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Antibacterial effects of banana pulp extracts based on different extraction methods against selected microorganisms

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Abstract

Various parts of banana have been shown to have antibacterial effect. Therefore, a comparative study on the antibacterial activity of the pulp extract of three different banana species, namely, Pisang Berangan (*Musa acuminate* AA/AAA), Pisang Mas (*Musa acuminate* AA) and Pisang Nipah (*Musa balbisiana* BBB) by different extraction methods was conducted against selected microorganisms (*Staphylococcus aureus*, *Streptococcus mutans*, Pseudomonas aeruginosa and Escherichia coli). The acetone, methanol and aqueous extracts of banana pulp were tested using agar disc diffusion method for antibacterial sensitivity testing. The solvent extraction data showed that acetone had the highest mean of banana pulp extract yield (15.16%), followed by methanol (13.73%) and aqueous solution (5.403%). The acetone and methanol extracts of all banana types showed an average with almost similar zone of inhibition activity at 10 mg/disc concentration against gram negative bacteria (Pseudomonas aeruginosa and Escherichia coli) ranging between 7 to 8.5 mm while no difference was seen in the case of gram positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*). With regard to aqueous extracts, all banana types did not show any inhibitory action against the tested microorganisms. In conclusion, the results implied that the pulp extracts of the three different banana species could be a potential source of antibacterial agents. However, further studies are needed to identify the bioactive components responsible for their antibacterial activity to maximize its therapeutic effect.

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INTRODUCTION

Plant extracts derived from their parts (roots, stems, leaves, fruits) are now increas-ingly used in research due to their widespread, immediate availability and cheaper cost, besides having potential medicinal properties and ability to manage certain health condi-tions which have been growing in recognition. Banana has long been used as a medicinal agent due to its nutritional rich properties. Banana is thought to have antibacterial activity, antioxidant activity and other biological activities such as antidiabetic, antidiarrheic, anti-tumoral, antimutagenic, antihelminthic and antiulcerogenic[1]. In Malaysia, the banana fruit is mostly considered as one of the major agriculture products grown for domestic purposes. There have been many studies reported on the potential prospect of banana as a therapeutic agent[2]. In the past studies, various parts of banana have been shown to have an inhibitory effect on pathogens making them as excellent candidates for the antibacterial as well as antioxidant sources. The phytochemical components of banana, tannins, eugenol and tyra-mine have been proven to have antibacterial effects[3]. Other active compounds present in banana such as alkaloids, glycosides, flavonoids, saponins, steroids, serotonin and dopa-mine also contribute to pharmacological effects[4].

Subrata et al. (2011) has reported that the root extract of banana has antibacterial properties. There is also evidence of antibacterial activity of Musa sapientum leaves extract in-vitro[5]. Another study on the extracts of pulp of Musa sapientum var. paradisiaca shows a significant healing effect due to their antioxidant activities[6]. A study revealed that peel and pulp of fully riped banana have potential antifungal and antibiotics properties[7].

Therefore, in this research, the banana pulp of three different banana types, namely Pisang Berangan (Musa Acuminata AA/AAA), Pisang Nipah (*Musa balbisiana* BBB) and Pisang Mas (Musa Acuminata AA) were tested against selected gram positive bacteria and gram negative bacteria by different extraction methods.

MATERIALS AND METHODS

Collection and preparation of plant extracts

Three types of banana pulp were obtained from Kubang Kerian, Kelantan. The banana pulps were washed thoroughly using tap water and wiped using a clean cloth. The succu-lent parts of the banana pulp were cut into about two centimeter thickness. Next, the cut pulp was allowed to dry in the oven at 50°C for three days. The dried pulp was powdered using electric blending machine and kept at 4°C in a tight-capped bottle.

Preparation of extracts: The extract preparation involved use of solvents namely, acetone, methanol and distilled water. Soxhlet apparatus technique was used to extract the crude compound from the banana pulp using methanol and acetone as its extraction solvent. Twenty gram of each banana pulp powder was placed inside a thimble made from thick filter paper. The thimble was loaded into the middle chamber of the soxhlet extractor. Three hundred millilitre of extraction solvent was added into a distillation flask and the rest of the soxhlet apparatus consisting of condenser and the middle chamber with thimble was attached to the flask. The solvent was heated to begin the distillation process and the cycle was allowed to stand for three days. Next, the extract was filtered using Whatman filter paper and transferred into a falcon tube. The solvents were removed by means of Concentrator Plus machine yielding the extracted compound.

As for the aqueous extract, distilled water was used as the solvent where 20 gram of pulp powder was dissolved in 300 ml of distilled water. The mixture was boiled for about 45 minutes. Next, the extract was filtered using Whatman filter paper and was subjected to freeze-drying. The extracts were stored in a sterile container at 4°C until further use. The extracts were weighed and freshly dissolved in sterile distilled water to a final concentra-tion of 50, 100 and 500 mg/ml respectively for agar disc diffusion test.

Bacterial strains

The bacterial species used in this study were obtained from the Medical Microbiolo-gy and Parasitology Laboratory, School of Medical Sciences, Universiti Sains Malaysia, comprising of gram positive bacteria, *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans* (*S. mutans*), and negative bacteria, Pseudomonas aeruginosa *(P. aeruginosa*), Escherichia coli (*E. coli*). All bacterial strains were grown and maintained by sub-culturing on Mac Conkey agar and sheep blood agar for gram negative and gram positive bacteria, respectively. All agar plates were incubated for 24 hours at 37ºC and maintained at 4°C.

Antibacterial assay

The antibacterial screening using agar disc diffusion technique was carried out based on Clinical and Laboratory Standards Institute[8] with some modifications. In-vitro antibacterial activity was examined for acetone, methanol and aqueous extracts from banana pulp. Col-onies from the plates were suspended into sterile Mueller Hinton broth to form a turbidity of 0.5 McFarland standards using nephelometry. Bacterial suspensions with approximately 1x CFU/ml suspension were streaked onto Mueller-Hinton agar plates using sterile swab stick. The test disc was prepared by incorporating 20 µL of each extract (50, 100 and 500 mg/ml) to 6 mm sterilized filter paper disc to give a final concentration of 1, 2 and 10 mg/disc. The discs were left to dry under

the biosafety cabinet overnight. Then, the im-pregnated disc, blank disc (negative control) and positive control discs were placed gently into the respective location. Imipenem (10 µg/disc) served as positive control against P. aeruginosa to confirm that their growth were inhibited by antibiotics; tetracyline (30 µg/disc) was used as a positive control to inhibit the growth of *E. coli* and S. aureus and ampicillin (30 µg/disc) was used as positive control to inhibit the growth of S. mutans. The culture plates were incubated at 37°C for 24 hours. Microbial growth was determined by measuring the diameter of zone of inhibition in mm.

Statistical analysis

Statistical analysis was made using one-way ANOVA. P values of less than 0.05 were considered significant.

RESULT

The mean of extract yields of sample obtained from different types of banana are pre-sented in Table 1. There was a statistically significant difference of yield percentage be-tween solvent extract as determined by one-way ANOVA (F (2, 6) = 9.876, p = 0.013) where acetone had the highest mean of banana pulp extract yield (15.16%), followed by methanol (13.73%) and aqueous solution (5.403%). Yield of different extracts of banana (Musa) pulp from different species are shown in Table 2. No difference was noticed in the different species of banana for each extraction methods.

The antibacterial activity of different extracts of banana pulps were analyzed in-vitro against gram positive and gram negative bacteria by disc diffusion method. The results are shown in Table 3. The aqueous extract of all types of banana pulp displayed no zone of inhibition indicating no antibacterial activity. Acetone and methanol extracts of all banana pulps exhibited antibacterial activity only against P. aeruginosa and *E. coli* at 10 mg/disc. The highest antibacterial activity was observed with methanol extract of banana pulps against *E. coli* with 8.5 mm zone of inhibition.

Variable	N	Mean (SD)	F statistics (df)	*p* value*
Methanol extract	3	13.73 (0.395)	9.876 (2,6)	< 0.05
Acetone extract	3	15.16 (4.99)		
Aqueous extract	3	5.403 (0.476)		

Table 1: Comparison of mean of percentage yield among extraction solvents (methanol, acetone and aqueous) of banana (*Musa*) pulp

Extraction solvent	Sample type	Weight 1 (g)	Weight 2 (g)	Percentage of yield (%)
Methanol	Pisang Berangan (*Musa acuminate*, **AA/AAA**)	2.675	20	13.38
	Pisang Nipah (*Musa balbisiana*, **BBB**)	2.832	20	14.16
	Pisang Mas (*Musa acuminate,* **AA**)	2.732	20	13.66
Acetone	Pisang Berangan (*Musa acuminate*, **AA/AAA**)	2.451	20	12.26
	Pisang Nipah (*Musa balbisiana*, **BBB**)	2.457	20	12.29
	Pisang Mas (*Musa acuminate*, AA)	4.185	20	20.92
Aqueous	Pisang Berangan (*Musa acuminate*, **AA/AAA**)	1.001	20	5.005
	Pisang Nipah (*Musa balbisiana*, **BBB**)	1.055	20	5.275
	Pisang Mas (*Musa acuminate,* **AA**)	1.186	20	5.930

Table 2: Yield of different extracts of banana (Musa) pulp.
*W1 was the weight of the extract after lyophilization/evaporation of solvent
*W2 was the weight of the plant powder

Test organism	Diameter of zone of inhibition (mm)										
	Distilled water (Negative control)	Standard antibiotics (Positive control)	Pisang berangan (*Musa acuminate*, AA/AAA) Conc. (mg/disc)			Pisang Mas (*Musa acuminate,* AA) Conc. (mg/disc)			Pisang Nipah (*Musa balbisiana*, BBB) Conc. (mg/disc)		
			1	2	10	1	2	10	1	2	10
ACETONE											
Staphylococcus aureus	-	24[b]	-	-	-	-	-	-	-	-	-
Streptococcus mutans	-	27[c]	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	20[a]	-	-	8.0	-	-	7.0	-	-	7.0
Escherichia coli	-	16[b]	-	-	7.0	-	-	7.0	-	-	7.0
METHANOL											
Staphylococcus aureus	-	24[b]	-	-	-	-	-	-	-	-	-
Streptococcus mutans	-	27[c]	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	20[a]	-	-	7.0	-	-	8.0	-	-	8.0
Escherichia coli	-	16[b]	-	-	7.0	-	-	8.5	-	-	8.0
AQUEOUS SOLUTION											
Staphylococcus aureus	-	24[b]	-	-	-	-	-	-	-	-	-
Streptococcus mutans	-	27[c]	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	20[a]	-	-	-	-	-	-	-	-	-
Escherichia coli	-	16[b]	-	-	-	-	-	-	-	-	-

Table 3: The diameter of inhibition zones of different concentration (1 mg/disc, 2 mg/disc and 10 mg/disc) of acetone, methanol and aqueous solution extract, from three types of banana species (*Musa acuminate* AA/AAA, *Musa acuminate* AA and *Musa balbisiana* BBB) against various organisms. -: No inhibition zone observed
*a: Imipenam, b: Tetracycline, c: Ampicillin

DISCUSSION

Medicinal plant and aromatic compounds have been found to be the sources of bioactive component that is responsible in inhibiting bacterial or fungal growth[9]. Vast research has been taken to determine the substances that have the capacity to inhibit pathogens growth without fa-voring the toxic effect towards the host cell. Many studies have reported the potential antibacte-rial activities of medicinal plant including tropical fruits such as banana[10]. Although some of the antibacterial activities of medicinal plant is well-documented, their antibacterial capacity in-vitro may have a wide degree of variation depending on several factors such as test medium, different methods, tested organisms and the different in nature of the plant[11].

From our results, acetone extract of banana pulp yielded the highest followed by methanol and aqueous solutions. The difference in yield of extracts might be influenced by the capability of extracting solvent to dissolve the bioactive compound and the polarities of various compounds present in the banana pulp[12]. Other factor that contributes to the variations of yield percentage is the presence of different extractable component[13]. Different species of banana showed similar yield of extraction among different extraction solvents suggesting that the chemical composition of the lipophilic extract of ripe pulp of banana fruit from several banana cultivars belonging to the *Musa acuminata* and *Musa balbisiana* species showed similar amounts of lipophilic extractives as well as qualitative chemical composition consistence with data reported by Vilela C. *et al* [14].

Agar disc diffusion test offers a means of qualitative measurement based on the diameter of zone of inhibition for early screening of antibacterial agent in the natural product. Disc diffu-sion assay method was conducted with some modifications[15].

Based on the results obtained, acetone, methanol and aqueous extracts of Pisang Berangan (Musa Acuminata

AA/AAA), Pisang Nipah (*Musa balbisiana* BBB) and Pisang Mas (Musa Acuminata AA) showed no inhibition against gram positive bacteria. Among the extracts tested, both acetone and methanol showed inhibition against gram negative bacteria at 10 mg/ disc con-centration in all types of banana.

Acetone extract of Pisang Berangan (Musa Acuminata AA/AAA) exhibited slightly larger inhibition zone diameter against P. aeruginosa compared to acetone extract of Pisang Nipah (*Musa balbisiana* BBB) and Pisang Mas (Musa Acuminata AA) of the same concentration. Simi-larly, the difference of inhibitory zone diameter was observed on methanol extract where the highest diameter of inhibition zone was seen on Pisang Mas (Musa Acuminata AA) extract at the concentration of 10 mg/disc with 8.5 mm measurement against *E. coli* . However, the present finding contradicted with the previous study, where they reported that the inhibition zone was most likely to be observed on gram positive bacteria due to their characteristics of having only peptidoglycan layer that function as a weak permeability barrier as compared to gram negative bacteria which possess an extra structure of lipopolysaccharides that makes their cell wall highly impermeable to lipophilic solutes[16]. This suggests that the banana pulp of acetone and methanol extract may have a broad range of antibacterial components.

Imam et al., (2011) found that the methanolic extract of Musa sapientum pulp (40 µg/disc) showed a good antibacterial activity against *E. coli* and P. aeruginosa with 17 mm and 16 mm of zone inhibition diameter respectively which are significantly higher than the present study[17]. The extract of the pulp of ripe Musa sapientum effectively inhibited *E. coli* and S. aureus with 25 mm and 27 mm diameter of zone inhibition, respectively[18]. In general, our study demonstrates an insignificant growth inhibition for most types of extract against various organ-isms which are not consistent with previous literature. This might be due to the differences in banana cultivation methods, geographical distribution of the plants as well as slight modification in the test methodologies.

The aqueous extract did not show any antibacterial activity which could be due to low phenolic content extracted by the water[19]. Previous study has found that water is more likely to have low selective properties as it unable to dissolve enough bioactive compounds for antibacte-rial activity. Inhibitory activity from aqueous extract showed relatively low with no activity against gram-negative bacteria[20]. This finding was supported by the fact that water extraction was more likely to dissolve non-bioactive polysaccharides[21] and it is not a good extractor for compound that bind to component made up of lipophylic such as membrane[22]. Therefore, highly polar solvent such as water might not be the best in extracting antibacterial bioactive component in this particular case.

CONCLUSIONS

Based on our results, it can be concluded that the studied plants namely Pisang Berangan (*Musa acuminate* AA/AAA), Pisang Mas (*Musa acuminate* AA) and Pisang Nipah (*Musa balbisiana* BBB) possess antibacterial activity against gram negative bacteria (P. aeruginosa and *E. coli*) without any difference among the species.

However, further research is needed to identify and determine the individual phenolic contents and phytochemicals present in the extracts.

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