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The antibacterial activities and chemical composition of extracts from *Carica papaya* cv. Sekaki/Hong Kong seed

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Abstract

Ten solvents were used to extract phytochemicals from the peel of *Carica papaya* cv. Sekaki/ Hong Kong to evaluate antibacterial activities and determine chemical composition of *Carica papaya* cv. Sekaki/Hong Kong seeds. The antibacterial activities of ten solvent extracts were tested against 14 microorganisms vis *Shigella sonnei, Salmonella typhimurium, Escherichia coli, Salmonella enteritidis, Vibrio vulnificus, Vibrio parahaemolyticus, Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Corynebacterium diphtheria, Clostridium perfringens, Streptococcus pneumoniae* and *Bacillus subtilis* using disk diffusion test (DDT). The *Carica papaya* seed of ACN and MeOH extracts inhibited 11 microorganisms, indicating their broad spectrum activity. The disk diffusion test exhibited moderate and clear inhibition on *C. diphtheria, S. pneumonia, B. subtilis* and *C. perfringens*. MeOH extract inhibited *S. enteritidis, V. vulnificus, P. mirabilis* and *B. cereus* with the lowest MIC at 11.25 mg/mL each, thus was chosen as the best extract. The hierarchy of extract potency can be ranked as MeOH > acetone > ACN > CHCL3 > hexane > DE = PE > EtOH > DCM based on clear and moderate inhibition and the lowest MIC. TPC and TFC of the extracts ranged between 4.83 to 22.59 mg GAE/g DW and 1.32 (water) and 17.15 mg QE/g DW respectively. The GC/MS analysis of MeOH extract identified potential antibacterial compounds such as isothiocyanatomethyl benzene, 9-octadecenoic acid, hexadecanoic acid and β-sitosterol. The *Carica papaya* seed cv. Sekaki/ Hong Kong possessed significant antibacterial activities when extracted by different solvents in particular MeOH solvent.

Introduction

Tropical fruit such pineapple, papaya and mango are consumed as dessert fruits or processed into value added products. Their peels and seeds which contain abundant beneficial phytochemicals are often discarded or underutilized. Phytochemicals from numerous seeds extracts and their antibacterial activities have been reported such as that of *Nephelium lappaceum* L. (Thitilertdecha *et al*., 2008), *Mesua ferrea* L. (Chanda *et al*., 2013), and hydrodistillate of Sphallerocarpus gracilis (Gao *et al.*, 2011). These activities have been attributed to flavonoids (Rauha *et al*., 2000) and phenolics (Majhenič *et al*., 2007). The antibacterial activities reported for the above extracts were from several different solvent extracts. Complete extraction of these phytochemicals by a single solvent may not be possible due to their chemical complexity, different distribution pattern throughout the plant and also due to the selectivity of the solvents themselves (Naczk and Shahidi, 2004; De Rijke *et al.*, 2006).

Only several reports on antibacterial activities from papaya *(Carica papaya)* seeds are available (Ayala-Zavala *et al.*, 2011). Pioneering work by Emeruwa (1982) reported significant antibacterial activity of protein precipitated from ethanol extracts of papaya seed against *Staphylococcus aureus, Bacillus cereus, Echerichia coli, Pseudomonas aeruginosa* and *Shigella flexneri*. The water extract from mixture of flesh, peel and seed of unripe *Carica papaya* also inhibited *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Osato *et al.*, 1993). Kermanshai *et al*. (2001) indicated that benzenic compounds and fatty acid (Koolen *et al*., 2013) from *Carica papaya* seed exhibited antibacterial activity.

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Carica papaya cv. Sekaki or Hong Kong co-exists with Eksotika I and Eksotika II varieties in Malaysia. The antibacterial properties of this variety has not been exhaustively studied, neither the composition of the resulting extracts reported. Therefore, this study was carried out to investigate the potential antibacterial activity of the discarded seed of the ripe papaya extracted by different solvents extractions against fourteen selected microbes of food spoilage and microorganisms. The chemical composition of the most potent extract was identified through GC/MS analysis using splitless mode.

Material and Method

Plant material

Carica papaya cv. Sekaki fruits at maturity stage of six (Sapii and Muda, 2005) were bought from an organic farm, Malaysia. Fruits were split into halves, seeds removed, washed thoroughly in distilled water, drained for 3 h, oven dried at 40°C for 3 days (Adejuwon *et al*. 2011), kept in airtight amber bottles and stored at -20°C until further analysis.

Extraction of phytochemicals

Hexane, petroleum ether (PE), diethyl ether (DE), chloroform ($CHCL_3$), dichloromethane (DCM), acetone, ethanol (EtOH), methanol (MeOH), acetonitrile (ACN) and distilled water were used as solvents in the extraction according to Alothman *et al.* (2009) with slight modification. Dried seeds were ground for 5 min in 240 W electrical blender (Panasonic MX-337, Malaysia) prior extraction. For each solvent extraction, a solid to solvent ratio of 1:2 was used except for water where the ratio of 1:6 was used to ensure complete seed immersion. Briefly, 50 g of dried ground *Carica papaya* cv. Sekaki seed powder was weighed into a conical flask and 100 mL of solvent was added. Extraction was carried out at room temperature (27°C) for 8 h in a shaker (100 rpm) which was followed by filtration through Whatman No.1 filter paper (GE Healthcare, UK). The filtrate was transferred into pre-weighed flat bottom flask, concentrated using rotary vacuum evaporator (Eyela N-1001, Japan) at 40°C and stored at 4°C for further analysis. Extractions were done in triplicate.

Disk diffusion test (DDT)

An amount of 0.05 g crude extracts were dissolved in Dimethyl sulfoxide (DMSO) (Fisher Scientific, UK) to a concentration of 0.1 g/mL extract solution and filtered through 0.45 µm cellulose membrane filter. The filtered extracts antibacterial activities were determined against *Shigella sonnei* (ATCC 29930), *Salmonella typhimuriu*m (ATCC 13311), *Escherichia coli* (ATCC 11229), *Salmonella enteritidis* (ATCC 13076), *Vibrio vulnificus* (ATCC 27562), *Vibrio parahaemolyticus* (ATCC 17802), *Proteus mirabilis* (ATCC 12453) *Staphylococcus aureus* (ATCC 12600), *Bacillus cereus* (ATCC 10875) and *Listeria monocytogenes* (ATCC 19111), *Corynebacterium diphtheria* (ATCC 13812), *Clostridium perfringens* (ATCC 13124), *Streptococcus pneumoniae* (ATCC 10015) and *Bacillus subtili*s (ATCC 11774) by modified disc diffusion method of Cattelan *et al*. (2013). A loopful of cells from the stock cultures (maintained at 4°C) was transferred into sterile tryptone soy broths (Oxoid, England) and incubated for 4 - 16 h at 37°C to achieve an inoculum concentration of 10^6 CFU/mL. An aliquot of 0.1 mL of the inoculum was spread on sterile Mueller-Hinton agar (MHA) (Oxoid, England). Sterile paper discs (6 mm diameter) containing 10 µL of the 0.1 g/mL extract solution were placed on the inoculated MHA. The plates were then incubated at 37°C for 24 h. Bacterial growth inhibition was determined as the diameter of the inhibition zones (mm) after subtracting 6 mm of paper disc diameter. DMSO (100%) and 10 mg/mL tetracycline hydrochloride (TCH) (Fisher Scientific, UK) were used as negative and positive controls, respectively.

Minimum inhibitory concentration identification (MIC)

A two-fold serial microdilution method of 96 multi-well microtitre plate (Turgis *et al*., 2012) was used for MIC determination with modifications. A 100 µL aliquot of TSB was added into each well. A volume of 100 µL of 50 mg/mL crude extracts in DMSO was added into the first well. A volume of 100 µL from first test well was pipetted into the second well of each microtiter row and then 100 µL of scalar dilution was transferred from the second until to the eleventh well. A volume of 100 µL from the last well was discarded. Ten microliters from each well was replaced with 10 µL of 106 CFU/mL bacterial suspensions to make up to concentration of 22.50 - 0.02 mg/mL. The optical density was measured at 600 nm in an Ultra Microplate Reader (Biotek Instruments, Winoo-ski, VT, USA) before (T_0) and after 24 h incubation (T_{24}) at 37°C. A MHA incubated with a target bacterium was used as a positive control of growth. The MIC was defined as the lowest concentration of antimicrobial agent showing a complete growth inhibition of the tested bacterial strain which was related to a difference absorbance of zero = $T_{24} - T_0 = 0$, i.e $T_{24} = T_0$ or T24 < T0.

To confirm the MIC, loophole of tested

concentrations which were equal and higher than MIC was streaked on MHA. The plates were then incubated at 37°C for 24 h. The least concentration of the extracts where no visible growth was observed on the agar plates was considered as MIC. All determinations were performed in triplicate.

Yield

Extraction yield (mg/g) on dry weight basis was calculated according to the equation below:

$$\text{Yield of extract (mg/g)} = \frac{\text{wt of concentrated extract (mg)}}{\text{dry weight of sample (g)}}$$

Quantitation of total phenolic content (TPC)

Total phenolic contents of Carica papaya crude extracts were determined by colorimetry assay with Folin-Ciocalteu according to Wong *et al.* (2013). An aliquot of crude extract containing 0.05 g of extract was diluted to 100 mL in a volumetric flask prior to analysis. An amount of 1 mL diluted crude extract was added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent (Sigma-Aldrich, Switzerland) in an aluminium foil wrapped 5 mL volumetric flask and vortexed for 10 s. The extract mixture was then incubated at 30°C for 5 min, followed by the addition of 1 mL of sodium carbonate (10%, w/v) solution (Sigma-Aldrich, Switzerland). The mixture was made up to volume and vortexed (VTX-3000L, Copens Scientific, Germany) for another 10 s and incubated in the dark at 30°C for 30 min. The absorbance of blue coloured aqueous layer which indicated the presence of phenolic compounds was measured at 747 nm using spectrophotometer (U-2810 Hitachi, Japan) against methanol as the blank. The same incubation procedure was used for the preparation of gallic acid standard (Sigma-Aldrich, Switzerland) in methanol to obtain a 0 - 10 mg/L standards including methanol as blank. A calibration curve was plotted and calibration equation for weight gained was $y = 0.0827x + 0.0007$ ($R^2 = 0.9999$). Readings were carried out in triplicate. Results were expressed as gallic acid equivalent (GAE) in mg/g of dry weight (DW) of sample (mg GAE / g DW).

Quantitation of total flavonoid content (TFC)

Total flavonoid contents of *Carica papaya* crude extracts were determined by colorimetry assay in accordance with Fariza *et al.* (2011). An aliquot of crude extract (containing 0.05 g of extract) was diluted in 100 mL volumetric flask prior to analysis. The diluted crude (1.25 mL) extract was added to 0.5 mL of 0.1 g/mL aluminium chloride solution (Sigma-Aldrich, Switzerland) and 0.5 mL of 1 M sodium acetate solution in an aluminium foil-wrapped 5 mL volumetric flask, made-up to volume and vortexed for 10 s which was followed by an incubation at 30 oC for 15 min. The appearance of a yellow colour solution, indicated the existence of flavonoids. The absorbance of the colored mixture was measured spectrophotometrically at 438 nm against ethanol as the blank. The calibration curve was established by replacing the *Carica papaya* seed diluted extracts with quercetin standards (Sigma-Aldrich, Switzerland). The same preparation and incubation procedure was given to the quercetin standards (in ethanol) to obtain seven working standards (0 - 12.5 mg/L) including ethanol as blank. The calibration equation obtained was $y = 0.0762x - 0.0126$ ($R^2 = 0.9994$). The measurements were carried out in triplicate. Results were expressed as quercetin equivalent per gram dry weight (QE) in mg per g of dry weight (DW) of sample (mg QE / g DW).

Gas chromatography mass spectrometry analysis (GC/MS)

Methanol extract was found to be the most potent extract. GC/MS analysis of the methanol extract was performed using an Agilent-Technologies 7890A GC system equipped with an Agilent-Technologies 5975 mass selective detector (Agilent Technologies, USA). The method of Tenore *et al.* (2011) with modification was adopted. For MS detection, the electron ionization mode with an ionization energy of 70 eV was used, with a mass range of m/z 50–550. An HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm) was used for peak separation. The column temperature ramp was programmed from 70°C for a min., then raised to 150°C at 15°C/min and held for 15 min and finally raised to 300°C at a rate of 15°C/min and held for 30 min. The GC injector and MS transfer line temperatures were set at 240°C and 23^{0}°C, respectively. Gas chromatography was performed in the splitless mode at 10:1 ratio. Helium was used as carrier gas at a flow rate of 1.2 mL/min. An injection volume of 1 µL was used for each diluted 0.01 g/mL extract. Essential compounds were identified by their retention times and mass fragmentation patterns of standards at National Institute of Standard (NIST) Mass Spectral 11 library.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate solvent extraction, disk diffusion test, TPC and TFC. One-way analysis of variance (ANOVA) with Tukey's test was conducted using XLSTAT-Pro (2014) statistical software (Addinsoft, Paris, France) to determine the significant difference

Table 1. Inhibition zone of *Carica papaya* seed extracts on gram-negative food pathogens by different solvent extracts

Solvents[3]	Total Inhibition[2], mm						
	S. sonnei	*S. typhimurium*	*E. coli*	*S. enteritidis*	*V. vulnificus*	*V. parahaemolyticus*	*P. mirabilis*
Hexane	na	na	3.06 ± 0.93[b]	na	na	na	2.91± 0.34[a]
PE	na	na	na	na	na	na	3.91 ± 0.88[b]
DE	na	na	3.87 ± 0.65[b]	na	na	na	2.69 ± 0.53[a]
CHCL3	na	4.40 ±0.56[c]	3.79 ± 0.60[b]	3.52 ± 0.28[b]	na	na	4.28 ± 0.37[c]
DCM	na	na	2.57 ± 0.22[a]	3.53 ± 0.25[b]	na	na	na
Acetone	2.58 ± 0.40[a]	3.89 ± 0.81[b]	2.29 ± 0.58[a]	3.49 ± 0.89[b]	na	na	3.23 ± 0.75[b]
EtOH	na	3.16 ± 0.77[b]	3.33 ± 0.65[b]	2.45 ± 0.86[a]	na	na	na
MeOH	2.79 ± 0.28[a]	6.09 ± 1.55[c]	2.07 ± 0.58[a]	3.45 ± 0.75[b]	3.04 ± 0.20[b]	na	3.80 ± 0.65[b]
ACN	1.93 ± 0.64[a]	4.22 ± 1.45[c]	4.65 ± 0.86[c]	4.43 ± 0.01[c]	2.93 ± 0.69[a]	na	2.55 ± 1.14[a]
Water[1]	na	na	na	na	na	na	na
DMSO	na	na	na	na	na	na	na
TCH	4.17 ± 0.76[c]	21.83 ± 0.29[c]	19.00 ± 0.00[c]	19.50 ± 0.50[c]	21.33 ± 0.58[c]	12.67 ± 1.15[c]	9.63 ± 1.91[c]

[1]Solid /solvent ratio of 1:6.
[2]Means ± S.D. are from triplicate measurements, after deduction of 6 mm disc diameter.
[3]PE - petroleum ether, DE- diethyl ether, $CHCL_3$-chloroform, DCM- dichloromethane, EtOH - ethanol, MeOH - methanol, ACN - acetonitrile.
[4]na - No antibacterial activity (inhibition zone of sample < 1 mm).
Different superscripts indicate antibacterial activities, [a]: Slight antibacterial activity (inhibition zone of sample 1–3 mm); [b]: Moderate antibacterial activity (inhibition zone of sample 3–4 mm); [c]: Clear antibacterial activity (inhibition zone of sample 4–10 mm).

between the means at 95% confidence level ($p < 0.05$) for solvent extraction, TPC and TFC.

Results and Discussion

Antibacterial screening by disk diffusion test

Test results for DDT are tabulated in Table 1 and 2 for gram-negative and gram-positive microorganisms, respectively. The interpretation of antibacterial activity classification was according to Rauha *et al.* (2000) where the zone of inhibition reported is excluding the disc diameter. Carica papaya seed extracts inhibited majority of tested microorganisms, except water extract (Table 1 and 2). Our results showed that gram-negative bacteria were more sensitive than the gram-positive bacteria to the papaya seed crude extracts (Table 2), thus contradicted that of Tiwari et al. (2011) claim. Among fourteen microorganisms, *C. diphtheria, E. coli* and *S. pneumonia* were the more sensitive microorganisms towards *Carica papaya* seed extracts. *S. typhimurium* commonly reported for raw and cooked meats spoilage was strongly inhibited particularly by MeOH, $CHCL_3$ and ACN extracts (Table 1). Even though wide spectrum inhibition can be observed among extracts, MeOH, $CHCL_3$, DCM, acetone and ACN clear inhibition were generally selective to one specific microorganisms such as *S. typhimurium, C. perfringens, S. pneumonia, C. diphtheria* and *L. monocytogenes*, respectively. *V. parahaemolyticus* was not inhibited by any of the extracts.

The highest number of inhibition was exhibited by MeOH and ACN extracts. ACN extract clearly inhibited *E. coli, S. typhimurium* and *S. enteritidis* which are frequently found in raw vegetables and fruits and unpasteurized fruit juices (Raybaudi-Massilia *et al*., 2009; Danyluk and Parish, 2012). Clear inhibitions were obtained from MeOH and $CHCL_3$ Carica papaya crude extracts against *S. typhimurium* which was similar compared to that of Chanda *et al*. (2013) antimicrobial studies of *Mesua ferrea* L. seed extract as well as *Sphallerocarpus gracilis* seed essential oil (Gao *et al*., 2011). PE, $CHCL_3$, acetone and MEOH extracts of *Mesua ferrea* L. seed were inactive against *E. coli* (Chanda *et al*., 2013) while our seed extract had slight to moderate inhibition, with PE extract as an exception. The $CHCL_3$ *Carica papaya* seed extract showed clear inhibition against *P. mirabilis*, similar to that of *Mesua ferrea* L. seed extract (Chanda *et al*., 2013). *L. monocytogenes* was moderately inhibited compared to strong inhibition by *Alpinia nigra* seed MeOH extract (Ghosh *et al.*, 2013). The broad spectrum inhibition of ACN and MeOH extracts against tested microorganisms proved the potential of *Carica papaya* seed as an antibacterial extract source. However, if considering clear and moderate inhibition only, MeOH and acetone extracts were the two most sensitive extracts.

Minimum inhibitory concentration identification (MIC)

Quantitative evaluation of the antimicrobial activity of *C. papaya* seed extract was carried out against selected microorganisms. The MICs, (mg/

Table 2. Inhibition zone of *Carica papaya* seed extracts on gram-positive food pathogens by different solvent extracts

Solvent[3]	Total Inhibition[2], mm						
	S. aureus	*B. cereus*	*L. monocytogenes*	*C. diphtheria*	*C. perfringens*	*S. pneumoniae*	*B. subtilis*
Hexane	na	na	na	3.10 ± 0.52[b]	3.84 ± 0.31[b]	3.16 ± 1.25[b]	na
PE	na	na	na	3.59 ± 0.45[b]	3.26 ± 0.72[b]	2.81 ± 0.70[a]	na
DE	na	na	na	3.10 ± 0.40[b]	na	3.39 ± 1.32[b]	na
CHCL3	na	na	na	2.77 ± 0.65[a]	4.02 ± 0.62[c]	na	na
DCM	na	na	na	na	na	4.08 ± 0.27[c]	na
Acetone	na	na	na	4.31 ± 0.45[c]	3.90 ± 0.02[b]	3.42 ± 0.98[b]	3.35 ± 0.70[b]
EtOH	na	na	na	3.66 ± 0.73[b]	2.22 ± 0.35[a]	2.52 ± 0.92[a]	na
MeOH	na	3.01 ± 0.77[b]	3.35 ± 1.10[b]	3.69 ± 0.70[b]	2.85 ± 0.46[a]	2.71 ± 0.33[a]	na
ACN	na	2.42 ± 1.03[a]	4.04 ± 0.73[c]	2.99 ± 0.69[a]	3.13 ± 1.17[b]	3.08 ± 0.51[b]	na
Water[1]	na	na	na	na	na	na	na
DMSO	na	na	na	na	na	na	na
TCH	27.83± 0.29[c]	15.00 ± 0.50[c]	na	12.44 ± 1.61[c]	14.78 ± 0.37[c]	11.61 ± 0.85[c]	14.35 ± 0.33[c]

[1]Solid/solvent ratio of 1:6.
[2]Means ± S.D. are from triplicate measurements, after deduction of 6 mm disc diameter.
[3]PE - petroleum ether; DE - diethyl ether; CHCL3 – chloroform; DCM – dichloromethane; EtOH – ethanol; MeOH – methanol; ACN - acetonitrile.
[4]na - No antibacterial activity (inhibition zone of sample < 1 mm).
Different superscripts indicate antibacterial activities, [a]: Slight antibacterial activity (inhibition zone of sample 1–3 mm); [b]: Moderate antibacterial activity (inhibition zone of sample 3–4 mm); [c]: Clear antibacterial activity (inhibition zone of sample 4–10 mm).

Table 3. Minimum inhibitory concentration (MIC) (mg/ml) of *Carica papaya* seed extracts on selected pathogens by different solvents

Pathogens[3]	MIC (mg/mL) for different solvents[1]								
	Hexane	PE	DE	CHCL3	DCM	Acetone	EtOH	MeOH	ACN
S. typhimurium	nd	nd	nd	5.63	nd	11.25	11.25	11.25	11.25
E. coli	11.25	nd	5.63	5.63	nd	nd	11.25	nd	11.25
S. enteritidis	nd	nd	nd	11.25	11.25	11.25	nd	5.63	11.25
V. vulnificus	nd	nd	nd	nd	nd	nd	nd	5.63	nd
P. mirabilis	nd	5.63	nd	11.25	nd	5.63	nd	5.63	nd
B. cereus	nd	nd	nd	nd	nd	nd	nd	5.63	nd
L. monocytogenes	nd	nd	nd	nd	nd	nd	nd	11.25	11.25
C. diphtheria	5.63	11.25	11.25	nd	nd	11.25	11.25	11.25	nd
C. perfringens	11.25	11.25	nd	11.25	nd	11.25	nd	nd	22.5
S. pneumoniae	11.25	nd	11.25	nd	22.5	11.25	nd	nd	22.5
B. subtilis	nd	nd	nd	nd	nd	11.25	nd	nd	nd

[1]PE - petroleum ether; DE – diethyl ether, $CHCL_3$ – chloroform; DCM – dichloromethane; EtOH - ethanol; MeOH – methanol; ACN – acetonitrile [2]nd - Not determined.
[3]Selection of pathogens and extracts based on clear and moderate inhibition from disk diffusion test result.

ml) of active extracts are as shown in Table 3. Only DDT results with moderate and clear zone inhibitions were chosen for MIC determination, of which five gram-positive and six gram-negative microorganisms fulfilled the inhibition zone criteria. The gram-positive bacteria, *P. mirabilis,* was the most sensitive microorganism to *Carica papaya* PE, acetone and MeOH extracts at 5.63 mg/mL (Table 2). MeOH extract at 5.63 mg/mL were inhibitory for *S. enteritidis, V. vulnificus, P. mirabilis* and *B. cereus* while ACN at 11.25 mg/mL for *S. typhimurium, E. coli, S. enteritidis* and *L. monocytogenes* even though MeOH and ACN extracts showed wide spectrum inhibition in the DDT result. This indicated that clear and moderate inhibitions from DDT results did not demonstrate lower MIC values (Klancnik *et al*., 2010). Hence, MeOH extract of *Carica papaya* seed was the most potent extract and *S. enteritidis, V. vulnificus, P. mirabilis* and *B. cereus* may be used as indicator microbes in the similar antibacterial study of *Carica papaya* seed extract. Based on clear and moderate inhibition and the lowest MIC, the extract potency was ranked as MeOH > acetone > ACN > $CHCL_3$ > hexane > DE = PE > EtOH > DCM.

Comparing to *Sphallerocarpus gracilis* seed essential oil against *B. cereus* and *S. enteritidis* (0.32 mg/mL of MIC) (Gao *et al.*, 2011) and mango seed kernel ethanolic extract against *Vibrio* spp.

Table 4. Extract yield, total phenolic and total flavonoid contents of *Carica papaya* seed extracts of different solvents

Solvents[3]		Yield[2] (mg/g sample)	TPC[2] (mg GAE / g DW)	TFC[2] (mg QE / g DW)
Non Polar	Hexane	119.15 ± 0.21^{bc}	8.55 ± 0.49^{g}	6.70 ± 1.08^{cd}
	PE	69.71 ± 0.09^{c}	11.00 ± 0.29^{f}	5.04 ± 0.28^{e}
	DE	115.80 ± 0.07^{c}	14.65 ± 0.78^{e}	7.02 ± 1.31^{c}
	CHCL3	63.59 ± 0.41^{bc}	4.83 ± 0.12^{j}	2.00 ± 0.14^{f}
	DCM	85.44 ± 0.40^{c}	7.01 ± 0.14^{h}	6.11 ± 0.12^{d}
Polar	Acetone	65.29 ± 0.10^{b}	22.59 ± 0.06^{a}	17.15 ± 0.25^{a}
	EtOH	24.80 ± 0.17^{bc}	15.45 ± 0.11^{d}	11.82 ± 0.00^{b}
	MeOH	20.56 ± 0.19^{bc}	16.66 ± 0.18^{c}	1.94± 0.24^{f}
	ACN	8.80 ± 0.14^{c}	19.73 ± 0.09^{b}	5.14 ± 0.07^{e}
	Water[1]	35.62 ± 0.35^{a}	5.57 ± 0.10^{i}	1.32 ± 0.00^{f}

[1]1:6 solid/solvent ratio.
[2]Means ± S.D. are from triplicate measurements.
[3]PE-petroleum ether; DE-diethyl ether; $CHCL_3$–chloroform; DCM–dichloromethane; EtOH–ethanol; MeOH-methanol; ACN–acetonitrile.
Different superscripts indicate significantly different mean ($p < 0.05$)

and *Bacillus* spp. (0.5 mg/mL MIC) (Kabuki *et al*., 2000), our MeOH extract had lower potency. Further fractionation and purification steps such of tetrahydrofuranyl diester, fractionated from petroleum ether extract of neem oil (*Azadirachta indica* A. Juss) had 2.5 mg/mL MIC compared to its volatile oil (5 mg/mL) against S. enteritidis (Zhang *et al*., 2010), may increase extract potency.

Extraction yield, total phenolic contents (TPC) and total flavonoid contents (TFC)

Higher yields (63.59 to 119.15 mg/g) were obtained in non-polar solvents as compared to polar solvents (8.80 to 65.29 mg/g) (Table 4). Among the 1:2 solid to solvent ratio extraction, hexane had the highest yield (~119.15 mg/g) while ACN had the lowest (~8.80 mg/g). Yield of extract from water extraction showed significant differences to the rest of evaluated solvents.

Table 4 shows TPC and TFC of crude extracts of different solvents. The TPC in the various crude extracts varied from 4.83 ($CHCL_3$) to 22.59 mg GAE/g DW (acetone) while TFC ranged from 1.32 (water) and 17.15 mg QE / g DW (acetone). However, the TFC from MeOH and ACN extracts were unproportioned with their TFC. Based on these collected data, it can be inferred that polar solvents are preferable for the extraction of phenolics and flavonoids; similar to Razali *et al.* (2012) conclusion on the extraction of *Tamarindus indica* L. seeds.

The high yield of extraction could not be equated to higher TPC, TFC and antibacterial activity. For instance, hexane crude extract which had the highest concentration of yield (119.15 mg/g) had low TPC, TFC and number of microorganisms inhibition compared to MeOH extract. Lower yield such as MeOH extract exhibited higher TPC and potent inihibitor. Our MeoH extract had five times higher concentration of TPC and three times greater TFC compared to Chilean papaya *(Vasconcellea pubescens)* seeds (Briones-Labarca *et al.*, 2015), and was considered had moderate TPC and TFC compared to *Chenopodium quinoa* (Miranda *et al*., 2010) and guarana seed *(Paullinia cupana)* (Majhenič *et al.*, 2007). Higher TPC may increase the inhibitory effect of MeOH extract as such work of Al-Zoreky (2009) on pomegranate peel.

GC/MS analysis of the methanol extract from Carica papaya seed

Twenty-five compounds (Table 5) were identified with more than 90 % similarity with the standard mass spectra in the NIST 11 library, representing 70 % of the relative area in the MeOH extract. The extract was characterized by the presence of benzyl groups, with benzyl nitrile (35.97%) having the highest percentage area, followed by 2-methyl-benzonitrile (7.78%) and isothiocyanatomethyl benzene (3.75%). Fatty acids and fatty acid methyl esters (FAME) groups (21.42 %) consisted of trans-9-octadecenoic acid (12.36%), hexadecanoic acid (2.79%), cis-9-octadecenoic acid, 2,3-dihydroxypropyl ester (1.45%), cis-9-octadecenoic acid methyl ester (1.19%), hexadecanoic acid trimethylsilyl ester (1.07%), and, cis-9-octadecenoic acid (1.05%) were also identified.

The likelihood of flavonoid in *Carica papaya* MeOH seed extract to be bactericidal is low due to very low TFC (Table 4). Higher concentration of anthocyanins than flavonoids was believed inhibited *E. coli* and *S. aureus* (Dib *et al*., 2013); however, our GC/MS analysis did not detect anthocyanin (Table 5). Pured form of flavonoids such as flavone, flavonols and naringenin and free flavonoids proved to inhibit successfully certain microorganisms (Rauha *et al*., 2000) in extent most probably in the latter form, but

Table 5. Chemical composition of the methanolic extract from *Carica papaya* seed

No.	Rt[a]	Compound[b]	% Area
1	5.196	2-methylbenzonitrile	7.78
2	5.403	2-phenylacetonitrile	35.97
3	7.397	Isothiocyanatomethyl benzene	3.75
4	7.810	Benzeneacetamide	0.58
5	17.714	Tetradecanoic acid	0.11[c]
6	22.750	Tetradecanoic acid trimethylsilyl ester	0.03[c]
7	23.132	Trifluoroacetate oleyl alcohol,	0.08
8	24.531	Hexadecanoic acid methyl ester	0.4[c]
9	24.874	Cis-9-Hexadecenoic acid	0.06[c]
10	25.394	Hexadecanoic acid	2.79[c]
11	26.227	Hexadecanoic acid, trimethylsilyl ester	1.07[c]
12	26.686	Trans-9, 12-octadecadienoic acid, methyl ester	0.1[c]
13	26.754	Cis-9-octadecenoic acid methyl ester	1.19[c]
14	26.991	Methyl stearate	0.07
15	27.404	Trans-9-octadecenoic acid	12.36[c]
16	27.488	Cis-9-octadecenoic acid	1.05[c]
17	27.771	11-trans-Octadecenoic acid trimethylsilyl ester	0.76[c]
18	28.810	Cis-9-octadecenamide	0.24
19	28.955	Cis-13-Octadecenoic acid	0.05[c]
20	29.490	Cis-7, 11-hexadecadien-1-yl acetate	0.12
21	29.559	Cis-9-octadecenal	0.09
22	30.667	Cis-9-octadecenoic acid, 2,3-dihydroxypropyl ester	1.45[c]
23	34.679	β-sitosterol	0.24
24	34.847	β-sitosterol trimethylsilyl ester	0.1
25	36.047	Stigmast-4-en-3-one	0.07

[a]Rt: Retention time (as min)
[b]Compounds identified at more than 90% similarity with the standard mass spectra in NIST 11 library.
[c]21.42 % total percentage peaks of fatty acids and fatty acid methyl esters group.

not within our experimental design. Phenolics may not be the only responsible antibacterial compounds since benzene compounds and fatty acids consisted in our MeOH seed extracts may demonstrate the same behavior as supported by Koolen *et al.* (2013). The GC/MS analysis of our *Carica papaya* MeOH seed (Table 5) showed that benzene groups and fatty acids were abundant. 2-phenylacetonitrile, although the highest compound detected, had not been reported as having antibacterial activities (Noumi *et al*., 2011; Sofrata *et al.*, 2011). Likewise, 2-methylbenzonitrile did not demonstrate the same inhibitory character. Isothiocyanatomethyl benzene or commonly known as benzyl isothiocyanates had bactericidal effect on food microorganisms (Kermanshai *et al*., 2001; Muhaidat *et al*., 2015). The pure form had reportedly inhibited *S. aureus, B. subtilis* and *E. coli* at 80, 200 and 200 (µg/mL) MIC, respectively (Al-Ani *et al*., 2015) as compared to our crude extract.

The unsaturated fatty acid, 9-octadecenoic acid was reported to exhibit wide spectrum antibacterial capacity (Desbois and Smith, 2010) against marine pathogenic bacteria such as *L. garvieae, V. anguillarium, V. harveyi* and *V. alginocolyticus* (Benkendorff *et al*., 2005). Hexadecanoic acid at 6.0% of *Salvia lanigera* was effective against *S. aureus* (Tenore *et al*., 2011); however, our MeOH extract had no such inhibition. The β-sitosterol, found in minute quantity in our seed extract, had also been reported to exhibit antibacterial activity (Dickson *et al*., 2007; Hajji *et al*., 2010).

Conclusion

In summary, MeOH extract of *Carica papaya* seed cv. Sekaki/ Hong Kong had demonstrated antibacterial activity against *S. enteritidis, V. vulnificus, P. mirabilis* and *B. cereus*. The MeOH extract contained isothiocyanatomethyl benzene, 9-octadecenoic acid, hexadecanoic acid and β-sitosterol as potential antibacterial compounds. Further fractionation and purification steps could shed further antibacterial potential of the MeOH extract.

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