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Effect of Preparation and Extraction Parameters of Banana (*Musa balbisiana* cv. Saba) Inflorescence on their Antibacterial Activities

(Kesan Penyediaan dan Parameter Pengekstrakan Jantung Pisang (*Musa balbisiana* cv. Saba) ke atas Aktiviti Antibakteria)

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ABSTRACT

*The study aimed to determine the influence of sample preparation and extraction parameters on the antibacterial activity of inflorescences from banana (*Musa balbisiaba *cv. Saba). Banana inflorescences were extracted using various solvent extractions and tested for antibacterial activity using agar-well diffusion assay against gram-positive bacteria* (Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes *and* Brochothrix thermosphacta) *and gram-negative bacteria* (Salmonella typhimurium, Salmonella enteritidis, Escherichia coli O157:H7, Enterobacter sakazakii, Yersinia enterocolitica *and* Vibrio parahaemolyticus) *The effects of geographical origin, drying methods and extraction parameters (sample-to-solvent ratio, extraction time and temperature as well as methanol to solvent ratio) on antibacterial activity of the banana by-product were carried out. Among all the extracts evaluated, methanolic extract from the buds showed significant higher inhibitory against all gram positive bacteria ranging from 12.56-13.54 mm. Interestingly, no significant different (*p>*0.05) was observed on the effect of geographical origin as well as extraction methods on the antibacterial capacity. Meanwhile, the extracts produced from 50°C oven dried sample seem to have comparable antibacterial activity with the freeze dried samples. Extraction parameters (sample-to-solvent ratio, extraction time and temperature as well as methanol to solvent ratio) were found responsible in determining the efficacy of the antibacterial. In conclusion, methanolic extracts from banana inflorescence buds could be a new source of natural antibacterial and further bioassay guided fractionation should be carried out to determine the bioactive compounds and their biological activities.*

Keywords: Antibacterial; banana by-product; extraction methods; methanolic

ABSTRAK

*Kajian ini bertujuan menentukan pengaruh penyediaan sampel dan faktor pengekstrakan ke atas aktiviti antibakteria jantung pisang (*Musa balbisiaba *cv. Saba). Jantung pisang telah diekstrak dengan menggunakan pelbagai pelarut dan diuji untuk aktiviti antibakteria menggunakan kaedah penyerapan agar terhadap bakteria gram positif* (Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes *dan* Brochothrix thermosphacta) *dan bakteria gram negatif* (Salmonella typhimurium, Salmonella enteritidis, Escherichia coli O157:H7, Enterobacter sakazakii, Yersinia enterocolitica *dan* Vibrio parahaemolyticus). *Kesan sampel yang diperoleh daripada kedudukan geografi, kaedah pengeringan dan kaedah pengekstrakan (nisbah sampel-ke-pelarut, masa pengekstrakan dan suhu serta metanol kepada nisbah air) yang berbeza ke atas aktiviti antibakteria produk sampingan pisang telah dilakukan. Ekstrak metanolik jantung pisang didapati memberikan kesan perencatan yang lebih kuat secara signifikan terhadap bakteria gram positif dengan julat 12.56-13.54 mm. Tidak ada perbezaan yang signifikan (*p>*0.05) diperhatikan kepada pengaruhan kedudukan geografi dan kaedah pengekstrakan ke atas kapasiti antibakteria. Sementara itu, ekstrak yang dihasilkan melalui pengeringan ketuhar pada suhu 50°C didapati mempunyai kesan antibakteria yang setara dengan sampel yang dikering sejukbeku. Faktor pengekstrakan (nisbah sampel-ke-pelarut, pengaruh masa dan suhu serta metanol kepada nisbah pelarut) didapati mempengaruhi kesan aktiviti antibakteria ini. Secara kesimpulannya, ekstrak metanolik daripada jantung pisang berpotensi dijadikan sumber baru antibakteria semula jadi dan kajian lanjut harus dilakukan untuk menentukan sebatian bioaktif serta aktiviti biologi mereka.*

Kata kunci: Antibakteria; kaedah pengekstrakan; metanolik; produk sampingan pisang

INTRODUCTION

Waste and by-products from agricultural industries are produced abundantly without profitable market values. However, waste management and promotion of green technologies have apparently driven the shift of public focus towards converting by-products into more value added products (Sim & Wu 2010). In Malaysia, nearly 34 thousand hectares of land had been used for bananas and plantains cultivation (Abdul Khalid et al. 2006) with the total production of 325353 tons in 2007 (DOA 2007) and producing by-products of approximately 220 tons metric per hectares every year. Currently, the potential utilization

of banana by-products is very limited and eventually causes open burning, which could lead to environmental pollutions (Oliveira et al. 2007). One of the efforts would be the exploration of natural products from banana by-products since banana is the world major food crops that could be an excellent source of raw material for bioactive supplies.

Extraction for plant bioactive compounds involves solvent diffusion into matrices and solubilized those natural compounds with similar polarity (Tiwari et al. 2009). However, the success in determination of plant bioactive compounds are largely dependent on the extraction parameters used which include the plant maturity (Mokbel & Hashinaga 2005), geographical origin (Jordan et al. 2013), handling and preparation of plant materials (Joshi et al. 2009), choice of solvents (Ao et al. 2008) and extraction conditions (Koh et al. 2009). Unfortunately, none of these is proven superior in isolating and separating biological active compounds from the broad diversity of structures and massive functionalities of molecules found in natural products.

Several studies suggested that extraction parameters of plant extracts could have significant effect on their bioactivity. However, the outcomes remain controversial and it is sample dependent. Moreover, there is very little information on the effect of extraction methods on the bioactivity of *Musa* spp. by-products. A recent study by Padam et al. (2012) indicating that methanol served as a better solvent in the extraction of antimicrobial and antioxidant from banana (*Musa paradisiacal* cv. Mysore) inflorescence. Therefore, there is a need to investigate the effect of extraction solvents, geographical differences and drying treatments on the antibacterial activity of banana inflorescence in order to provide better insight of the factors that could affects the quality of bioactive extracts.

Materials and Methods

Materials

The samples of banana inflorescence (*Musa balbisiana* cv. Saba) were obtained from the local plantations in the areas of Kota Belud, Kota Marudu, Tuaran and Tamparuli in Sabah. The banana variety was authenticated by a botanist from the Sabah Agriculture Department and voucher specimen (BORH 1519) was deposited in BORNENSIS Herbarium, Institute of Tropical Biology and Conservation, Universiti Malaysia Sabah. *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 12932, *Brochothrix thermophacta* ATCC 11509, *Salmonella Enteritidis* ATCC 13076, *Salmonella Typhimurium* ATCC 13311, *Enterobacter sakazakii* ATCC 51329, *Vibrio parahaemolyticus* ATCC 17802 and *Yersinia enterolitica* ATCC 23715 were obtained from Microbiologics, USA. Prior to use, the bacteria were inoculated into nutrient broth (Oxoid, England) and incubated for 24 h. A single colony was picked and streaked on a slant nutrient agar (Oxoid, England) as stock culture. The stock culture was kept at 4°C prior to further preparation.

Sample Preparation

The banana inflorescence buds were separated from the bracts, cleaned and dried at 50°C using a factory scale dehydrating oven (Memmert, Germany) for 72 h until the sample's moisture content remains stable at 10.0±0.5%. The moisture content of the dried inflorescence samples were examined using a moisture analyser (Metler Toledo, Switzerland). The samples were ground into powder using an electric blender (Panasonic, Japan) and sieved through a mesh at the size of approximately 1.0 mm^2. The powder was kept in airtight plastic bags at 4°C until further use.

Solvent Extraction of Samples

Banana inflorescence buds (10 g) was individually extracted using 100 mL of petroleum ether, hexane, chloroform, ethyl acetate, isopropanol, ethanol, methanol and deionized water respectively by direct infusion using thermostated water bath (Wisebath, Korea) at 30°C for 24 h with 125 rpm constant shaking. All extracts were vacuum filtered using Whatman filter paper No.1 and evaporated under reduced pressure at 40°C using a rotary evaporator (Buchi, Switzerland). Dried extracts were kept at 4°C prior to be assayed for antibacterial activity. Methanolic extracts of oven dried inflorescence buds of *Musa balbisiana* cv. Saba obtained from different geographical locations (Kota Belud, Kota Marudu, Tuaran and Tamparuli districts) were extracted at similar conditions and extracts were kept at -20°C until further analysis.

Determination of Antibacterial Activity of *Musa Balbisiaba* cv. Saba Extracts

Preparation of Bacterial Cultures

The bacteria were transferred to a fresh nutrient agar and incubated at 35°C for 24 h. A single colony of each bacterium was picked and inoculated into sterile tryptone soy broth (Merck, Germany) and incubated at 35°C for 18 h. Following the incubation, the broth containing cultures was centrifuged at 2700 × g (Kubota, Japan) for 5 min and the resulting pallet was washed and re-suspended using sterile ringer's solution. Optical density of each culture was adjusted (based on a predetermined standard growth curve of the bacteria against absorbance measured at 540 nm) to achieve suspension containing approximately 10^7 CFU/mL. The diluted bacterial suspension was used for the antibacterial assay.

Antibacterial Assay using Well Diffusion Method

1 mL of bacterial suspension (10^7 CFU/mL) was dispensed into plates containing approximately 20 mL sterilized tryptone soy agar and mixed evenly. The agar plates were left to solidify at room temperature before four

equidistant wells (6 mm diameter) were cut out using a sterile cork borer. The by-product extracts were diluted in their respective extraction solvents to yield a concentration of 100 mg/mL and 25 µL of each extract was pipetted into a well in duplicate with 5 mg extract each. The plates were incubated at 35°C for 24 h (Valgas et al. 2007). All assays were done in triplicate. The diameters of inhibition zones were measured in mm (including the 6 mm well diameter).

Effect of Extraction Techniques

Conventional extraction was performed in shaking water bath for 24 h with temperature controlled at 30°C while reflux at 65°C for 3 h with condenser tube equipped. Microwave assists extractions were performed using home-used microwave (Sharp, Japan) and heated at high energy option on microwave while ultrasonic assist extractions were done using ultrasonic bath (Bransonic, USA) at 43 khz, both for 15 min. All the extractions were carried out using methanol at sample to solvent ratio of 1: 10. The extracts were then filtered and dried under vacuum rotary evaporator and kept at -20°C until further analysis.

Effect of Drying Methods

Banana inflorescence buds were subjected to 3 different drying methods: oven dry (50°C and 80°C), sun dry and freeze dry. In the sun-drying method, banana inflorescences were dried under the sun light. In the freeze-drying treatment, banana inflorescence samples were lyophilized at -20°C overnight before being transferred into in a freeze-dryer (Christ Alpha 1–4, UK) (0.125 mbar and -80°C). All dried banana inflorescences were standardised at moisture content of approximately 10.0±1.0%. The dried banana inflorescences (buds and bracts) were extracted using methanol at sample to solvent ratio of 1:10 in shaking water bath at 30°C for 24 h. The extracts were then filtered and dried using vacuum rotary evaporator and kept at -20°C until further analysis.

Effect of Extraction Parameters on Antibacterial Activity

In the previous section, oven dried sample at 50°C was selected to evaluate the extraction parameters on the antibacterial activity using assay guided method. The method is based on the logical steps of general liquid-solid extraction for each step, the parameter which shows the best antibacterial activity (highest inhibition zone) for most bacterial tested was used to the subsequent steps in which the previous parameter was applied as the fixed variable. However, in the case where there is more than one parameter within a variable that shows high antibacterial activity with no significance differences, the parameter which deemed efficient (less energy, short time or low cost) is selected.

Solvent to Sample Ratio

Extraction of banana inflorescence samples was carried out by varying sample to methanol (solid-liquid) ratio at 1:5, 1:10, 1:15 and 1:20 w/v, respectively. The drying temperature is fixed at 50°C.

Duration of Extraction

In the extraction time, the duration studied was 1, 3, 5, 8, 12, 24 h, respectively. The drying method and solvent to sample ratio were fixed at 50°C oven dried and sample to methanol ratio of 1:10 w/v, which were preselected based on the highest antibacterial activity in the previous section.

Temperature of Extraction

The extraction temperatures of banana inflorescences varied from the room temperature (25°C) to the near boiling point of the selected extraction solvent with an interval of 10°C. The sample undergone 50°C oven dried with sample to methanol ratio of 1:10 w/v and 3 h of extraction time were preselected based on the highest antibacterial activity determined in the previous section.

Aqueous Mixtures of Solvent

The effect of the alcohol solvent-water mixtures on the antibacterial activity of extracts was determined. The methanol-water mixtures were set at 20, 40, 60, 80 and 100% v/v, respectively. The drying method (50°C oven dried), sample to solvent ratio 1:10 w/v, duration of extraction (3 h) and temperature of extraction (35°C) were fixed based on the preselected best method showing the highest antibacterial activity.

Results and Discussion

Effect of Different Extraction Solvents and Geographical Origin

Methanol is proved to be the best solvent for the extraction of antibacterial compounds from banana (*Musa balbisiana* cv. Saba) inflorescence buds with the inhibition ranged from 7.56 to 13.54 mm against all tested bacteria (Table 1). This is probably due to the solvent consisted of a very short hydrocarbon with an attachment of a negatively charged hydroxyl ion which makes it possess a good range of extracting ability based on its high polarity and diffusion coefficient (Lee & Li 1991). It penetrates through the membrane cells by selectively dissolving polar compounds that are having antibacterial properties from the banana inflorescence. Similar finding was reported by Ao et al. (2008) in extracting antibacterial from an ornamental plant *(Ficus microcarpa)*.

Non-polar organic solvent extractions such as hexane and petroleum ether extracts show very low antibacterial activity (6.47 to 10.64 mm) on *Staphylococcus aureus* (SA) and *Bacillus cereus* (BC). Muthuvelan and Raja (2008) suggested that the polarity distribution of antibacterial

TABLE 1. Antibacterial activity of *Musa balbisiana* cv. Saba buds extracts using various extraction solvents

Solvent	Diameter of inhibition zone (mm)					
	SA	BC	LM	VP	YE	BT
H2O	$8.21\pm0.34_{b}$	$9.14\pm0.65_{b}$	$8.15\pm0.24_{b}$	na	na	$8.34\pm0.45_{b}$
MeOH	$13.22\pm0.44_{d}$	$13.54\pm0.75_{d}$	$12.67\pm0.51_{d}$	$12.43\pm0.43_{b}$	$7.56\pm0.33_{a}$	$12.56\pm0.56_{d}$
EtOH	$9.59\pm0.38_{c}$	$10.64\pm0.53_{c}$	$8.44\pm0.43_{b}$	$6.74\pm0.34_{a}$	na	$9.25\pm0.34_{c}$
Isopropanol	$9.21\pm0.23_{c}$	$8.65\pm0.56_{b}$	$8.34\pm0.63_{b}$	na	na	$8.57\pm0.56_{b}$
Acetone	$8.42\pm0.62_{b}$	$8.34\pm0.23_{b}$	$7.64\pm0.24_{a}$	na	na	$7.53\pm0.12_{b}$
EtoAc	$8.72\pm0.24_{b}$	$9.38\pm0.25_{b}$	$8.23\pm0.65_{b}$	na	na	$8.45\pm0.45_{b}$
CHCl3	$7.34\pm0.56_{a}$	$6.47\pm0.72_{a}$	na	na	na	na
Hexane	$7.31\pm0.37_{a}$	$7.53\pm0.62_{a}$	na	na	na	na
Pet. Ether	$7.12\pm0.49_{a}$	$6.78\pm0.54_{a}$	na	na	na	na

Data represent the means ± SD (n=3) including the well diameter
Subscripts represent significance differences between extraction solvents at p<0.05
'na' No Activity
H_2O: Deionized water, MeOH: Methanol, EtOH: Ethanol, EtoAc: Ethyl Acetate, CHCl: Chloroform, Pet. Eth: Petroleum ether
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), YE: *Yersinia enterocolytica* (gram -), BT: *Brohroctrix termosphacta* (gram + anaerobic)
Extracts showed no activity against gram negative bacteria

compounds in plants varies depending on species and plant families. Non-polar solvents may not be a good choice in extracting antibacterial metabolites in this case since most of the targeted metabolites are at the moderately polar end of the spectrum.

Banana inflorescences obtained from different plantations in Kota Belud, Kota Marudu, Tuaran and Tamparuli were evaluated for their antibacterial activity under the same conditions. However, there is no significant difference (p>0.05) between the antibacterial activities of samples (buds) cultivated in different locations. The result is in agreement to the report by Jordan et al. (2013) who found that the geographical origin of the samples do not influence the antimicrobial activity of rosmaric extracts.

EFFECT OF EXTRACTION METHODS

In this study, no significant difference (p>0.05) was observed in antibacterial activity of the extracts obtained from conventional and reflux extraction but their inhibitory activities were significantly higher (p<0.05) than the extracts obtained from emerging ultrasonic and microwave assisted extraction (Table 2). Paniwynk et al. (2001) proposed that reduced recovery of bioactive compounds through ultrasound assisted extraction could be possibly due to the oxidation caused by the highly reactive hydroxyl radicals formed during sonication of aqueous solvent. Microwave assisted extraction uses cells internal water as conductive medium for microwave dielectric, generates heat that might cause thermal degradation of bioactive compounds, thus reduce the bioactivity of the extracts (Omirou et al. 2009). Generally, application of extraction methods with optimum parameters is varied depending on the target of bioactive compounds as well as the type of plants (Biesaga 2011).

EFFECT OF DRYING METHODS

Among the drying methods tested, oven dried (50°C) and freeze-dried inflorescence extract seem to have a stronger antibacterial activity (7.23-13.43 mm) against all tested bacteria (Table 3). However, no significant difference (p<0.05) was found between these method of preparations. Joshi et al. (2009) reported similar retention of epigallocatechin, chlorogenic acid and quercetin glycosides in both freeze-dried and oven-dried 'Redfield' apple which could probably due to the stability of those phenolics in the sample. On the other hand, sun dried buds extract showed a significant lower antibacterial activity. Direct exposure of bioactive compounds to sunlight could

TABLE 2. Effect of extraction methods on antibacterial activity of the methanolic extracts of *Musa balbisiana* cv. Saba buds

Methods	Diameter of inhibition zone (mm)				
	SA	BC	LM	BT	VP
Conventional	$14.34\pm0.69_{b}$	$15.23\pm1.63_{b}$	$12.35\pm1.39_{b}$	$14.25\pm1.22_{b}$	$8.32\pm0.44_{a}$
Reflux	$13.54\pm0.23_{ab}$	$12.63\pm0.55_{a}$	$12.64\pm0.62_{b}$	$14.06\pm0.37_{b}$	$8.24\pm0.52_{a}$
Ultrasonic	$12.62\pm0.62_{a}$	$12.22\pm0.27_{a}$	$10.24\pm0.32_{a}$	$13.14\pm0.58_{a}$	$7.88\pm1.09_{a}$
Microwave	$12.25\pm0.75_{a}$	$12.06\pm1.25_{a}$	$9.43\pm0.63_{a}$	$13.28\pm0.11_{a}$	$7.22\pm1.35_{a}$

Data represent the means ± SD (n=3) including the well diameter
Subscripts represent significance differences between extraction parameters at p<0.05
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), BT: *Brohroctrix termosphacta*(gram + anaerobic)
Extracts showed no activity against other gram negative bacteria

TABLE 3. Effects of various drying methods on the antibacterial activity of *Musa balbisiana* cv. Saba buds extracts

Drying methods	Diameter of inhibition zone (mm)					
	SA	BC	LM	VP	YE	BT
OD 50°C	$13.13\pm0.34_{c}$	$13.45\pm0.43_{c}$	$12.15\pm0.76_{c}$	$13.65\pm0.45_{c}$	$7.23\pm0.34_{a}$	$13.52\pm0.35_{c}$
OD 80°C	$9.34\pm0.52_{a}$	$9.46\pm0.23_{a}$	$7.17\pm0.53_{a}$	$7.23\pm0.23_{a}$	$7.14\pm0.56_{a}$	$8.23\pm0.43_{a}$
FD	$13.13\pm0.45_{c}$	$13.43\pm0.34_{c}$	$12.05\pm0.25_{c}$	$12.53\pm0.64_{c}$	$8.34\pm0.65_{a}$	$12.73\pm0.67_{c}$
SD	$10.56\pm0.37_{b}$	$10.23\pm0.23_{b}$	$9.33\pm0.54_{b}$	$9.23\pm0.32_{b}$	$7.24\pm0.44_{a}$	$9.23\pm0.23_{b}$

Data represent the means ± SD (n=3) including the well diameter
OD: Oven drying, FD: Freeze drying, SD: Sun drying
Subscripts represent significance differences between drying methods at $p<0.05$
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), YE: *Yersinia enterocolytica* (gram -), BT: *Brohroctrix termosphacta* (gram + anaerobic)
Extracts showed no activity against gram negative

facilitate oxidation process which correlated with the reduction of bioactivity (Joshi et al. 2009). Besides, sun drying is commonly associated with prolonged drying times allow degradation of bioactive compounds by enzymatic reaction (polyphenol oxidase and glycosidase) (Shahidi & Naczk 2004). This experiment indicates that oven drying is more practical to be used for banana by-product extraction.

EFFECT OF EXTRACTION PARAMETERS ON ANTIBACTERIAL ACTIVITY

The extraction of antibacterials using sample-to-solvent ratio of 1:5 w/v gave significant lower ($p>0.05$) inhibitory probably due to the limited amount of solvent to dissolve the possible antibacterial compounds. The results showed a ratio of 1:10 w/v between sample and methanol is the best option with the observable inhibition zone of 7.34 to 13.45 mm on all bacteria tested (Table 4). The impact of solid-to-solvent ratio is one of the factors that always been overlooked as a wide range of ratios have been employed for the extraction of phytochemicals from various plant matrices (Mukhopadhyay et al. 2006). Nevertheless, an optimised sample-to-solvent ratio could pin-pointed the important of the extractability of target compounds/ extracts.

Oven dried inflorescence samples (50°C) were extracted using methanol to sample ratio of 10:1 (v/w) at various extraction times to evaluate the effect on its antibacterial activity. The antibacterial activity was at the peak at 3 h of extraction (7.73-13.36 mm) for all tested bacteria and the inhibitory activity did not improve significantly ($p>0.05$) after exposed to the subsequent hours of extraction (Table 5). A slight decline in inhibitory was observed at the extended time of extraction (up to 24 h) probably due to oxidation of the bioactive compounds (Biesaga 2011). Thus, an extraction time of 3 h was selected for the subsequent process to evaluate the effect of temperature on the antibacterial activity of the banana inflorescence extract.

A pure methanol coupled with 50°C oven dried sample ratio of 1:10 w/v and 3 h extraction time were used to evaluate the effect of temperatures on the extraction efficiency of *Musa balbisiana* cv. Saba buds. The increase in extraction temperature at 35°C significantly increases the antibacterial activity of the banana buds extracts against all the bacteria with 8.26-14.25 mm diameter of inhibition (Table 6). This could be due to the increase of kinetic energy as the induction of heat which yields higher due to better solubility (Anne 2008) as well as plant structure disruption (Prasad et al. 2011). However, the increment of high temperature may denature some heat sensitive compounds (Durling et al. 2007). Generalization the effects of temperature may not be suitable in all cases since it is strongly dependent on the typology of the desired compound in the sample (Spigno et al. 2007).

Table 7 shows banana inflorescence extracted with 80 and 100% methanol gave the strongest antibacterial activity against all tested bacteria. The addition of water increase the polarity of the extracting solvent makes it more selective and more antibacterial compounds from the

TABLE 4. Effects of solvent to sample ratios on the antibacterial activity of *Musa balbisiana* cv. Saba buds extracts

Solid to solvent ratio	Diameter of inhibition zone (mm)					
	SA	BC	LM	VP	YE	BT
1:20	$13.22\pm0.35_{b}$	$13.34\pm0.34_{b}$	$12.33\pm0.32_{b}$	$13.53\pm0.63_{b}$	$7.63\pm0.39_{a}$	$13.34\pm0.23_{b}$
1:15	$13.34\pm0.45_{b}$	$13.24\pm0.45_{b}$	$12.23\pm0.34_{b}$	$13.75\pm0.29_{b}$	$7.54\pm0.23_{a}$	$13.25\pm0.26_{b}$
1:10	$13.23\pm0.54_{b}$	$13.45\pm0.35_{b}$	$12.13\pm0.51_{b}$	$13.03\pm0.23_{b}$	$7.34\pm0.83_{a}$	$13.34\pm0.32_{b}$
1:5	$12.23\pm0.23_{a}$	$12.33\pm0.65_{a}$	$11.23\pm0.12_{a}$	$12.23\pm0.31_{a}$	$6.39\pm0.24_{a}$	$12.73\pm0.36_{a}$

Data represent the means ± SD (n=3) including the well diameter
Subscripts represent significance differences between extraction parameters at $p<0.05$
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), YE: *Yersinia enterocolytica* (gram -), BT: *Brohroctrix termosphacta* (gram + anaerobic)
Extracts showed no activity against gram negative

TABLE 5. Effects of extraction time on the antibacterial activity of *Musa balbisiana* cv. Saba buds extract

Extraction time (h)	Diameter of inhibition zone (mm)					
	SA	BC	LM	VP	YE	BT
1	$12.25 \pm 0.24_a$	$12.23 \pm 0.23_a$	$11.35 \pm 0.45_a$	$11.23 \pm 0.24_a$	$6.63 \pm 0.53_a$	$12.23 \pm 0.45_a$
3	$13.34 \pm 0.53_b$	$13.36 \pm 0.33_b$	$12.46 \pm 0.23_b$	$12.62 \pm 0.34_b$	$7.73 \pm 0.24_b$	$13.34 \pm 0.23_b$
5	$13.18 \pm 0.34_b$	$13.52 \pm 0.21_b$	$12.26 \pm 0.87_b$	$12.26 \pm 0.43_b$	$7.87 \pm 0.78_b$	$12.13 \pm 0.25_b$
8	$13.34 \pm 0.23_b$	$13.16 \pm 0.14_b$	$12.22 \pm 0.24_b$	$12.73 \pm 0.32_b$	$7.76 \pm 0.35_b$	$12.64 \pm 0.64_b$
12	$12.25 \pm 0.27_b$	$13.83 \pm 0.27_b$	$12.73 \pm 0.13_b$	$12.35 \pm 0.36_b$	$7.33 \pm 0.75_b$	$12.43 \pm 0.34_b$
24	$12.52 \pm 0.16_b$	$13.23 \pm 0.12_b$	$12.26 \pm 0.32_b$	$12.63 \pm 0.41_b$	$7.72 \pm 0.57_b$	$12.87 \pm 0.32_b$

Data represent the means ± SD (n=3) including the well diameter
Subscripts represent significance differences between extraction times at $p<0.05$
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), YE: *Yersinia enterocolytica* (gram -), BT: *Brohroctrix termosphacta* (gram + anaerobic)
Extracts showed no activity against gram negative

TABLE 6. Effects of extraction temperatures on the antibacterial activity of *Musa balbisiaba* cv. Saba buds extract

Extraction temperature (°C)	Diameter of inhibition zone (mm)					
	SA	BC	LM	VP	YE	BT
25	$13.23 \pm 0.40_b$	$13.24 \pm 0.94_b$	$12.45 \pm 0.67_b$	$12.26 \pm 0.46_b$	$7.24 \pm 0.48_a$	$13.38 \pm 0.63_b$
35	$13.46 \pm 0.39_c$	$14.25 \pm 0.23_c$	$12.23 \pm 0.62_c$	$13.23 \pm 0.17_c$	$8.46 \pm 0.27_b$	$13.47 \pm 0.37_c$
45	$13.34 \pm 0.36_c$	$13.23 \pm 0.57_c$	$12.57 \pm 0.34_b$	$13.24 \pm 0.27_c$	$7.57 \pm 0.73_a$	$12.46 \pm 0.53_b$
55	$12.23 \pm 0.34_a$	$13.57 \pm 0.45_a$	$11.21 \pm 0.53_a$	$11.43 \pm 0.34_a$	$7.28 \pm 0.87_a$	$12.83 \pm 0.73_b$

Data represent the means ± SD (n=3) including the well diameter
Subscripts represent significance differences between extraction temperatures at $p<0.05$
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), YE: *Yersinia enterocolytica* (gram -), BT: *Brohroctrix termosphacta* (gram + anaerobic)
Extracts showed no activity against gram negative

TABLE 7. Effects of methanol to water ratio on the antibacterial activity of *Musa balbisiana* cv. Saba buds extract

Methanol to water (%)	Diameter of inhibition zone (mm)					
	SA	BC	LM	VP	YE	BT
100	$13.53 \pm 0.23_c$	$14.34 \pm 0.83_c$	$12.83 \pm 0.53_c$	$13.73 \pm 0.13_c$	$8.32 \pm 0.23_a$	$13.25 \pm 0.64_c$
80	$13.46 \pm 0.64_c$	$14.98 \pm 0.27_c$	$12.34 \pm 0.34_c$	$13.34 \pm 0.23_c$	$8.23 \pm 0.34_a$	$13.74 \pm 0.34_c$
60	$9.28 \pm 0.23_b$	$8.46 \pm 0.53_b$	$8.23 \pm 0.23_b$	$8.64 \pm 0.56_b$	na	$8.26 \pm 0.32_b$
40	$9.45 \pm 0.73_b$	$9.34 \pm 0.23_b$	$7.23 \pm 0.64_a$	$7.23 \pm 0.53_a$	na	$8.15 \pm 0.57_b$
20	$7.25 \pm 0.83_a$	$7.23 \pm 0.34_a$	$7.23 \pm 0.35_a$	na	na	$7.63 \pm 0.35_a$

Data represent the means ± SD (n=3) including the well diameter
Subscripts represent significant differences between aqueous methanol at $p<0.05$
'na' No Activity
SA*: Staphylococcus aureus* (gram +), BC: *Bacillus cereus* (gram +), LM: *Listeria monocytogenes* (gram +), VP: *Vibrio parahaemolyticus* (gram -), YE: *Yersinia enterocolytica* (gram -), BT: *Brohroctrix termosphacta* (gram + anaerobic)
Extracts showed no activity against gram negative

samples becoming soluble (Kim et al. 2007). However, the addition of more water to alcohol ratio also increases the retention of highly polar non-bioactive polysaccharides which explain the reduction of antibacterial activity on gram negative bacteria (Koh et al. 2009).

CONCLUSION

The results signify preparation and extraction parameters could affect antibacterial activity of banana buds extract. Thus, combination of methanol, 50°C dried sample, sample to solvent ratio of 1:10 v/w and 3 h extraction time were found adequate in extracting desirable quality of antibacterial extracts from the banana by-product. This study also demonstrated that banana inflorescence could be a new potential source of natural antibacterial and further study on the chemical profiles of the effective fraction should be performed.

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REFERENCES

Abdul Khalid, H.P.S., Siti Alwani, M. & Mohd Omar, A.K. 2006. Chemical composition, anatomy, lignin distribution and cell wall structure of Malaysia plant waste fibers. *Bioresources* 1: 220-232.

Anne, S. 2008. Evaluation and optimization of methods for identification of milk thistle. *J. AOAC Int*. 91: 278-286.

Ao, C., Li, A., Elzaawely, A.A., Xuan, T.D. & Tawata, S. 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. *Food Control* 19: 940-948.

Biesaga, M. 2011. Influence of extraction methods on stability of flavonoids. *J. Chromatogr. A*. 1218: 2505-2512.

DOA. 2007. *Report on Crops Hectarage and Production in Sabah 2007*. Department of Agriculture (DOA), Kuala Lumpur, Malaysia.

Durling, N.E., Catchpole, O.J., Grey, J.B., Webby, R.F., Mitchell, K.A., Foo, L.Y. & Poerry, N.B. 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. *Food Chem*. 101: 1434-1441.

Jordan, M.J., Lax, V., Rota, M.C., Loran, S. & Sotomayor, J.A. 2013. Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control* 30: 463- 468.

Joshi, A.P.K., Rupasinghe, H.P.V. & Khanizadeh, S. 2009. Impact of drying process and bioactive phenolics, vitamin C and antioxidant capacity of red-fleshed apple slices. *J. Food Process. Pres*. 35: 453-457.

Kim, J.M., Chang, S.M., Kim, I.H., Kim, Y.E., Hwang, J.H. & Kim, K.S. 2007. Design of optimal solvent for extraction of bio-active ingredients from mulberry leaves. *Biochem. Eng. J*. 37: 271-278.

Koh, G.Y., Chou, G.X. & Liu, Z.J. 2009. Purification of a water extract of Chinese sweet tea plant (*Rubus suavissimus* S. Lee) by alcohol precipitation. *J. Agr. Food Chem*. 57: 5000-5006.

Lee, Y.E. & Li, S.F.Y. 1991. Binary diffusion coefficient of the methanol/water system in the temperature range 30-40°C. *J. Chem. Eng. Data* 36: 240-243.

Mokbel, M.S. & Hashinaga, F. 2005. Antibacterial and antioxidant activities of banana (*Musa* AAA cv. Cavendish) fruits peel. *Am. J. Biochem. Biotech*. 1: 126-132.

Mukhopadhyay, S., Luthria, D.L. & Robbins, R.J. 2006. Optimization of extraction process for phenolics acids from black cohosh (*Cimicifuga racemosa*) by pressurized liquid extraction. *J. Sci. Food Agr.* 86: 156-162.

Muthuvelan, B. & Raja, R.B. 2008. Studies on the efficiency of different extraction procedures on the antimicrobial activity of selected medicinal plants. *World J. Microb. Biotech*. 24: 2837-2842.

Oliveira, L., Cordeiro, N., Evtuguin, D., Torresa, I.C. & Silvestre, A.J.D. 2007. Chemical composition of different morphological parts from 'Dwarf Cavendish' banana plant and their potential as a non-wood renewable source of natural products. *Ind. Crop. Prod*. 26: 163-172.

Omirou, M., Papastylianou, I., Iori, R., Papastephanou, K.K., Ehaliotis, C. & Karpouzas, D.G. 2009. Microwave-assisted extraction of glucosinolates from *Erura sativa* seeds and soils: Comparison with existing methods. *Phytochem. Analysis* 20: 214-220.

Padam, B.S., Tin, H.S., Chye, F.Y. & Abdullah, M.I. 2012. Antibacterial and antioxidative activities of the various solvent extracts of banana (*Musa paradisiaca* cv. Mysore) inflorescences. *J. Biol. Sci*. 12: 62-73.

Paniwynk, L., Beaufoy, E., Lorimer, J.P. & Mason, T.J. 2001. The extraction of rutin from flower buds of *Sophora japonica*. *Ultrason. Sonochem*. 8: 299-301.

Prasad, K.N., Hassan, F.A., Yang, B., Kong, K.W., Ramanan, R.N., Azlan, A. & Ismail, A. 2011. Response surface optimization for the extraction of phenolic compounds and antioxidant capacities of underutilized *Mangifera pajang* koserm. peels. *Food Chem*. 128: 1121-1127.

Shahidi, F. & Naczk, M. 2004. *Phenolics in Food and Neutraceuticals*. Boca Raton: CRC Press.

Sim, E.Y. & Wu, T.Y. 2010. The potential reuse of biodegradable municipal solid wastes (MSW) as feedstock in vermicomposting. *J. Sci. Food Agri*. 90: 2153-2162.

Spigno, G. & Tramelli, L. & De Faveri, D.M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng*. 81: 200-208.

Tiwari, B.K., Valdramidis, V.P., O'Donnel, C.P., Muthukumarappan, K., Bourke, P. & Cullen, P.J. 2009. Application of natural antimicrobials for food preservation. *J. Agr. Food Chem*. 57: 5987-6000.

Valgas, C., de Souza, S.M., Smania, E.F.A. & Artur Smania, J. 2007. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microb*. 38: 369-380.

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