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 balbisiana* 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

**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 these by-products is limited.
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 BORNENIS Herbarium, Institute of Tropical Biology and Conservation, Universiti Malaysia Sabah. Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 11778, Listeria monocytogenes ATCC 12932, Brochothrix thermophaacta 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 at 35°C for 18 h. Following the incubation, the broth containing cultures were centrifuged at 2700 × g (Kubota, Japan) for 5 min and the resulting pellet was washed and re-suspended using sterile ringer’s solution. Optical density of each culture was read by direct infusion using thermostated water bath (Wisebath, Korea) at 30°C. The cultures were diluted 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 suspended using sterile tryptone soy broth (Merck, Germany) and incubated at 35°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 4°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 BALBISIANA 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 pellet 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 (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
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 rosmary 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 1. Antibacterial activity of Musa balbisiana cv. Saba buds extracts using various extraction solvents**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>SA</th>
<th>BC</th>
<th>LM</th>
<th>VP</th>
<th>YE</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>8.21±0.34 a</td>
<td>9.14±0.65 a</td>
<td>8.15±0.24 b</td>
<td>na</td>
<td>na</td>
<td>8.34±0.45 a</td>
</tr>
<tr>
<td>MeOH</td>
<td>13.22±0.44 a</td>
<td>13.54±0.75 a</td>
<td>12.67±0.51 b</td>
<td>12.43±0.43 a</td>
<td>7.56±0.33 a</td>
<td>12.56±0.56 a</td>
</tr>
<tr>
<td>EtOH</td>
<td>9.59±0.38 a</td>
<td>10.64±0.53 a</td>
<td>8.44±0.43 a</td>
<td>6.74±0.34 a</td>
<td>na</td>
<td>9.25±0.34 a</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>9.21±0.23 a</td>
<td>8.65±0.56 a</td>
<td>8.34±0.63 a</td>
<td>na</td>
<td>na</td>
<td>8.57±0.56 a</td>
</tr>
<tr>
<td>Acetone</td>
<td>8.42±0.62 a</td>
<td>8.34±0.23 a</td>
<td>7.64±0.24 a</td>
<td>na</td>
<td>na</td>
<td>7.53±0.12 a</td>
</tr>
<tr>
<td>CHCl3</td>
<td>7.34±0.56 c</td>
<td>6.47±0.72 a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Hexane</td>
<td>7.31±0.37 a</td>
<td>7.53±0.62 a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Pet. Ether</td>
<td>7.12±0.49 a</td>
<td>6.78±0.54 a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Data represent the means ± SD ($n=3$) including the well diameter

Subscripts represent significance differences between extraction solvents at $p<0.05$


SA: Staphylococcus aureus (gram +), BC: Bacillus cereus (gram +), LM: Listeria monocytogenes (gram +), VP: Vibrio parahaemolyticus (gram -), YE: Yersinia enterocolitica (gram -), BT: Brochothrix thermosphacta (gram + anaerobic)

Extracts showed no activity against gram negative bacteria

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

<table>
<thead>
<tr>
<th>Methods</th>
<th>SA</th>
<th>BC</th>
<th>LM</th>
<th>VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>14.34±0.69</td>
<td>15.23±1.63</td>
<td>12.35±1.39</td>
<td>14.25±1.22</td>
</tr>
<tr>
<td>Reflux</td>
<td>13.54±0.23</td>
<td>12.63±0.55</td>
<td>12.64±0.62</td>
<td>14.06±0.37</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>12.62±0.62</td>
<td>12.22±0.27</td>
<td>10.24±0.32</td>
<td>13.14±0.58</td>
</tr>
<tr>
<td>Microwave</td>
<td>12.25±0.75 a</td>
<td>12.86±1.25</td>
<td>9.43±0.63</td>
<td>13.28±0.11 b</td>
</tr>
</tbody>
</table>

Data represent the means ± SD ($n=5$) 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: Brochothrix thermosphacta (gram + anaerobic)

Extracts showed no activity against other gram negative bacteria
Extracts showed no activity against gram negative (gram -), BT:

- Subscripts represent significance differences between extraction parameters at p<0.05
- OD: Oven drying, FD: Freeze drying, SD: Sun drying
- Data represent the means ± SD (n=3) including the well diameter
- Subscripts represent significance differences between drying methods at p<0.05
- Effects of various drying methods on the antibacterial activity of Musa balbisiana cv. Saba buds extracts

<table>
<thead>
<tr>
<th>Drying methods</th>
<th>SA (mm)</th>
<th>BC (mm)</th>
<th>LM (mm)</th>
<th>VP (mm)</th>
<th>YE (mm)</th>
<th>BT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD 50°C</td>
<td>13.13±0.34</td>
<td>13.45±0.43</td>
<td>12.15±0.76</td>
<td>13.65±0.45</td>
<td>7.23±0.34</td>
<td>13.52±0.35</td>
</tr>
<tr>
<td>OD 80°C</td>
<td>9.34±0.52</td>
<td>9.46±0.23</td>
<td>7.17±0.53</td>
<td>7.23±0.23</td>
<td>7.14±0.56</td>
<td>8.23±0.43</td>
</tr>
<tr>
<td>FD</td>
<td>13.13±0.45</td>
<td>13.43±0.34</td>
<td>12.05±0.25</td>
<td>12.53±0.64</td>
<td>8.34±0.65</td>
<td>12.73±0.67</td>
</tr>
<tr>
<td>SD</td>
<td>10.56±0.37</td>
<td>10.23±0.23</td>
<td>9.33±0.54</td>
<td>9.23±0.32</td>
<td>7.24±0.44</td>
<td>9.23±0.23</td>
</tr>
</tbody>
</table>

Data represent the means ± SD (n=3) including the well diameter

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 (Mukhopadhay 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>
<thead>
<tr>
<th>Solid to solvent ratio</th>
<th>SA (mm)</th>
<th>BC (mm)</th>
<th>LM (mm)</th>
<th>VP (mm)</th>
<th>YE (mm)</th>
<th>BT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:20</td>
<td>13.22±0.35</td>
<td>13.34±0.34</td>
<td>12.33±0.32</td>
<td>13.53±0.63</td>
<td>7.62±0.39</td>
<td>13.34±0.23</td>
</tr>
<tr>
<td>1:15</td>
<td>13.34±0.45</td>
<td>13.24±0.45</td>
<td>12.23±0.34</td>
<td>13.75±0.29</td>
<td>7.54±0.23</td>
<td>13.25±0.26</td>
</tr>
<tr>
<td>1:10</td>
<td>13.23±0.54</td>
<td>13.45±0.35</td>
<td>12.13±0.51</td>
<td>13.03±0.23</td>
<td>7.34±0.83</td>
<td>13.34±0.32</td>
</tr>
<tr>
<td>1:5</td>
<td>12.23±0.23</td>
<td>12.33±0.65</td>
<td>11.23±0.12</td>
<td>12.23±0.31</td>
<td>6.39±0.24</td>
<td>12.73±0.36</td>
</tr>
</tbody>
</table>
Extracts showed no activity against gram negative bacteria (Koh et al. 2009). which explain the reduction of antibacterial activity on gram negative (gram -), BT:

Extracts showed no activity against gram negative (gram -), BT:

Subscripts represent significance differences between extraction times at p<0.05

Data represent the means ± SD (n=3) including the well diameter

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.

CONCLUSION

The results signify preparation and extraction parameters could affect antibacterial activity of banana buds extract.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Science, Technology and Innovation, Malaysia (MOSTI) for the financial support (Science Fund, No. 02-01-10-SF0061) and postgraduate scholarship under National Science
Fellowship (NSF). The technical assistance by the Department of Agriculture Sabah on the banana species authentication is greatly appreciated.

REFERENCES


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Received: 22 July 2014
Accepted: 25 May 2015