International Food Research Journal 24(3): 976-984 (June 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

In vitro antibacterial activities and composition of *Carica papaya* cv. Sekaki/ Hong Kong peel extracts

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Article history

Received: 26 April 2016
Received in revised form: 7 June 2016
Accepted: 24 June 2016

Keywords

Solvent extraction
Antibacterial activity
Peel
GC/MS analysis

Abstract

Ten solvents were used to extract phytochemicals from the peel of *Carica papaya* cv. Sekaki/Hong Kong to determine their antibacterial activities. Moderate to clear inhibition on *Corynebacterium diphtheriae, Streptococcus pneumoniae, Bacillus subtilis* and *Clostridium perfringens* were obtained from the disk diffusion test out of fourteen pathogens tested. Petroleum ether extract, the most potent extract, showed moderate inhibition towards *C. diphtheriae* and *S. pneumoniae* at MIC of 5.63 mg/mL and 1.40 mg/mL. Polar solvents gave higher yield, total phenolic and total flavonoid contents than nonpolar solvents. Extract yields were 10.9 to 84.1 mg/g in polar solvents and 3.9 to 20.3 mg/g in non-polar solvents. Twenty eight compounds were identified in petroleum extracts through GC/MS analysis. Among the compounds identified were fatty acids, esters, alkane, tocopherols and sterols. 9,12,15-octadecatrienoic acid was the most abundant compound.

Introduction

Antibacterial compounds are used in food as preservatives to extend shelf life and in non-food as sanitizing agents. Natural antimicrobial active ingredients are known to have less health implications, and hence agro-base wastes such as peels and seeds of fruits could be a cheaper source for these preservatives supporting the global move to reduce waste. The search for beneficial phytochemicals from fruit peels and seeds extracts, and their antibacterial activities have been reported by several researchers such as that on *Nephelium lappaceum* L. methanolic extract (Thitilertdecha *et al*., 2008), 80% methanolic extract of pomegranate (*Punica granatum* L.) fruit peels (Al-Zoreky, 2009), and *Citrus sinensis* (acetone extract) and *Citrus limon* ethyl acetate peels extracts (Ashok Kumar *et al*., 2011) among others. The antibacterial activities reported were from several different solvent extracts since complete extraction of these phytochemicals by a single solvent may not be possible due to their chemical complexity and different distribution pattern throughout the plant besides the selectivity of the solvents themselves (Naczk and Shahidi, 2004; De Rijke *et al*., 2006). Reports on antibacterial activities from papaya *(Carica papaya)* peels and seeds are still scanty as evident by the recent review on agro-industrial potential of exotic fruit by-products (Ayala-Zavala *et al.*, 2011). Pioneering work by Emeruwa (1982) reported the very significant antibacterial activity of protein precipitated from ethanol extracts of epicarp (peel), endocarp and seeds of papaya against *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella flexneri.* The water extract from mixture of flesh, seed and peel of unripe *Carica papaya* inhibition on *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* was reported by Osato *et al.*, (1993) and antibacterial properties from peel of *Carica papaya* involving peptides and secondary metabolites such as phenolic compounds (Nayak *et al*., 2007) have also been reported. Organic solvents are also known to be toxic to bacterial cells as they bind to the cell membrane and render inhibition or cell rupture thus causing cell fatality (Torres *et al*., 2011). Hence, the effect of solvent inhibition must be taken into account in any inhibition studies to obtain the true inhibition capacity of the active components.

To date, the information regarding *Carica papaya* peel composition is limited. Rivera-Pastrana *et al*. (2010) identified the papaya fruit composition

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by high pressure liquid chromatography-mass spectrometry while Canini *et al*. (2007) scrutinized the leaf composition by gas chromatography mass-spectrometry (GC/MS), not the peel. Due to this scanty information, the chemical composition of the most potent extract was also identified through GC/MS analysis.

Carica papaya cv. Sekaki or Hong Kong co-exists with Eksotika I and Eksotika II varieties in Malaysia. The antibacterial activities of these varieties have not been exhaustively studied, neither the composition of the resulting extracts reported. Therefore, this study was carried out to determine the antibacterial activity of the discarded peel of the ripe papaya as affected by different solvents extractions against fourteen selected pathogens.

Materials and Methods

Preparation of plant materials

Carica papaya cv. Sekaki fruits at maturity stage of six (Sapii and Muda, 2005) were bought from D'Lonek Sdn. Bhd. Organic Farm, Rembau, Negri Sembilan, Malaysia. A voucher of herbarium specimen numbered as SK 2368/14 was deposited at the Herbarium of Institute of Bioscience, Universiti Putra Malaysia as part of identification process. The peels (0.4 ± 0.1 cm) were washed thoroughly in distilled water, drained for 3 hr and oven dried at 40°C for 3 days (Adejuwon *et al.*, 2011). Dried peels were ground for 5 min in 240 W electrical blender (Panasonic MX-337, Malaysia), 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. 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 peel immersion. Briefly, 50 g of dried ground Carica papaya cv. Sekaki peel 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 flasks and concentrated using rotary vacuum evaporator (Eyela N-1001, Japan) at 40°C. Extracts were then suspended in 5 mL respective solvents and stored at 4°C in amber reagent bottles for further analysis. Extractions were done in triplicate.

Disk diffusion test (DDT)

An aliquot of each diluted crude extract (containing 0.1 g crude extract) was blown with N_2 to dryness until a constant weight was reached. The resultant dried 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 antibacterial activity were determined against *Shigella sonnei* (ATCC 29930), *Salmonella* Typhimurium (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), *Listeria monocytogenes* (ATCC 19111), *Corynebacterium diphtheriae* (ATCC 13812), *Clostridium perfringens* (ATCC 13124), *Streptococcus pneumoniae* (ATCC 10015) and *Bacillus subtilis* (ATCC 11774) (Microbiologics® Minnesota, U.S) by modified disc diffusion method of Cattelan *et al.* (2013). The inoculums were prepared by transferring a loopful of cells from the stock cultures (maintained at 4°C) into sterile tryptone soy broths (TSB, Oxoid, England) and incubated between 4 - 16 h at 37°C to achieve inoculum containing 10^6 CFU/mL. An aliquot of 0.1 mL of the inoculum was spread on sterile Mueller Hinton agar (MHA, Oxoid, England) which was followed by placing sterile paper discs (6 mm diameter) containing 10 µL of the 0.1 g/mL extract solution pipetted on them. The plate agars 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 and tetracycline hydrochloride (TCH, Fisher Scientific, UK) were used as negative and positive controls, respectively. The interpretation of antibacterial activity classification was according to Rauha *et al.* (2000).

Minimum inhibitory concentration identification (MIC)

A two-fold serial microdilution method of 96 multi-well microtitre plate, following Turgis *et al.* (2012) was used for MIC determination with modifications. Briefly, 100 µL of TSB, supplemented with tween 80 at concentration of 1% (v/v) was pipetted into each well. A volume of 100 µL of 1 x 10^5 µg/mL crude extracts in DMSO was then added into the first well. This was followed by pipetting

the first test well was pipetted into the second well of each microtiter row and this serial dilution was repeated until eleventh well. A volume of 100 µL from the last well was discarded. An aliquote of 90 µL from each well was mixed with 10 µL of 106 CFU/mL bacterial suspensions (*C. diphtheriae, S. pneumoniae, C. perfringens,* and *B. subtilis*) which will make the extract concentrations to be 45 - 0.044 mg/mL in the first well until the lowest concentration in the eleventh well. The microtitre plate was incubated at 37°C for 24 h. Then, the optical density was measured at 600 nm in an Ultra Microplate Reader (Biotek instruments, Winoo- ski, VT, USA) before (T_0) and after (T_{24}) incubation. TSB was used as positive control for growth. The MIC is defined as the lowest concentration of antimicrobial agent showing a complete growth inhibition of the tested bacterial strains which is related to the difference in absorbance of T_{24} and T_0 (T_{24}- T_0) that is equal to zero or negative values. In order to identify the bactericidal of bacteriostatic characteristics of the extracts, inoculums from the wells with concentration equal to or greater than MIC were streaked on MHA medium and were incubated at 37°C for an additional of 24 h. All determinations were performed in triplicate.

Yield

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

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

Quantitation of total phenolic content (TPC)

Total phenolic content of Carica papaya crude extracts were determined by colorimetry assay with Folin-Ciocalteu in accordance to Wong *et al*. (2013). An aliquot of crude extract, (containing 0.05 g of extract) were diluted in 100 mL 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 vortex for 10 s. After incubation at 30°C for 5 min, 1 mL of sodium carbonate (10%, w/v) solution (Sigma-Aldrich, Switzerland) was added into the mixture. The mixture was made up to volume and vortexed (VTX-3000L, Copens Scientific, Germany) for 10 s and incubated in dark environment at 30°C for 30 min. Two layers of solution may occur in non-polar solvent mixtures and the blue coloured aqueous layer which indicated the existence of phenolic compounds was taken for measurement. The absorbance of the mixture was measured at 747 nm using spectrophotometer (U-2810 Hitachi, Japan) and the respective solvent was used as blank. The calibration curve was established by replacing the *Carica papaya* diluted extracts with 1 mL gallic acid standards (Sigma-Aldrich, Switzerland). The same incubation procedure was given to the gallic acid standard (in methanol) to obtain nine working standards (0 - 10 mg/L) including methanol as blank. The calibration equation for weight gained was $y = 0.0827x + 0.0007$ ($R^2 = 0.9999$). The measurements were carried out in triplicate. The results were expressed as gallic acid equivalent (GAE) in mg per g of dry weight (DW) of sample (mg GAE / g DW).

Quantitation of total flavonoid content (TFC)

Total flavonoid content of Carica papaya crude extracts were determined by colorimetry assay in accordance to 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. An amount of 1.25 mL diluted crude extract was added to 0.5 mL of 0.1 g/mL aluminum chloride solution (Sigma-Aldrich, Switzerland) and 0.5 mL of 1 M sodium acetate solution in an aluminium foil-wrapped 5 mL volumetric flask, make up to volume with ethanol, vortexed for 10 s and incubated at 30°C for 15 min. After which, 1 mL of the mixture was taken for measurement. The absorbance of the mixture was read off spectrophotometrically at 438 nm and respective solvent was used as blank. The calibration curve was established by replacing the *Carica papaya* peel 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 gained was $y = 0.0762x - 0.0126$ ($R^2 = 0.9994$). The measurements were carried out in triplicate. The 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 analysis of the petroleum ether extract was performed using an Agilent-Technologies 7890A GC system equipped with an Agilent-Technologies 5975 mass selective detector (Agilent Technologies, USA). This 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 separation. The

Table 1. Inhibition zone of *Carica papaya* peel on gram-negative food pathogens

Solvents[3]	Total Inhibition[2, 5], mm						
	S. sonnei	*S.* Typhimurium	*E. coli*	*S.* Enteritidis	*V. vulnificus*	*V. parahaemolyticus*	*P. mirabilis*
Hexane	na[4]	na	na	na	$1.33 \pm 0.58^{a}_{B}$	na	$1.45 \pm 0.11^{a}_{B}$
PE	na	$1.83 \pm 0.29^{a}_{B}$	$1.33 \pm 0.58^{a}_{B}$	$1.83 \pm 0.29^{a}_{B}$	na	$1.50 \pm 0.87^{a}_{B}$	$1.53 \pm 1.05^{a}_{B}$
DE	$1.00 \pm 0.00^{a}_{B}$	$1.67 \pm 0.58^{a}_{B}$	$1.17 \pm 0.29^{a}_{B}$	$1.33 \pm 1.15^{a}_{B}$	na	$2.50 \pm 0.50^{a}_{B}$	$1.53 \pm 1.02^{a}_{B}$
$CHCL_3$	$1.67 \pm 0.58^{a}_{B}$	$1.67 \pm 0.58^{a}_{B}$	na	$1.83 \pm 0.29^{a}_{B}$	$1.17 \pm 0.76^{a}_{B}$	na	na
DCM	$1.83 \pm 0.29^{a}_{B}$	$1.33 \pm 0.58^{a}_{B}$	$1.50 \pm 0.50^{a}_{B}$	$2.33 \pm 0.76^{a}_{B}$	$1.33 \pm 0.29^{a}_{B}$	$1.33 \pm 0.76^{a}_{B}$	$1.27 \pm 0.28^{a}_{B}$
Acetone	$1.00 \pm 1.00^{a}_{B}$	na	$1.33 \pm 0.58^{a}_{B}$	$1.00 \pm 0.00^{a}_{B}$	$2.00 \pm 0.00^{a}_{B}$	na	$1.54 \pm 0.53^{a}_{B}$
EtOH	$1.67 \pm 0.29^{a}_{B}$	$1.33 \pm 0.58^{a}_{B}$	$2.00 \pm 0.87^{a}_{B}$	$1.17 \pm 0.29^{a}_{B}$	na	$2.17 \pm 0.76^{a}_{B}$	$1.01 \pm 0.66^{a}_{B}$
MeOH	$1.33 \pm 0.58^{a}_{B}$	na	na	na	$2.67 \pm 1.15^{a}_{B}$	na	na
ACN	$1.33 \pm 0.58^{a}_{B}$	$1.33 \pm 0.58^{a}_{B}$	na	na	na	na	$1.52 \pm 0.28^{a}_{B}$
Water[1]	na	na	na	na	na	na	$1.67 \pm 0.31^{a}_{B}$
DMSO	na	na	na	na	na	na	na
TCH	$4.17 \pm 0.76_{A}$	$21.83 \pm 0.29_{A}$	$19.00 \pm 0.00_{A}$	$19.50 \pm 0.50_{A}$	$21.33 \pm 0.58_{A}$	$12.67 \pm 1.15_{A}$	$9.63 \pm 1.91_{A}$

[1]Solid /solvent ratio of 1:6.
[2]Means with different subscript capital letter are significantly difference ($p < 0.05$).
[3]PE - petroleum ether, DE- diethyl ether, CHCL3-chloroform, DCM- dichloromethane, EtOH - ethanol, MeOH – methanol and ACN - acetonitrile.
[4]na - No antibacterial activity (inhibition zone of sample < 1 mm).
[5]Different subscripts 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) and [c]Clear antibacterial activity (inhibition zone of sample 4 – 10 mm).

column temperature ramp was programmed from 70°C/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 15oC/min and held for 30 min. The GC injector and MS transfer line temperatures were set at 240°C and 230°C, respectively. GC 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 0.1 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. The n-alkanes standard containing 5 µg/mL each of 33 n-alkane hydrocarbons (C_8 to C_{40}) were used as reference points in the calculation of linear retention indices (LRI), described by Zhao *et al*. (2005) for future LRI database reference.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate solvent extraction and disk diffusion. 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 between the means at 95% confidence level ($p < 0.05$) for solvent extraction, TPC and TFC.

Results and Discussion

Disk diffusion test

Pure solvents were reported to have antibacterial effect on the pathogens tested; hence, this effect must be removed to avoid misinterpretation (Nakatsu *et al.*, 2000). In our study, the solvents were evaporated off prior to the DDT test to eliminate solvent antibacterial effect. Test results for DDT are tabulated in Table 1 and Table 2 for gram-negative and gram-positive pathogens, respectively. Generally, gram-positive bacteria were more sensitive than the gram-negative bacteria to the papaya peel crude extracts (Table 2) where all crude extracts slightly inhibited the gram-negative pathogens as compared to gram-positive pathogen inhibition which was moderately to clear inhibition. *C. diphtheriae, C. perfringens* and *S. pneumoniae* which were reported for vegetables and dairy products spoilage were moderately inhibited by the extract on the overall. *B. subtilis* frequently associated with pungent off-odour development of sterilized desiccated coconut was also moderately inhibited. We would anticipate a stronger inhibition of the pathogens once the crude extracts are further purified and currently we are working on this aspect.

DE extract showed statistically significant difference of inhibition against *B. cereus* and *B. subtilis*. The water extract slightly inhibited *P. mirabilis* (Table 1) and B. subtilis (Table 2). The later inhibition was also reported by Al-Zoreky (2009) for pomegranate peel. PE extract although was the lowest yield, contained about 7.87 mg GAE/g was able to inhibit nine pathogens vis *S.* Typhimurium, *E. coli, S.* Enteritidis, *V. parahaemolyticus, P. mirabilis, B. cereus, C. diphtheriae* (moderate), *S. pneumoniae* (moderate) and *B. subtilis*. Similarly, DCM which had flavonoid was able to exhibit slight to moderate inhibition of all tested food pathogens except *L. monocytogenes*. The inhibition of *S.* Typhimurium by flavonoid was also observed by Mandalari *et al.* (2007). Chloroform ($CHCL_3$) extract showed slight to almost moderate inhibition of mainly gram-positive

Table 2. Inhibition zone of *Carica papaya* peel on gram-positive food pathogens

Extract[3]	Total Inhibition[2, 5], mm						
	S. aureus	*B. cereus*	*L. monocytogenes*	*C. diphtheria*	*C. perfringens*	*S. pneumoniae*	*B. subtilis*
Hexane	na[4]	na	na	2.39 ± 0.64^{aB}	na	2.45 ± 0.33^{aB}	na
PE	na	1.00 ± 0.0^{aC}	na	3.35 ± 0.61^{bB}	na	3.84 ± 1.80^{bB}	2.98 ± 0.34^{aBC}
DE	1.83 ± 0.29^{aB}	2.67 ± 0.58^{aB}	na	2.85 ± 0.79^{aB}	2.18 ± 1.35^{aB}	2.69 ± 0.77^{aB}	4.34 ± 0.64^{cB}
$CHCL_3$	1.67 ± 0.58^{aB}	1.17 ± 0.29^{aBC}	na	3.33 ± 1.15^{bB}	2.05 ± 0.58^{aB}	2.51 ± 0.34^{aB}	2.63 ± 0.39^{aBC}
DCM	1.50 ± 0.50^{aB}	1.33 ± 0.29^{aBC}	na	1.94 ± 0.83^{aB}	3.30 ± 1.15^{bB}	3.21 ± 1.01^{bB}	2.53 ± 0.33^{aBC}
Acetone	na	1.67 ± 1.15^{aBC}	na	3.14 ± 0.52^{bB}	2.41 ± 0.25^{aB}	3.08 ± 0.53^{bB}	1.45 ± 0.81^{aBC}
EtOH	1.33 ± 0.29^{aB}	1.33 ± 0.29^{aBC}	na	1.25 ±1.09^{aB}	1.77 ± 1.58^{aB}	2.97 ± 0.99^{aB}	2.41 ± 0.51^{aBC}
MeOH	1.33 ± 0.58^{aB}	1.33 ± 0.58^{aBC}	1.33 ± 0.58^{aA}	2.83 ± 0.37^{aB}	2.62 ± 1.26^{aB}	1.55 ± 0.41^{aB}	2.76 ± 0.45^{aBC}
ACN	na	na	na	2.12 ± 0.34^{aB}	2.65 ± 0.56^{aB}	2.59 ± 0.60^{aB}	2.81 ± 0.31^{aBC}
Water[1]	na	na	na	na	na	na	2.13 ± 1.97^{aBC}
DMSO	na	na	na	na	na	na	na
TCH	27.83± 0.29^{A}	15.00 ± 0.50^{A}	na	12.44 ± 1.61^{A}	14.78 ± 0.37^{A}	11.61 ± 0.85^{A}	14.35 ± 0.33^{A}

[1]Solid /solvent ratio of 1:6.

[2]Means with different subscript capital letter are significantly difference ($p < 0.05$).

[3]PE - petroleum ether, DE- diethyl ether, CHCL3-chloroform, DCM- dichloromethane, EtOH - ethanol, MeOH – methanol and ACN - acetonitrile.

[4]na - No antibacterial activity (inhibition zone of sample < 1 mm).

[5]Different subscripts 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) and [c]Clear antibacterial activity (inhibition zone of sample 4 – 10 mm).

pathogens, but no antibacterial activity was reported for similar extract in Punica granatum peel by Negi and Jayaprakasha (2003). This could probably due to the different active components present in these two extracts. Acetone had slight to moderate inhibition against all pathogens tested except for *S. aureus, L. monocytogenes, S.* Typhimurium and *V. parahaemolyticus.*

The inhibition of *E. coli* by EtOH and *L. monocytogenes* and by MeOH crude extracts, respectively, could be due to the antibacterial activity of alkaloid and/or terpenoid. Terpenoid was reported to have inhibitive effect on *E. coli* and *L. monocytogenes* (Oussalah *et al*., 2007; Gutierrez *et al.*, 2008). MeOH extract although had high TPC (~20 mg GAE/g of DW) only slightly inhibited 9 pathogens tested vis. *S. sonnei, V. vulnificus, S. aureus, B. cereus, L. monocytogenes, C. diphtheriae, C. perfringens, S. pneumoniae* and *B. subtilis*. MeOH extract was more bactericidal to gram-positive than gram-negative pathogens. Crude non-polar extracts were bactericidal to bigger spectrum of pathogens as seen from the overall findings above.

Minimum inhibitory concentration

Quantitative evaluation of the antimicrobial activity of Carica papaya peel was carried out against selected microorganisms. The MIC (mg/mL) of the most active extract from the different solvents are presented in Table 3. The selection of pathogens and extracts were based on disk diffusion test results whereby only moderate and clear zone inhibitions were chosen. Clearly, of fourteen pathogens, four gram-positive pathogens fulfilled the inhibition zone criteria. It has been stated that gram-negative bacteria are more resistant to various antimicrobials than gram-positive microorganisms due to their outer lipopolysaccharide membranes (Al-Zoreky, 2009). The gram-positive bacteria, *S. pneumoniae*, was the most sensitive organism to *Carica papaya* PE extract with only 1.40 mg/mL (Table 3) compared to $CHCL_3$ and DCM extracts. In a previous DDT, the DCM extract of peels showed wide spectrum of inhibition, but only gave 45 mg/mL of MIC against *S. pneumoniae*. *C. diphtheriae* was most sensitive to PE extract (5.63 mg/mL) but was less sensitive towards $CHCL_3$ and acetone extracts (MIC for each extract = 11.25 mg/mL). The MIC value for DE extract against *B. subtilis* was 22.5 mg/mL while DCM extract against *C. perfringens* was 11.25 mg/mL. PE extract was bactericidal to both *C. diphtheriae* and *S. pneumonia* since the growth of these pathogens were not observed after 48 h incubation. Further fractionation could probably result in a lower MIC (Mcgaw and Jager, 2002). From these findings, PE extract was identified as the most potent extract while *C. diphtheriae* and *S. pneumoniae* were chosen as bioindicators for further antibacterial studies.

Extraction yield, total phenolic and total flavonoid contents

Extraction yields, total phenolic and total flavonoid contents of extracts and screening are as in Table 4. Lower yields (3.94 to 20.30 mg/g) were obtained in non-polar solvents as compared to polar solvents (10.92 to 84.05 mg/g). Water gave the highest extraction yield at 123.33 mg/g, at normalized solid to solvent ratio of 1:2. Among the 1:2 solid/solvent ratio extraction methods, acetone had the highest yield (~ 84 mg/g) while PE had the lowest (~ 3.9

Table 3. Inhibition zone of *Carica papaya* peel on gram-positive food pathogens

Extract[1]	MIC[2] (mg/mL)				MBC[2] (mg/mL)			
	C. diphtheria	*S. pneumoniae*	*B. subtilis*	*C. perfringens*	*C. diphtheria*	*S. pneumoniae*	*B. subtilis*	*C. perfringens*
PE	5.63	1.40	nt	nt	5.63	1.40	nt	nt
$CHCL_3$	11.25	nt	nt	nt	22.5	nt	nt	nt
Acetone	11.25	5.63	nt	nt	11.25	5.63	nt	nt
DCM	nt	45	nt	11.25	nt	45	nt	g[3]
DE	nt	nt	22.5	nt	nt	nt	22.5	nt

[1]PE - petroleum ether, CHCL3 – chloroform, DCM – dichloromethane and DE – diethyl ether.
[2]nt - Not tested.
[3]Bacterial growth was observed on all concentration of extracts.

Table 4. Extract yield, total phenolic content and total flavonoid content of *Carica papaya* peel extracts

Extract[3]	Yield[2] (mg/g sample)	TPC[2] (mg GAE / g DW)	TFC[2] (mg QE / g DW)
Hexane	20.30 ± 0.46^{bc}	5.42 ± 0.22^{f}	4.41 ± 0.18^{a}
PE	3.94 ± 0.10^{c}	7.87 ± 1.49^{e}	7.17 ± 0.49^{b}
DE	11.47 ± 0.10^{c}	1.73 ± 0.71^{g}	1.71 ± 0.05^{c}
$CHCL_3$	19.36 ± 0.50^{bc}	8.29 ± 0.32^{e}	8.08 ± 0.21^{d}
DCM	10.99 ± 0.01^{c}	5.60 ± 0.00^{f}	5.27 ± 0.31^{e}
Acetone	84.05 ± 0.81^{b}	27.51 ± 0.12^{a}	25.28 ± 0.10^{f}
EtOH	61.83 ± 0.60^{bc}	25.17 ± 0.07^{b}	23.25 ± 0.06^{g}
MeOH	59.05 ± 0.30^{bc}	19.97 ± 0.07^{c}	18.74 ± 0.06^{h}
ACN	10.92 ± 0.12^{c}	17.42 ± 0.99^{d}	16.52 ± 0.86^{i}
Water[1]	123.33 ± 7.26^{a}	0.47 ± 0.06^{g}	0.34 ± 0.03^{j}

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

mg/g). Yield of extract from water extraction showed significant difference from other solvents.

Table 4 shows TPC of crude extracts of different solvents. Acetone contained the highest TPC (27.51 mg GAE/g DW) while water had the lowest (0.47 mg GAE/g DW) despite having the highest yield. Although PE had the lowest extraction yield, it still contained moderately high TPC (7.87 mg GAE/g DW). The overall results for the TPC in this study are higher than that reported for Tunisian quince (Cydonia oblonga Miller) peel extract compared on per 100 g basis as reported by Fattouch *et al.* (2007). TPC reported for *Nephelium lappaceum* L. (Thitilertdecha *et al.,* 2008) seemed to be higher than the papaya extracts but direct comparison could not be made since the TPC was expressed as catechin equivalent and not as galic acid equivalent. Based on our study, it can be inferred that the non-aqueous extraction of *Carica papaya* peel was inefficient for the extraction of phenolic compounds and polar solvents are more preferable.

The total flavonoid contents in different crude extracts varied from 0.34 to 25.28 mg QE/g DW (Table 4) with acetone extract being the highest (25.28 mg/g) followed by ethanol (23.25 mg/g), methanol (18.74 mg/g) and acetonitrile (16.52 mg/g). The findings also reaffirmed the TPC results, where extracts from higher solvent polarity also had higher TFC.

Carica papaya peel composition by GC/MS

Result of the GC/MS analysis of PE extract, the most potent extract, is as shown in Table 5 where 28 compounds were identified having greater than 90% similarity with the standard mass spectra in the library, representing 54% of the relative peak area of the chromatogram. This could be perhaps due to inability to identify thermally stable and non-volatile compounds since sample derivatization was not carried out. The *Carica papaya* peel compositions which were the first ever reported in this study, had characterized the presence of fatty acids and esters, alkane, tocopherols and sterols. 9, 12, 15-octadecatrienoic acid (12.84%) was the most abundant compound detected, followed by α-tocopherol (7.01%), β-sitosterol (6.66%), palmitic acid (5.24%), campesterol (3.82%) and stigmasterol (3.07%).

Comparison between calculated LRI of HP5-MS and retention indices (RI) from different columns showed different values except for squalene since HP5-MS column used in this study was a semi-polar column compared to non-polar columns of the database (Table 5). The RI also known as Kováts indices were calculated from isothermal programming while LRI values were calculated from temperature programming separation for more complex plant metabolites (Zhao *et al*., 2005). It is important to build LRI database of plant volatiles such as for our

Table 5. GC/MS peak identification of *Carica papaya* peel using HP5-MS column

No.	Retention time	Linear retention indices[a]	Retention indices[b]	Compound[c]	Area (%)
1	4.654	1094	1072[g]	Tetramethylpyrazine	0.01
2	5.250	1151	1103[e]	2-Phenylacetonitrile	0.02
3	10.867	1545	1532[g]	4,4,7a-Trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one	0.06
4	11.387	1566	1556[i]	Dodecanoic acid	0.12
5	14.810	1666	1655[j]	Triethyl citrate	0.03
6	17.630	1725	1714[g]	Methyl tetradecanoate	0.04
7	20.557	1774	1772[e]	Tetradecanoic acid	0.43
8	23.950	1822	1842[l]	Tert-Butyl(dimethyl)silyl myristate	0.25
9	25.027	1911	1886[l]	Methyl 9-hexadecenoate	0.04
10	25.394	1935	1909[k]	Methyl palmitate	0.19
11	26.327	1956	1942[j]	Palmitic acid	5.24
12	27.603	2111	2098[g]	Methyl 9,12,15-octadecatrienoate	1.87
13	28.275	2186	2113[h]	9,12,15-octadecatrienoic acid	12.84
14	30.491	2499	2002[n]	Methyl 8,11,14-heptadecatrienoate	2.03
15	31.057	2600	2593[d]	1-hexacosene	1.11
16	32.494	2848	2847[a]	Squalene	0.95
17	32.838	2902	2026[e]	1-Iodohexadecane	0.85
18	33.418	2987	2951[m]	α-Tocopherol acetate	0.45
19	33.533	3002	2770[m]	Hexacosyl pentafluoropropionate	1.26
20	34.144	3080	3043[m]	β-tocopherol	0.76
21	34.290	3098	3055[m]	δ-tocopherol	1.50
22	34.374	3108	2800[n]	Octacosane	1.89
23	34.909	3166	3040[h]	Stigmastan-3,5-diene	0.71
24	35.084	3185	3138[m]	α-tocopherol	7.01
25	36.093	3281	3080[j]	Ergosterol	0.43
26	36.376	3306	3105[f]	Campesterol	3.82
27	36.727	3333	3142[f]	Stigmasterol	3.07
28	37.545	3396	3194[f]	β-sitosterol	6.66

[a]Compounds listed in order of retention indices calculated against n-alkanes on HP5-MS column.

[b]Compounds listed in order of retention indices based on NIST 11 library by different columns; [d]CP Sil 8 CB, [e]DB-1, [f]E-301, [g]HP-1, [h]OV-101, [i]RTX-1, [j]SE-30, [k]SPB-1, [l]Ultra-1, mVF-5MS and nEstimated value from library

[c]Compounds identified at 90% similarity with the standard mass spectra in NIST 11 library.

Carica papaya peel as chromatographic separation reference since HP5-MS column is currently opted for separation due to its low stationary phase bleed for better sensitivity and performance at elevated temperature (Antolovich *et al*., 2000). Besides, LRI reports on these volatiles are presently limited.

Claimed of antibacterial activity of several identified compounds have been reported by Mcgaw and Jager (2002) such on 9,12,15-octadecatrienoic acid in leaf of Schotia brachypetala have strong antibacterial activity against the Gram-positive bacteria *B. subtilis* and *S. aureus*. However, only slight inhibition on *B. subtilis* was obtained in our study. Hexadecanoic acid in *Salvia lanigera* was reported to inhibit *S. aureus* (Kristmundsdottir and Skulason, 2011; Tenore *et al.*, 2011), but not in our finding. Antibacterial activities of stigmasterol (18.29%) and β-sitosterol (60.73%) have also been reported by Hajji *et al.* (2010) in Mirabilis jalapa tubers and Dickson *et al.* (2007) in root bark of *Caesalpinia benthamiana.* Alpha-tocopherol exerted substantial antioxidative effect but insignificant antibacterial effect against Enterobacteriaceae, *Pseudomonas* and lactic acid bacteria (Georgantelis *et al.*, 2007) which could be due to the antagonistic effects of the compound to antibiotics or drugs and thus, preventing their antibacterial activity (Broniatowski *et al*., 2015). Campesterol (3.82%) from mango seed kernel had been attributed to possess antibacterial properties against coliforms (Abdalla *et al*., 2007). However, we could not confirm or make any further verifications since our samples were not fractionated and tested individually. Nonetheless, we believe that PE extract from *Carica papaya* peel could be a potential source of antibacterial compounds for *C. diphtheriae* and *S. pneumonia* based on findings reported here.

Since the 9,12,15-octadecatrienoic acid in pure form gave lower MIC (1.53 mg/mL) than our crude extract (22.5 mg/mL) against *B. subtilis* (Mcgaw and Jager, 2002), further fractionation steps to obtain pure compounds should be taken in order to achieve better inhibition (Tanaka *et al.*, 2013). Hence, PE extract from *Carica papaya* peel could be a potential source of antibacterial compounds for *C. diphtheriae* and *S. pneumoniae*. Since not all compounds could be successfully identified at this stage, we would think this could open up a new avenue for future work.

Conclusion

Higher recovery of phytochemicals was obtained in extracts obtained from polar solvents showing varying inhibition capacity towards selected pathogenic microbes. Although PE extract had the lowest TPC content, it was able to moderately inhibit *C. diphtheriae* and *S. pneumoniae*. PE extract, which was the most potent extract gave MIC of 5.63 mg/mL and 1.40 mg/mL against *C. diphtheriae* and *S. pneumoniae,* respectively. The GC/MS analysis of PE extract identified 9,12,15-octadecatrienoic acid, α-tocopherol, β-sitosterol, palmitic acid, campesterol and stigmasterol as potential antibacterial compounds from *Carica papaya* peel. Hence, *Carica papaya* peel could be a potential source for extracting antibacterial compounds against these pathogenic microbes.

Acknowledgement

This work is supported by the Research University Grant (RUGS) vote number 9328200 of Universiti Putra Malaysia, Serdang, Selangor. Authors declare no conflict of interest.

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