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Analysis of Fatty Acid Composition Using GC-MS Method and Antibacterial Activity Test of n-Hexane Extract from Petai Seeds (*Parkia speciosa* Hassk.)

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Abstract. Analysis of fatty acid composition using GC-MS method and antibacterial activity test of n-hexane extract from Petai seed (*Parkia speciosa* Hassk.) has been performed. Fatty acid was extracted from petai seed by maceration method using n-hexane solvent to produce n-hexane extract converted into fatty acid methyl ester through a methanolysis reaction. Then fatty acid composition was analyzed using the GC-MS method. The result of GC-MS analysis showed that the fatty acid composition found in 760 gr petai seeds which produced 11.16 g of fatty acids, was 1.0088 gr palmitic acid methyl ester (9.04%), 1.2700 gr linoleic acid methyl ester (11.38 %), 7.3556 gr oleic acid methyl ester (65.91%) and 1.4887 gr stearic acid methyl ester (13.34%). The antibacterial activity test showed that all concentrations of n-hexane extracts of 50 mg/ml, 150 mg/ml, and 250 mg/ml tested were able to inhibit the growth of E. *coli* and S. *aureus* bacteria in n-hexane extract, wherein the inhibited zone by the n-hexane extract is included in the weak category (≤14 mm) except at the concentration of 250 mg/ml in the S. aureus test bacteria belonging to the medium type (15-19 mm).

Keywords: Antibacterial, Petai Seed, Diffusion Agar, GC-MS.

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1. Introduction

The petai plant is thought to have originated from Malaysia. However, this plant has long been cultivated in Indonesia, especially in Java. This plant grows a lot in areas with a dry season that is not too extreme. The most important part of the petai plant to use is the seed. Petai seeds are very popular with many people even though they have a distinctive smell because they stimulate appetite. Petai can be eaten raw as a salad, boiled, fried, or baked. Many are also used as food flavouring (Setianingsih, 1995).

According to Aminudin from the Department of Physiology, Faculty of Medicine, Universitas Kebangsaan Malaysia, petai can be used as a remedy for anaemia, mosquito bites, stress, premenstrual syndrome, depression, stomach ache, liver, diabetes, intestinal worms, and several other health problems. According to research in " The New England Journal of Medicine, "eating petai as part of the daily diet will have an excellent effect on digestion because it has a soft and

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smooth texture and can neutralize stomach acid and reduce irritation by coating the inner surface of the stomach (Susilo, 2012). However, if it is too much, it makes it difficult to urinate (Sunarjono, 2016).

Petai seeds contain minerals, nutrients, and vitamins. In 100 grams of fresh petai seeds, there is an energy source of 142 kcal, water content 60.5 g, protein 10.4 g, fat 2.0 g, carbohydrates 22.0 g, calcium 95 mg, phosphorus 115 mg, iron 1, 2 mg, 200 SI of vitamin A, 0.17 mg of vitamin B1 and 36 mg of vitamin C (Sunanto, 1992).

Kataren (1986) states that edible fats and oils are produced by nature and can be sourced from vegetable or animal sources. In plants or animals, the oil serves as a reserve energy source. Fats and oils contain mixed triglycerides, esters of glycerol, and long-chain fatty acids. Vegetable oils are found in fruits, nuts, seeds, plant roots, and vegetables. In animal tissue, fat is found throughout the animal's body, but the most significant amount is found in adipose tissue and bone marrow. The oil is extracted from the seeds or kernels by grinding them and using a solvent, including dissolving it in a low boiling solvent and then separating it by evaporation (Gaman, 1981). According to Huda (1997), fatty acids can be used for antibacterial activity. Saturated and unsaturated fatty acids can bind to electron carriers on the bacterial cell membrane, and bonds to unsaturated fatty acids cause the breaking of the carbon chain so that the membrane fluidity becomes unstable. In contrast, saturated fatty acids can reduce membrane fluidity and damage electron transport within the membrane.

Bacterial metabolism can cause harm because it can infect, cause disease, and damage food. Antibacterials are included in antimicrobials that inhibit bacterial metabolism growth (Fardiaz, 1992).

Based on the results of research (Kurniawati, 2014) in a journal entitled "Antibacterial Activity of Petai Peel Extract (Parkia speciosa Hassk) Against Escherichia coli and Staphylococcus aureus " states that the simplicia powder of petai peel extracted by ultrasonication in stages using n-hexane, ethyl acetate solvent, and 70% ethanol to obtain compounds that can inhibit bacterial growth such as alkaloids, saponins, flavonoids, tannins, steroids, and triterpenoids. The extraction results were n-hexane, ethyl acetate, and 70% ethanol extract from petai peel, respectively concentrations of 50, 100, 150, 200, 250, and 300 mg/ml by dissolving them with DMSO solvent. Then the study results reported that n-hexane, ethyl acetate, and 70% ethanol extract from petai peel at 50, 100, 150, 200, 250, and 300 mg/ml sample concentrations had the potential as an antibacterial. Ethyl acetate extract of petai peel at a concentration of 300 mg/ml has an antibacterial ability four times that of 10 mg/ml streptomycin against S. *aureus* bacteria and 2.8 times against E. *coli* bacteria.

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Then from the results of subsequent research by (Verawaty, 2016) in the journal "The Effect of Concentration of Ethanol Extract of Petai Peel and Seeds (Parkia speciosa Hassk) on Escherichia coli Bacteria " states that dried petai skins and seeds are macerated with an ethanol solvent to obtain compounds that can inhibit the growth of bacteria such as flavonoids and maceration results in a rotary evaporator to obtain a thick ethanol extract of the skin and seeds of petai. The condensed ethanol extracts of the skin and seeds of petai were made at a concentration of 5 %, 10%, and 20%, respectively. The solvent used to dissolve the viscous ethanol extract is DMSO because DMSO can dissolve polar and non-polar compounds. Besides, this solvent also does not inhibit bacterial growth, so it does not interfere with the results of observations of antibacterial activity. Then the study results reported that the ethanol extract from the skin and seeds of petai had an inhibitory effect on the growth of Escherichia coli bacteria at concentrations of 5%, 10%, and 20%. Where the higher the concentration of the extract, the wider the inhibition zone that is generated.

Based on the description above and some literature related to petai plants, the authors are interested in researching the composition of fatty acids found in petai seeds and testing their antibacterial activity against bacteria. Escherichia coli and Staphylococcus aureus by agar diffusion method.

2. Materials and Methods

2.1 Equipment

The tools used in this study were: UV-Visible Spectrophotometer, Gas Chromatography-Mass Spectrometry (GC-MS), Rotary Evaporator, Oven, Incubator, Refrigerator, Blender, Test Tube, Beaker Glass, Erlemeyer, Separating Funnel, Analytical Balance, Micro Pipette, Aluminum Foil, Disc Paper, Needle Ose, Autoclave, Caliper, Hot Plate, Stir Bar, Measuring Cup, Petri Dish, Water Bath, Dropper Pipette, Vortex, Filter Paper, Glass Jar, Heater, Cotton Buds.

2.2 Materials

The material used includes The materials used in this study were: Petai seed powder, n-hexane, Aquadest, 98% H_2SO_4, $CHCl_3$, Na_2SO_4, TLC Plate, Dimethyl Sulfoxide (DMSO), Nutrient Agar (NA), Mueller Hinton Agar (MHA), *Staphylococcus aureus* bacteria, *Escherichia coli* bacteria, 30 mg chloramphenicol, 70% ethanol.

2.3 Provision of Samples

Petai seeds are peeled and separated from the seed coat. Samples were sliced thinly and then air-dried ± 2 days, then pulverized with a blender.

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2.4 Preparation of n-Hexane Extract of Petai Seeds

Weighed 760 grams of petai seed powder, macerated using 3 litres of n-hexane for 3×24 hours. Then it was filtered, the n-hexane filtrate obtained, evaporated using a rotary evaporator and then concentrated in a water bath until a solvent-free n-hexane extract was obtained.

2.5 Preparation of Fatty Acid Methyl Esters

Put 20 mg of n-hexane extract into the sample bottle, then add 4 ml of solvent with the ratio, Methanol (1.7 ml): H_2SO_4 98% (0.3 ml): Chloroform (2 ml). Close the bottle tightly, then homogenize using a vortex for 15 minutes. Then heated using a heating block at 90ºC for 90 minutes. After heating, cool the sample bottles to room temperature for ± 5-6 hours. After cooling, 1 ml of distilled water was added and homogenized using a vortex for ± 1 minute. Leave it for a while until two layers are formed. Separate the bottom layer containing the fatty acid methyl ester into another sample bottle. Then add Na_2SO_4 until it does not dissolve. After that, it was filtered using filter paper, then the filtrate was transferred to a vial, and the sample was ready for GC-MS analysis.

2.6 Antibacterial Activity Test of n-Hexane Extract

2.6.1 Preparation of Mueller Hinton Agar (MHA) Media

As much as 11.4 grams of Mueller Hinton Agar powder was put in an Erlenmeyer, then dissolved with 3 50 ml of distilled water and heated until all dissolved and boiled. Then sterilized in an autoclave at 121°C for 15 minutes.

2.6.2 Preparation of Mueller Hinton Agar (MHA) Media

A total of 9.8 grams of Nutrient Agar was put in an Erlenmeyer, then dissolved in 350 ml of distilled water and heated until all dissolved and boiled. Then sterilized in an autoclave at 121°C for 15 minutes.

2.6.3 Preparation of Agar Media and Bacterial Culture Stock

Into a sterile test tube, 3 ml of sterile Nutrient Agar medium was put in and allowed to stand at room temperature until it solidified in an oblique position forming an angle of 30-45°. The bacterial culture of E.*coli* from the primary strain was taken with a sterile loop needle and then inoculated on the surface of the Nutrient Agar medium by scraping, then incubated at 35°C for 18-24 hours. The same thing was also done on the bacterial culture of S. *aureus*.

2.6.4 Preparation of Bacterial Inoculum

A total of 10 ml of distilled water was put into a test tube, sterilized in an autoclave at 121°C for 15 minutes. Colonies of E. *coli* bacteria were taken from the bacterial culture stock with a bent loop needle, put into sterile distilled water, and then homogenized with a vortex. The absorbance value of the blank in the form of sterile aquadest was measured with a wavelength of 600 nm. The absorbance value of the bacterial suspension was measured at a wavelength of 600 nm. The same thing was done for S. *aureus* bacteria colonies.

2.6.5 Preparation of Variation of Concentration of n-Hexane extract

n-hexane extract from petai seeds was prepared in various concentrations by weighing the extract as much as 50, 150, and 250 mg, respectively, then dissolved in 1 ml of DMSO each. Extract concentrations were 50 mg/ml, 150 mg/ml, and 250 mg/ml.

2.6.7 Extract Antibacterial Activity Test of n-Hexane Extract of Petai Seeds

Mueller Hinton Agar medium was inserted into a sterile petri dish with a temperature of 45-50°C, then left until the media solidified. A sterile cotton bud is taken, and then the bacterial inoculum is dipped and streaked onto the solidified MHA media. Insert the paper disc that has been sterilized into the media. Pipette each 10µL of n-hexane extract with various concentrations of 50 mg/ml, 150 mg/ml, and 250 mg/ml onto disc paper. Then incubated at 35°C for 18-24 hours. Next, the diameter of the clear zone around the paper disc was measured with a vernier caliper.

3 RESULTS AND DISCUSSION

3.1 GC-MS Chromatogram Data Analysis of n-Hexane Extract of Petai Seeds

Fatty acid extraction from 760 g of petai seeds by maceration method using n-hexane solvent produced 11.16 g of n-hexane extract containing fatty acids. 20 mg of n-hexane extract was converted into fatty acid methyl esters through a methanolysis reaction and then analyzed using the GC-MS method.

The GC-MS chromatogram from the extraction of fatty acids from petai seeds showed four peaks with respective retention times of 26.645; 30.514; 30,668; and 31.353 minutes. As shown in Figure 1.

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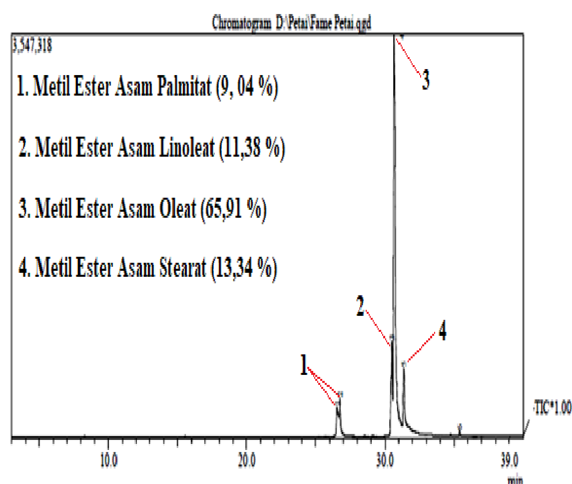

Figure 1. The FTIR spectrum of edible fil

Based on a comparison between the mass spectrum of the sample and the data library, it can be concluded that the compounds from each component are as shown in Table 1 below :

Table 1. Composition of fatty acids contained in the n-hexane extract of petai seeds

No	Retention Time	Fatty Acid	Molecular Formula	Area (%)
1	26.645	Palmitic Acid Methyl Esters	$C_{17}H_{34}O_2$	9.04
2.	30.514	Linoleic Acid Methyl Esters	$C_{19}H_{34}O_2$	11.38
3.	30.668	Acid Methyl Esters oleate	$C_{19}H_{36}O_2$	65.91
4.	31.353	Stearic Acid Methyl Esters	$C_{19}H_{38}O_2$	13.34

From the results of the analysis of the GC-MS chromatogram data in Figure 1, it shows that the composition of the fatty acids contained in 760 grams of petai seeds which yielded 11.16 grams of n-hexane extract containing fatty acids, showed four peaks, namely:

1. At peak number 1, it can be seen that it consists of 2 peaks given by palmitic acid methyl ester with a retention time of 26.645 minutes with an area of 1.0088 gr (9.04%). The emergence of these two peaks is probably caused by the GC-MS operating conditions, which are determined by temperature, pressure, mobile phase concentration, and column dimensions. In addition, it is also influenced by the accuracy of the selection of the stationary and mobile phases (Hutami, 2012).
2. Peak number 2 is given by linoleic acid methyl ester with a retention time of 30.514 minutes with an area of 1.2700 gr (11.38%).
3. Peak number 3 is given by oleic acid methyl ester with a retention time of 30.668 minutes with an area of 7.3556 gr (65.91%).
4. Peak number 4 is given by stearic acid methyl ester with a retention time of 31.353 minutes with an area of 1.4887 gr (13.34%).

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3.2 Analysis of GC-MS Mass Spectrum Data of n-Hexane Extract of Petai Seeds

As shown in Figure 1, the peaks with retention times have a mass spectrum. The mass spectrum displays a fragmentation pattern, with the most (100% abundant) detected ion being the base ion. The mass spectrum of the sample can be the basis for estimating the compound at a specific retention time when compared to the mass spectrum of the MS library data, which has a high similarity index (SI) value (Hutami, 2012), where the n-hexane extract of petai seeds contains four types of fatty acids namely palmitic acid methyl ester (9.04%), linoleic acid methyl ester (11.38%), oleic acid methyl ester (65.91%) and stearic acid methyl ester (13.34%). The comparison of the mass spectra of the samples corresponds to the mass spectra of the data library for the four types of fatty acids and will be fragmented for each of these fatty acids.

3.2.1 Peak (peak) number 1 with a retention time of 26.645

This spectrum is a compound with the molecular formula $C_{17}H_{34}O_2$. Mass spectrum data showed molecular ion peaks m/e = 270, 227, 171, 143, 129, 101, and 74. Comparison of the sample mass spectrum data with the mass spectrum obtained in the library, which detected more Palmitic Acid Methyl Esters

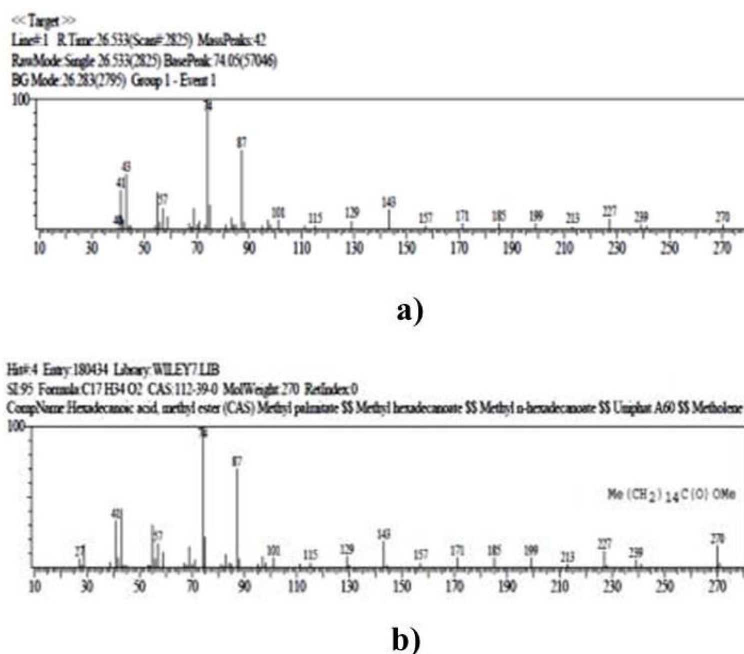

Figure 1. Mass spectrum of palmitic acid methyl ester Note: (a) sample (b) standard library

This compound is in Figure 2, where the MS results give a molecular ion peak at a molecular weight of m/e = 270 followed by fragmentation peaks with a molecular weight of m/ e as follows 227, 171, 143, 129, 101, and 74. This spectrum is a compound with the molecular formula $C_{17}H_{34}O_2$., a palmitic acid methyl ester. The fragmentation patterns of palmitic acid methyl esters are fragmentation-releasing molecules m/e= 43 (C_3H_7) forming molecules with m/e= 227 $(C_{14}H_{27}O_2)^+$, fragmenting releasing molecules m/e= 56 (C_4H_8) forms a molecule with m/e= 171

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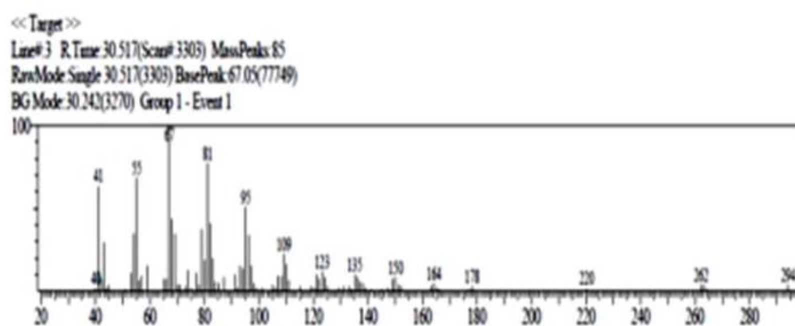
$(C_{10}H_{19}O_2)^+$, fragmentation releases a molecule m/e= 28 (C_2H_4) forms a molecule with m/e= 143 $(C_8H_{15}O_2)^+$, fragmentation releases molecules m/e= 14 (CH_2) forms molecules with m/e= 129 $(C_7H_{13}O_2)^+$, fragments release molecules m/e= 28 (C_2H_4) forms a molecule with m/e= 101 $(C_5H_9O_2)^+$, and fragmentation releases a molecule m/e= 27 (C_2H_3) forms a molecule with m/e= 74 $(C_3H_6O_2)^+$.

3.2.2 Peak number 2 with a retention time of 30.514

This spectrum is a compound with the molecular formula $C_{19}H_{34}O_2$. Mass spectrum data showed molecular ion peaks m/e = 294, 262, 220, 178, 150, 135, 109, 67, and 41. Comparison of the sample mass spectrum data with the mass spectrum obtained in the library, which detected more Linoleic Acid Methyl Esters



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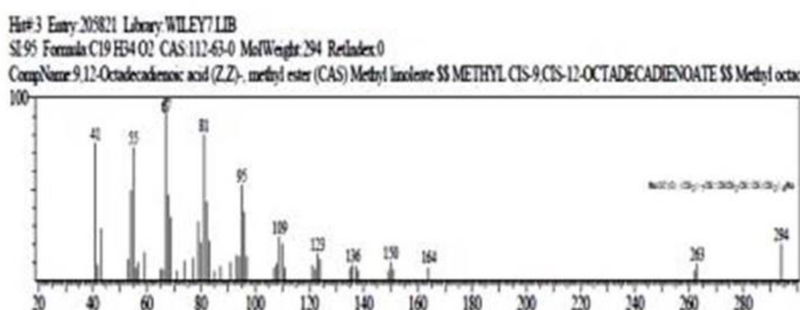


d)

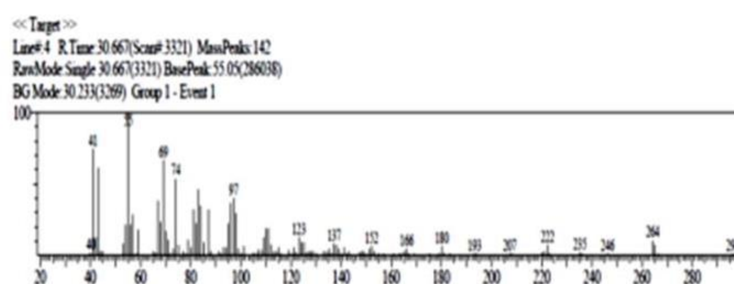
.**Figure 2**. Mass spectrum of linoleic acid methyl ester Note: (a) sample (b) standard library

This compound is in Figure 3, where the MS results give a molecular ion peak at a molecular weight m/e= 294 followed by fragmentation peaks with a molecular weight m/e as follows m/e= 262, 220, 178, 150, 135, 109, 67, and 41. This spectrum is a compound with the molecular formula $C_{19}H_{34}O_2$, a linoleic acid methyl ester. The fragmentation patterns of linoleic acid methyl esters are fragmentation-releasing molecules m/e= 32 (CH_3OH) forming molecules m/e= 262 $(C_{18}H_{30}O)^+$, fragmenting releasing molecules m/e= 42 (C_3H_6) forms a molecule with m/e= 220 $(C_{15}H_{24}O)^+$, fragmentation releases a molecule m/e= 42 (C_3H_6) forms a molecule with m/e= 178 $(C_{12}H_{18}O)^+$, fragmentation releases molecules m/e= 28 (C_2H_4) forms molecules with m/e= 150 $(C_{10}H_{14}O)^+$, fragments release molecules m/e= 15 (CH_3) forms molecules with m/ e= 135

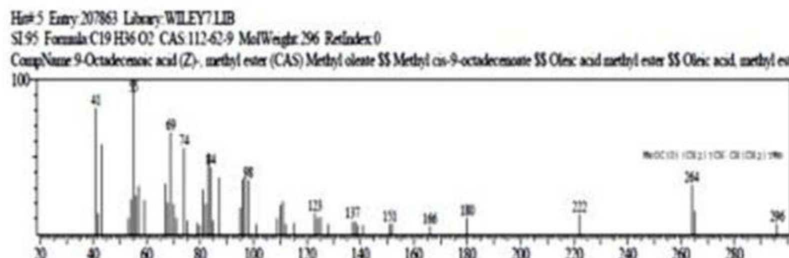
$(C_9H_{11}O)^+$, fragmentation releases molecules m/e= 26 (C_2H_2) forms molecules with m/e= 109 $(C_7H_9O)^+$, fragments releases molecules m/e= 42 (C_3H_6) forms a molecule with m/e= 67 $(C_4H_3O)^+$, and fragmentation releases a molecule m/e= 26 (C_2H_2) forms a molecule with m/e= 41 $(C_2HO)^+$.

3.2.3 Peak number 3 with a retention time of 30.668

This spectrum is a compound with the molecular formula $C_{19}H_{36}O_2$. Mass spectrum data showed molecular ion peaks m/e = 296, 264, 222, 180, 137, 55, and 41. Comparison of the mass spectrum data of the sample with the mass spectrum obtained in the library, which detected more oleic acid methyl esters.



e)



f)

Figure 3. Mass spectrum of acid methyl ester oleic Note: (a) sample (b) standard library

This compound is in Figure 4, where the MS results give a molecular ion peak at a molecular weight of m/e = 296 followed by fragmentation peaks with a molecular weight of m/e as follows 264, 222, 180, 137, 55, and 41. This spectrum is a compound with the molecular formula $C_{19}H_{36}O_2$, the oleic acid methyl ester. The fragmentation patterns of oleic acid methyl esters are fragmentation-releasing molecules m/e= 32 (CH_3OH) to form molecules with a molecular weight m/e= 264 $(C_{18}H_{32}O)^+$, fragmentation releasing molecules m/e= 42 (C_3H_6) forms a molecule with a molecular weight of m/e= 222 $(C_{15}H_{26}O)^+$, fragmentation releases a molecule m/e= 42 (C_3H_6) form a molecule with m/e= 180 $(C_{12}H_{20}O)^+$, fragmentation releases molecules m/e= 43 (C_3H_7) forms molecules with m/e= 137 $(C_9H_{13}O)^+$, fragments release molecules m/e= 82 (C_6H_{10}) forms a molecule m/e= 55 $(C_3H_3O)^+$, and fragmentation releases a molecule m/e= 14 (CH_2) to form a molecule m/e= 41 $(C_2HO)^+$.

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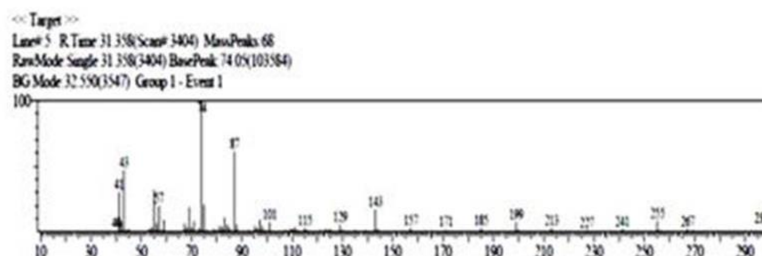
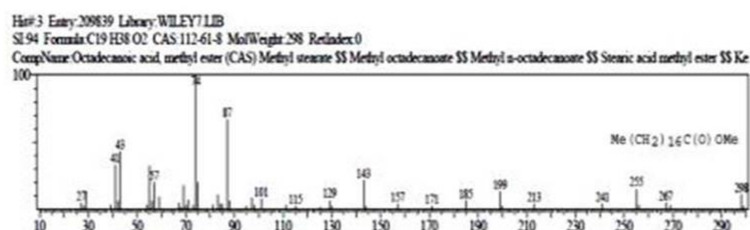
3.2.4 Peak number 4 with a retention time of 31.353

This spectrum is a compound with the molecular formula $C_{19}H_{38}O_2$. The mass spectrum data showed molecular ion peaks m/e = 298, 255, 199, 143, 101, and 74. Comparing the sample mass spectrum data with the mass spectrum obtained in the library detected more Stearic Acid Methyl Ester.



g)



h)

Figure 4. Mass spectrum of stearic acid methyl ester Note: (a) sample (b) standard library

This compound is in Figure 5, where the MS results give a molecular ion peak at a molecular weight of m/e= 298 followed by fragmentation peaks with a molecular weight of m/e as follows 255, 199, 143, 101, and 74. This spectrum contains the formula molecule $C_{19}H_{38}O_2$, a stearic acid methyl ester. The fragmentation patterns of stearic acid methyl esters are fragmentation-releasing molecules m/e= 43 (C_3H_7) forming molecules with a molecular weight m/e = 255 $(C_{16}H_{36}O_2)^+$, fragmenting releasing molecules m/ e= 56 (C_4H_8) forms a molecule with m/e= 199 $(C_{12}H_{23}O_2)^+$. Fragmentation releases a molecule m/e=56 (C_4H_8) forms a molecule with m/e= 143 $(C_8H_{15}O_2)^+$, and fragmentation releases molecules m/e= 42 (C_3H_6) forms molecules with m/e= 101 $(C_5H_9O_2)^+$, fragments releases molecules m/e= 27 (C_2H_3) forms a molecule with m/e= 74 $(C_3H_6O_2)^+$.

Fatty acids are carboxylic acids with long, saturated and unsaturated aliphatic chains. Analysis of fatty acids in petai seeds shows that the content of fatty acids in petai seeds is classified as saturated fatty acids and unsaturated fatty acids. Based on the analysis of the GC-MS data, the fatty acid composition of 760 g of petai seeds yielded 11.16 g of n-hexane extract, which contained fatty acids consisting of saturated fatty acids, namely: 1.0088 g of palmitic acid methyl ester (9.04 %), 1.4887 gr stearic acid methyl ester (13.34 %) and unsaturated fatty acids namely: 1.2700 gr linoleic acid methyl ester (11.38 %), 7.3556 gr oleic acid methyl ester (65, 91 %).

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Each fatty acid has different or the same benefits in the human body as oleic acid, which has a function in the body as a source of energy and antioxidants to inhibit cancer, lower cholesterol levels, and a solvent for vitamins A, D, E, K. Deficiency of oleic acid can cause visual disturbances, decreased memory, and impaired brain cell growth in fetuses and infants (Al-Saghir et al., 2004). While stearic acid shows a neutral effect on whole blood (Beef Facts, 2007) besides that, another use of stearic acid is to prevent oxidation (Swern, 1979).

Linoleic acid plays an essential role in fat transport and metabolism, immune function, and maintaining the function and integrity of cell membranes. According to Berkow (1977), Linoleic acid is an essential fatty acid, namely a fatty acid that is not produced by the body but must be consumed from outside. If the milk given to children contains low linoleic acid, they will experience slow growth and skin diseases. Regarding nutrition, palmitic acid is an essential source of calories but has low antioxidant power (Ketaren, 1986).

3.2 Antibacterial Activity Test on n-Hexane Extract of Petai Seeds

The n-hexane extract obtained from petai seeds containing fatty acids was tested for antibacterial activity at 50 mg/ml, 150 mg/ml, and 250 mg/ml. Antibacterial activity test of the n-hexane extract of petai seeds at concentrations of 50 mg/ml, 150 mg/ml and 250 mg/ml showed the presence of inhibition zones on the growth of the test bacteria, namely E. *coli* and S. *aureus*. It can be seen from the results of measuring the diameter of the inhibition zone formed, which is in the form of a clear area around the disc paper containing the n-hexane extract of petai seeds which can be seen in Figure 6 below:

The results of measuring the diameter of the inhibition zone for the antibacterial activity of the n-hexane extract of petai seeds against E. *coli* and S. *aureus* bacteria can be seen in Table 1.

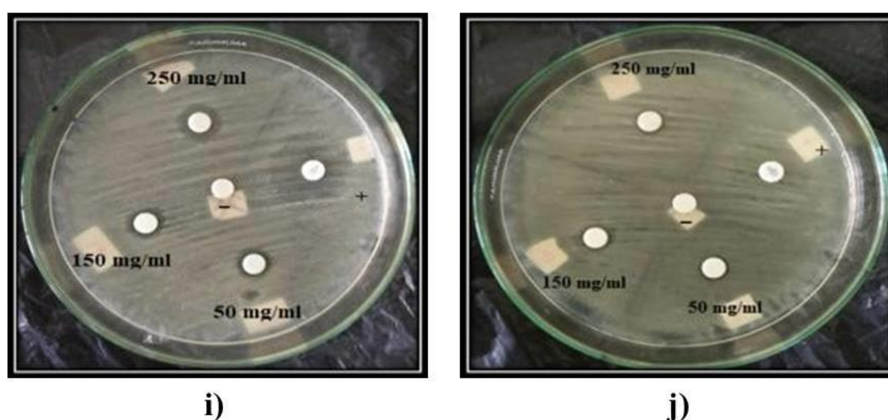

Figure 4. Mass spectrum of stearic acid methyl ester Note: (a) sample (b) standard library

The results analysis of the nutritional content of edible film from the pectin peel of sweet orange (*Citrus sinensis l.*) with the addition of tapioca flour and glycerin as plasticizer

Table 2. Nutritional Contents of Edible Film

Treatment	**Diameter of Inhibition Zone (mm) of n-hexane Extract**	
	E.coli	***S. aureus***
Negative Control	0.00	0.00
Positive Control	25.60	28.50
Concentration 50 mg/ml	10.00	11.50
Concentration 150 mg/ml	11.00	12.00
Concentration 250 mg/ml	13.00	15.00

Information :

- Negative control used disc paper soaked with DMSO.
- The positive control used the antibiotic chloramphenicol 30 mg.

Antibacterial activity testing aims to determine bacterial activity on the test sample, namely the n-hexane extract of petai seeds which contain fatty acids. Antibacterial activity test was carried out by the agar diffusion method by attaching paper discs to E . *coli* and S. *aureus*, which had been grown on Nutrient Agar medium and then pipetted 10 µL of n-hexane extract with various concentrations of the extract, then dripped onto disc paper. Antibacterial activity was determined by measuring the zone of inhibition formed around the disc paper. The n-hexane extract from petai seeds was prepared in various concentrations by weighing the extract as much as 50, 150, and 250 mg, respectively, then dissolved in 1 ml of DMSO each. Extract concentrations were 50 mg/ml, 150 mg/ml, and 250 mg/ml. The negative control used was disc paper soaked in DMSO solvent. Verawaty (2016) stated that the choice of DMSO solvent was because DMSO is a solvent that can dissolve almost all compounds, both polar and non-polar. DMSO solvent also does not inhibit bacterial growth, so it does not interfere with the results of observations of antibacterial activity. In comparison, the positive control used was the antibiotic chloramphenicol. The diameter of the inhibition zone resulting from the antibacterial activity test can be seen in Table 2.

Based on the Clinical and Laboratory Standards Institute (2012) states that an inhibition zone diameter of ≤14 mm has a weak antibacterial activity, an inhibition zone diameter of 15-19 mm has a medium antibacterial activity, and an inhibition zone diameter of ≥20 mm has an intense antibacterial activity. The research results in Table 4.2 show that all concentrations tested could inhibit the growth of the test bacteria in the n-hexane extract. The concentration of n-hexane extract inhibited the growth of E. *coli* The largest coli was at 250 mg/ml with an inhibition zone diameter of 13.00 mm. Meanwhile, the concentration of n-hexane extract showed inhibition of *S.aureus* was the largest at 250 mg/ml with an inhibition zone diameter of 15.00 mm. The

inhibition zone formed by the n-hexane extract was included in the weak category except at a 250 mg/ml concentration in the S. *aureus* bacteria test belonging to the moderate category.
Petai seeds in the n-hexane extract had the most potent antibacterial activity in inhibiting the growth of S. *aureus* bacteria, a gram-positive bacteria, compared to E. *coli*, a gram-negative bacteria. The bacterial cell wall influences differences in the sensitivity of bacteria to antibacterials. Pelczar and Chan (1986) stated that the structure of the cell wall of gram-positive bacteria is relatively simple, making it easier for antibacterial compounds to enter the cell and find targets to work on, while the cell wall of gram-negative bacteria is more complex. The cell wall structure of gram-positive bacteria is single-layered with a low lipid content of 1-4%, while gram-negative bacteria have a high lipid content of 11-22% (Fardiaz, 1992), and the outer membrane consists of 3 layers, namely lipopolysaccharide, lipoprotein and phospholipid (Tortora, 2001).

The negative control used was disc paper soaked with DMSO solvent, which did not show the diameter of the inhibition zone. It indicates that the solvent used had no antibacterial activity or effect on the antibacterial test. While the positive control for chloramphenicol showed that the diameter of the inhibition zone had vigorous antibacterial activity, it was widely used as an antibiotic that inhibited bacterial growth.

4 Conclusion

Based on the results of the research that has been done, it can be concluded as follows :
1. The fatty acid composition of 760 gr of petai seeds yields 11.16 gr of n-hexane extract, which contains fatty acids consisting of saturated fatty acids, namely: 1.0088 gr palmitic acid methyl ester (9.04%), 1.4887 gr stearic acid methyl ester (13.34%) and unsaturated fatty acids namely: 1.2700 gr linoleic acid methyl ester (11.38%), 7.3556 gr oleic acid methyl ester (65.91%).
2. The results of the antibacterial activity test showed that all concentrations tested, namely 50 mg/ml, 150 mg/ml, and 250 mg/ml, could inhibit the growth of E. *coli* and S. *Aureus* bacteria on n-hexane extract, where The concentration of n-hexane extract showed inhibition of the growth of E . the largest coli was at 250 mg/ml with an inhibition zone diameter of 13.00 mm. Meanwhile, the concentration of n-hexane extract showed S. *aureus* inhibition was the largest at 250 mg/ml with an inhibition zone diameter of 15.00 mm. The inhibition zone formed by the n-hexane extract was included in the weak category except at a 250 mg/ml concentration in the S. . *aureus* bacteria test, belonging to the moderate category.

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