International Food Research Journal 19(1): 77-84 (2012)

Short Communication
Antibacterial property of *Hylocereus polyrhizus* and *Hylocereus undatus* peel extracts

Nurmahani, M.M., *Osman, A., Abdul Hamid, A., Mohamad Ghazali, F. and Pak Dek, M.S.

Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, 43400, Malaysia

Abstract: Food-borne diseases have always been a major concern to the consumers, food safety authorities and food industries. *Hylocereus* spp or Pitaya peels are normally treated as wastes and will be discarded during processing. In this study, the antibacterial activity of ethanol, chloroform and hexane extracts from *Hylocereus polyrhizus* (red flesh pitaya) and *Hylocereus undatus* (white flesh pitaya) peels against nine pathogens was evaluated using disc diffusion method and broth micro-dilution method. Result from disc diffusion method showed that chloroform extracts from *H. polyrhizus* and *H. undatus* peel were found to exhibit good antibacterial activity where almost all the pathogens studied were successfully inhibited. Result of the minimum inhibitory concentration (MIC) showed that all extracts inhibit the growth of bacteria in the range of 1.25-10.00 mg/mL for all bacteria while their minimum bacteriacidal concentrations (MBC) indicated double of the MICs concentration except for *B. cereus*, *L. monocytogenes* and *C. jejuni*. Even though there is no clear trend indicating which bacteria were sensitive most to the extract, it can be concluded that chloroform extract of both *H. polyrhizus* and *H. undatus* peel showed the most potent antibacterial activity. Thus, these findings could be used further to understand the antibacterial property of the peel of pitaya fruits.

Keywords: Antibacterial, food-borne pathogens, *Hylocereus* peel, disc diffusion, MIC

Introduction

In the past decade interest on the topic of antibacterial property of plant extracts has been growing (Lee *et al*., 2007). Food processors and agencies are very concerned with the high and growing number of food-borne outbreaks and illnesses associated with microorganisms especially bacteria. Bacteria also have become far more resistant to many antibacterial agents. For instance, of two million people who acquired bacterial infection in United States of America (USA) hospitals annually, 70% of the cases involved the strains that are resistant to at least one antibacterial agent (Cushnie and Lamb, 2005). The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics (Mahida and Mohan, 2006). Consumers too have questioning the safety of foods containing the synthetic antibacterial agent as preservatives (Shan *et al*., 2007). Therefore, there has been increasing interest in developing new types of highly effective and non-toxic antibacterial agents from natural sources (Shan *et al*., 2007). Over the past two decades, scientists have turned back to traditional folk medicines or natural products to uncover the scientific basis of remedial effects such as antibacterial agents (Haslam, 1996).

Beside plants, fruits also have become the main subject for researchers to be investigated since their bioactive compounds close related with herbs, commonly referred as phytochemicals such as carotenoids, polyphenols and anthocyanins that are abundantly present in fruits and vegetables such as tomatoes, grapes, pomegranates and strawberries are gaining lot of interest due to their functional property (Jayaprakasha *et al*., 2001; Li *et al*., 2006; Rao and Rao, 2007). Furthermore, natural compounds in fruits and vegetables such as polyphenols such as flavonoids and tannins have shown very promising results in combating bacteria, fungus and viral (Ahmad and Beg, 2001; Cushnie and Lamb, 2005).

Hylocereus species or better known as dragon fruit or pitaya from the Cactaceae family had become interest subject to many researchers mainly due to its unique taste, shape and the flesh colour (Mizrahi *et al*., 1997). Many studies have been conducted to investigate the chemistry of betalains, the major bioactive compounds in *H. polyrhizus* (Wybraniec *et al*., 2001; Wybraniec and Mizrahi, 2002; Stintzing, 2004). Furthermore, *H. polyrhizus* and *H. undatus* seeds have been reported to contain high level of essential fatty acid, namely linoleic acid and linolenic acids (Ariffin *et al*., 2009). The functionality of *H. polyrhizus* seed are including antioxidant property

*Corresponding author.
Email: *azosman@putra.upm.edu.my, azizahmasahuling@yahoo.com*
Tel: +603 89468373; Fax: +603 89423552

(Adnan *et al*., 2010) and the uses of different part of *H. undatus* in promoting wound healing in diabetic rats also have been reported (Perez *et al*., 2005). However, investigations on the antibacterial activity on the fruit peels are lacking due to less popular in commercial application (Soong and Barlow, 2004).

Therefore the present study was aimed to evaluate the antibacterial properties of ethanol, chloroform and hexane extracts from *H. polyrhizus* and *H. undatus* peel using disc diffusion and broth macro-dilution methods in determining minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC). The findings obtained in the study could support the potential application of pitaya peels as a natural source of antibacterial agent rather than being discarded as domestic waste as currently practiced.

Materials and Methods

Chemicals and reagents

Ethanol, chloroform and n-hexane were purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide, Nutrient agar, Mueller-Hinton agar and 96-well microplate were bought from Fisher Scientific (Leicestershire, UK), Difco (Maryland, USA), Oxoid (Hampshire, England), and Nunco™ Surface (Rosklide, Denmark), respectively.

Bacterial strains and growth conditions

Nine bacterial strains were used in this study were *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), *Enterococcus faecalis* (ATCC 14506), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Yersinia enterocolitica* (ATCC 23715) and *Campylobacter jejuni* (ATCC 29428) were obtained from ATCC (American Type and Collection Centre). Bacterial strains were cultivated at 37°C and maintained on a nutrient agar slant at 4°C. The working cultures were activated on nutrient agar at 37°C for 24 hours.

Sample preparation

Red flesh pitaya fruits (*H. polyrhizus*) were obtained from a pitaya farm in Sepang, Selangor, Malaysia while the white flesh pitaya fruits (*H. undatus*) were obtained from a local supermarket, imported from Vietnam. The fruits were rinsed with distilled water and hand peeled. The peel was then freeze-dried using a freeze dryer (Labconco, Missouri, USA) and was ground into powder using a domestic blender (Pensonic, Selangor, Malaysia). The powdered peel was stored at -20°C.

Preparation of extracts

The extraction of the freeze-dried peel powder was conducted according to the method of Siddhuraju and Manian (2007) with some modifications. Twenty-grams of the grounded peel were extracted using different solvents namely 95% ethanol, chloroform and n-hexane. The extractions were carried out in an Innova 4000 incubator shaker (New Brunswick Scientific, New Jersey, USA) at 30°C for 2 hours prior to filtration using Whatman No. 4 cellulose filter paper. The filtrates were then concentrated under vacuum pressure (Büchi, Flawil, Switzerland) at 40°C. For the antibacterial study, the dried extracts of ethanol (EE) were dissolved in 95% ethanol. Chloroform (CE) and n-hexane (HE) extracts were dissolved in 5% dimethyl sulfoxide (DMSO). The final concentrations of all extracts were standardized at 10 mg/mL.

Antibacterial activity

Disc diffusion assay

The disc diffusion assay was adopted from Clinical and Laboratory Standards Institute, CLSI (2009) was employed to observe the inhibitory spectrum of the extracts against nine pathogenic bacteria. Four or five well isolated colonies (depending on the size of the colonies) were picked from an overnight plate culture and inoculated into saline suspension at room temperature. The turbidity was then adjusted to 0.5 McFarland standards (10^8 CFU/mL) and streaked onto Mueller-Hinton agar plates using sterile cotton swabs. Whatman No. 1 filter paper discs of 6mm diameter were impregnated with 20 µL of the extracts. Chloramphenicol disc was used as positive control. Extraction solvents (ethanol, chloroform and hexane) were used as negative controls. The plates were incubated at 37°C for 24 hours. The screening of antibacterial activity was assessed based on the diameter of the clear zone surrounding the paper disc (including the disc diameter) in millimetre (mm). The tests were conducted in triplicates and repeated for three times.

Minimum inhibitory concentration and minimum bacteriacidal concentration

Minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC) of the different *H. polyrhizus* and *H. undatus* peel extracts were conducted using micro-dilution method as proposed by Preuss *et al.,* (2005). For (MIC), each extract was individually diluted to two-fold serial

dilution. The bacteria's turbidity was initially adjusted to 0.5 McFarland standards. Aseptically, 100 µL of each extract from each dilution was added with 95 µL of nutrient broth and 5 µL of the bacteria in the sterilised 96 wells microplate. The cultures were then incubated at 37°C for 24 hours in a metabolic rotary shaker (New Brunswick Scientific, Missouri, USA) and the growth was monitored spectrophotometrically (Bio Rad, Perth, UK) at 600 nm. Determination of MBC was done by sub-culturing 5 µL of suspension drawn from the wells with the lowest concentration that showed inhibition of the bacteria tested on nutrient agar and further incubated for another 24 hours. Chloramphenicol was used as positive control.

Results and Discussion

Disc diffusion assay

In the present study, nine food borne pathogens were tested for their sensitivity to different extracts of *H. polyrhizus* and *H. undatus* pitaya peel using disc diffusion method. Table 1 shows the diameters of inhibition zone exhibited by each extract towards the selected bacteria. All six extracts; ethanol extract of *H. polyrhizus* (REE), chloroform extract of *H. polyrhizus* (RCE), hexane extract of *H. polyrhizus* (RHE), ethanol extract of *H. undatus* (WEE), chloroform extract of *H. undatus* (WCE) and hexane extract of *H. undatus* (WHE), exhibited inhibition zones of about 7-9 mm against certain bacteria, indicating a broad spectrum activity against both gram positive and gram negative bacteria. Negative controls which were 95% ethanol and 5% DMSO did not show any inhibition zones.

RCE inhibited almost all of the bacteria tested except for *C. jejuni* while REE managed to inhibit six of the bacteria studied except for *S. aureus*, *B. cereus* and *C. jejuni*. RHE inhibited four bacteria tested which were *S. aureus*, *E. coli*, *S. typhimurium* and *K. pneumoniae*. With respect to the different solvents used in this assay, RCE showed a better antibacterial activity followed by REE and finally RHE. Similarly for *H.undatus*, WCE inhibited six bacteria except for *E. faecalis*, *S. typhimurium* and *K. pneumoniae* while WEE successfully inhibited five bacteria, namely *S. aureus*, *L. monocytogenes*, *E. faecalis*, *S. typhimurium* and *Y. enterocolitica*. However, WHE inhibited four out of nine bacteria tested, which were *S. aureus*, *E. coli*, *S. typhimurium* and *K. pneumoniae*. As compared to the *H. polyrhizus* peel, RCE exhibited better antibacterial activity than WCE for the fact that it only inhibited six bacteria combated. WEE showed lower antibacterial activity than REE where it only inhibited five bacteria including *S. aureus*, *L. monocytogenes*, *E. faecalis*, *S. typhimurium* and *Y. enterocolitica*. RHE and WHE showed similar trend in which four bacteria were successfully inhibited including *S. aureus*, *E. coli*, *S. typhimurium* and *K. pneumoniae*. *C. jejuni* was the most resistant bacteria strain since WCE was the only extract that managed to mildly inhibit its growth. Overall, the antibacterial activity using this assay revealed that *H. polyrhizus* peel has a better activity as compared to *H. undatus* peel.

Gram-positive bacteria are considered to be more sensitive as compared to Gram-negative because of the differences in their cell wall structures (Ahmad and Beg, 2001). But in this study, there was no clear trend observed for Gram-positive and Gram-negative bacteria. This finding is in agreement with the finding observed by Negi and Jayaprakasha (2003) where no clear trend was found on the different types of bacteria on the antibacterial activity of *Punica granatum* peel extracts. The effect of the different types of solvents used during extraction was clearly observed in the present study. Al-Zoreky (2009) stated that the differences in the antibacterial activity of pomegranate peel extracts could partially due to variations in phenolic content of extracts, strain sensitivity and antibacterial procedures adopted in the tests. In the present study, RCE successfully inhibited the growth of all Gram-positive bacteria and all Gram-negative bacteria except for *C. jejuni*.

Even though there are many studies reporting the presence of various bioactive compounds like flavonoids and phenols in fruits peels such as grape, citrus, mangosteen and pomegranate (Wang *et al*., 2008; Al-Zoreky, 2009; Katalinic *et al.*, 2010, Palakawong *et al*., 2010), there are no reports available on the antibacterial activity of the different varieties of pitaya peel.

Like any other Gram-negative bacteria cell wall, *C. jejuni* cell wall composed of lipopolysaccahrides that are broken down to secrete enterotoxins (Skirrow, 1994). Other possible virulent factors of *C. jejuni* include flagella, microbial adherence and invasion of intestinal epithelial cells (Bourke *et al.*, 1998) while the curved shape and motility of *Campylobacter facilitate* its penetration through the gastrointestinal mucus (Wallis, 1994). In the present study, it is interesting to note that WCE could only inhibit the growth of *C. jejuni* while other extracts failed. *C. jejuni* has been the leading cause of human gastroenteritis (Hani and Chan, 1995). Similarly, chloroform extracts were the only extracts that managed to inhibit the growth of *B. cereus*. The presence of spore of *B. cereus* may possibly increase its resistance towards bioactive compounds. Other was defined as the lowest

Table 1. Antibacterial activity of *H. polyrhizus* (red flesh pitaya) *H. undatus* (white flesh pitaya) peel extracts of using disc diffusion method

Bacteria strains	Mean diameter of inhibition zone (mm)							Negative control		
	Extract[a] (0.2mg/disc)						Chlorampenicol (10µg/disc)	Ethanol	Chloroform	Hexane
	REE	RCE	RHE	WEE	WCE	WHE				
Gram (+) bacteria										
Staphylococcus aureus	NI	9.0±0.10	7.0±0.05	8.0±0.10	8.0±0.25	7.0±0.07	30.0±0.28	NI	NI	NI
Bacillus cereus	NI	8.0±0.10	NI	NI	7.0±0.15	NI	29.0±0.34	NI	NI	NI
Listeria monocytogenes	9.1±0.20	9.0±0.20	NI	7.0±0.1	8.0±0.27	NI	26.0±0.41	NI	NI	NI
Enterococcus faecalis	8.0±0.10	7.1±0.05	NI	7.0±0.11	NI	NI	30.0±0.22	NI	NI	NI
Gram (-) bacteria										
Escherichia coli	7.0±0.20	7.0±0.15	8.0±0.20	NI	7.0±0.17	7.0±0.12	8.0±0.33	NI	NI	NI
*Salmonella T*yphimurium	9.0±0.05	8.2±0.21	9.0±0.35	7.0±0.10	NI	8.0±0.15	28.0±0.25	NI	NI	NI
Campylobacter jejuni	NI	NI	NI	NI	7.1±0.13	NI	30.0±0.41	NI	NI	NI
Yersinia enterocolitica	7.1±0.21	8.5±0.21	NI	7.0±0.15	8.0±0.05	NI	28.0±0.24	NI	NI	NI
Klebsiella pneumoniae	7.0±0.25	8.0±0.10	8.5±0.23	NI	NI	7.0±0.16	28.0±0.27	NI	NI	NI

[a] Values are expressed as mean value ± standard deviation (n = 3). R= red flesh pitaya; W= white flesh pitaya; EE= ethanol extract; CE= chloroform extract; HE= hexane extract; NI= no inhibition.

resistance are genetic makeup of the sporulating species, sporulating conditions, core water content, spore coats and spore mineral content (Nicholson *et al*., 2000). Russell (2005) stated that many antibacterial agents are bacteriastatic rather than bacteriacidal and sporostatic at low concentrations and not being sporocidal at high concentrations. Increasing the concentration of one extract does not necessarily increase the antibacterial activity (Rota *et al*., 2008).

The same trend of result was also found in the screening of *H. undatus* peel. Chloroform extract of *H. undatus* peel (WCE) appeared to inhibit six out of the nine bacteria tested except for *E. faecalis, S. typhimurium* and *K. pneumoniae*. It is interesting to note that WCE successfully inhibited the growth of *C. jejuni* as compared to other extracts. A similar study by Kirbaşlar *et al*., (2009) reported that some essential oils extracted from grapefruit, bitter orange, orange, mandarin, lemon and bergamot peel showed a high antibacterial activity against bacteria and selected fungi. A study on green banana peel by Mokbel and Hashinaga (2005) revealed that the ethyl acetate of green banana peel extracts displayed high antibacterial and antioxidant activity and these activities may be due to 12-hydroxystrearic acid and succinic acid present in the banana peel. Therefore, the bioactive compounds such as some essential oils and fatty acids extracted from semi-polar solvents like chloroform and ethyl acetate might have contributed to the antibacterial activity as exhibited by RCE and WCE in this particular study.

In a study conducted by Mohamed *et al*. (1994) on ethanol extract of *Nephelium lappaceum* peel revealed that its antibacterial activity on *S. typhi* using the same assay was good with the inhibition zone of 26 mm but no inhibition was detected in *Lansium domesticum* peel extract. This was also observed in this study where REE and WEE successfully inhibited *S. Typhimurium* growth at the disc potency of 0.2 mg/disc. It could be said that the inhibition of *S.* Typhimurium differs with different sample used in a study. *S.* Typhimurium caused salmonellosis which developed diarrhea, fever, vomiting and abdominal cramps.

Like any other methods, the disc diffusion method used in the present study also has some limitations. Despite its simplicity, various parameters in the test could affect the accuracy of the result such as the volume of antimicrobial agent on the paper discs, the thickness of the agar layer and the solvent of dilution used (Burt, 2004). Different possible methods such as hole-plate method and cylinder method could be adopted to improvise this study. In the present study, the screening of antibacterial properties of different extracts of *H. polyrhizus* and *H. undatus* peels indicated that extraction with chloroform gave the best antibacterial activity.

Minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC)

MIC was defined as the lowest concentration of the extract to inhibit the growth of the bacteria at the end of 24 hours incubation. In contrast, MBC

concentration of an antibacterial agents needed to kill the microorganisms after 48 hours incubation (Preuss *et al.*, 2005). The antibacterial activity of the six different extracts derived from pitaya peels was determined by broth micro-dilution method using the 96 well microplates. The MIC and MBC values are expressed in mg/mL as tabulated in Table 2. All the six extracts exhibited good antibacterial activity.

In the present study, there is no definite trend being observed on the inhibition of different type of bacteria. *B. cereus, L. monocytogenes* and *C. jejuni* were found to be the most resistant bacteria to all extracts tested. RCE successfully inhibited the growth of all bacteria at 1.25 mg/mL. Similarly, the inhibition of *L. monocytogenes, E. coli* and *S. typhimurium* was also observed to be at 1.25 mg/mL for all REE, RCE and RHE. However, RHE was found to be better than REE because the inhibition of *S. aureus, B. cereus, C. jejuni* and *K. pneumoniae* was at least 2-4 folds less concentrated than REE. Although *S. aureus, E. coli* and *C. jejuni* were found to be the most resistant to Chloramphenicol (MIC 32 µg/mL), they were inhibited by RCE at 1.25 mg/mL. The MBC were noted to be double of the MIC concentration except for *B. cereus, L. monocytogenes* and *C. jejuni* where it requires more than 80.00 mg/mL to be completely killed. The same trend was observed in WCE whereby *E. coli, C. jejuni* and *Y. enterocolitica* were inhibited at 1.25 mg/mL while most of the Gram-positive bacteria such as *S. aureus, B. cereus* and *L. monocytogenes* including one Gram-negative bacteria *S. Typhimurium* were inhibited at 2.5 mg/mL. In this study, *K. pneumoniae* and *E. faecalis* were the most resistant bacteria since the MIC and MBC values for WCE was found to be 10 mg/mL.However it is interesting to note that WHE and WEE could inhibit the growth of *K. pneumoniae* at 5 mg/mL, two-fold lower than WCE. According to the result obtained, WHE was found to exhibit better activity after WCE as compared to WEE. This could be observed on the inhibition of *S. aureus, S. typhimurium, E. coli* and *Y. enterocolitica* where WEE required double dose to inhibit the bacteria. As compared to RCE, WCE required a higher dosage to inhibit the growth of certain bacteria. The MIC of WCE for S. aureus, B. cereus, L. monocytogenes and S. typhimurium were at least two-fold higher than the MIC value of RCE. For K. pneumoniae, WCE required 10 mg/mL to inhibit their growth as compared to only 1.25 mg/mL for RCE. The same pattern was also observed for WHE and WEE whereby their MIC value was at least two-fold higher as compared to that of RHE and REE concentrations. Hence, results from broth dilution method revealed that the antibacterial the *H. polyrhizus* peel extracts was generally higher than the *H. undatus* peel extracts.

In the present study, the most potent antibacterial extract was RCE. The MIC values for inhibition of *S. aureus* and *K. pneumoniae* were 1.25 mg/mL. Negi and Jayaprakasha (2003) found that acetone extract of pomegranate fruit peel inhibited the growth of *B. cereus, S. aureus* and *E. coli* at 0.2 mg/mL. Choi *et al*., (2009) reported that phenolic compounds present in the ethanol extract of pomegranate fruit peel may be responsible for its remarkable antibacterial activity against Salmonella. A study on pomegranate fruit peel by Voravuthikunchai *et al*., (2005) indicated that enterohemorrhagic *E. coli* O157:H7 was inhibited in the presence of 0.5-3.0 mg/mL of ethanol extracts and Al-Zoreky (2009) found that 80%-methanol extract of pomegranate fruit peel inhibited the growth of *E. coli* at 1 mg/mL. In the present study, *E. coli* were inhibited by both RCE and WCE at 1.25 mg/mL.

For the peel extracts of both *H. polyrhizus* and *H. undatus* fruits observed in this study, there was a general trend where the order of antibacterial activity would be CE > HE > EE. Despite the finding observed by Tian *et al.,* (2009) on Gala chinensis which revealed that the different polarity does affect the bioactivities (i.e: antioxidant and antibacterial activities) where with the increasing polarity of one solvent, the antioxidant and antibacterial activities will decreased, this was not observed in this present study. In the present study, chloroform extract showed the most potent antibacterial activity followed by hexane and ethanol extracts. The synergistic between bioactive compounds in chloroform have been suggested to cause such activity when the authors reported that kaempferol and luteolin showed synergy against Herpes Simplex Virus (Cushnie and Lamb, 2005). Therefore in this study, it could be suggested that synergistic interaction between bioactive compounds had caused chloroform extract to exhibit such antibacterial activity against all bacteria tested.

Nurliyana *et al.,* (2010) further reported that the peels of both *Hylocereus* species contained high number of total phenolic compounds (TPC) rather than the pulp. It was reported that the antibacterial of fractions extracted from 100% (v/v) ethanol was more effective as compared to fractions derived from 70% (v/v) ethanol (Mandalari *et al*., 2007). Wojdylo *et al.,* (2007) further supported that polyphenolic compounds were commonly found in both edible and inedible plants. However, the non-flavonoids compounds can be found in the pulp but the flavonoids compounds were found in the peel, seed and stem (Paixão *et al*., 2007). In addition, Li *et al.,* (2006) revealed that the TPC of pomegranate peel extracts

Table 2. Minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC) of *H. polyrhizus* (red flesh pitaya) and *H. undatus* (white flesh pitaya) peel extracts

Bacteria strains	MIC (mg/mL) Extract[a]						Chloramphenicol (µg/mL)
	REE	RCE	RHE	WEE	WCE	WHE	
Gram (+) bacteria							
Staphylococcus aureus	5.0 [b]*10.0*	1.25 *2.5*	1.25 *2.5*	5.0 *5.0*	2.5 *10.0*	2.5 *5.0*	32.0
Bacillus cereus	5.0 *>80.0*	1.25 *>80.0*	2.5 *>80.0*	10.0 *>80.0*	2.5 *>80.0*	10.0 *>80.0*	4.0
Listeria monocytogenes	1.25 *>80.0*	1.25 *>80.0*	1.25 *>80.0*	5.0 *>80.0*	2.5 *>80.0*	1.25 *>80.0*	4.0
Enterococcus faecalis	1.25 *2.5*	1.25 *5.0*	5.0 *10.0*	10.0 *10.0*	10.0 *10.0*	5.0 *10.0*	8.0
Gram (-) bacteria							
Escherichia coli	1.25 *2.5*	1.25 *2.5*	1.25 *1.25*	10.0 *10.0*	1.25 *2.5*	2.5 *10.0*	32.0
Salmonella Typhimurium	1.25 *2.5*	1.25 *2.5*	1.25 *1.25*	10.0 *10.0*	2.5 *2.5*	1.25 *2.5*	8.0
Campylobacter jejuni	5.0 *>80.0*	1.25 *>80.0*	1.25 *>80.0*	5.0 *>80.0*	1.25 *>80.0*	5.0 *>80.0*	32.0
Yersinia enterocolitica	5.0 *5.0*	1.25 *2.5*	5.0 *10.0*	5.0 *10.0*	1.25 *10.0*	2.5 *5.0*	8.0
Klebsiella pneumoniae	5.0 *10.0*	1.25 *2.5*	1.25 *1.25*	5.0 *10.0*	10.0 *10.0*	5.0 *10.0*	8.0

[a] Values are expressed as mean value ± standard deviation (n = 3).
R= red flesh pitaya;
W= white flesh pitaya;
EE= ethanol extract;
CE= chloroform extract;
HE= hexane extract;
NI= no inhibition.
[b] Values in italic are minimum bactericidal concentration (MBC)

was found 10 times higher than the pulp extracts, suggesting phenolics might be one of the responsible compounds that contributing to its high antimicrobial activity (Negi and Jayaprakasha, 2003). However, even though flavonoids, tannins and terpenoids will be found in the aqueous phase, but they are more often obtained in less polar solvents including chloroform (Cowan, 1999). Therefore it is possible to postulate that the antibacterial activity of both *H. polyrhizus* and *H. undatus* in the present study may be due to these same compounds. The antibacterial activity of plant extracts may be due to the capability of bioactive compounds to form a complex with extracellular and soluble proteins, inhibit enzyme activity and also affect bacteria cell walls (Cowan, 1999). Those bioactive compounds may also disrupt bacteria membranes (Tsuchiya *et al.*, 1996).

Conclusion

Different types of bacteria responded differently towards the different extracts. The present study revealed that all the bacteria tested were sensitive to different extracts from red and white flesh pitaya fruit peels. It can be concluded that, chloroform extracts of both *Hylocereus* species peel showed greatest antibacterial activity with *H. polyrhizus* peel being greater than *H. undatus* peel. With respect to both extracts, chloroform extract of red flesh pitaya peel (RCE) can be classified as a good source of potent natural antibacterial agent for both, Gram-positive and Gram-negative bacteria. Therefore, the potentials of these extracts as antibacterial alternatives could be further studied and the *Hylocereus* fruit peels could be utilised as a natural source of antibacterial agent that will benefit the society as a whole rather than being discarded as currently practiced.

Acknowledgements

The authors would like to thank Universiti Putra Malaysia for the financial aid under Research University Grant Scheme (RUGS) (Project No. 02/01/07/0015RU) and Faculty of Food Science and Technology for providing the facilities.

References

Adnan, L., Osman, A. and Abdul Hamid, A. 2010. Antioxidant activity of red pitaya (Hylocereus polyrhizus) seed. International Journal of Food Properties. (D.O.I 10.1080/10942911003592787).

Ahmad, L. and Beg, A. Z. 2001. Antibacterial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology (74): 113-133.

Al-Zoreky, N. S. 2009. Antibacterial activity of pomegranate (*Punica granatum L.*) peels. International Journal of Food Microbiology (134): 244-248.

Ariffin, A. A., Bakar, J., Tan, C. P., Abdul Rahman, R., Karim, R., Loi, C. C. 2009. Essential fatty acids of pitaya (dragon fruit) seed oil. Food Chemistry (114): 561-564.

Barreira, J. C. M., Ferreira, I. C. F. R., Oliveira, M. B. P. P. and Pereira, J. A. 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skin and fruit. Food Chemistry (107): 1106-1113.

Bourke, B., Chan, V. L. and Sherman, P. 1998. *Campylobacter upsaliensis*: waiting in the wings. Clinical Microbiology Review (11): 440–449.

Burt, S. A. 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. International Journal of Food Microbiology (94): 223-253.

Caro, A. D. and Piga, A. 2007. Polyphenol composition of peel and pulp of two Italian fresh fig fruits cultivars (*Ficus carica* L.). European Food Research and Technology (226): 715-719.

Choi, J., Kang, O., Lee, Y., Chae, H., Oh, Y., Brice, O., Kim, M., Sohn, D., Kim, H., Park, H., Shin, D., Rho, J. and Kwon, D. 2009. *In vitro* and *In vivo* Antibacterial Activity of *Punica granatum* Peel Ethanol Extract against *Salmonella.* Evidence-based Complementary and Alternative Medicine (eCAM) (10): 1-8.

Clinical and Laboratory Standards Institute. 2009. Performance Standards for Antibacterial Disk Susceptibility Tests Approved Standards. CLSI document M2-A7. Wayne, PA.

Cowan, M. M. 1999. Plant Products as Antibacterial Agents. Clinical Microbiology Reviews (12): 564-582.

Cushnie, T. P. and Lamb, A. J. 2005. Antibacterial Activity of Flavonoids. International Journal of Antibacterial Agents (26): 343-356.

Hani, E. K. and Chan, V. L. 1995. Expression and characterisation of *Campylobacter jejuni* benzolglycine amidohydrolase (hippuricase) gene in *Escherichia coli.* Journal of Bacteriology (177): 2396-2402.

Haslam, E. 1996. Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action. Journal of Natural Product (59): 205-215.

Jayaprakasha, G. K., Singh, R. P. and Sakariah, K. K. 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation model in vitro. Food Chemistry (73): 285-290.

Kirbaşlar, F. G., Tavman, A., Dülger, B. and Türker, G. 2009. Antibacterial activity of Turkish citrus peel oils. Pakistan Journal of Botany (41): 3207-3212.

Katalinić, V., Možina, S. S., Skroza, D., Generalić, I., Abramović, Miloš, M., Ljubenkov, I., Piskernik, S., Pezo, I., Terpinc, P. and Boban, M. 2010. Polyphenolic profile, antioxidant properties and antibacterial activity of grape skin extracts of 14 *Vitis vinfera* varieties grown in Dalmatia (Croatia). Food Chemistry (119): 715-723.

Lee, S., Chang, K., Su, M., Huang, Y. and Jang, H. 2007. Effects of some Chinese medicinal plant extracts on five different fungi. Food Control (18): 1547-1554.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J. and Cheng, S. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry (96): 254–260.

Lim, H. K., Tan, C. P., Karim, R., Ariffin, A. A. and Bakar, J. 2010. Chemical composition of DSC thermal properties of two species of *Hylocereus cacti* seed oil: *Hylocereus undatus* and *Hylocereus polyrhizus*. Food Chemistry (119): 561–564.

Mahida, Y. and Mohan, J. S. S. 2006. Screening of Indian Plant Extracts for Antibacterial Activity. Pharmaceutical Biology (44): 627–631.

Mandalari, G., Bennett, R. N., Bisignan, G., Trombetta, D., Saija, A., Faulds, C. B., Gasson, M. J. and Narbad, A. 2007. Antibacterial activity of flavonoids extracted from bergamot (*Citrus bergamia Risso*) peel, a byproduct of the essential oil industry. Journal of Applied Microbiology (103): 2056–2064.

McCarrell, E., Gould, S., Fielder, M., Kelly, A., El Sankary, W. And Naughton, D. 2008. Antibacterial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C. BMC Complementary and Alternative Medicine (8): 64.

Mizrahi, Y., Nerd, A. and Nobel, P.S. 1997. Cacti as crops. Horticultural Reviews (18): 291–320.

Mohamed, S., Hassan, Z., Abdul Hamid, N. 1994. Antimicrobial Activity of some Tropical Fruit Wastes (Guava, Starfruit, Banana, Papaya, Passionfruit, Langsat, Duku, Rambutan and Rambai), Pertanika Journal Tropica Agriculture Science (17): 219–227.

Mokbel, M. S. and Hashinaga, F. 2005. Antibacterial and Antioxidant Activities of Banana (*Musa*, AAA cv. Cavendish) Fruits Peel. American Journal of Biochemistry and Biotechnology (1): 125–131.

Negi, P. S. and Jayaprakasha, G. K. 2003. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. Journal of Food Science (68): 1473–1477.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. and Setlow, P. 2000. Resistance of *Bacillus* Endospores to Extreme Terrestrial and Extraterrestrial Environments. Microbiology and Molecular Biology Reviews (64): 548–572.

Nurliyana, R., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M. R. and Kamarul Rahim, K. 2010. Antioxidant study of pulps and peels of dragon fruits: a comparative study. International Food Research Journal (17): 367–375.

Paixão, N., Perestrelo, R., Marques, J.C. and Câmara, J.S. 2007. Relationship between antioxidant capacity and total phenolic content of red, rose and white wines. Food Chemistry (105): 204–214

Palakawong, C., Sophonadora, P., Pisuchpen, S. and Phongpaichit, S. 2010. Antioxidant and antibacterial activities of crude extracts from mangosteen (*Garcinia mangostana* L.) parts and some essential oils. International Journal of Food Research (17): 583–589.

Perez, R. M., Vargas, R. S. and Ortiz, Y. D. H. 2005. Wound Healing Properties of *Hylocereus undatus* on Diabetic Rats. Phytotherapy Research (19): 665–668.

Preuss, H. G., Echard, B., Enig, M., Brook, I. and Elliot, T. B. 2005. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Molecular and Cellular

Biochemistry (272): 29–34.

Rao, A. V. and Rao, L. G. 2007. Carotenoids and human health. Pharmacological Research (55): 207–216.

Rota, M. C., Herrera, A., MartÍnez, R. M., Sotomayor, J. A. and Jordan, M. J. 2008. Antibacterial activity and chemical composition of T*hymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Journal of Food Control (19): 681–687.

Russell, A. D. 2005. Mechanisms of action, resistance and stress adaptation. In Davidson, P. M., Sofos, J. N. and Branen, A. L. (Eds). Antimicrobials in Food, Third Edition, p. 633–657. Boca Raton: Taylor & Francis.

Shan, B., Cai, Y., Brooks, J. D. and Corke, H. 2007. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology (117): 112–119.

Siddhuraju, P. and Manian, S. 2007. The antioxidant activity and free-radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chemistry (105): 950–958.

Skirrow, M. B. 1994. Diseases due to *Campylobacter, Helicobacter* and related bacteria. Journal Comparative Pathology 111(2): 113–149.

Soong, Y. and Barlow, P. J. 2004. Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry (88): 411–417.

Stintzing, F. C. and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food and in human nutrition. Trends in Food Science & Technology (15): 19–38.

Tian, F., Li, B., Ji, B., Yang, J., Zhang, G., Chen, Y. and Luo, Y. 2009. Antioxidant and antibacterial activities of consecutive extracts from *Galla chinesis*: The polarity affects the bioactivities. Food Chemistry (113): 173–179.

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T. and Iinuma, M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of Ethnopharmacology (50): 27–34.

Voravuthikunchai, S., Sririrak, T., Limsuwan, S., Supawita, T., Lida, T. and Honda, T. 2005. Inhibitory effects of active compounds from *Punica granatum* pericarp on verocytotoxin production by entereohemmorhagic *Escherichia col*i O157:H7. Journal of Health Sciences (51): 590–596.

Wallis, M. R. 1994. The pathogenesis of *Campylobacter jejuni*. Br Journal Biomedical Science (51): 57–64.

Wang, Y., Chuang, Y. and Hsu, H. 2008. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chemistry (106): 277–284.

Wojdylo, A., Oszmiański, J. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry (105): 940–949.

Wybraniec, S. and Mizrahi, Y. 2002 Fruit flesh betacyanin pigments in *Hylocereus cacti*. Journal of Agricultural and Food Chemistry (50): 6086–6089.

Wybraniec, S., Platzner, I., Geresh, S., Gottlieb, H. E., Haimberg, M., Mogilnitzki, M. and Mizrahi, Y. 2001. Betacyanins from vine cactus *Hylocereus polyrhizus*. Phytochemistry (58): 1209–1212.