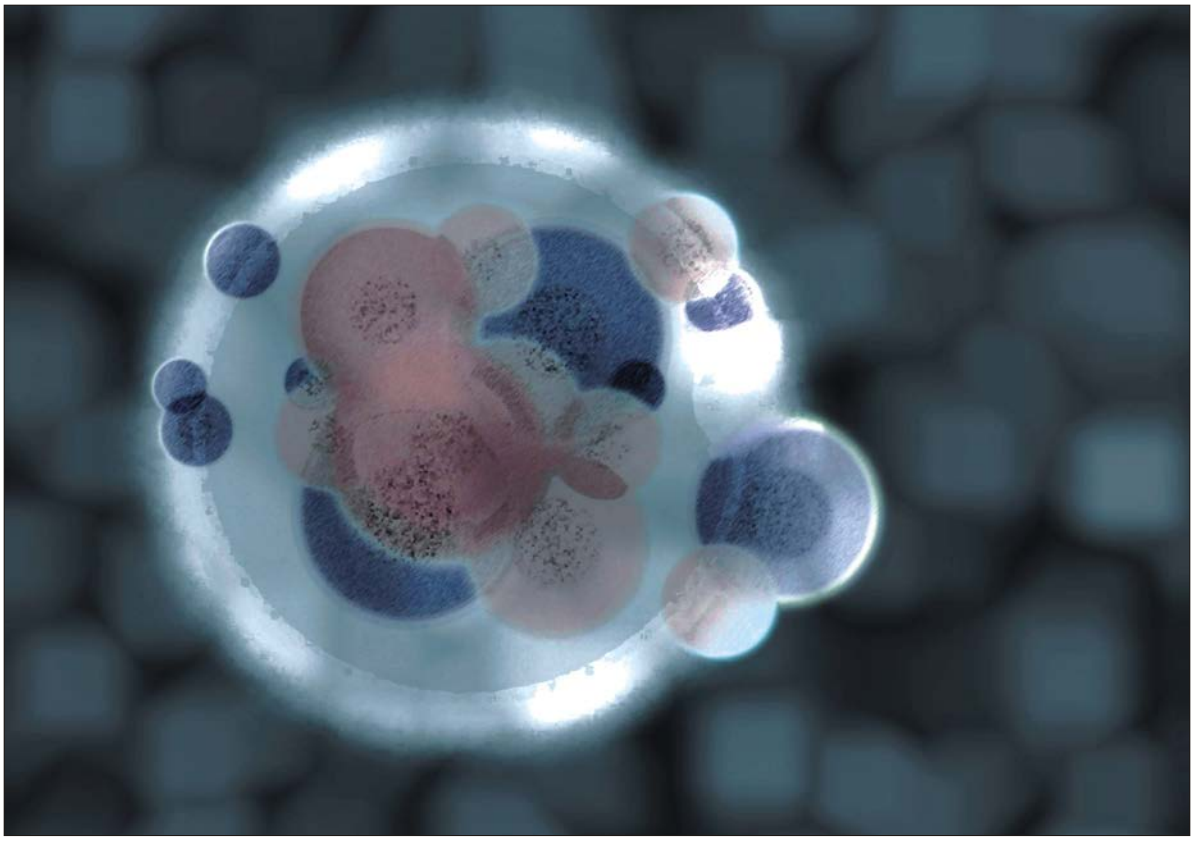

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Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (*Musa paradisiaca* cv. Mysore) Inflorescences

B.S. Padam, H.S. Tin, F.Y. Chye and M.I. Abdullah
School of Food Science and Nutrition, Universiti Malaysia Sabah,
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

Abstract: The inflorescence of Mysore banana (*Musa paradisiaca* cv. Mysore) was investigated for its antibacterial and antioxidant activities using various solvent extractions. Buds and bracts of the inflorescence showed a wide spectrum of inhibition against foodborne pathogenic bacteria such as *Staphylococcus aureus* (SA), *Bacillus cereus* (BC), *Listeria monocytogenes* (LM) and *Vibrio parahaemolyticus* (VP). The antioxidant activity was found higher in extracts with higher polarity. Methanolic extract of buds proves to have the strongest antibacterial and antioxidant activities. The 1 mg mL^{-1} of the methanolic buds extracts were able to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical up to 77.8% and inhibit the Lipid Peroxidation (LPO) at 67.2%. Meanwhile, the TEAC, FRAP and TPC values of the bud extracts were 137.71 μmol g^{-1} extract Trolox equivalent, 2114.70 μmol g^{-1} extract Fe^{2+} equivalent, 122.03 GAE mg g^{-1} extract, respectively. Pearson's correlation indicated significant positive correlation ($r>0.9$, $p<0.01$) between antibacterial activity, antioxidant values and TPC. The Minimum Inhibitory Concentration (MIC) values of the buds methanolic extracts were determined at 16.5 and 31.0 mg mL^{-1} against SA and LM. It is concluded that banana inflorescence extracts could be potentially be exploited as a source of natural antibacterial and antioxidants.

Key words: Banana byproducts, natural antibacterial, antioxidants, phenolics

INTRODUCTION

Plant secondary metabolites are well known for their bioactive properties and have been used for centuries as drugs and preservatives (Duru and Onyedineke, 2010; Tiwari *et al*., 2009). These metabolites are produced within the plants for many reasons and some known metabolites have been found to play a very important role in the plants defense against various types of stress, which includes climatic stress (Munne-Bosch *et al*., 2001), microbial infestations (Huang *et al*., 2006) and attack by herbivores (Sa *et al*., 2008). Recently, many plants have been identified containing antibacterial and antioxidant constituents, indicating the potential to be exploited in the field of crop bio-control (Oyelana *et al*., 2011) pharmaceutical, medicine food and beverage as well as food and beverage industry (Chye and Sim, 2009; Tiwari *et al*., 2009). The synthesis of large numbers of antibiotics over the past decades has caused complacency about the threat of bacterial resistance to both humans and animals (Adeleke and Omafuvbe, 2011; Frederick, 2011; Ynalvez *et al*., 2012). Bacteria become resistant to antimicrobial agents due to chromosomal changes or the exchange of genetic materials via plasmids and transposons (Adeleke and Omafuvbe, 2011; Manikandan *et al*., 2011). Therefore, there is always be a demand for novel and natural antibacterial to fill the gap within the known industrial applications (Davidson and Branen, 2005; Sundaram *et al*., 2011).

The demand for natural additives to be used in foods has led to the expansion of interest towards plant-based antibacterials (Tiwari *et al*., 2009) and their potential should not be underestimated. However, the sustainability of these natural antibacterials depends significantly on the abundance and the availability of the source of raw materials (McChesney *et al*., 2007). Byproducts from the food crops such as banana could be a feasible source for natural products since banana is one of the most important fruit in the world with an estimated annual production of 120 million tones including the plantain varieties (FAO, 2008). The byproducts of banana, which is estimated at about 220 tones of plant mass per hectare (Shah *et al*., 2005) however, remain untapped and readily to be explored and utilized instead of being treated as agriculture wastes. The inflorescence, which is one of the byproducts of banana, is a unique structure that protrudes out and remains at the terminal part of the fruit bunch until the fruit matures. It is consumed as a

Corresponding Author: F.Y. Chye, School of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia Tel: +60 088 320 257 Fax: +60 088 320 259

vegetable either cooked or raw by some ethnics in the Asian region. It is readily produced worldwide and a suitable candidate to be screened for the existence of antibacterials. Previous studies done on some parts of banana byproducts of different varieties revealed the great potential of antibacterials (Mokbe and Hashinaga, 2005) and antioxidants from the fruit peels (Someya *et al*., 2002) and flowers (Roobha *et al*., 2011) as well as antifungal (Ho *et al*., 2007) from the fruits.

Extraction of desired metabolites and components from plants generally utilized solid-liquid interaction using organic solvents, water or liquefied gasses. The bioactivity and quality of plant extracts depend heavily on the nature of the extracting solvent of the plant sample (Chan *et al*., 2009; Sultana *et al*., 2009). Solid-liquid extraction such as solvent infusion is one of the most widely used conventional methods to extract bioactives and valuable components from plant sources due to its cost competitiveness (Sayyar *et al*., 2011; Taha *et al*., 2011). The polarity of solvent and its capacity to dissolve desired active components plays a very important factor in selecting a good extraction solvent (Sultana *et al*., 2009; Tiwari *et al*., 2011). Extraction solvents used must representing as much as possible the whole polarity spectrum of the available metabolites in order to dissolve desirable bioactive compounds from the plant matrices. Only through a thorough screening using multiple solvents can we fully justify the target compound groups exhibiting desired activity.

This study presents the evaluation of the antibacterial and antioxidant potential of banana inflorescences using various solvent extraction systems as well as to determine the possible relationship between constituents and the bioactivities.

MATERIALS AND METHODS

Chemicals, solvents, media and cultures: Petroleum ether, hexane, chloroform, ethyl acetate, acetone, isopropanol, ethanol, methanol (ACS grade) were purchased from Merk, Germany. Microbiological media were purchased from Oxoid, England. Bacterial stock cultures (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 13932, *Salmonella typhiminium* ATCC 13311, *Salmonella enteritis* ATCC 13076, *Enterobacter sakazakii* ATCC 51329, *Yersinia enterocolitica* ATCC 23715 and *Vibrio parahaemolyticus* ATCC 17802) were obtained from American Type Culture Collection (ATCC). Folin-Ciocalteu Reagent, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), β-Carotente, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), Tween 40, Butylated hydroxyl toluene (BHT), Trolox, Gallic acid, Iron (II) sulfate were from Sigma, USA.

Sample preparation: Banana inflorescences (*Musa paradisiaca* cv. Mysore) were obtained from local plantations from the area of Kota Belud, Sabah. The samples were authenticated by a botanist from the Agriculture Department of Sabah. The buds of the inflorescence were separated from the bracts, cleaned and dried at 50°C under constant ventilation using a dehydrating oven (Thermoline, Australia) for 48 h until constant moisture content at 10.0%+0.5. Samples were ground into powder using an electric blender (Panasonic, Malaysia) with a mesh size of approximately 1 mm^2. Samples were kept in an airtight polythene bags at 4°C prior to further analysis.

Solvent extraction: Banana inflorescence (buds and bracts) (10 g) were individually extracted using 100 mL of petroleum ether, hexane, chloroform, ethyl acetate, acetone, isopropanol, ethanol, methanol and deionized water by direct infusion in a thermostated water bath (Wisebath, Korea) at 30°C for 24 h with 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 until assayed for antibacterial and antioxidant activity as described in a later section.

Preparation of bacterial cultures: All bacteria were sub-cultured onto tryptone soy agar and a pure culture was then kept in slant agar as stock. Upon usage, the bacteria were transferred by streaking on a fresh sterile agar and incubated at 35°C for 24 h. A single colony of each culture was inoculated into sterile tryptone soy broth and incubated at 35°C for 18 h. Following incubation, the broth containing bacterial suspension culture was centrifuged at 3500 rpm for 5.0 min and the resulting pallets were washed and re-suspended using sterile ringer's solution. Optical density of each culture was adjusted (based on a predetermined growth curve of bacteria at 540 nm) to achieve dilutions containing approximately 10^7 CFU mL^{-1}. The diluted bacterial suspensions were used for the antibacterial assay.

Antibacterial assay using well diffusion method: Sterile 20 mL tryptone soy agar was dispensed into plates containing 1.0 mL of bacterial suspension (10^7 CFU mL^{-1}) and mixed evenly. Agar was left to solidify at room temperature for 20 min and four equidistant wells (6 mm diameter) were cut out from each plate using a sterile cork borer. Extracts were diluted in methanol to yield the

concentration of 100 mg mL^{-1} and 25 μL of the extracts were pipetted into the wells so that each well contains 2.5 mg of extracts. Methanol and kanamycin was used as a negative control and positive control, respectively. All assays were done in triplicate. The plates were incubated at 35°C for 24 h (Al-Zoreky, 2009; Valgas *et al*., 2007). The diameters of inhibition zones were measured in millimeters (including the 6 mm well diameter).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): Banana extracts with significant inhibition zone based on the well-diffusion method were pre-evaluated for their inhibitory ranges using two-fold broth dilution method. One milliliter of extract prepared at the concentration of 100 mg mL^{-1} in TSB was serially diluted two-fold in sterile universal bottles containing 1 mL of similar broth to achieve concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.563 mg mL^{-1}. Bacterial inoculums (0.1 mL of 10^7 CFU mL^{-1}) were pipetted into the universal bottles containing the extracts and TSB before incubation at 35°C for 24 h. After incubation, 0.1 mL of the inoculated TSB were transferred and spread onto a fresh TSA to determine the range of inhibitory concentration based on the visible growth on the agar. Further dilutions were done using smaller factors in order to obtain more specific inhibitory ranges for each tested bacteria. The narrow ranges of concentrations were subjected to time-kill assay to determine the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) which were defined as the lowest concentration to kill 90% of bacterial population and the lowest concentration to kill 99% of bacterial population within 24 h (Chye and Sim, 2009). Decimal Reduction Time (DRT) for banana inflorescence extract was calculated based on a linear regression lines, using the linear portion of the survivor curves (Log CFU mL^{-1} against time of exposure at a constant temperature) and is defined as the time resulting in the reduction of Log_{10} reduction (90% killings) of CFU mL^{-1} of the tested strains.

Total phenolic content: The total phenolic content of banana extracts was determined by using the Follin-Ciolcateu method as described by Lim and Murtijaya (2007). Calibration curve was prepared using 0.3 mL of reference gallic acid solutions in methanol, which was later mixed with 1.5 mL of standard Follin-Ciocateu reagent, diluted with distilled water (1:10 ratio) and added 1.2 mL of 7.5% sodium carbonate solution in water subsequently. The absorbance was read on a spectrophotometer at 765 nm after 15 min at 45°C. The extracts of banana 0.3 mL (1.0 mg mL^{-1}) in methanol were determined using the similar procedures. Pure water was used as a blank. All experiments were performed in triplicate. The total content of phenolics was determined as gallic acid equivalence (mg GAE g^{-1} extract).

DPPH scavenging inhibition assay: The scavenging ability of the banana inflorescence extracts were determined based on method described by Ao *et al*. (2008) with modifications. Methanolic solution (0.1 mL) containing 1 mg of extracts of banana byproduct was added to a methanolic solution of DPPH (0.025 g L^{-1}, 3.9 mL) and left idle at room temperature for 20 min. BHT and BHA were used as positive controls and an equal amount of methanol was used as a negative control. The absorbance of this solution was measured at 517 nm using Uv-Vis spectrophotometer. The measurement was performed in triplicate. The scavenging activity was calculated using the formula below:

$$\text{DPPH radical scavenging (\%)} = \frac{(OD_{control} - OD_{sample})}{OD_{control}} \times 100$$

$Od_{control}$ = Initial concentration of DPPH, OD_{sample} = DPPH containing banana extracts.

The selected that give the highest DPPH radical scavenging activity was evaluated for its EC_{50} value which corresponds to concentration of sample required to scavenge 50% of the DPPH free radicals.

Lipid Peroxidation (LPO) inhibition using the β-carotene linoleate model system: LPO inhibition activity of banana inflorescence extract was evaluated by the β-Carotene linoleate model system as described by Barros *et al*. (2007). One mL solution of β-carotene in chloroform (0.1 mg mL^{-1}) was mixed with 20 mg of linoleic acid and 200 mg of tween-40 in a round bottom flask. The chloroform was then removed by a rotary evaporator (45°C, 4 min) and 50 mL of oxygenated distilled water was added slowly with vigorous agitation to the residue subsequently to form an emulsion. Aliquots (4.8 mL) of the emulsion were added to 0.2 mL methanolic solutions banana inflorescence extracts (1.0 mg mL^{-1}). Samples were kept in 50°C water bath for 120 min and their absorbance values were recorded at 470 nm. Measurements at 20 min intervals were carried out for kinetics study and the experiments were repeated twice. The LPO inhibition activity was evaluated using the formula:

$$\text{LPO inhibition} = \frac{\text{β-carotene content after 2 h of assay}}{\text{Initial β-carotene content}} \times 100$$

BHT and BHA were used as positive controls and blank samples (without β-carotene) were prepared as background subtraction. The extract that shows the highest LPO inhibition activity was evaluated for its EC_{50} value, which corresponds to concentration of sample required to retain 50% of the β-carotene.

ABTS (TEAC) assay: ABTS radical scavenging activity was determined according to the method by Re *et al.* (1999). ABTS radicals were produced by reacting 7.0 mM ABTS in H_2O and 2.45 mM potassium persulfate and storing it in the dark room for 12 h. Prior to usage, the ABTS solution was diluted to an absorbance of 0.700±0.025 at 734 nm with phosphate buffer (0.1 M, pH 7.4). Stock solutions of extracts and Trolox was diluted with methanol to obtain different concentrations of 0.25-1.0 mg mL^{-1} and 0.08-0.5 mmol L^{-1}, respectively. ABTS solution (3.0 mL) was added to 0.03 mL of extracts and Trolox at various concentrations and the absorbance at 734 nm were recorded after 6 min. All experiments were carried out at least three times and in triplicate. Methanol was used as negative the control. The concentration of antioxidants giving the same percentage change of absorbance of the $ABTS^{\cdot}$ as that of 1.0 mM Trolox was regarded as Trolox Equivalent Antioxidant Capacity (TEAC) value.

FRAP assay: Ferric Reduction Antioxidant Power assay was carried out according to Benzie and Strain (1996). The extracts of banana byproduct were diluted with methanol to obtain different concentrations. Ferric-TPTZ reagent was prepared by mixing 2.5 mL of 20 mmol mL^{-1} 2,4,6-tripyridil-s-triazine solution in 40 mmol L^{-1} HCl with the addition of 2.5 mL of 20 mmol L^{-1} $FeCl_3.6H_20$ and 25 mL of 0.3 mmol L^{-1} acetate buffer (pH 3.6). FRAP reagent (900 μL), freshly prepared and incubated at 37°C will be mixed with 90 μL of distilled water and 30 μL of banana inflorescence samples. BHT and methanol were used as controls. All assays (in triplicate) were incubated at 37°C in a water bath for 4 min and the absorbance was measured at 593 nm. The final dilution of the test sample in the mixture was 1/34. Methanolic solutions of known Fe^{2+} concentration ($FeSO_4.7H_2O$), ranging from 100-2500 μmol L^{-1} were used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC1) was defined as the concentration of extract having a ferric-TPTZ reducing ability equivalent to that of 1 μmol L^{-1} $FeSO_4.7H_2O$. Results were expressed as Fe^{2+} equivalents.

Statistical analysis: Results were expressed as Mean±SD for three replication. One way Analysis of Variance (ANOVA) (SPSS version 16) was applied on the values obtained from the experiments. Pearson's correlation analysis was used to determine the relationship between antibacterial, antioxidant and TPC. Statistical significance was defined at $p<0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Antibacterial activity of banana (*Musa paradisiacal* cv. Mysore) inflorescence extracts: Individual extraction of banana inflorescence using solvents with different polarity shows a wide spectrum of bacterial inhibition activities. Table 1 shows the antibacterial activity of the banana inflorescence was greatly affected by solvent polarity. It seems that extracts from organic solvents with higher polarity such as methanol and ethanol gave significant stronger inhibitory activity against *Staphylococcus aureus* (SA), *Bacillus cereus* (BC), *Listeria monocytogenes* (LM) and *Vibrio parahaemolyticus* (VP). No inhibitory respond was observed for other bacteria used in the study. The antimicrobial activity of the extracts increased as the polarity of the extracting solvents increased. This is in agreement with the study by Sundaram *et al.* (2011) who discovered that *Withania somnifera* extracts from the polar solvents showed much higher antibacterial activity than extracts from non-polar solvents. Methanolic extract of the banana inflorescence (buds) showed profoundly distinct antibacterial activity by having observable inhibition ranges from 12.02 to 13.23 mm on all susceptible bacteria as compared to 7.23 to 8.23 mm inhibition by the extracts from the bracts. The same extracts from the bracts, however, did not show any inhibitory activity towards gram-negative bacteria (VP). Methanol has unique physical properties than other organic solvents where the molecules consist 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 high polarity, low viscosity and high diffusion coefficient (Lee and Li, 1991). It penetrates through the cell and organelle membranes selectively dissolving polar compounds having antibacterial properties from the banana inflorescence. It was reported by Philip *et al.* (2009) that methanol is the best solvent in screening and extracting for antibacterials form plant materials. Besides, it has been used in extracting polar active compounds such as polyphenols, phenones, flavones, anthocyanins, saponins, tannins and xanthoxyllines (Al-Zoreky, 2009; Ogundare, 2007; Tiwari *et al.*, 2011). Similar to the report by Ogundare (2007), the methanolic extract of buds in the current study was shown to have broad spectrum activity as it inhibits both gram-positive and gram-negative bacteria although gram-negative bacteria are generally more resistant than gram-positive bacteria in respond to chemical inhibitors (Munyendo *et al.*, 2011).

Table 1: Antibacterial screening of banana inflorescence using various extraction solvent against selected food pathogens

		Diameter of inhibition (mm)			
Extract	Yield % (Dry extract)	SA	BC	LM	VP
Buds					
H_2O	15.74±0.17	8.43±0.40^{bc}	8.97±0.25^{b}	7.90±0.36^{b}	na
MeOH	8.26±0.17	12.97±0.45^{e}	13.23±0.45^{d}	12.02±0.43^{c}	12.90±0.44^{b}
EtOH	6.69±0.12	9.85±0.56^{d}	10.50±0.30^{c}	8.40±0.17^{b}	6.90±0.36^{a}
Isopropanol	6.35±0.21	9.07±0.12^{cd}	8.77±0.25^{b}	8.07±0.12^{b}	na
Acetone	6.54±0.23	8.57±0.40^{bc}	8.77±0.25^{b}	7.00±0.00^{a}	na
EtoAc	3.53±0.07	8.83±0.29^{cd}	9.17±0.29^{b}	8.34±0.29^{b}	na
$CHCl_3$	4.65±0.14	7.67±0.58^{ab}	6.83±0.58^{a}	na	na
Hexane	2.40±0.11	7.17±0.29^{a}	7.07±0.12^{a}	na	na
Pet. Ether	2.20±0.08	7.00±0.50^{a}	6.67±0.29^{a}	na	na
Bracts					
H_2O	12.93±0.16	7.25±0.25^{ab}	7.67±0.29^{b}	na	na
MeOH	8.84±0.17	8.23±0.25^{c}	8.17±0.29^{b}	7.23±0.25	na
EtOH	8.26±0.22	7.60±0.17^{b}	7.67±0.29^{b}	na	na
Isopropanol	8.97±0.24	7.40±0.17^{ab}	7.73±0.25^{b}	na	na
Acetone	6.69±0.25	7.17±0.29^{ab}	7.67±0.29^{b}	na	na
EtoAc	4.89±0.21	7.67±0.29^{bc}	7.50±0.50^{ab}	na	na
$CHCl_3$	4.71±0.2	6.83±0.29^{ab}	6.83±0.29^{a}	na	na
Hexane	3.53±0.16	na	na	na	na
Pet. Ether	2.34±0.05	na	na	na	na
Kanamycin					
(3 μg well^{-1})		17.50±0.71	21.00±0.00	17.50±0.00	18.50±0.71

Data represent the Means ± SD (n = 3) including the well diameter. Subscripts represent significance differences between extraction solvent at $p<0.05$. 3na-no activity. H_2O: Deionized water, MeOH: Methanol, EtOH: Ethanol, EtoAc: Ethyl acetate, $CHCl_3$: Chloroform, Pet. Eth: Petroleum ether. SA: *Staphylococcus aureus* (gram+), BC: *Bacillus cereus* (gram+), LM: *Listeria monocytogenes* (gram+), VP: *Vibrio parahaemolyticus*. [na]Results not shown: No inhibition was detected for *Salmonella typhimurium* (ST, gram-), *Salmonella enteritis* (SE, gram-), *Enterobacter sakazakii* (ES, gram-) and *Yersinia enterocolitica* (YE, gram-)

In Table 1, the yield is directly proportionate to the polarity of the solvent and the lower the polarity of the extracting solvent, the lower the extraction yield. Water seems to be a good extracting medium based on the yield (15.88%) but may not be selective enough in dissolving the desired antibacterial compounds from the inflorescence as the observed inhibitory activity is relatively low (7.25 to 8.43 mm) with no activity against gram-negative bacteria. Previous studies have found that water extraction is heavily dependant on the hydrosolubility of bioactives (Louis *et al*., 2011), poor in extracting bioactives that bind with lipophylic structures such as membranes (Ghisalberty, 2008) and was prone to dissolves more non-bioactive polysaccharides (Koh *et al*., 2009). Inflorescence obtained from other moderate polarity organic solvents such as ethanol (6.90-10.50 mm), isopropanol (7.60-9.07 mm), acetone (7.00-8.77 mm), ethyl acetate (7.50-9.17 mm) and chloroform (6.83-7.67 mm) showed fair antibacterial activities on selected gram-positive bacteria. The non-polar extracts (hexane and petroleum ether) show no antibacterial activity except for the buds extracts which has a very low activity (6.67 mm to 7.17 mm) on SA and BC. These results contradict earlier reports by Muthuvelan and Raja (2008), where they revealed that non-polar hexane and diethyl ether was the best solvent in extracting antibacterials from *Azadiracta indica, Pongamia pinnata* and *Aloe barbadensis*. This suggests that the polarity distribution of antibacterial compounds in plants varies depending on species and plant families. Thus, non-polar solvents may not be a good choice in extracting antibacterial metabolites in this case since most of the targeted bioactive metabolites from plants are at the polar end of the spectrum. Methanol is proves to be the best extracting solvent for harnessing bacterial inhibitors from banana inflorescence.

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of banana buds methanolic extract: Extract which shows significant inhibition diameter (>12 mm) were used to determined its Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The MIC and MBC of the bud's methanolic extracts are shown in Table 2. BC is the most susceptible among tested bacteria giving the lowest MIC and MBC values of 16.5 and 18.5 mg mL^{-1}, respectively based on the time kill assay. According to Fig. 1, the Decimal Reduction Time (DRT) for BC at MIC level was calculated to be 13.7 h. The DRT for the methanolic bud extracts were determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the survivor curves (Chye and Sim, 2009). A slightly higher MIC and MBC values was shown towards SA at the concentration of 25.5 and 27.5 mg mL^{-1} with the DRT determined at 15.4 h. The methanolic extract seems to have the highest MIC and MBC values at 42.0 and 46.0 mg mL^{-1}, respectively against VP, indicating VP is the most resistant bacterial

Table 2: Antibacterial (MIC, MBC and DRT) and antioxidant activities (EC_{50} of DPPH Scavenging and LPO Inhibition) of banana (Mysore) buds methanolic extract

Minimum inhibitory concentration			
SA	BC	LM	VP
(Minimum bactericidal activity) (mg mL^{-1})			
25.5	16.5	31.0	42.0
(27.5)	(18.5)	(34.0)	(46.0)
Decimal reduction time at MIC (MBC) h			
15.4	13.7	18.4	9.8
(7.1)	(6.0)	(7.6)	(7.1)

Antioxidant activities	
EC_{50} DPPH Scavenging mg mL^{-1}	EC_{50} LPO Inhibition mg mL^{-1}
0.77	0.93

SA: *Staphylococcus aureus* (gram+), BC: *Bacillus cereus* (gram+), LM: *Listeria monocytogenes* (gram+), VP: *Vibrio parahaemolyticus*. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; DRT: Decimal reduction time (h)

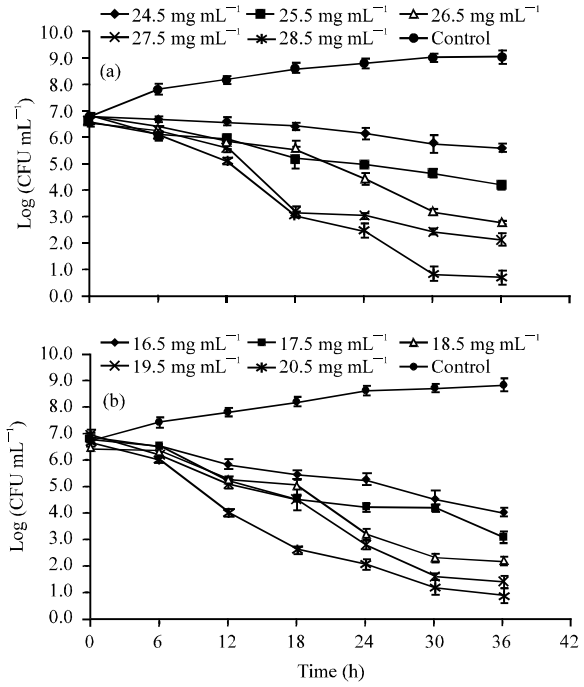

Fig. 1(a-b): Effects of methanol buds extracts on the growth of (a) SA and (b) BC at pre-selected concentration

pathogens among the selected bacteria. However, the DRT value for VP at MIC level was 9.8 h, which is lower than the DRT value for BC. This suggests that the methanolic bud extract could reduce the viable count of VP in a short time exposure due to its fast diffusion into the bacteria membrane, but the extract lost its effectiveness at prolonged exposure (Hakkim *et al*., 2012).

The MIC and MBC values are inversely proportional to the antibacterial activity and the lower the MIC and MBC values, the higher the antibacterial activity. The relatively higher MIC and MBC values between VP and the rest of the tested bacteria (SA, BC and LM) might be contributed to its cell wall properties. Gram-negative bacteria are protected by their lipopolysaccharide layer (LPS) hindering the direct exposure of inner membrane layer to the activities of natural antibacterials (Munyendo *et al*., 2011). A few earlier studies concur with these results and reported that plant-derived antibacterials exhibit similar pattern in gram susceptibility (Ogundare, 2007; Renukadevi and Suhami Sultana, 2011; Shan *et al*., 2007). Antibacterials derived from agricultural byproducts such as the banana inflorescence proves to be useful in controlling SA, BC, LM and VP and could be potentially developed further into novel food preservatives to ensure food safety.

Antioxidant activity of banana (*Musa paradisiaca* cv. Mysore) inflorescence extracts: DPPH scavenging activity: Similar to the antibacterial activity, solvents with higher polarity are much favorable towards extracting the antioxidants from banana inflorescence. Water, alcohols (methanol, ethanol and isopropanol), acetone and ethyl acetate prove to be better extracting solvent compared to chloroform, hexane and petroleum ether in which the extracts show almost no antioxidant activity. In the DPPH assay, buds extracts from alcohols (methanol, ethanol and isopropanol), acetone, ethyl acetate and water able to scavenge more than 50% of DPPH radicals at the concentration of 1.0 mg mL^{-1} (Fig. 2a). The trend is almost similar in the bract extracts with a slightly lower scavenging capacity. Roobha *et al*. (2011) reported that the bract of *Musa acuminata* contains significant amount cyanidin rutinoside, which is a substantial antioxidant. The degree of the discoloration of DPPH radical was contributed by the capacity of the sample that acts as a hydrogen donor (Ao *et al*., 2008). Methanolic extract of buds and bracts show the highest DPPH radical scavenging activity of 77.8 and 44.0%, respectively with the buds activity almost comparable to BHT and BHA. The radical scavenging activity shown was similar with lotus rhizome extracts where extracts from methanol, ethanol, acetone and ethyl acetate gave higher DPPH scavenging activity while low activity seen on the petroleum ether extract (Yang *et al*., 2007). The EC_{50} value of the methanolic extract of the inflorescence buds was found to be 0.77 mg mL^{-1} which is lower than that of pangi seed extract which may be the result of hydrogen atom transfer from the polyphenol aromatic rings to stabilize the DPPH radicals during chain reaction (Chye and Sim, 2009).

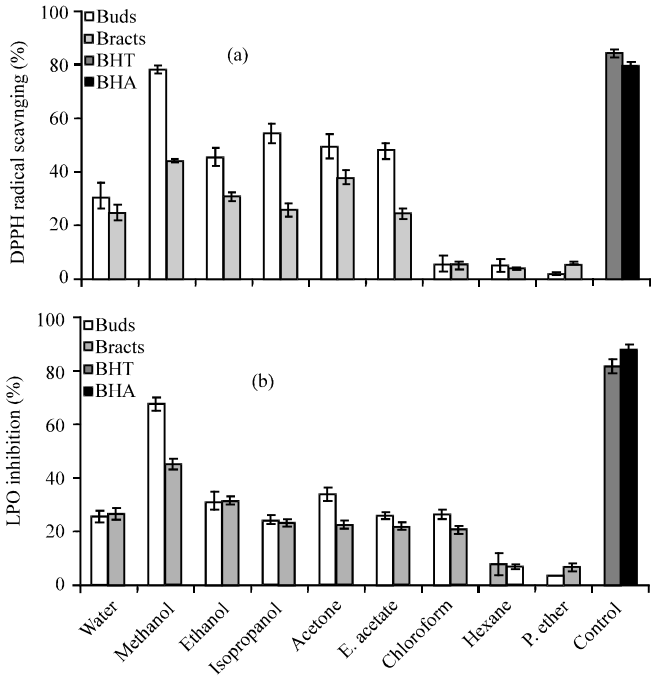

Fig. 2(a-b): Antioxidant activity of banana (Mysore) buds and bracts extracts according to different solvent extraction based on (a) DPPH assay and (b) LPO inhibition using β-carotene model

LPO inhibition activity: Buds and bracts extracts from alcohols (methanol, ethanol, isopropanol), acetone, ethyl acetate, chloroform and water show a fair activity in inhibiting the bleaching of b-carotene while almost no bleaching inhibition activity (<10%) was detected for non-polar extracts (hexane and petroleum ether). Inhibition of Lipid Peroxidation (LPO) up to 67.2 and 45.2% was achieved by buds and bracts methanolic extracts, respectively (Fig. 2b). The addition of heat to the linoleate system rapidly creates linoleate acid-free radicals, which automatically attacks the highly unsaturated β-carotene while the presence of other antioxidant components from the methanolic buds extracts could reduce the destruction of β-carotene by neutralizing linoleate acid-free radicals and other free radicals in the system (Bareira *et al*., 2008). BHT and BHA (at the concentration 1.0 mg mL^{-1}) show a more superior b-carotene bleaching inhibition capacity, able to preserve 81.5 and 87.4% of the b-carotene. Methanol buds extract exhibit EC_{50} values of 0.93 mg mL^{-1}, which indicates a notable LPO inhibition activity.

ABTS assay: Organic solvent (methanol, ethanol and acetone) extracts of the buds show significant trolox equivalent values of 137.7, 96.4 and 94.1 μmol g^{-1} extracts, respectively (Fig. 3a). Buds extracts show lower trolox equivalent (<50 μmol g^{-1} extract) with almost no activity from extracts of chloroform, hexane and petroleum ether (<10 μmol g^{-1} extract). The antioxidant activity in the ABTS system measures the capacity of an extract to react and neutralize the ABTS-radical cation generated in the assay system (Biglari *et al*., 2008) and by comparing it with a trolox standard to get a comparison based on the improved Trolox Equivalent Antioxidant Capacity (TEAC) value. A higher TEAC value simply means a better antioxidant capacity present in the extract in reacting with the ABTS-radical cation. However, the TEAC value alone cannot represent the overall antioxidant capacity of an extract and it must be accompanied by other assays as the presence of reaction products in the ABTS system might react faster than the parent compounds in the extract thus giving an outstandingly higher TEAC value (Arts *et al*., 2004; Everette and Islam, 2012).

FRAP assay: The highest FRAP values (2.11 mmol Fe^{2+} g^{-1} extracts) was also shown by methanolic extract of buds which is two times higher than the FRAP values for extracts obtained from ethanol, isopropanol, acetone, ethyl acetate and water (Fig. 3b). FRAP values from bracts of methanol, ethanol, isopropanol, acetone, ethyl acetate and water extracts show moderate reduction of Fe^{3+} to Fe^{2+} with the average value of less than 1.0 mmol Fe^{2+} g^{-1} extract. In contrast to the TEAC assay, Ferric Reducing Antioxidant Power (FRAP) assay evaluates the antioxidant activity of an extract based on its capacity to

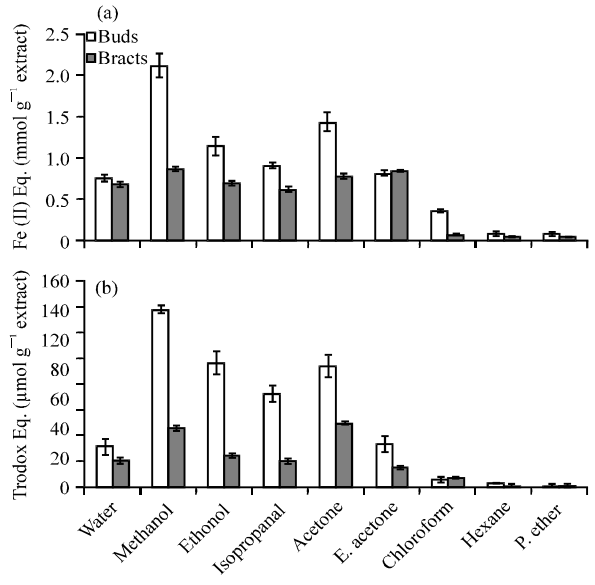

Fig. 3(a-b): Antioxidant activity of banana (Mysore) buds and bracts extracts according to different solvent extraction based on (a) FRAP assay and (b) Trolox equivalent antioxidant capacity

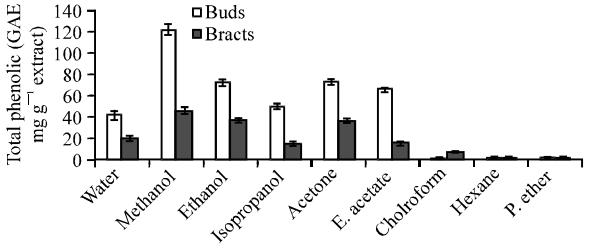

Fig. 4: Total phenolic content of banana (Mysore) buds and bract extracts according to different solvent extraction

reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), comparing it with a known concentration Fe^{2+} standard in this assay (Benzie and Strain, 1996). The higher the reduction of Fe^{3+} ions by a reducing agent (plant extract), the better the antioxidant capacity of that particular extract. Polyphenolic and phenolic compounds, which are often associated with having high antioxidative capacity (Biglari *et al*., 2008; Chan *et al*., 2009; Chye and Sim, 2009) may have been present in the banana inflorescence extracted with methanol.

Total Phenolic Content (TPC) and it correlation towards antibacterial and antioxidant activity: The solvent extracts of buds and bracts showed a very wide range in their Total Phenolic Content (TPC) values. The TPC values for buds and bracts were shown at Fig. 4. Methanol buds extract exhibit the highest TPC values (125.75 mg GAE g^{-1} extract) followed by ethanol, acetone and ethyl acetate extract. Chloroform, hexane and petroleum ether extract showed a very low TPC values for both buds and bracts (1.58-6.95 mg GAE g^{-1} extract). This is due to the lower polarity nature of the solvents and solvents like chloroform and petroleum ether generally extracts components such as terpenoids, alkaloids and fatty acids (Tiwari *et al*., 2011). Bracts extracts have a lower TPC values compared to the buds except for chloroform extract. The TPC value of the buds were higher than *Trianthema decandra* (74.60 GAE mg g^{-1} plant extract) (Geethalakshmi *et al*., 2010) and comparable to the TPC value of *Ficus microcarpa* leaves and fruit (128.12-179.68 GAE mg g $^{-1}$ plant extract) (Ao *et al*., 2008).

The bivariate correlations between the antibacterial, antioxidant properties and the Total Phenolic Content (TPC) were analyzed through Pearson's correlation and the results are summarized in Table 3. There is a strong positive correlation ($r>0.8$; $p<0.01$) between the TPC and all the antioxidant properties of the banana inflorescence extract. FRAP values (r = 0.9603) and the DPPH radical scavenging activity (r = 0.9554) gave the highest correlation coefficient with TPC values for banana buds and bracts respectively. It is therefore indicate the phenolic compounds within the inflorescence extract were responsible for their high antioxidative properties and the higher the total phenolic content, the higher the FRAP, TEAC, DPPH radical scavenging and LPO inhibition values. These results were in accordance to some current literatures where phenolic compounds such as monophenols, anthocyanins, flavonoids, tannins were indisputably found as good antioxidants (Pinelo *et al*., 2005; Ballistreri *et al*., 2009; Chye and Sim, 2009; Hodzic *et al*., 2009). Meanwhile significant correlations were also reported between TPC values with DPPH radical scavenging, FRAP (Vaquero *et al*., 2010), LPO inhibition Barros *et al*. (2007) and TEAC (Li *et al*., 2008) in their respective studies.

Likewise, there was a strong positive correlation between the TPC of the banana inflorescence extract (both buds and bracts) with its antibacterial inhibition properties (SA, BC and LM). A slightly lower correlation (r = 0.7120) was observed between TPC of buds to the VP inhibition and the TPC values of bracts which showed a slightly lower correlation against inhibition on SA (r = 0.7790) and BC (r = 0.7770), respectively. Therefore, it can be justified that the phenolic compounds present within the banana inflorescence extracts contributed significantly to its antioxidant and antibacterial properties. This could be validated by several other

Table 3: Correlation between antibacterial inhibition zone, antioxidant properties and total phenolic content of banana inflorescence methanolic extracts

	FRAP	TEAC	BCI	DPPH	TPC	VP	LM	BC	SA
Buds									
SA	0.9023**	0.8705**	0.9129**	0.8592**	0.9021**	0.8723**	0.9452**	0.9514**	1.0000
BC	0.8995**	0.8797**	0.8887**	0.8823**	0.9410**	0.8296**	0.9562**	1.0000	
LM	0.8620**	0.8062**	0.8770**	0.8714**	0.9031**	0.8622**	1.0000		
VP	0.7393**	0.6923**	0.8433**	0.6241**	0.7120**	1.0000			
TPC	0.9603**	0.9261**	0.8766**	0.9554**	1.0000				
DPPH	0.9239**	0.8973**	0.8255**	1.0000					
LPOI	0.9209**	0.8451**	1.0000						
TEAC	0.9518**	1.0000							
FRAP	1.0000								

	FRAP	TEAC	BCI	DPPH	TPC	BC	SA
Bracts							
SA	0.8700**	0.6950**	0.8640**	0.8360**	0.7790**	0.8920**	1.0000
BC	0.8850**	0.7740**	0.8180**	0.8740**	0.7770**	1.0000	
TPC	0.8020**	0.9190**	0.8270**	0.9310**	1.0000		
DPPH	0.9250**	0.9340**	0.7960**	1.0000			
LPOI	0.7420**	0.7210**	1.0000				
TEAC	0.7930**	1.0000					
FRAP	1.0000						

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). SA: *Staphylococcus aureus* (gram+), BC: *Bacillus cereus* (gram+), LM: *Listeria monocytogenes* (gram+), VP: *Vibrio parahaemolyticus* (gram-) TPC: Total phenolic content, DPPH: DPPH radical scavenging assay, BCI: β-carotene bleaching inhibition assay, TEAC: Trolox equivalent antioxidant power, FRAP: Ferric reduction antioxidant power

studies suggesting the similar correlation between TPC values, antibacterial and antioxidants (Chye and Sim, 2009; Shan *et al*., 2007; Vaquero *et al*., 2010). TPC value has always been associated to the antioxidant activities (Ballistreri *et al*., 2009; Hayouni *et al*., 2007; Oboh, 2008; Pinelo *et al*., 2005) but not necessary positively related towards antibacterial activities. Phenolic compound such as rutin is known to be good antioxidant but did not show any antibacterial activity (Arima *et al*., 2002). There have been similar reports indicated there were no significant correlation between TPC and antibacterial activity (Borawska *et al*., 2010; Fattouch *et al*., 2008). This suggests that the variation between phenolic constituents and its bioactive activities could be due to the different plant species studied, the type of bacteria tested and the type of antioxidant assays might also influence the relationship.

In this study, a wide range of correlation coefficients values were observed (0.6241-0.9129) between the antioxidant assays and the susceptibility (inhibition diameter) of different bacteria. β-Carotene bleaching inhibition and FRAP values were strongly correlated ($p<0.01$, $r = 0.9129$ and $r = 0.9023$, respectively) with the inhibition against SA while the lowest correlation coefficient value is between DPPH assay and VP inhibition. The wide variation of correlation might be contributed to the type of antioxidant activity and bacterial species (Borawska *et al*., 2010; Shan *et al*., 2007) while at this level of study the possible synergism between compounds within the extracts as well as with other artifacts within the antioxidant assay system are unknown (Arts *et al*., 2004; Fattouch *et al*., 2008). The strong correlation between the bacterial inhibition for buds and bracts indicate the bacterial susceptibility to similar compound within the extracts (Taguri *et al*., 2006). There was also strong correlation of extracts antioxidant activity ($p<0.01$, $r>0.9$) between the antioxidant assays (DPPH, TEAC and FRAP) implying the versatility of the modes of action of antioxidant compounds within the banana inflorescence extracts as they can be good free radical scavengers, electron donors and reducers (ferric ions) (Dudonne *et al*., 2009; Everette and Islam, 2012).

CONCLUSION

The results presented in this paper signify the potential of banana inflorescence (*Musa paradisiaca* cv. Mysore) which is one of the agricultural byproducts to be a source of natural antibacterials and antioxidants. Methanol has been proven to be the best extraction solvent based on the significant antibacterial and antioxidant activities, which is tied to the high concentration of phenolics. Bud extracts contains relatively potent antibacterials and antioxidants compared to the bract extracts. Further study should focuses on the optimization of the extraction method, isolation and the characterization of the antibacterial and antioxidative compounds responsible for these activities.

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