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## Antibacterial Activity of Mango Kernel Extracts

<sup>1,2</sup>M.E.S. Mirghani, <sup>1,2</sup>F. Yosuf, <sup>1</sup>N.A. Kabbashi, <sup>3</sup>J. Vejayan and <sup>1</sup>Z.B.M. Yosuf

<sup>1</sup>Department of Biotechnology Engineering, Kulliyah of Engineering,  
International Islamic University Malaysia (IIUM), Gombak, P.O. Box 10, 50728 Kuala Lumpur, Malaysia

<sup>2</sup>Biomolecular Engineering Research Unit (BioMERU), Kulliyah of Engineering, IIUM, Malaysia

<sup>3</sup>Department of Biochemistry, Faculty of Medicine and Health Sciences, Monash University,  
Sunway Campus, Malaysia

**Abstract:** This study is emphasized specifically on the potential of the mango *Mangifera Indica* seed kernel by discovering the prospective usage of mango seed kernels as a source of antibacterial compounds against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains. For this study, three types of mango were used; Blackgold, Lemak and Waterlily from Malaysia and Thailand. The approach adopted for this purpose was by implementing agar well diffusion method. The results were expressed as the average diameter of bacterial inhibition zones surrounding the wells. The required solvents for the extraction were ethanol, methanol, acetone and phosphate buffer solutions were prepared in a different concentration; 0.10M, 0.05M and 0.01M. The results showed that Lemak gave relatively high antibacterial activity among other types of mango ranging between 1.40 and 2.23 cm. For the known antibiotic, which was Tetracycline, under the same conditions the diameter of inhibition zones were between 2.30 to 3.30. Then, the minimum inhibition concentration tests were conducted for Lemak for two solvents extract that gave the highest inhibition zones which were methanol and acetone. The results showed that the minimum inhibition concentration of extracts that inhibited the growth of *Escherichia coli* and *Bacillus subtilis* was after 10X dilution (1 mM). The results obtained confirmed the antibacterial potential of mango kernels extracts and this would probably become an alternative source of new and natural antibacterial agents.

**Key words:** Anti-bacterial activity, *Bacillus subtilis*, *Escherichia coli*, Mango *Mangifera indica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

### INTRODUCTION

Mango, *Mangifera indica* L., is a member of the family Anacardiaceae. Mango has become naturalized and adapted throughout the tropics and subtropics. There are over 500 classified of mango varieties; some of them have evolved and have been described throughout the world. The genus of *Mangifera* consists of 69 species and mostly restricted to tropical Asia (Ian, 2006).

The highest variety of mango occurs in Malaysia, particularly in peninsular area and about 28 species are found in this region (Ian, 2006). Malaysia lies wholly within the tropics, which encompasses heavy precipitation, high temperatures and high humidity, which are the favoring factors for mango vegetation. There are several varieties of mango grown in Malaysia; the better known cultivars are Golek (MA 162), Masmuda (MA 204), Maha 65 (MA 165) and Chok Anan (MA 224). The

domestic consumption increased from 42,634 MT (2002) to 55,901 MT (2005). The significant increases of mango consumption in domestic activity lead to the accumulation of waste. Mango seed kernel usually wasted when processing. However, with appropriate treatment and study, the kernel might be possibly used as a food ingredient and even in other purposes.

After consumption or industrial processing of mangoes, considerable amounts of mango kernels (seeds) are discarded as waste (Sandhu and Lim, 2007), approximately 40 to 60% waste is generated during processing of mangoes; 12 to 15% consists of peels and 15 to 20% of kernels. According to mango varieties, the seed represents from 10 to 25% of the whole fruit weight. The kernel inside the seed represents from 45 to 75% of the seed and about 20% of the whole fruit. However, more than one million tons of mango seeds are being treated as wastes.

**Corresponding Author:** M.E.S. Mirghani, Department of Biotechnology Engineering, Kulliyah of Engineering,  
International Islamic University Malaysia (IIUM), Gombak, P.O. Box 10,  
50728 Kuala Lumpur, Malaysia

**Compositional quality of mango seed kernel:** In a study for Egyptian mango by-product to proximate its compositional quality of mango seed kernel (Abdalla, 2006), mango seed kernels contained a considerable amount of total phenolic compounds, total lipid, unsaponifiable matter and a low amount of crude protein, but the quality of protein was good because it was rich in all essential amino acids with highest values of leucine, valine and lysine. Eight phenolic compounds were identified which tannin and vanillin were in highest amounts. Unsaponifiable matter showed the occurrence of high amounts of squalene followed by sterols and tocopherols. Stearic acid was the main saturated fatty acid, while oleic acid was the major unsaturated fatty acid in all lipid classes. The fatty acid composition of total lipid and neutral lipid was similar, while phospholipid had a high amount of palmitic, linoleic and linolenic acids (Fig. 1).

Depending on their variety, mango seed kernels contain on a dry weight average 6.0% protein, 11% fat, 77% carbohydrate, 2.0% crude fiber and 2.0% ash. Mango seed kernels were shown to be a good source of polyphenols, phytosterols as campesterol,  $\beta$ -sitosterol and tocopherols. Extracted and fractionated total lipids from Alphonso mango kernel into three lipid classes. Total lipid (11.6% of dry kernel) consisted of 96.1% neutral and 3.9% polar lipids, which comprised 2.9% glycolipids and 1.0% phospholipids. Extracted and fractionated total lipids from three different mango seed kernel varieties namely golek, pairi and hindi. The neutral lipids varied from 95.2 to 96.2%, phospholipids from 2.7 to 3.3% and glycolipids from 1.1 to 1.4%. Triglycerides constituted the major fraction of the neutral lipids, for all varieties and accounted from 93.7 to 96.4%. On the nutritional and toxicological studies of the mango seed kernel, indicated that mango seed kernel fat is promising and a safe source of edible oil and was found to be nutritious and non-toxic so that it could be substituted for any solid fat without adverse effects (<http://wapi/mctweb.dll/>). Burman and Liljequist (2001) concluded that the Nigerian mango kernel has a good source of high

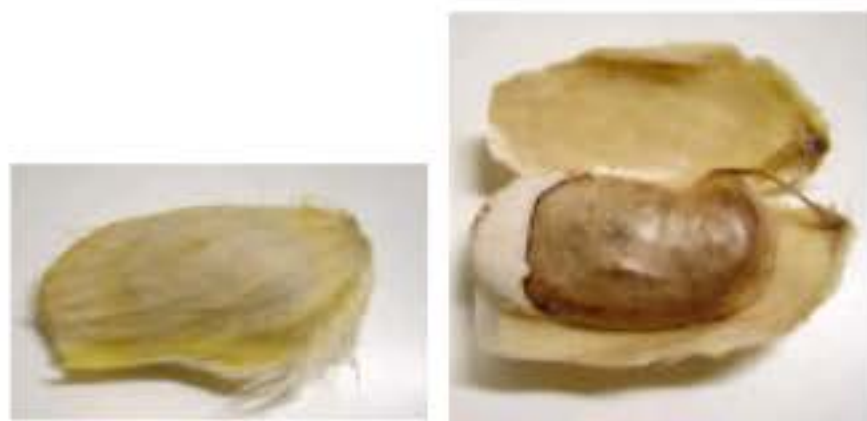


Fig. 1: Mango seed kernel

quality fat and protein as well as tannin. When extracted, these components could be valuable commercially in the vegetable oil industry, in confectionary and in tanning. Similarly, the processed flour could be principal ingredient in the diets of babies and adults in Nigeria. Karp (2009) suggested that mango seed kernel could be used as a potential source for functional food ingredients due to its high quality of fat and protein as well as high levels of natural antioxidants. Furthermore, It is published that soaking and boiling treatments had a great impact in reducing the anti-nutritional factors (<http://en.wikipedia.org/wiki/>).

Depending on their variety, mango seed kernels contain on a dry weight average 6.0% protein, 11% fat, 77% carbohydrate, 2.0% crude fiber and 2.0% ash (Sandhu and Lim, 2007). Since, the mango seed kernel contains valuable elements, it is considerable to utilize it in some way so that hazards could be eliminated and probably valuable products could be produced.

This research focuses exclusively on antibacterial activity of mango kernel extracts against several of Gram-positive and Gram-negative bacterial strains.

## MATERIALS AND METHODS

**Agar well diffusion method:** The antibacterial properties of mango seed kernel extracts can be investigated by using the agar well diffusion method. Through this method, the Minimum Inhibitory Concentration (MIC) of the mango seed kernels against food-borne pathogenic bacteria can be determined. The MIC is the lowest concentration of a drug or antibiotic that prevents growth of a particular pathogen.

The principle of this assay technique is fairly simple. When an antibiotic is placed inside the well of suitable agar medium previously inoculated with the test bacterium, the antibiotic diffuses radially outward through the agar, producing an antibiotic concentration gradient. The antibiotic is present at high concentrations near the well and affects even minimally susceptible microorganisms. As the distance from the well increases, the antibiotic concentration drops and only more susceptible pathogens are harmed. A clear zone or ring is present around an antibiotic well after incubation if the agent inhibits bacterial growth. The wider the zone surrounding a well, the more susceptible the pathogen is.

The extracts of mango seed kernels display a broad antibacterial spectrum and are more effective against gram-positive than the gram-negative bacteria. The antibacterial activity of the mango seed kernels is stable against heat (121°C, 15 min), freezing (-20°C, 16 h)

and pH treatment (pH 3 to 9) normally used in food processing (Kabuki *et al.*, 2000).

## METHODOLOGY

### Experimental materials

**Raw material:** Three types of mango fruits were purchased from Selayang wet market. The seed kernels were taken out from each fruits manually and grinded. They were dried before being used.

**Bacterial strains:** The bacterial strains were obtained from Molecular Biology Laboratory, IIUM. The used strains were:

- Gram-positive:- *Staphylococcus aureus* and *Bacillus subtilis*
- Gram-negative:- *Escherichia coli* and *Pseudomonas aeruginosa*

**Preparation of mango seed kernels:** The seeds were washed and air-dried and the kernels were removed manually from the seeds. The kernels were chopped or cut into small pieces by using grinder. After that, they were dried in a freezer at -80°C and transferred to freeze dryer to remove the excessive moisture for several days. Finally, the samples were stored at 4°C until utilization for the next procedures.

**Preparation of extraction reagents and extracts:** The required solvents for the extraction (85% ethanol, 85% methanol, 85% acetone and 0.1 M phosphate buffer of pH 7) were prepared prior to the extraction process. (Sambrook and Russell, 2001) the pH of the buffer was measured again by pH meter.

0.2 g of each sample was weighted and put into a falcon tube. Each solvent was measured according to Table 1, following to the concentration required. The solvent was pipetted into the tubes containing the sample. The tubes were mixed by using vortex mixer for 30 sec and centrifuged at 13,000 rpm for 15 min. After centrifugation, the supernatants were transferred into another centrifuge tube to be tested against the selected bacteria. The extracts were stored at -20°C if not used.

Table 1: Antibacterial activity of *Mangifera indica* kernel extracts against *Bacillus subtilis*

Solvent	Diameter of inhibition zone (cm)				Positive control	Negative control
	Blackgold	Waterlily	Lemak			
Ethanol	1.933	1.567	1.633	2.700	0	
Methanol	1.677	1.500	1.967	2.867	0	
Acetone	1.600	1.633	2.233	2.700	0	
PBS	1.400	1.000	1.600	2.600	0	

### Preparation of the media

**LB Media:** Thirty seven gram of LB agar powder were dissolved in 1 L of distilled water inside 1 L of Schott bottle. The pH was adjusted to 7.0 before being sterilize by autoclaving it for 20 min at 15 psi (1.05 kg cm<sup>-2</sup>) on liquid cycle. Plates were poured directly from the Schott bottle under laminar flow for about 20-25 mL per plate. After the medium hardened completely, the plates were inverted and stored at 4°C. When needed, they were removed from storage 1 to 2 h before use.

To prepare 1 L of LB liquid broth, 25 g of LB broth powder were dissolved in 1 L of distilled water inside 1 L of Schott bottle. The pH was then adjusted to 7.0 before being sterilized by autoclaving it for 20 min at 15 psi (1.05 kg cm<sup>-2</sup>) on liquid cycle. Then, it was stored at 4°C (Sambrook, and Russell, 2001).

**Top agar:** For 1 L of top agar, LB agar and LB broth were mixed together in the ratio of 3:1. They were dissolved in 1 L of distilled water. The pH was then adjusted to 7.0 before being sterilize by autoclaving it for 20 min at 15 psi (1.05 kg cm<sup>-2</sup>) on liquid cycle. After autoclaving, the medium was allowed in 40-45°C dryer prior to use or storage (Sambrook, and Russell, 2001).

### Antibacterial assays

**Preparation of cell culture:** The bacterial strains were kept in the eppendorf tubes that contain glycerol in -80°C freezer. Prior to use, the eppendorf tubes were thawed in room temperature for several minutes. Then, a dip of each bacterial strain was transferred into different bijou bottles that contain 10 mL of LB liquid broth by using a wire loop. The bijou bottles were put into incubator shaker at 200 rpm and 37°C for overnight. In the next day, a dip of the content of each bijou bottles were transferred and distributed evenly onto the LB agar plates and put inside incubator at 37°C for overnight. After there were cell growth on the agar and there were no contaminations, the plates were stored in the chiller at 4°C until next utilization (Denyer *et al.*, 2004).

The subcultures must be freshly prepared. A single colony was taken from each plate by using a wire loop. Then, it was transferred into bijou bottle that contains 10 mL LB liquid broth and was put into incubator shaker at 200 rpm and 37°C, for overnight. In the next day, the Optical Density (OD) of the bacterial culture was measured at wavelength 600 nm with LB liquid broth as the blank. If the OD value was greater than 0.5, the dilution was performed by adding LB liquid broth into the bacterial culture in the bijou bottle.

**Antibacterial screening:** The agar well diffusion method was used to analyze and evaluate the antibacterial activity for each extract. About 50 µL of bacteria subcultures were

put into each shake flask. Then, 4 mL of top agar (35 to 40°C) were added into the same flasks and shake gently. The top agar containing the bacterial cultures were poured and distributed evenly onto the LB agar plates. The plates were transferred into incubator at 37°C. After 15 min, 5 wells (5 mm diameter) were made in each plate using sterile pipette tips. Then, about 50 µL of each solvent extracts were loaded into each well. As a negative control, 50 µL of each solvent (acetone, methanol, ethanol and phosphate buffers) alone were used. For the positive control, 50 µL of tetracycline was used. All the plates are then were covered with lids and incubated overnight at 37°C. The presences of inhibition zones on LB plates containing bacterial culture around the well were observed. The diameters of each inhibition zone were measured. The type of sample and concentration of solvent that gave the optimum result were identified (Denyer *et al.*, 2004).

**Minimum Inhibition Concentration (MIC):** The dilutions for the solvents extract were prepared for 10x, 100x and 1000x. 500 µL of 0.01 M sample was pipetted into a Falcon tube. The tube was mixed by using vortex mixture before the previous steps were repeated in order to obtain another dilute sample. Then, the antibacterial screening was performed towards all the different dilute samples to obtain the minimum inhibition concentration. The presences of inhibition zones on LB plates were observed and the diameters of each inhibition zone were measured and recorded.

**RESULTS AND DISCUSSION**

The antibacterial activities of *Mangifera indica* kernel extracts were investigated by using agar well diffusion method. This study involved different solvents and concentrations in order to evaluate and compare the antibacterial activity for various circumstances. Antibacterial activities of each solvents extract were expressed in terms of the average diameter of the presence inhibition zone. The results were shown as in the Table 2-6.

From the observation and measurement of diameter of inhibition zones formed, it has been demonstrated that the extracts from the *Mangifera indica* kernel had significant potential as an antibacterial compounds. The results shown above were for the extracts concentration of 0.10 M against four types of bacterial strains that signify Gram-positive and Gram-negative bacteria. The results clearly justified that all of the extracts have shown antibacterial activity. For the known antibiotic, which was the Tetracycline, the diameter of the inhibition zones were

between 2.300-3.300. These measurements were relatively high compared to diameters of inhibition zones for the sample extracts. This is because Tetracycline is already recognized as the wide-spectrum antibiotic that inhibits the growth of both Gram-positive and Gram-negative bacteria. The extracts from Lemak gave great results as larger inhibition zones were formed compared to other types of mango kernel extracts. Thus, further analysis of antibacterial activity was performed for this specific type of kernel extracts at different concentrations. Later on, the Minimum Inhibition Concentration (MIC) of the extracts was determined (Table 5-9).

From the above results, as the extracts concentration decreases, the size of the inhibition zone also decreases. This means that the concentration of the extracts relative to the antibacterial activity. Besides that, the antibacterial activity against Gram-positive bacteria gave higher results compared to the Gram-negative bacteria. One of the reasons that could possibly contribute to this phenomenon was the observation that Gram-negative

Table 2: Antibacterial activity of *Mangifera indica* kernel extracts against *Staphylococcus aureus*

Solvent	Diameter of Inhibition Zone (cm)				
	Blackgold	Waterlily	Lemak	Positive control	Negative control
Ethanol	1.700	1.533	2.000	3.133	0
Methanol	1.700	1.333	1.933	2.567	0
Acetone	1.833	1.500	1.200	2.700	0
PBS	1.567	0.900	1.933	2.600	0

Table 3: Antibacterial activity of *Mangifera indica* kernel extracts against *Pseudomonas aeruginosa*

Solvent	Diameter of Inhibition Zone (cm)				
	Blackgold	Waterlily	Lemak	Positive control	Negative control
Ethanol	1.300	1.267	1.400	3.167	0
Methanol	1.500	1.800	2.000	3.200	0
Acetone	1.500	1.400	1.333	3.300	0
PBS	1.600	1.500	1.700	3.167	0

Table 4: Antibacterial activity of *Mangifera indica* kernel extracts against *Escherichia coli*

Solvent	Diameter of Inhibition Zone (cm)				
	Blackgold	Waterlily	Lemak	Positive control	Negative control
Ethanol	1.400	1.500	1.567	2.600	0
Methanol	1.500	1.333	1.867	2.500	0
Acetone	1.700	1.400	1.533	2.600	0
PBS	1.400	1.300	1.600	2.300	0

Table 5: Inhibition zone diameter of the antibacterial activity of Lemak extracts at different concentrations against *Staphylococcus aureus*

Solvent	Diameter of inhibition zone (cm)				
	0.10 M	0.05 M	0.01 M	Positive control	Negative control
Ethanol	1.500	1.300	1.233	2.600	0
Methanol	1.800	1.500	1.400	2.700	0
Acetone	1.500	1.400	1.300	2.700	0
PBS	1.800	1.400	1.400	2.700	0

Table 6: Inhibition zone diameter of the antibacterial activity of Lemak extracts at different concentrations against *Escherichia coli*

Solvent	Diameter of Inhibition Zone (cm)			Positive control	Negative control
	0.10M	0.05M	0.01M		
Ethanol	1.500	1.400	1.300	2.533	0
Methanol	1.500	1.333	1.200	2.300	0
Acetone	1.433	1.367	1.233	2.433	0
PBS	1.467	1.333	1.300	2.200	0

Table 7: Minimum Inhibition Concentration (MIC) of antibacterial activity of Lemak extracts against *Bacillus subtilis*

	Dilution			
	0.01 M	10x	100x	1000x
Methanol	1.500	1.200	0	0
Acetone	2.550	1.067	0	0

Table 8: Minimum Inhibition Concentration (MIC) of antibacterial activity of Lemak extracts against *Escherichia coli*

	Dilution			
	0.01 M	10x	100x	1000x
Methanol	1.500	1.067	0	0
Acetone	1.550	1.000	0	0

Table 9: Antibacterial activity of protein extracts

	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>
Blackgold	1.200	1.467
Waterlily	0	0
Lemak	0	0

bacteria are more resistant to antibiotics than are Gram-positive bacteria due to its complex outer membrane (Prescott *et al.*, 2005). For minimum inhibition concentration (MIC), the results demonstrated that the MIC for the Lemak kernel extracts was at 10x dilution which the concentration was 1 mM. The extract concentrations below than 1 mM did not show any antibacterial activity against both Gram-positive and Gram-negative bacteria.

In order to have a better evaluation and ease of understanding, graphs of inhibition zones diameter caused by the extracts of all samples (blackgold, waterlily and lemak) against four bacterial strains have been plotted. The graphs were constructed as to compare the antibacterial activity among all the samples and to identify which sample gave the great result. The graphs were plotted based on the data shown in Table 1-4; they are shown in Fig. 2-5.

From the graph plotted, it has been identified that Lemak extracts gave the highest antibacterial activity compared to other sample extracts. Also, it was found that methanol extract has given the optimum activity compared among the solvents.

The antibacterial activity towards protein extracts that were obtained from other ongoing research on mango kernel protein was also been analyzed. From the result, only Blackgold showed antibacterial activity against *E. coli* and *B. subtilis* whereas the other type of sample

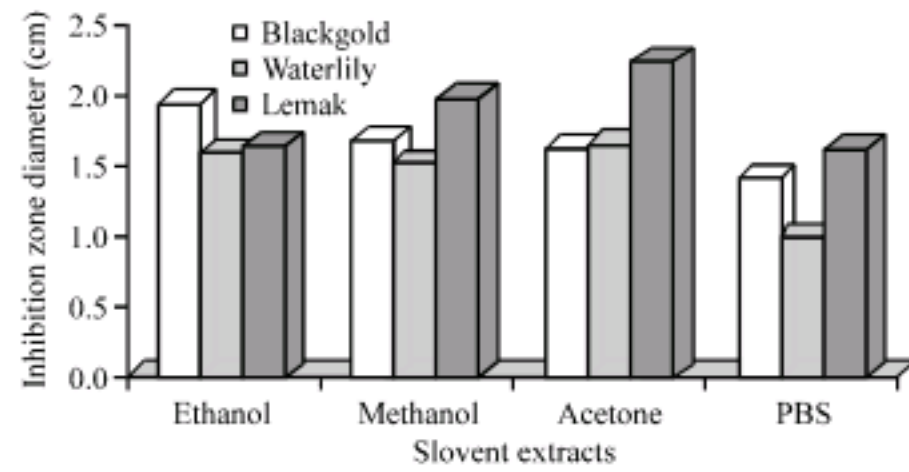


Fig. 2: Graph of antibacterial activity against *B. subtilis*

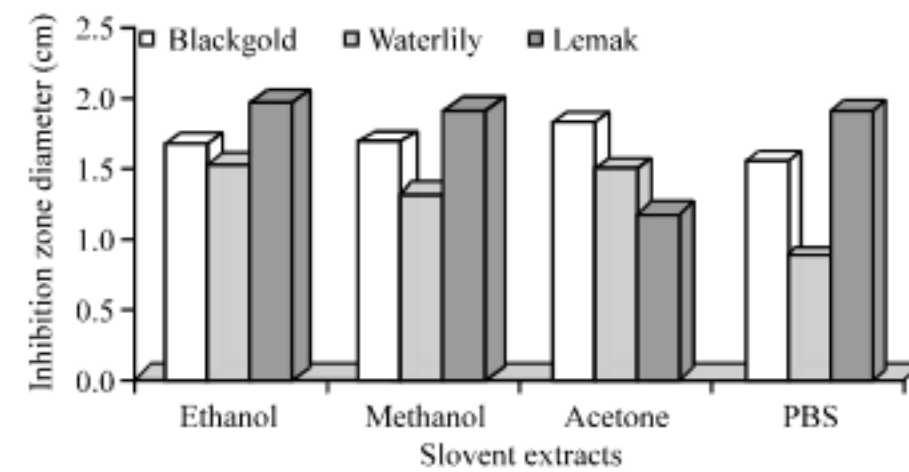


Fig. 3: Graph of antibacterial activity against *S. aureus*

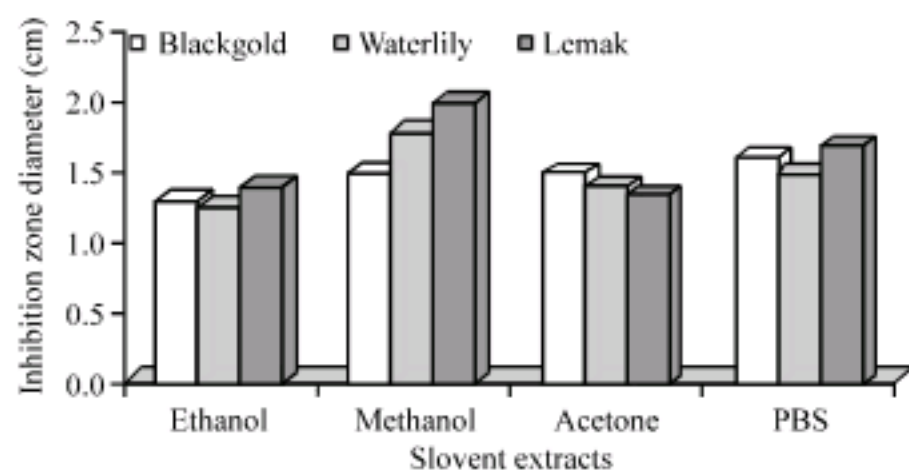


Fig. 4: Graph of antibacterial activity against *P. aeruginosa*

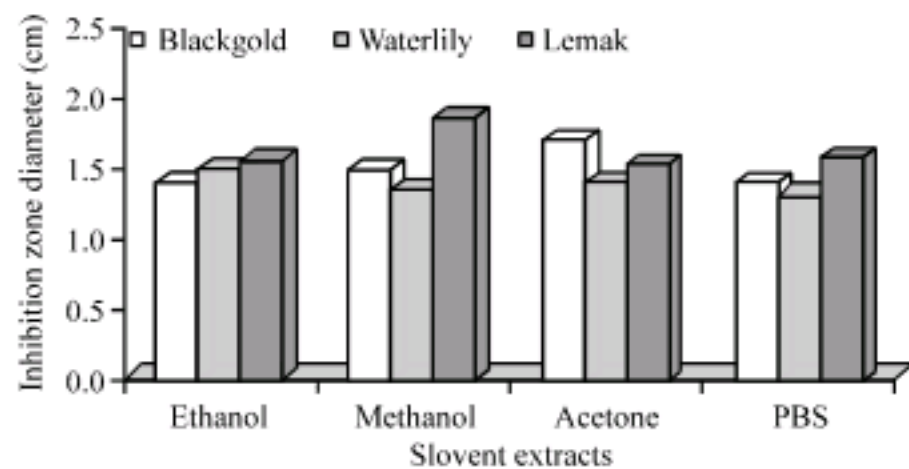


Fig. 5: Graph of antibacterial activity against *E. coli*

did not show any antibacterial activity. Thus, it can be said that protein did not contribute to the antibacterial activity for Lemak and Waterlily extracts. The antibacterial activity might come from other components in the extracts.

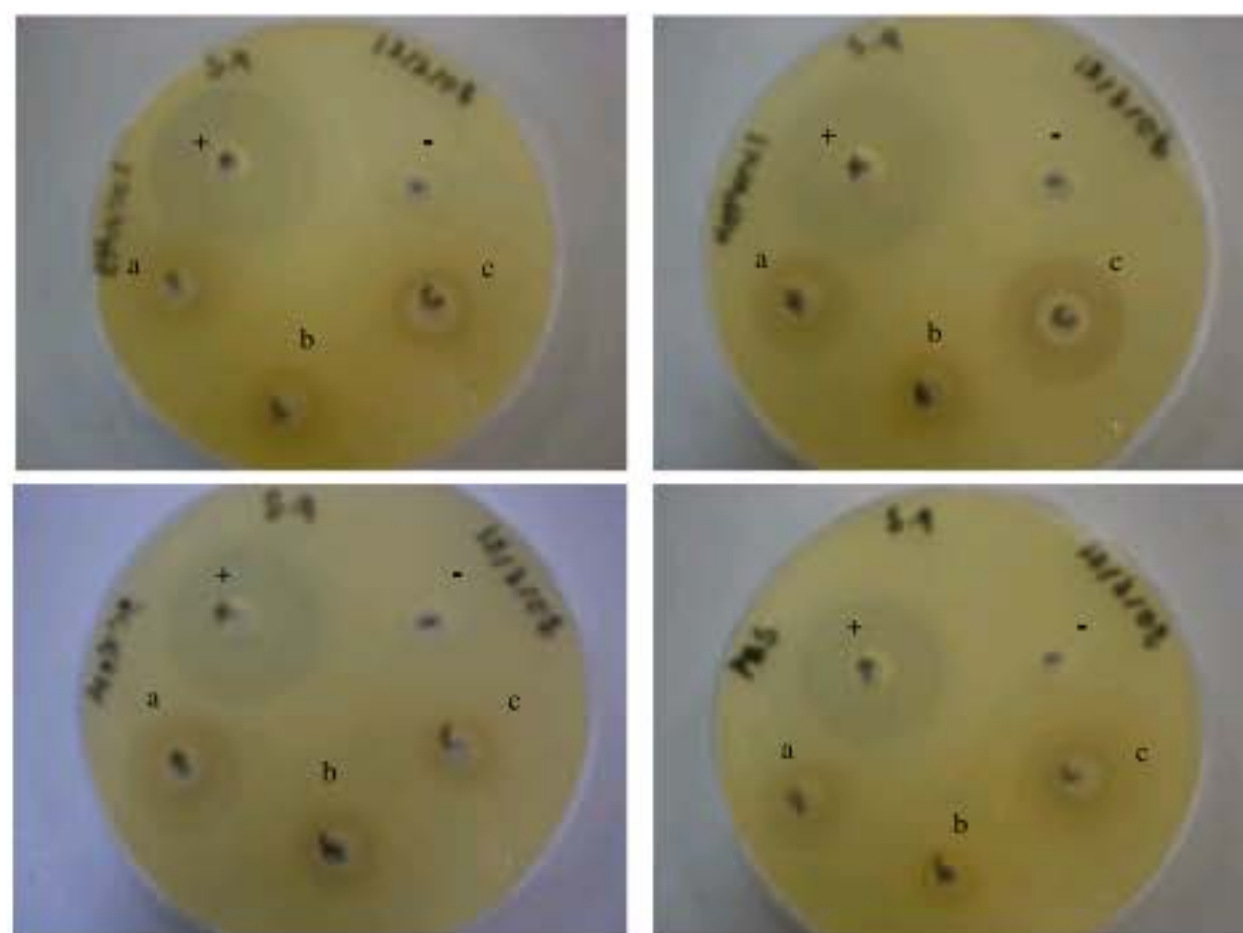


Fig. 6: Formation of inhibition zones of *Mangifera indica* kernel extracts for different solvents against *S. aureus*. Plate 1(a), 1(b), 1(c) and 1(d) represent the solvent extracts of ethanol, methanol, acetone and phosphate buffer, respectively

The inhibition zones formed for all samples extracts surrounding the wells on LB plates containing bacterial culture were shown in Fig. 6 as one example. Where, (+) sign indicates the positive control (tetracycline), (-) as negative control (the solvent alone), (a) as blackgold extract, (b) as waterlily extract and (c) as lemak extract.

### CONCLUSIONS

This research is mainly used to optimize the usage of mango seed kernel by implementing the design of its extraction for prospective applications that will be beneficial to people. Also, it will help to play a role in minimizing waste generation worldwide.

In the beginning of this project, several objectives had been set; to perform solvents extraction from the *Mangifera indica* seed kernel and to find out any antibacterial activity of the extracts. This project essentially investigated the antibacterial properties of the *Mangifera indica* kernel extracts. From the results obtained, the aims of this research have been successfully achieved. The extracts from *Mangifera indica* kernel have been proven to contain antibacterial properties that capable to inhibit the growth of pathogenic bacteria. Through this project, three types of mango have been used; blackgold, waterlily and lemak. The antibacterial activities of four solvents extract (ethanol, methanol,

acetone and phosphate buffers) have been tested against four pathogenic bacteria which signify Gram-positive and Gram-negative bacteria.

As the results showed constructive values, there is intense possibility for the compound in *Mangifera indica* kernel extracts can be utilized as an alternative antibacterial agent in the treatment of infectious disease caused by pathogenic bacteria. For recommendation in order to improve the results in the future, numerous studies must be made in order to identify the active compounds in the sample extracts which contribute to the antibacterial activity. It is crucial to discover the effects of the compounds towards animals and human cells, including the toxicity, the positive or negative reaction and the action mechanisms, to make sure that the compounds are safe and has no bad effects on the health. Besides that, the study must involve various extraction reagents at different concentration so that better comparisons can be achieved.

In conclusion, this project has been successfully finished within the time given. All the objectives have been accomplished as satisfactory results are obtained. Many knowledge and precious experiences obtained throughout this project especially regarding to the laboratory works. As the mango seed kernel can be utilized as antibacterial compounds, thus its usage can be optimized and indirectly can reduce the waste produced by the mango seed kernels.

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