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Analysis of Fatty Acid Composition Using GC-MS and Antibacterial Activity Test of *n*-Hexane Extract from Jengkol Seeds (*Pithecellobium lobatum Benth.*)

Surya Graha P. Siahaan, Lamek Marpaung*
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia
*Corresponding Author: toyamaikayakadai@yahoo.co.id

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ABSTRACT
Analysis of fatty acid composition using GC-MS and antibacterial activity test of *n*-hexane extract from Jengkol seeds (*Pithecellobium lobatum Benth*) was performed with the maceration method to obtain *n*-hexane extract of Jengkol seeds. The n-hexane extract of Jengkol seeds was first esterified using methanol (1.7 ml): 98% H_2SO_4 (0.3 ml): Chloroform (2 ml), then analyzed its fatty acid composition by GC-MS method. The results of fatty acid composition analysis found in Jengkol seeds are saturated fatty acids, namely: Margaric Acid (1.10%), Palmitate Acid (2.64%), stearic acid (12.01%), and unsaturated fatty acids, namely: Linolelaidate acid (10.02%) %), and oleic acid (74.23%). The antibacterial activity test against *Escherichia coli* and *Staphylococcus aureus* using n-hexane extract of Jengkol seed was done by agar diffusion method. The concentration variations were 50 mg/ml, 150 mg/ml, and 250 mg/ml. From the results of this test, n-hexane Jengkol seeds have an antibacterial activity that can inhibit bacterial growth but is weak (≤14 mm).

Keywords: Agar Diffusion, Antibacterial, GC-MS, Jengkol Seeds.

ABSTRAK
Analisis komposisi asam lemak dengan GC-MS dan uji aktivitas antibakteri *n*-heksana dari biji jengkol (*Pithecellobium lobatum Benth*) dilakukan dengan metode maserasi untuk mendapatkan ekstrak n-heksana dari biji jengkol. ekstrak n-heksana biji jengkol terlebih dahulu diesterifikasi menggunakan metanol (1,7 ml) : H_2SO_4 98% (0,3 ml) : Kloroform (2 ml) kemudian dianalisis komposisi asam lemaknya dengan metode GC-MS. Hasil analisis komposisi asam lemak yang terdapat pada biji jengkol adalah asam lemak jenuh yaitu: Asam Margarat (1,10%), Asam Palmitat (2,64%), Asam Stearat (12,01%), dan asam lemak tidak jenuh yaitu: Asam Linolelaidat (10,02%). %) %) %), dan asam oleat (74,23%). Untuk uji aktivitas antibakteri terhadap *Escherichia coli* dan *Staphylococcus aureus* menggunakan ekstrak *n*-heksan biji jengkol, dilakukan dengan metode difusi agar, dan variasi konsentrasi 50 mg/ml, 150 mg/ml dan 250 mg/ml. Dari hasil pengujian tersebut, *n*-heksana biji jengkol memiliki aktivitas antibakteri yang dapat menghambat pertumbuhan bakteri, namun lemah (≤14 mm).

Keyword: Antiibakteri, Biji Jengkol, Difusi Agar, GC-MS.

1. Introduction

Jengkol (*Pithecellobium lobatum Benth.)* has long been grown in Indonesia and other areas in western Indonesia. The Jengkol plant grew wild in the past, but now it is cultivated by many people, especially in rural areas, in the yard, yards, and even on hillsides [1]. Most Indonesians favor Jengkol as a companion to the staple food of rice consumed in a new form as fresh vegetables or various other processed forms [2]. Jengkol seeds, ancient ones, are part of the plant most widely used as food. In addition, this plant can also be used as a medicinal ingredient. Jengkol is known to prevent diabetes, is a diuretic, and is suitable for heart health [3].

Edible fats and oils (edible fat) are produced by nature and can be sourced from vegetable or animal materials. In plants or animals, the oil serves as a reserve energy source. Vegetable oil can be obtained from fruits, nuts, seeds, plant roots, and vegetables [4]. Vegetable oil can be obtained by extracting it using a solvent

that has a low boiling point and then separating the solvent by evaporation. One of the requirements for a compound to be analyzed using GC-MS is that the compound is volatile. A long-chain fatty acid has a high boiling point because it has a carboxylic group which causes hydrogen bonds to occur. While also increasing the chain hydrocarbons, the boiling point increases. So, the boiling point of the analyzed compound is the low transformation of the carboxylic group into an ester group with an acid catalyst using methanol produces fatty acid methyl ester compounds, which GC-MS can analyze.

Bacterial metabolism can cause harm because of its ability to infect and cause disease and damage materials and food. Antibacterials are included in antimicrobials which are used to inhibit the growth of bacterial metabolism [5]. According to [6], Fatty acids can be used for antibacterial activity. Where saturated and unsaturated fatty acids can bind to electron carriers on the bacterial cell membrane, bonds to unsaturated fatty acids cause the breaking of the carbon chain so that the membrane's fluidity becomes unstable. In contrast, saturated fatty acids can reduce membrane fluidity and damage electron transport within the membrane.

Based on the results of previous research [7] tested the antibacterial activity of extracts from the peel of the Jengkol fruit (*Pithecellobium lobatum Benth*) of the Fabaceae tribe against *Escherichia coli*, *Shigella dysenteriae,* and *Salmonella typhimurium* bacteria. The smallest concentration at which Jengkol peel extract can inhibit *Escherichia coli* bacteria is 20 mg/ml. For *Shigella dysenteriae* bacteria, it is 40 mg/ml, while against *Salmonella typhimurium* bacteria, it is 60 mg/ml.

Inhibition test of the ethanol extract of Jengkol peel (*Pithecellobium lobatum Benth*) against Methichilin-resistant growth *of Staphylococcus aureus* bacteria. Test inhibition was carried out using the disc diffusion method. The parameters observed were the area of the inhibition zone formed. The results showed that the ethanol extract of Jengkol rind with a concentration of 12.5%, 25%, 50%, and 75% inhibited the growth of MRSA with an average inhibition zone of 13.4 mm, 14.8 mm, and 16.2 mm, 17.4 mm, respectively [8]. Based on the description above and some literature related to Jengkol plants, the authors are interested in researching the composition of the fatty acids found in Jengkol seeds and testing their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria using the diffusion method.

2. Materials and Methods

2.1. Equipment

The tools used in this study include UV-Visible Spectrophotometer, Gas Chromatography-Mass Spectrometry (Simadzu), rotary evaporator, oven, incubator, refrigerator, blender, test tube, beaker glass, Erlenmeyer, funnel, analytical balance, micropipette, aluminum foil, disc paper, loop needle, autoclave, caliper, hot plate, stir bar, measuring cup, petri dish, water bath, pipette, vortex, filter paper, glass jar, heater, cotton bud.

2.2. Materials

The materials used in this study included: Jengkol seed powder, *n*-hexane, aquadest, 98% H_2SO_4, $CHCl_3$, Na_2SO_4, DMSO, Nutrient Agar (NA), Muller Agar (MHA), 30 mg chloramphenicol, 70% ethanol, *Staphylococcus aureus,* and *Escherichia coli* bacteria.

2.3. Provision of Samples

The Jengkol seeds are peeled and separated from the seed coat. They were sliced thinly, dried in the sun for ± 2 days, and then pulverized with a blender.

2.4. Manufacture Extract n-Hexane Jengkol Seeds

As much as 1010 g of Jengkol seed powder was weighed and macerated using 4 liters of n-hexane for 3×24 hours and then filtered. The n-hexane filtrate obtained was evaporated using a rotary evaporator and then concentrated back on the water bath until n -hexane extract is solvent-free.

2.5. Preparation of Fatty Acid Methyl Esters

In order to continue to the next step, put 20 mg of the sample into the sample bottle, then add 4 ml of solvent by comparison, methanol (1.7 ml): H_2SO_4 98% (0.3 ml): Chloroform (2 ml). Closed bottles tightly, then homogenized using a vortex for 15 minutes. It was 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 Fatty Acid Methyl Esters into another sample bottle. Then add Na 2 SO 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. Equipment Sterilization

The tools are washed thoroughly, dried, and covered tightly with parchment paper. Then put it in the autoclave and tightly closed it. They were sterilized at 121°C for 15 minutes.

2.7. Production of Mueller Hinton Media Agar (MHA)

A total of 11.4 g of Mueller powder Hinton Agar entered Erlenmeyer, then dissolved with 350 ml of distilled water and heated until all were dissolved and boiling, then sterilized in an autoclave at 121 ° C for 15 minutes. Nutrient Agar (NA) Media, As much as 9.8 f NA was put in Erlenmeyer, then dissolved in 350 ml of distilled water, heated until all dissolved and boiling, and then sterilized in an autoclave at 121°C for 15 minutes.

2.8. Preparation of Agar Media and Bacterial Culture Stock

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

2.9 Preparation of Bacterial Inoculums

A total of 10 ml of aquadest was included in test tubes and sterilized in an autoclave at 121°C for 15 minutes. The 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.

2.10 Preparation of Variation of Extract Concentration of n-Hexane from Jengkol Seeds

The n-hexane extract of Jengkol seeds was made in various concentrations by weighing as much as 50, 150, and 250 mg each, then dissolved each with 1 ml of DMSO. Extract concentrations were 50 mg/ml, 150 mg/ml, and 250 mg/ml.

2.11 Antibacterial Activity Test of n-Hexane Extract

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, then the bacterial inoculum is dipped, then it is streaked onto the solidified MHA media. Insert the paper disc that has been sterilized into the media. Pipette 10µL of n-hexane extract with various concentrations onto paper discs. It was then incubated at 35°C for 18 to 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 Analysis of n-hexane Extract of Jengkol Seeds

Extraction of fatty acids from Jengkol seeds was performed by maceration method using n-hexane solvent produced by the n-hexane extract of Jengkol seed. 20 mg of n-hexane extract was esterified into fatty acid methyl esters, then analyzed using Shimadzu GC-MS. Because the GC-MS method is effective for determining the structure of compounds because it requires fast analysis time, good separation power, and can perform qualitative and quantitative analysis.

One of the requirements for a compound to be analyzed using GC-MS is that the compound is volatile. A long-chain fatty acid has a high boiling point because it has a carboxylic group which causes hydrogen bonds to occur. In addition, as the number of hydrocarbon chains increases, the boiling point increases. So that the boiling point of the compound to be analyzed is the low transformation of the carboxylic group into an ester group with a catalyst H_2SO_4 98 % using methanol produces fatty acid methyl ester compounds, which GC-MS can analyze.

The *n*-hexane extract of Jengkol seeds showed five peaks with a retention time of 26.501 each; 26.761; 30.519; 30.654; and 31.365 minutes, as seen in Figure 1.

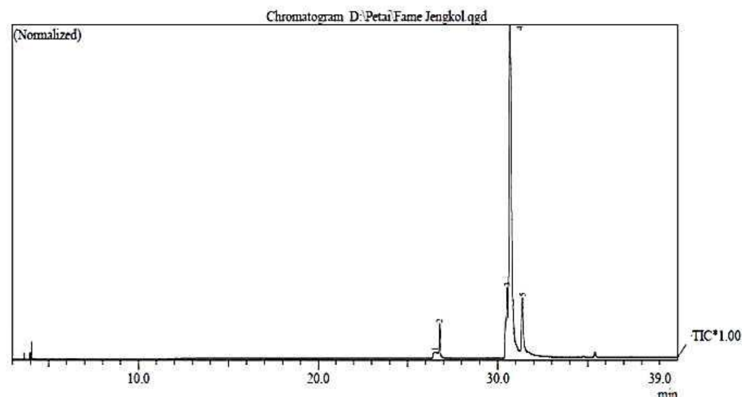


Figure 1. Chromatogram *n*-hexane extract of Jengkol seeds

Figure 1 shows that there are five peaks with different retention times, abundances, and peak areas, so there are five other methyl ester compounds obtained by comparing the MS unknown spectrum with library data. It can be concluded compounds of each component as shown in Table 1 below:

Table 1. The composition contained in the *n*-hexane extract of Jengkol seed

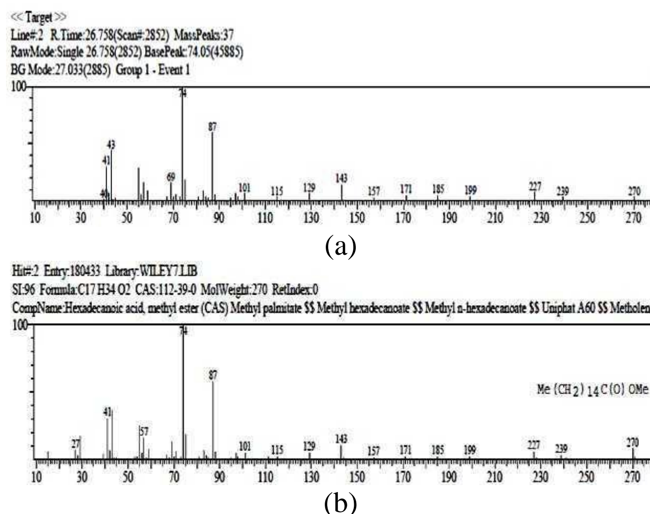
No. Peak	Retention Time	Name	Molecular Formula	Area %
1	26.501	Methyl Esters Sour Margarik	$C_{17}H_{34}O_2$	1.10
2	26.761	Methyl Esters Palmitic Acid	$C_{17}H_{34}O_2$	2.64
3	30.519	Methyl Acid Esther Linolelaidate	$C_{19}H_{34}O_2$	10.02
4	30.654	Methy Esther Oleic Acid	$C_{19}H_{36}O_2$	74.23
5	31.365	Methyl Acid Esters Stearate	$C_{19}H_{38}O_2$	12.01

3.2. Analysis of GC-MS Spectrum Data

To determine the fatty acid composition, the results of the mass spectrum of each *unknown peak* are compared with the mass spectrum of the compounds in the *library list*. MS data analysis from each peak of each of these compounds can be carried out by observing M^+ prices and the fragmentation pattern based on m/e prices. The typical peak of an unbranched long chain methyl ester is at m/e: 74 due to the Mc Laferty rearrangement, which produces the cation $[H_3CCOOCH_3]^+$, another typical breakdown pattern of the methyl ester is M-32 which occurs due to the release of the CH_3 molecule OH, the pattern of m/e fractions of 28, 42 and 56 provides information on the possibility of releasing the alkene, M-31, which is CH_3O [9]. The following is the fractionation pattern of the methyl ester compound obtained.

(a) Peak with a retention time of 26.758

This spectrum is a compound with the molecular formula C17H34O2. The mass spectrum data shows ion peaks m/e = 270, 227, 171, 143, 129, 101. and 74. By comparing the unknown spectrum data with the mass spectrum obtained with spectrum data in the library, which detects more is Palmitic Acid Methyl Ester.



(a)

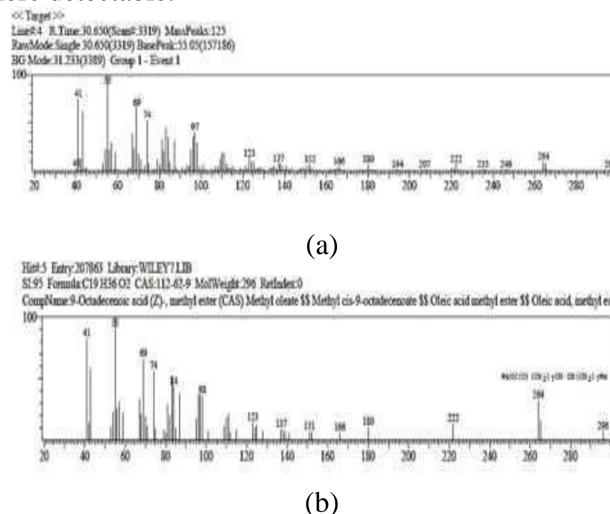
(b)

Figure 2. The spectra of Palmitic Acid Methyl Esters with a retention time of 26.758 (a) Sample (b) Standard *library*

This compound is at peak number 2 with a retention time 26.758 at 2.64%. MS results give molecular ion peaks at m/e = 270 (M) + followed by fragmentation peaks with mass m/e as follows 270, 227, 171, 143, 129, 101. and 74 (100%). The spectra are compounds with the molecular formula $C_{17}H_{34}O_2$ which is Palmitic Acid Methyl Ester. The fragmentation patterns are followed by the release of a molecule with a molecular weight of m/e = 43. The loose molecule (C_3H_7) forms a molecule with a mass weight of m/e = 227 $(C_{14}H_{27}O_2)^+$, then the fragmentation releases a molecule m/ e= 56 loose molecules (C4H8) form molecules with mass weight m/e= 171 $(C_{10}H_{19}O_2)^+$, Fragmentation loose molecules m/e =28 loose molecules (C_2H_4) form molecules with mass weight m/e= 143 $(C_8H_{15}O_2)^+$, Fragmentation of molecular dissolution m/e= 14 detached molecule (CH_2) forms a molecule with mass weight m/e= 129 $(C7H13O2)^+$, Fragmentation of the loose molecule m/e= 28 loose of the molecule (C2H4) to form a molecule with mass weight m/e= $(C5H9O2)^+$, and fragmentation dislodged molecule m/e= 27 detached molecule (C2H3) forms a molecule with m/e = 74 $(C3H6O2)^+$

(b) Peak with a retention time of 30.650

The mass spectrum data shows molecular ion peaks m/e = 296, 264, 222, 194, 152, 123, 97, 69, and 55. By comparing the unknown spectrum data with the mass spectrum obtained with the spectrum data in the library, Methyl Oleic Acid Esters are more detectable.



(a)

(b)

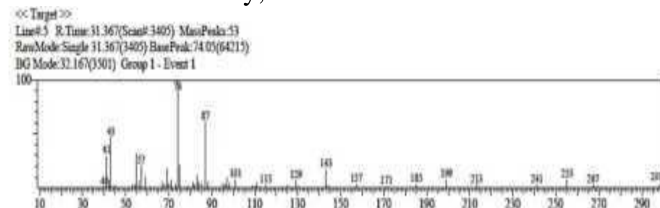
Figure 3. The spectra of Oleic Acid Methyl Esters with a retention time of 30,650 (a) Sample (b) Standard *library*

This compound is at peak number 4 with a retention time 30.650 at 74.23%. MS results give molecular ion peaks at molecular weight m/e = 296 (M+) followed by fragmentation peaks with molecular weight m/e as follows 296, 264, 222, 194, 152, 123, 97, 69, and 55 (100 %), this spectrum is a compound with the molecular formula $C_{19}H_{36}O_2$ which is an Oleic Acid Methyl Ester. The fragmentation patterns are followed by the release of the molecule with a molecular weight of m/e= 32 the loose molecule (CH3OH) forms a molecule with

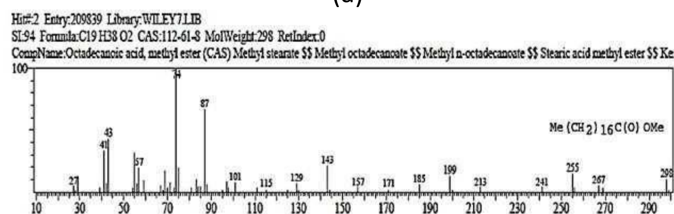
weight mass m/e= 264 $(C_{18}H_{32}O)^+$, then Fragmentation release of molecule m/e=42 the loose molecule (C_3H_6) forms a molecule with mass/e=222 $(C_{15}H_{26}O)^+$,Fragmentation of molecule release m/e= 28 loose molecule (CO) forms a molecule with mass weight m/e= 194 $(C_{14}H_{26})^+$, Fragmentation of loose molecule m/e= 42 loose molecule (C_3H_6) forms a molecule with mass weight m/e = 152 $(C_{11}H_{20})^+$, Fragmentation of loose molecule m/e= 29 loose molecule (CH_3CH_2) comprises a molecule with mass weight m/e= 123 $(C_9H_{15})^+$, Fragmentation releases molecules m/e = 26 molecules escapes (C_2H_2) forms molecules with mass weight m/e= 97 $(C_7H_{13})^+$, Fragmentation release of molecule m/e = 28 release of molecule (C_2H_4) forms a molecule with mass weight m/e= 69 $(C_5H_9)^+$, And Fragmentation release of molecule m/e =14 release of molecule (CH_2)forms a molecule with a mass weight of m/e 55 $(C_4H_7)^+$.

(c) Peak with a retention time of 31.367

This spectrum is a compound with the molecular formula $C_{19}H_{38}O_2$. The mass spectrum data shows ion peaks m/e = 298, 255, 199, 185, 143, 101, and 74. By comparing the unknown spectrum data with the mass spectrum obtained with spectrum data in the library, which detects more Stearic Acid Methyl Ester



(a)



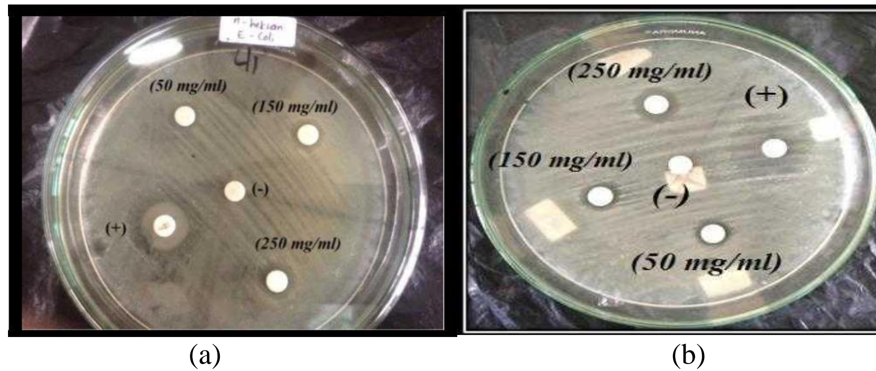
(b)

Figure 4.The spectra of Stearic Acid Methyl Esters with a retention time of 31.367
Note: (a) Sample (b) Standard *library*

This compound is at peak number 5 with a retention time 31.365 at 12.01%. The MS results gave a molecular ion peak at a molecular weight of m/e = 298 (M+) followed by fragmentation peaks with a molecular weight of m/e 298, 255, 199, 185, 143, 101, and 74 (100 %). The spectra are compounds with the molecular formula C19H38O, Stearic Acid Methyl Ester. The fragmentation patterns of stearic acid are fragmentation releasing molecules m/e= 43 (C_3H_7) forming molecules with a molecular weight m/e= 255 $(C_{16}H_{31}O_2)$ + , fragmenting releasing molecules m/e= 56 loose molecules (C_4H_8) form molecules with m/e= 199 $(C_{12}H_{23}O_2)^+$, fragmentation loose molecules m/e= 56 loose molecules (C_4H_8) form molecules m/e= 143 $(C_8H_{15}O_2)^+$, fragmentation releases molecules m/e= 42 (C_3H_6) forms molecules with m/e= 101 releases molecules $(C_5H_9O_2)^+$, and fragments releases molecules m/ e= 27 (C_2H_3) forms a molecule with m/e= 74 $(C_3H_6O_2)^+$.

3.3. Results of Antibacterial Activity Test on n-Hexane Extract of Jengkol Seeds

The antibacterial activity test of the n-hexane extract of Jengkol seeds showed zones inhibition of bacterial growth. Antibacterial activity testing aims to determine the activity of bacteria on the test sample. The antibacterial activity test was carried out by the agar diffusion method using disc paper. The extract was pipetted 10μL with various concentration variations onto the paper discs. Then the disc paper was placed on the surface of the NA media, as in Figure 5.



(a) (b)

Figure 5. Bacterial inhibition zone with extracts *n*- hexane on: (a) *E.coli* , (b) *S.aureus*

Antibacterial activity was determined by measuring the zone of inhibition formed around the disc paper. The extracts used for the antibacterial activity test had concentrations of 250 mg/ml, 150 mg/ml, and mg/ml (w/v), which were dissolved in 1 ml of DMSO. The negative control used was disc paper soaked in DMSO solvent. The choice of DMSO solvent is 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 the observation 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.

Table 2. Diameter of clear zone *n*-hexane extract of Jengkol seeds against bacteria *E. coli* and *S. aureus.*

Treatment	**Zone Diameter Resistance(mm)**	
	Extract *n* -Hexane	
	E. coli	***S. aureus***
Negative Control	0.00	0.00
Positive Control	25	19
Concentration 50 mg/ml	8	10
Concentration 150 mg/ml	9	11
Concentration 250 mg/ml	10	11.5

Description: - Negative control used disc paper soaked with DMSO.
- The positive control used chloramphenicol 30 mg.

Table 2 shows that the negative control does not show the diameter of the inhibition zone. It shows that the solvent used has no antibacterial activity or effect on the antibacterial test. While the positive control, namely chloramphenicol, showed that the diameter of the inhibition zone had a very strong antibacterial activity. Based on the [10] that a compound has antibacterial activity with an inhibition zone of ≤14 mm, which is categorized as weak (resistant), 15 - 19 mm is classified as medium (intermediate), and ≥20mm is categorized as strong. The table above shows that the n-hexane extract of Jengkol seeds can inhibit the growth of weak categorical bacteria (inhibition zone ≤14 mm).

Based on the chemical composition, bacteria comprise 70% water, 30% solids of 3% DNA, 12% RNA, 5% protein, 5% polysaccharides, and 5% fat [11]. Bacteria have a cell membrane composed of double-layered phospholipids and proteins [12]. This cell membrane functions as a selective barrier for the entry and exit of chemical compounds from outside and inside the cell. Material that passes through the cell membrane is divided into groups of macromolecules and micromolecules which are then broken down as nutrients and energy for the bacteria [12]. The amphipathic structure of fatty acids provides detergent properties that damage the bacterial cell's cell membrane, causing fatty acids to enter the interior of the bacterial cell. Fatty acids that enter the interior of the bacterial cell in bacterial cells cause inhibition of bacterial growth or death of bacterial cells [13]. Saturated and unsaturated fatty acids can bind carrier electrons to the bacterial cell membrane; bonds in unsaturated fatty acids cause the breaking of carbon chains so that the fluidity of the membrane becomes

unstable, while saturated fatty acids can reduce membrane fluidity and damage electron transport within the membrane [6]. The n-hexane extract of Jengkol seeds has the best antibacterial activity against *S.aureus* bacteria compared to *E.coli* bacteria, classified as gram-negative bacteria. The cell walls of gram-positive bacteria are relatively simple, making it easier for antibacterial compounds to enter the cell and find targets to work on. In contrast, the cell walls of gram-negative bacteria are more complex [14].

4. Conclusion

Analysis of the fatty acid composition of the n-hexane extract of Jengkol seeds which was first esterified using Methanol (1.7 ml): $H2SO4$ 98% (0.3 ml): Chloroform (2 ml) was carried out by the GC-MS. The results of the fatty acid composition found in Jengkol seeds are saturated fatty acids, namely: Margaric Acid (1,10 %), Palmitic Acid (2.64%), and Stearic Acid (12.01%), and unsaturated fatty acids, namely: Linolelaidic Acid (10.02%), and Oleic Acid (74.23%). Antibacterial activity test results with *n*-hexane extract of Jengkol seeds showed that all concentrations tested were 50 mg/ml, 150 mg/ml, and 250 mg/ml able to inhibit the growth of *Escherichia* coli and *Staphylococcus aureus* bacteria, where the zone inhibition formed by the *n*-hexane extract of Jengkol seeds against *E. coli* and *S. aureus* is included weak category (≤14 mm).

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

References

[1] Pitojo, S. 1992. Cultivation and Benefits Jengkol. Yogyakarta.
[2] Alatas, F. 2017. The Effect of Giving Extracts Ethanol 96% Jengkol (Pithecollobiumlobatum) on histopathological features of kidney tissue and increased levels of urea creatinine in white male rats (Rattus norvegicus) Sprague Dawley Strain. Bandar Lampung: Lampung University.
[3] Roswaty, A. 2010. All About Jengkol & Petai. Jakarta: PT Gramedia Pustaka.
[4] Ketaren S, 1986. Food Oil and Fat. Jakarta: UI-Press.
[5] Fardiaz S. 1987. Antimicrobial compounds. Bogor: PAU.
[6] Huda, S. 1997. Study on Dental Plaque Activity Of Mace And Betel Nut And Their Active Components Japan. Ph.D. Thesis
[7] Rahmawati. 2010. Simplicia Characterization and Antibacterial Activity Test of Extracts Jengkol Fruit Skin (Pithecellobium lobatum Benth.) Against Escherichia coli, Shigella dysenteriae, and Salmonella Typhimurium bacteria. University North Sumatera.
[8] Yusra, RF 2012. Extract Inhibitory Test Jengkol Fruit Peel Ethanol (Pithecellobium lobatum Benth) on Bacterial Growth Methichilin-resistant Staphylococcus aureus. Aceh: Faculty of Medicine Unsiyah
[9] Silverstein RM, Bassler GC, Morrill TC, 1984. Spectrometric Investigations of Organic Compounds. Fourth Edition. Translated by AJ Hartomo, et al and Anny Victor Purba. Jakarta: Erlangga.
[10] Clinical and Laboratory Standard Institute. 2012. Performance Standard for Antimicrobial Disk Susceptibility Test: Approved Standards - Eleventh Edition.
[11] Philip. LC 1967. Microbiology. London: WB Saunder Company
[12] Pratiwi, ST. 2008. Pharmaceutical Microbiology. Jakarta: Erlangga
[13] Chamberlain NR., Mehrtens BG., Xiong Z., Kapral FA., Boardman JL., Rearick JI. (1991) Correlation of carotenoid production, decreased membrane fluidity, and resistance to oleic acid killing in Staphylococcus aureus18Z. Infect Immun. Volume 59.
[14] Pelczar, MJ, Chan ECS. 1986. Basics Microbiology, Volume 1. Jakarta: UI Press