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Phytochemical Screening And In Vitro Antibacterial Activity Of Pulp And Peel Extracts From Musa Acuminata Under Different Extraction Conditions

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ABSTRACT

The objectives of the study are to identify and compare the antibacterial activities of the methanolic extracts of pulp and peel of Musa acuminata from Soxhlet and maceration technique. This study compares the percentage yield, phytochemical constituents and antibacterial activities between the pulp and peel of Musa acuminata extracted at different conditions against three types of bacteria;Staphylococcus aureus, Streptococcus pyogenes and Escherichia coli. Four concentrations (25% w/v, 50% w/v, 75% w/v, 100% w/v) were used in the evaluation of antibacterial activity. The percentage yield of both methanolic pulp and peel extracts were found to be greater in Soxhlet extraction (EM01 & EM02) than the maceration method (EM03 & EM04). The phytochemical constituents found in Musa acuminata peel were terpenoids, flavonoids, phenols, tannins, alkaloids, and coumarins while only terpenoids, phenols, tannins and alkaloids were found in Musa acuminata pulp. Reducing sugar was found in both pulp and peel extracts. The results showed significant difference ($p<0.05$) between the effect of each concentrations towards the inhibition of gram-positive bacteria; S. aureus and S. pyogenes while no significant effect was found against gram-negative bacteria, E. coli. The pulp extract from EM01 showed the best antibacterial activities against gram-positive bacteria strains as compared to EM03. In conclusion, the pulp and peel extracts of Musa acuminata Colla (AA group) cv. Pisang Berangan showed antibacterial activities against gram-positive bacteria but not against gram-negative bacteria.

Key words: Musa acuminata, Antibacterial, Soxhlet extraction, Maceration, Phytochemical screening

INTRODUCTION

Majority of the acute bacterial skin infections are caused by Staphylococcus aureus and Streptococci organisms (Gabillot-Carre and Roujeau, 2007).Based on clinical information from Hospital Kuala Lumpur (HKL) Malaysia in year 2006, there were 244 cases of enterococci-infected patients making it the top three most common bacteria causing nosocomial infections (Shalaby et al., 2016).Recent studies conducted in different countries have proven the effectiveness of plant extracts with known antimicrobial substances.Phytochemical constituents play a major role in plant protection against infections by microorganisms. Main antibacterial phytochemicals are polyphenols (eg., flavonoids, terpenes, phenolics acids and coumarins) andalkaloids (Savoia, 2012).Studies have shown that all parts of bananas possess various pharmacological and therapeutic actions towards certain diseases (Joseph et al., 2014).

MATERIALS AND METHODS

Chemicals, Equipment and Materials

Extract preparation involved the use of methanol as solvent. Soxhlet apparatus, water bath, incubator shaker, electric blender machine, Buchner funnel, Whatman filter paper No. 1, rotary evaporator machine (Buchi Rotavapor, Model R-210), freeze-drier machine (Freeze-drier, Alpha 1-2LD plus), conical flasks, scintillated glass vials and amber bottles and beakers were used during the extraction procedures.

Collection and Preparation of Pulp and Peel Extracts

The banana fruits (Musa acuminata Colla (AA group) cv. Pisang Berangan) were obtained from nearby local market in Seri Kembangan, Selangor. Verification has been done by certified Arborist with the voucher number SK1008/16. Matured Musa acuminata fruits weighing approximately 3 kilograms were used in this experiment. The Musa acuminata peel and pulp were washed thoroughly, cut into small pieces and dried in the oven at 45°C for approximately 21 days. The dried banana peel and pulp were made into powders using the electric grinder machine and were kept in airtight amber bottles at 4^{o}C prior to use (Jalani et al., 2014).

Extraction Methods

Two different methods were used for extraction of Musa acuminata which were Soxhlet and maceration extraction. For Soxhlet extraction, 30 g of the banana peel and pulp powders were placed inside a thimble covered in a muslin cloth. The thimble was loaded into the middle chamber of the Soxhlet apparatus. Methanol was added into a distillation flask and was heated at 80^{o}C in a water

bath to initiate the distillation process. For maceration extraction, the banana peel and pulp powders were being macerated with methanol using an incubator shaker at 25^{o}C at 140 rpm for 48 hours. Each pulp and peel were placed inside a conical flask and sealed. The ratio between the powders for extraction to solvent is 1:10 (Gunavathy et al., 2014).Both extracts were then filtered before evaporated using rotary evaporator machine (Buchi Rotavapor, Model R-210) (Ehiowemwenguan et al., 2014).The extracts were converted into powder form by using a freeze-drier machine (Freeze-drier, Alpha 1-2LD plus) at -50^{o}C for 48 hours. The weight of the powdered extracts was measured then the extract was stored tightly in airtight container at -80^{o}C prior to use. Powdered extracts of Soxhlet pulp (EM01) and peel (EM02) and maceration pulp (EM03) and peel (EM04) were calculated for their percentage yield by applying the following formula:

$$\frac{\textbf{Weight of the extract after lyophilisation}}{\textbf{Weight of the powdered plant material}} \times \mathbf{100}$$

Phytochemical Screening and Identification of Reducing Sugar in Musa acuminata Extracts

The methanolic extracts of Musa acuminata were then analysed for terpenoids, flavonoids, phenols, saponins, tannins, alkaloids, glycosides, sterols, lipids and coumarins contents. Fehling's test was used to identify reducing sugar contents in the peel and pulp of Musa acuminata. Each test was conducted in triplicate to ensure the validity of the result.

Test for terpenoids

0.5 g of the extract was mixed with 2 mL of chloroform. Then, 3 mL of concentrated sulphuric acid was carefully added to form a layer. The presence of terpenoids is indicated by a reddish-brown colouration of the interface (Tiwari et al., 2011).

Test for flavonoids

Extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (Tiwari et al., 2011).

Test for phenols

2 mL of the extract was added to 3 mL of 5% ferric chloride solution. Then, 5 drops of ferric-chloride potassium ferrycyanide was added to the mixture. The presence of phenol is indicated by the presence of dark green precipitate (Kujur et al., 2010).

Test for saponins

0.5 g of extract was shaken with 2 mL of water. If foam produced persists for 10 minutes, this indicated the presence of saponins (Tiwari et al., 2011).

Test for tannins

0.5 g of the extract was added to 0.1% ferric chloride. The presence of gallic tannins is indicated by a blue black colouration whereas the presence of cathecholic tannins is indicated by a brownish or green black colouration (Ayoola et al., 2014).

Test for alkaloids

1 mL portions of each extracts was acidified with 3 drops of 1M hydrochloric acid and treated with 5 drops of Mayer's regent (potassium mercuric Iodide). Formation of a yellow or white coloured precipitate or turbidity indicated the presence of alkaloids (Belay and Sisay, 2014).

Test for glycosides

Glacial acetic acid was added to the extract. Then, a few drops of 5% chloride and concentrated sulphuric acid were added. The formation of a reddish-brown colouration at the junction of two layers and bluish green colour in the upper layer indicated the presence of glycosides (Ahirrao et al., 2011).

Test for sterols

2 mL of concentrated sulphuric acid was added to a small quantity of extract. The presence of sterols is indicated by the formation of purple ring at the upper surface (Ahirrao et al., 2011).

Test for lipid

Two drops of extract was placed on a filter paper. The presence of oil on filter paper indicated the presence of lipid (Ahirrao et al., 2011).

Test for coumarins

3 mL of sodium hydroxide was added to 2 mL of the extract. The formation of yellow colour indicated the presence of coumarins (Savithramma et al., 2011).

Test for Fehling's

The extract was dissolved in 5 mL of distilled water and then filtered. The filtrate was boiled with 5-8 drops of Fehling's solution A and B for a few minutes. An orange red or brick red precipitate formation indicated the presence of reducing sugars(Tiwari et al., 2011).

Microorganisms and Reference Antibiotics

Musa acuminata peel and pulp were used for the preparation of antibacterial screening against three types of microorganism;Staphylococcus aureus, Streptococcus pyogenes, and Escherichia coli. All of the bacteria were obtained from Medical Science Laboratory, Cyberjaya University College of Medical Sciences, Cyberjaya, Malaysia. Mueller-Hinton agar and broth had been selected to be used in this study. Gentamicin (10µg/disc) was used as the positive control against Staphylococcus aureus, Streptococcus pyogenes, and Escherichia coli to confirm that their growths were inhibited by antibiotics. Methanol was used as negative control group.

Antibacterial Screening

Kirby-Bauer disc diffusion method for anti-microbial susceptibility testing was carried out to assess the presence of antibacterial activity of Musa acuminata peel and pulp extracts. A blank sterilised filter paper disc was used. The test discs were prepared by incorporating 100µL (1g/ml) of each extracts to 10 mm of sterilised filter paper disc (Whatman filter paper No. 1). The final disc concentrations were impregnated with four different concentrations of 25% w/v, 50% w/v, 75% w/v and 100% w/v. The discs were fully soaked into the respective concentrations and were incubated at 37°C for 24 hours. The impregnated test discs were used immediately. The zone of inhibition was further analysed by its strength of antibacterial activity. There are four category of strength of activity and each of them wascharacterised by inhibition ability such that, strong activity was indicated for an inhibition of more than 15 mm, moderate activity ranging from 10.00 to 14 mm, weak activity when diameter of inhibition was less than or equal to 9 mm. No inhibition was considered as zero activity.

RESULTS

Methanolic Extraction

Table 1 illustrates the percentage yield of extracts from Soxhlet extraction method which showed higher percentage than the maceration technique.

Table 1. The final weight and percentage yield of Musa acuminata pulp and peel crude extracts from two different methods

Extraction Code	Extraction Yield (g)	% Yield
EM01	5.72	38.13
EM02	4.69	31.27
EM03	2.45	8.17
EM04	2.34	7.80

Phytochemicals and Reducing Sugar Analysis

Table 2 describes the phytochemicals present in the peel extracts which are greater than the pulp extracts.

Table 2. The phytochemical screening of peel and pulp of Musa acuminataColla (Group AA) cv. Pisang Berangan methanolic extracts

Test	EM01	EM02	EM03	EM04
Terpenoids	+++	+++	+++	+++
Flavonoids	---	+++	---	+++
Phenols	+++	+++	+++	+++
Saponins	---	---	---	---
Tannins	+++	+++	+++	+++
Alkaloids	+++	+++	+++	+++
Glycosides	---	---	---	---
Sterols	---	---	---	---
Lipids	---	---	---	---
Coumarins	---	+++	---	+++

Fehling's (Reducing sugar)	+++	+++	+++	+++

Evaluation of Antibacterial Properties of Musa acuminataPeel and Pulp Extracts

As shown in **Table 3**, for antibacterial activity of EM01 and EM02, gram-positive bacteria strain Staphylococcus aureus exhibited a zone of inhibition of antibacterial activity with mean ± SEM ranging from 13 mm to 16 mm from all of the EM01 concentrations. As for Streptococcus pyogenes, inhibition of bacteria strain was observed in EM01 of concentration 100% w/v with mean ± SEM of 21.8 mm of inhibition diameter. The mean difference is significant when compared with other concentrations, the positive control and the negative control with ($p<0.001$). A strong inhibition of growth of S.aureus was also observed in the EM02 at concentration of 100% w/v with mean ±SEM of 16.9 mm. Musa acuminata peel extracts were also shown to inhibit S. pyogenes at concentration of 50% w/v with mean ± SEM of 15.5 mm in diameter. However, zero inhibition activities were presented by both EM01 and EM02 extracts in gram-negative strain Escherichia coli.

Meanwhile,EM03 and EM04 extracts showed moderate inhibition of gram-positive bacteria strain Staphylococcus aureus with a zone of inhibition ranging from 10 to 14 mm in diameter for EM03 while the 100% w/v and 75% w/v of EM04 inhibited at 17 mm and 14 mm respectively as depicted in **Table 4**. As for Streptococcus pyogenes, inhibition of bacteria strain was observed in EM03 extract at concentration of 75% w/v and 100% w/v with mean ± SEM diameter of 12.9 mm and 14.1 mm respectively. The EM04 extract at concentration of 50% had shown to inhibit Streptococcus pyogenes moderately with a mean ± SEM diameter of 13.9 mm. In the meantime, no inhibition activities were presented by gram-negative strain Escherichia coli in both EM03 and EM04 extracts.

Table 3. The mean ± SEM, antibacterial activity of various concentrations from Soxhlet extracts of pulp and peel against the selected bacteria

Bacteria	Zone of inhibition (mm)									
	EM01				EM02				Positive Control	Negative control
	25%	50%	75%	100%	25%	50%	75%	100%		
Gram-positive										
Staphylococcus aureus	13.8±0.2^{d,i,j}	14.6±0.2^{i,j}	15.5±0.7^{i,j}	16.1±0.2^{a,i,j}	0^{h,i}	0^{h,i}	0^{h,i}	16.9±0.2^{e,f,g,h,i,j}	22.05^{a,b,c,d,e,f,g,h,j}	0^{a,b,c,d,h,i}
Streptococcus pyogenes	0^{d,i}	0^{d,i}	0^{d,i}	21.8±1.0^{a,b,c,i,j}	0^{f,i}	15.5±0.0^{e,g,h,i,j}	0^{f,i}	0^{f,i}	24.75^{a,b,c,d,e,f,g,h,j}	0^{d,f,i}
Gram-negative										
Escherichia coli	0	0	0	0	0	0	0	0	25.10	0

[a] statistically significant when compared to pulp 25%, [b] statistically significant when compared to pulp 50%, [c] statistically significant when compared to pulp 75%, [d] statistically significant when compared to pulp 100%, [e] statistically significant when compared to peel 25%, [f] statistically significant when compared to peel 50%, [g] statistically significant when compared to peel 75%, [h] statistically significant when compared to peel 100%, [I] statistically significant when compared to positive control, [j] statistically significant when compared to negative control.

Table 4. The mean ± SEM, antibacterial activity of various concentrations from maceration extracts of pulp and peel against the selected bacteria

Bacteria	Zone of inhibition (mm)									
	EM03				EM04				Positive Control	Negative control
	25%	50%	75%	100%	25%	50%	75%	100%		
Gram-positive										
Staphylococcus aureus	10.2±0.2[b,c,d,i,j]	13.3±0.1[a,d,i,j]	13.9±0.1[a,d,i,j]	14.9±0.2[a,b,c,i,j]	0[g,h.i.j]	0[g,h,i,j]	14.5±0.1[e,f,h,i]	17.2±0.6[g,h,i]	22.05[a,b,c,d,e,f,g,h,i,j]	0a,b,c,d,g,h,i
Streptococcus pyogenes	0[c,d,i]	0[c,d,i]	12.9±0.1[a,b,d,i,j]	14.1±0.2[a,b,c,i,j]	0[f,i]	13.9±0.2[e,g,h,i,j]	0[f,i]	0[f,i]	24.75[a,b,c,d,e,f,g,h,j]	0[c,d,f,i]
Gram-negative										
Escherichia coli	0	0	0	0	0	0	0	0	25.10	0

[a] statistically significant when compared to pulp 25%, [b] statistically significant when compared to pulp 50%, [c] statistically significant when compared to pulp 75%, [d] statistically significant when compared to pulp 100%, [e] statistically significant when compared to peel 25%, [f] statistically significant when compared to peel 50%, [g] statistically significant when compared to peel 75%, [h] statistically significant when compared to peel 100%, [I] statistically significant when compared to positive control, [j] statistically significant when compared to negative control.

DISCUSSION

Extraction method that involves heating does produce greater yield than the cold extraction technique. The quantity of extracts obtained was influenced by different extraction conditions. An increase in temperature during extraction procedure produced higher yield (Shehadi et al., 2014). Furthermore, the presence of different extractable compounds had contributed to variations in percentage of yield (Jalani et al., 2014). It may be due to the capability of the solvent to dissolve the compounds under different conditions. Moreover, some bioactive components dissolve in a various favourable conditions. Highest yield was commonly observed when using methanol or ethanol and their mixture with water as solvent for extraction (Nur Syukriah et al., 2014).

The bioactive compounds present in both extraction methods are equal. In respect to different extraction temperature effects, the presence or absence of phytochemicals were not influenced by a change in temperature (Shehadi et al., 2014). The phytochemical analysis has revealed the presence of terpenoids, flavonoids, phenols, tannins, alkaloids, coumarins and reducing sugar in the peel extracts. This shows that these compounds are polar components and are abundant in nature, despite of a change in extraction temperature. Simultaneously, it is also found that, polar solvents such as methanol and butanol extracted the most effective bioactive component; alkaloids and flavonoids (Alabri et al., 2014).

Additionally, the pulp extracts showed lesser amount of compound than the peel extracts. The phytochemical analysis of pulp extracts revealed the presence of terpenoids, phenols, tannins, alkaloids, and reducing sugar while flavonoids and coumarins are absent. A previous study involving phytochemical screening of Musa rasthalifruit revealed the absence of coumarins in the pulp extracts but shows a positive result for flavonoids test (Frederick and Mani, 2016).Varieties in banana cultivar show different levels of flavonoids and total phenolic contents (Babu et al., 2012).This may support the result of an absence in flavonoids from pulp extracts of Musa acuminataColla (AA group) cv. Pisang Berangan. On the other hand, Fehling's test was conducted on Musa rasthali fruit and shows the presence of reducing sugar (Frederick and Mani, 2016).Saponins, glycosides, sterols, and lipids do not show positive results throughout the observation of the phytochemical screening. This is observed in both peel and pulp from both extraction methods. However, saponins, glycosides,and steroids are present in the methanol extracts of unripe green fruit peel of Musa acuminata (Gunavathy et al., 2014).Apart from that, an ethanolic extracts of Musa sapientumpeel also showed the presence of glycosides and saponins (Ehiowemwenguan et al., 2014).In another study, there

were absence of saponins from peel of methanolic extracts of Musa acuminataColla and Musa sapientum(Audu et al., 2015).

The absence of saponins from pulp and peel are further supported by a study showing the absence of saponins from the methanolic extracts of the skin and flesh of Musa paradisiaca(Bashir et al., 2015).Recent study has shown the absence of saponins from Musa paradisiacal butanolic peel extracts (Kamali et al., 2016).Some researchers stated that saponins are difficult to be isolated in its purest form due to its high molecular weight (Doughari, 2012).Dehydration of banana fruits during the drying procedure may cause volatile substances to be lost, degraded or oxidised thus influencing the preservation of phytochemicals (Arvanitoyannis and Mavromatis, 2009; Azwanida, 2015).Besides, the solubility of phytochemicals in the solvent methanol alone is lesser when compared to the utilisation of several solvents for extraction. The use of different solvent polarity may somehow affect the solubility of the extracted compounds.Based on the observation from antibacterial activity of pulp and peel extracts from both Soxhlet and maceration methods, the methanolic peel extracts of Musa acuminata have shown significant effects towards gram-positive bacteria but the results was different on types of bacteria strain. A significant increase in diameter of inhibition was shown from effects of pulp and peel extracts against gram-positive bacteria S. aureus and S. pyogenes.

However, the zones of inhibition of EM03 extracts have shown lesser diameter of inhibition when compared to EM01 extracts. This could probably be due to improper and inadequate diffusion of extracts into the disc. There is a possibility that some extracts are sticking to the base of plate used to contain the impregnated disc, thus less absorption and diffusion of extracts into the disc. Most likely additional extraction (eg., ethy acetate) is required to remove small glucosides and proteins in extracts.Staphylococcus aureus strain was susceptible to be inhibited at highest concentration of the peel extracts. There was exception in S. pyogenes strains. The EM02 extracts showed inhibition against gram-positive S. pyogenes with diameter of zone of inhibition 15.6 mm at concentration 50% w/v. This could be due to a probability of having optimum concentration for inhibition at that particular dose.

Above all, no inhibition of E. coli was recorded from extracts of banana peel and pulp from both extraction methods. The resistance of E. coli towards any concentration of both pulp and peel extracts is consistent with a prior study stating that the macerated ethanolic extracts from rambutan peel, mango peel, mango seed and tamarind seed coat showed no inhibition effects towards E. coli and C. albicans while the hot water banana peel extracts showed no antibacterial activity towards other gram-negative bacteria strain P. aeruginosa(Tewtrakul et al., 2008).This shows that gram-

negative bacteria are resistance towards antibacterial substances due to the presence of lipopolysaccharide layer which limits the penetration of antimicrobial agents (Simoes et al., 2009).

Based on the aforementioned antibacterial activity of both extraction methods, it shows that Musa acuminata pulp and peel extracts possess antibacterial activities towards gram-positive bacteria S. aureus and S. pyogenes. The susceptibility of the pulp and peel extracts of Musa acuminata towards gram-positive bacteria could be due to the alteration of the membrane structure of the bacterial cell wall that breaks hydrogen bond that functions in maintaining rigidity and permeability of the cell (Modarresi-Chahardehi et al., 2012). The inhibition of gram-positive bacteria S. aureus and S. epidermidis was also shown in a data collection stating that banana peel extracts showed antibacterial activity towards gram-positive bacteria strains; S. aureus and S. epidermidis(Sukatta et al.,2010).

As observed from the results obtained, the antibacterial activity differs from previous study stating that the methanolic extracts of pulp from three different cultivars of bananas namely, Pisang Berangan (Musa acuminata AA/AAA), Pisang Mas (Musa acuminata AA) and Pisang Nipah (Musa balbisianaBBB) showed positive antibacterial activity against gram-negative bacteria of strains E. coli with 8.6 mm zone of inhibition and P. aeruginosa with 8.0 mm zone of inhibition but no inhibition was recorded towards gram-positive bacteria S. aureus and S. mutans(Jalani et al., 2014).

Analysis of antibacterial effects of Musa sapientumpeel stated that growth of gram-positive bacteria S. aureus was inhibited at higher minimum bactericidal concentration (MBC) when compared to inhibition of gram-negative bacteria E. coli and K. pneumonia(Ehiowemwenguan et al., 2014).This shows that gram-negative bacteria have higher susceptibility towards ethanolic extracts of banana peel. Unpredictable and inconsistent antibacterial activity towards inhibition of bacteria between studies might be due to the absence in other important antimicrobial agents such as saponins, glycosides, steroids, coumarins and flavonoids.

It can be concluded that the methanolic pulp and peel extracts produced from Soxhlet extraction gives better result as compared to using maceration technique for extraction. Soxhlet extraction technique gives greater percentage yield than the latter. Specifically, pulp extracts from Soxhlet extraction shows the best antibacterial activity. The manipulation of temperatures may affect the constituents present in plant materials but there is possibilities that at even higher temperature, the antibacterial activities of banana extracts be greater and may somehow be at equal level as the action of commonly used antibiotics. However, the phytochemical analysis showed equal

constituents present in pulp and peel extracts from different extraction conditions. The peel and pulp of Musa acuminata extracts showed antibacterial effects towards gram-positive bacteria but not against gram-negative bacteria. The characterisation of cell wall structure between gram-positive and gram-negative bacteria could be a factor for inhibition activity.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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