 3. The mass-to-charge ratio (m/z) spectra of the molecules from compounds that produce total ion abundance peaks shown in the chromatogram Figure 1

The time of retention and mass ratio (m/z) spectra vary and are typical. This makes it possible to be able to identify secondary metabolite compounds in Moringa leaves. This compound was identified by matching the values of these parameters with the same parameter values found in the National Institute of Standards and Technology Mass Spectral Database (NIST-MS). The identification results can be immediately seen in the GC-MS results because the GC-MS QP2010 Ultra tool used had been integrated with the NIST-MS system. Table 2 shows the results of identifying secondary metabolite compounds along with retention time, molecular formula, and molecular weight of each compound. There are 25 kinds of secondary metabolite compounds. Of the 25 secondary metabolite compounds, there are five dominants successively compounds from the largest content, namely 9-Octadecenoic acid (29,64%), Hexadecanoic acid (18,42%), Octadecanoic acid (7,07%), Oleic Acid (5,88%), and 14-Methyl-8-hexadecyn-1-ol (5,47%).

Table 2. Results of identification of secondary metabolite compounds of Moringa leaves

Peak	Retention Time (minutes)	Compound Name	Molecular Formula	Molecular Weight
1	3.024	5-Hexen-2-ol, 5-methyl-	$C_7H_{14}O$	114
2	17.600	TETRADECAMETHYLCYCLOHEPTASILOXANE	$C_{14}H_{42}O$ 7 Si_7	518
3	23.806	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	242
4	27.794	2,15-Hexadecanedione	$C_{19}H_{36}O_3$	312
5	28.456	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270
6	30.400	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
7	31.430	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240
8	31.997	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	296
9	32.380	Octadecanoic acid, methyl ester (CAS)	$C_{19}H_{38}O_2$	298
10	32.737	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252
11	33.090	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	$C_{19}H_{34}O_2$	294
12	33.302	Oleic Acid	$C_{18}H_{34}O_2$	283
13	33.563	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	$C_{19}H_{34}O_2$	294
14	33.925	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	296
15	34.640	Hexadecanoic acid, (3-bromoprop-2-ynyl) ester	$C_{19}H_{33}BrO_2$	372
16	34.947	1H-Indene, 1-(1,5-dimethyl-2-hexenyl) octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha.]	$C_{18}H_{32}$	248
17	35.541	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	$C_{35}H_{68}O_5$	568
18	36.026	1-Ethyl- 3,cis -(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol	$C_{13}H_{26}O_2$	214
19	36.515	Tetratetracontane (CAS)	$C_{44}H_{90}$	618
20	36.735	Tetratetracontane (CAS)	$C_{44}H_{90}$	618
21	37.435	methyl dihydromalvalate	$C_{19}H_{36}O_2$	296
22	37.722	9-Octadecenal, (Z)- (CAS)	$C_{18}H_{34}O$	266
23	38.574	9-Octadecenal, (Z)- (CAS)	$C_{18}H_{34}O$	266
24	38.965	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	$C_{39}H_{76}O_5$	624
25	39.340	Tricosanoic acid, methyl ester (CAS)	$C_{24}H_{48}O_2$	368

Measurement of Nanoparticle Synthesis Results

After identifying secondary metabolite compounds in Moringa leaves, the process of synthesizing nanoparticles from these secondary metabolite compounds was carried out by following the steps described in the methodology section. The size distribution of nanoparticle particles was measured using the HORIBA SZ-100 brand *Particle Size Analyzer* (PSA).

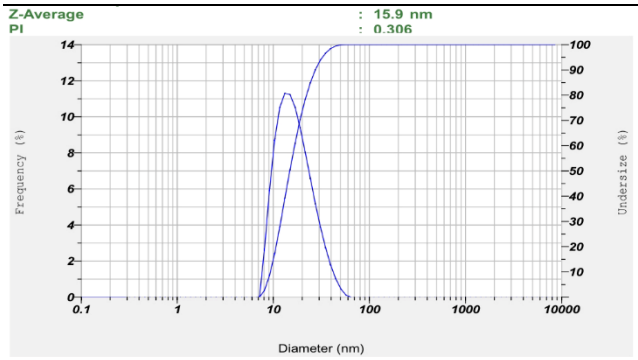


Figure 4. Test results of Particle Size Analyzer (PSA) sample 1

Figure 4. shows the results of the Moringa Leaves Extract Nanoparticle test was measured using a Particle Size Analyzer (PSA) during the test within 30 seconds of ± 15.9 nm and a polydispersity index of 0.306.

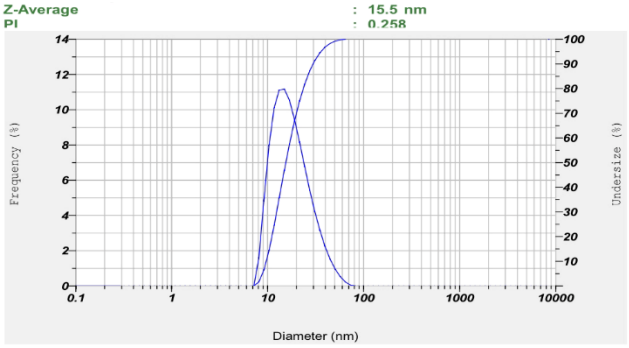


Figure 5. Test results of Particle Size Analyzer (PSA) sample 2

Figure 5. The results of the Moringa Leaves Extracts Nanoparticle test were measured using a Particle Size Analyzer (PSA) during the test within 30 seconds of ± 15.5 nm and a polydispersity index of0.258.

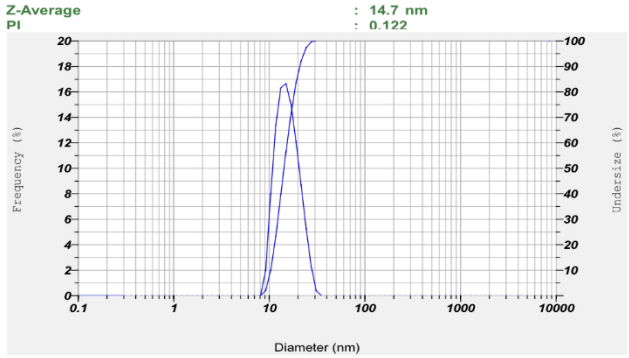


Figure 6. Test results of Particle Size Analyzer (PSA) sample 3

Figure 6. shows the results of the Moringa Leaves Extracts Nanoparticle test was measured using a Particle Size Analyzer (PSA) during the test within 30 seconds of ± 14.7 nm and a polydispersity index of 0.122.

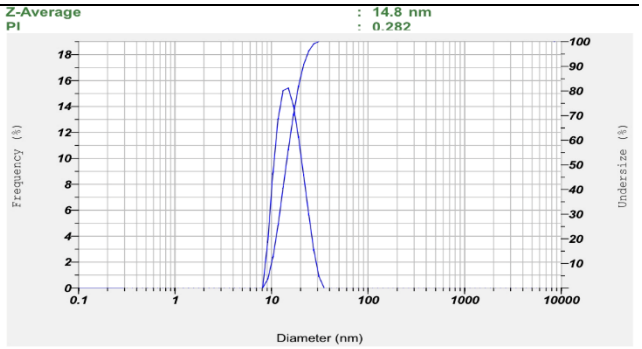


Figure 7. Test results of Particle Size Analyzer (PSA) sample 4

Figure 7. shows the results of the Moringa Leaves Extracts Nanoparticle test was measured using a Particle Size Analyzer (PSA) during the test within 30 seconds of ± 14.8 nm and a polydispersity index of 0.282.

Discussion

The amount of area or percent can be seen from each peak in Table 1. From the results of the examination above to show that the area is large, the percent area is also large, in the GC-MS results on Moringa leaf extract it can be seen that each secondary metabolite compound contained in Moringa leaves have different percentages of area at each peak. Kitson et al., (1996) stated that the size of this area percent indirectly describes the amount of the compound content in the extract concentrate.

The results of this study indicated that there were 25 types of secondary metabolites contained in Moringa leaves. Of the 25 secondary metabolites, there are 5 dominant metabolites, namely 9-Octadecenoic acid (29.64%), Hexadecanoic acid (18.42%), Octadecanoic acid (7.07%), Oleic Acid (5.88%), and 14-Methyl-8-hexadecyn-1-ol (5.47%) so that the benefits of each of these metabolites can be identified. 9-Octadecenoic acid, Hexadecanoic acid, Octadecanoic acid and Oleic Acid are natural fatty acid compounds that are abundant in plants and animals. This compound can modulate the function of neutrophils so that it affects the inflammatory process. The compound can also inhibit the action of protein kinase C and lymphocytes so that it can reduce cardiovascular disease, lower blood pressure, rheumatoid arthritis and some cancers. 25-29 Whereas the compound 14-Methyl-8-hexadecyn-1-ol in Binku's research (2021) found that this compound has antimicrobial and antiproliferative effects.

The process of making nanoparticle compounds - bioactive compounds resulting from ethanol extract using spontaneous emollation (Zhao, Y., & Sun, M. 2019). molecules of bioactive compounds produced through the extract results, can gather and form nanometer-sized particles in a beaker which is stirred until homogeneous and then added aquadest slowly with using a syringe so that it can produce nanometer-sized particles (Sahu, P. K., & Jena, P. K. 2018).

These nanometer-sized particles were previously encapsulated using tween 80 surfactant.

Tween 80 is a non-ionic surfactant consisting of polysorbate 80. This surfactant has two properties, namely hydrophilic (attracts water) and lipophilic (attracts oil/fat). The hydrophilic nature of Tween 80 is due to its high molecular polarity, so it can dissolve easily in air. Meanwhile, the lipophilic nature of Tween 80 is caused by the presence of a long carbon chain in its molecule which can interact with oil or fat (Aboelwafa, A.A et al ., 2020 , Singh, P et al., 2018).

From the results of nanoparticle synthesis of Moringa leaf extract using the spontaneous emulsion method, after being tested using PSA, results were obtained with a particle size of 15.9 nm in sample 1 with a concentration of 2 mL with a polydispersity index of 0.306; 15.5 nm in sample 2 with a concentration of 1.5 mL with a polydispersity index of 0.258; 14.7 nm in sample 3 with a concentration of 1 mL with a polydispersity index of 0.122; and 14.8 nm in sample 4 with a concentration of 0.5 mL with a polydispersity index of 0.282.

Characterization of nanoparticles from Moringa leaves was carried out by examining their size and distribution using a particle size analyzer (PSA). The results of the analysis showed that the size of the Moringa leaf nanoparticles was 15.9 nm in sample 1 with a concentration of 2 mL with a polydispersity index of 0.306; 15.5 nm in sample 2 with a concentration of 1.5 mL and a polydispersity index of 0.258; 14.7 nm in sample 3 with a concentration of 1 mL with a polydispersity index of 0.122; and 14.8 nm in sample 4 with a concentration of 0.5 mL with a polydispersity index of 0.282. The nanoparticle size of Moringa leaves contains small nanoparticles. This is in accordance with the theory that compounds with a size of 1-100 nm are called nanoparticles. The high polydispersity index value of moringa leaf nanoparticles indicates high heterogeneity. Non-uniform particle size and distribution can result from the formation of larger particles.

Conclusion

The process of synthesizing nanoparticles of secondary metabolite compounds of Moringa leaves ethanol extracts by spontaneous emulsion method has produced small particles. This allows the development of synthesis results used in its various fields, such as in the pharmaceutical for drugs development.

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