Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

ANTIBACTERIAL ACTIVITY OF FLESH AND PEEL METHANOL FRACTIONS OF RED PITAYA, WHITE PITAYA AND PAPAYA ON SELECTED FOOD MICROORGANISMS

*MOHD ADZIM KHALILI, R.[1], CHE ABDULLAH, AB.[2] AND ABDUL MANAF, A.[3]

[1]Faculty of Medicine and Health Sciences, [2]Faculty of Food Technology, University Sultan Zainal Abidin (UniSZA), Kampus Kota, Jalan Sultan Mahmud, 20400 Kuala Terengganu, [3]Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin (UniSZA), Kampus Gong Badak, 21300 Kuala Terengganu, Terengganu, Malaysia. Email: mohdadzim@unisza.edu.my

Received: 23 Dec 2011, Revised and Accepted: 24 Feb 2012

ABSTRACT

Red pitaya is good source of antioxidants, minerals and vitamins associated with promoting health and reducing the risk for chronic diseases. Based on ethno pharmacological and taxonomic information, antibacterial activities of methanol fractions of peel and flesh of red pitaya, white pitaya and papaya were determined by discs diffusion assay method against some Gram positive bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*, and Gram negative bacteria *Salmonella enterica typhi*, *Serratia marcescens*, *Shigella flexneri*, *Klebsiella*, *Pseudomonas aeruginosa* and *Escherichia coli*. The methanolic extract of red pitaya showed positively high exhibited antimicrobial activity against all tested food microorganisms especially on *Staph. epidermidis, Staph. Aerues, Pseudo. Aeruginosa* and *Salmonella typhi*. It has been showed that the methanol extracts may contain the active components. The present study supports, red pitaya flesh and peel has potential to be used as antibacterial treatment against pathogenic food microorganisms.

Keywords: Antimicrobial activity, Discs diffusion assay, Minimum inhibition concentration (MIC), Minimum bactericidal concentration (MBC) and Fruits.

INTRODUCTION

The most critical problem related with health and causes of mortality rates in society is infectious disease[1]. Since last decade, there has been an increasing evidence of bacterial and fungal infections due to the population explosion, pollution, changed environmental conditions and wastes from different sources, which may affect food with perfect nutrition value[2]. This factor causes less immunogenicity in human beings and animals, this fact coupled with the resistance developed in microorganisms to allopathic agents, antibiotics with increased toxicity in human being and animal during prolonged treatment with several antimicrobial drugs[3].

Bacterial diseases continue to present a major threat to human health. Tuberculosis, for instance, rank among the world's leading causes of death. *Streptococcus*, another bacterium, continues to be a frequent cause of life threatening infections during the first two months of life. Food-borne and water-borne bacteria such as *Salmonella* and *Campylobacter* are responsible for a recent troubling increase in diarrhea related diseases. Meanwhile, during the last decade, scientists have discovered many new organisms and new strains of many familiar bacteria, such as *Escherichia coli*. Emerging bacterial diseases present a clear challenge to biomedical researchers[4,5]. The frequency of serious nosocomial bacterial and fungal infections is rising due to the use of newer and more powerful antimicrobial agents.

Recently, the extracts of plants including from fruit have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects of oxidants. Particularly, the antioxidative and antimicrobial activities of plant and fruit extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies[6]. Because of the possible toxicities of the synthetic antioxidants, Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed toward natural antioxidants[7]. As additional new antimicrobial agents are being found, microorganisms become more resistant to existing chemotherapies. Thus, there is continuous need to develop novel antimicrobial compounds that would be effective against these and other pathogens[8].

Red pitaya a tropical fruit may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant[9]. Red pitaya should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease[10]. Therefore, attention in recent times has been focused on the isolation, characterization and utilization of natural antioxidants, especially growing interest in polyphenols as potential disease preventing agents. As these compounds are predominantly found in most of fruit tissues, it would be worthwhile investigating the nature of polyphenols that are present in red pitaya peel.

The trend to use natural products like fruits, vegetables and plants extract which may act as natural antimicrobials or antioxidant preservatives may also influence health of consumers as well prolog the shelf-life of relevant food products[11,12,13]. Fruits and vegetables however, contain many different antioxidant and antimicrobial components. The majority of the antioxidant capacity of a fruit or vegetable may be from compounds such as other vitamin C, vitamin E or β-carotene. The study was aim to investigate the potential effect of antibacterial activity of flesh and peel methanol fractions of red pitaya, white pitaya and papaya on selected food microorganisms, which might have possible application in the treatment of infectious diseases.

MATERIALS & METHODS

Extraction Procedure

The method of Adel S. Alzubairi[14] has been used for the extraction process. Red pitaya, white pitaya and papaya fruits were obtained from a local plantation in Lembah Bidong Setiu, Terengganu, Malaysia. The fruits were extracted with 95% methanol solvent. The fruits were carefully washed under running tap water, dried with a soft cloth and the skin peeled; the fresh flesh was then cut into small pieces (1.5 cm x 1.5 cm x 1.5 cm) and macerated in methanol for 7 days with occasional shaking and the process was repeated three times. Methanol extract were filtered through Whatman ® No. 41 filter paper (pore size 20-25 µm) and was then concentrated with rotary evaporator (Buchi, Sigma Aldrich) under reduced pressure at 40^{0}C and store at -20^{0}C until were used for the analysis. To screen the extracts of red pitaya, white pitaya and papaya compound were dissolved in 1 mL of DMSO to give a stock solution of extract at 100 mg/mL. All extracts were kept at 4^{0}C throughout the experiments.

Test microorganisms

The antimicrobial activity of methanol extract peel and flesh of red pitaya, white pitaya and papaya was evaluated included the Gram positive bacteria *Staphylococcus epidermidis (*ATCC12228), *Staphylococcus aureus* (ATCC25179), *Enterococcus faecalis* (ATCC 10100) and *Listeria monocytogenes* (ATCC 13932), and Gram negative bacteria *Salmonella enterica typhi* (ATCC 10749), *Serratia marcescens* (ATCC 8100), *Shigella flexneri* (ATCC 25923), *Klebsiella* (ATCC 10273), *Pseudomonas aeruginosa* (ATCC 14149) and *Escherichia coli* (ATCC 85218). All the microorganisms' strains were purchased from American Type Culture Collection (ATCC), Manassas, USA. All strains were carefully identified and grown using standard microbiological methods. The bacterial isolates were first sub-cultured in Mueller-Hinton broth (Merck, Germany) and incubated at 37°C for 18 hours.

Antimicrobial activity assay

Antimicrobial activity of methanol extract peel and flesh of red pitaya, white pitaya and papaya was determined against ten bacterial pathogens by the agar disc diffusion assay method as described by the National Committee of Clinical Laboratory Standards[15]. The bacterial strains were prepared from an overnight broth cultures were diluted using sterile normal saline to give an inoculums size about 10^8 cfu/mL. The density of bacterial suspension of the cultures was standardized turbidometrically to 500 000 – 1 000 000 colony forming units per milliliter (cfu/mL) at wavelength of 600 nm. A total of 100 µL of suspension containing 10^8 cfu/mL of bacteria, spread on nutrient agar.

The crude methanol extract were dissolved initially in *dimethyl sulfoxide* (DMSO), diluted to a concentration of 100 mg/mL and filtered using 0.25 µm millipore filters. Sterile discs were impregnated with the 30 µL of extract solutions (100 mg/mL) and placed on the inoculated agar. Negative controls were prepared using DMSO, while Penicillin G (10 µg/dics) and Gentamicin (10 µg/disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. The diameter of inhibition zone was measured in millimeters (mm) by Vernier calipers. All tests were repeated ten times to minimize test error. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity[16].

Minimum inhibitory concentration

Minimum inhibition concentration of methanol extracts peel and flesh of red pitaya, white pitaya and papaya was determined by combination methods introduced by Vollekova[17] with slightly modification by Usman[18]. MIC was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, the microorganisms were prepared using the broth dilution technique. The stock extract concentration of 100 mg/mL was made by dissolving 10 g of the extract in 100 mL of DMSO and the working concentrations were prepared by four-fold serial dilution technique that range from 0.39 mg/mL to 25 mg/mL using Muller-Hinton broth (Merck, Germany) and later inoculated with 0.2 mL suspension of the test organisms. After 24 hours of incubation at 37°C, the tubes were observed for turbidity with visually naked eye check and measuring the optical density (O.D) at 600 nm. The lowest concentrations where no turbidity were observed was determined and noted[18]. Solvent blanks and positive controls were also included and all tests were performed in tenth replicate and repeated for five times to minimize test error.

Minimum bactericidal concentration

Minimum bactericidal concentration of methanol extract peel and flesh of red pitaya, white pitaya and papaya was determined by a modification of the method of Spencer and Spencer[19], and Smith-Palmer[20]. The tube containing 5 mL nutrient broth with different concentration of isolated Samples were inoculated were 50 µL of the bacterial suspension (10^5 CFU/mL) was placed aseptically in 5 mL of nutrient broth separately and incubated for 24 hours at 37°C. The growth was observed both visually and by measuring optical density (O.D) at 600 nm at regular intervals followed by 20 µL taken from broth with no visible growth in the MIC assay and sub-cultured on freshly prepared Muller Hinton agar plates and later incubated at 37°C for 48 hours respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plate. Solvent blanks and positive controls were also included and all tests were performed in tenth replicate and repeated for five times to minimize test error.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of ten replicates. Statistical analysis was performed with single factor and one way ANOVA to identify the significant differences ($p<0.05$) and Duncan test for coefficient variation on antibacterial effects methanol extract peel and flesh of red pitaya, white pitaya and papaya.

RESULTS

Plants and fruits are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive [21,22]. Antioxidant vitamins in fruits are some of the important nutrients besides other vitamins, mineral, flavonoids and phytochemicals, which have been reported to contribute to health. In recent years, great interest has been focused on antioxidant properties particularly because of their likely role in prevention of coronary heart disease and cancers[23,24]. The level of the essential antioxidant compound in contrast to other antioxidative defenses is determined mainly by their dietary supply[25].

Fruits and vegetables are the main source of antioxidant vitamins, making these foods essential to human health. In the recent years much attention has been devoted to natural antioxidant and their association with health benefits[26]. The phenolic compounds are commonly found in both edible and non-edible plants and fruits, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers[27]. The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food[28]. Several fruits and vegetable have been reported to exhibit antioxidant activity, majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins are known to possess antioxidant potential[29,30]. Microorganisms including Gram positive and Gram negative bacteria have been recognized as the main causers of various human infections[31]. Though effective antimicrobials have been developed over the years, there has been increased development of antimicrobial drug resistance to presently available antimicrobials[32].

Antimicrobial activity assay

The results of the *in-vitro* antimicrobial activity of methanol extract of red pitaya flesh against four Gram positive bacteria and sixth Gram negative bacteria are shown in **Table 1**. The red pitaya flesh extract showed very high activity of inhibition against tested Gram positive bacteria; *Staphylococcus epidermidis (*15.00 ± 0.03 mm), *Staphylococcus aureus (*19.00 ± 0.43 mm), *Enterococcus faecalis (*18.50 ± 0.80 mm), *Listeria monocytogenes (*16.00 ± 1.84 mm), and Gram negative bacteria k*lebsiella (*19.50 ± 0.10 mm), *Pseudomonas aeruginosa (*15.00 ± 0.50 mm), *Salmonella entrica typhi* (14.50 ± 0.90 mm), *serratia marcescens* (15.80 ± 0.04 mm) and *Escherichia coli* (14.50 ± 0.04 mm). Low antibacterial activity of red pitaya flesh extract with inhibition zones less than 14.00 mm was observed against *Shigella flexneri* (12.50 ± 0.90 mm).

The white pitaya flesh extract showed low antibacterial activity on *Staphylococcus epidermidis (*10.00 ± 0.30 mm), *Staphylococcus aureus (*9.00 ± 0.90 mm) and *Enterococcus faecalis (*11.00 ± 0.15 mm) for Gram-positive bacteria, and *serratia marcescens* (8.50 ± 0.10 mm), *Escherichia coli* (7.00 ± 0.70 mm) and *Shigella flexneri* (10.50 ± 0.30 mm) as compare with the red pitaya flesh extract. The white pitaya flesh extract did not show any inhibition activity on

Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa and *Salmonella entrica typhi.* The papaya flesh extract also showed significant inhibitory effects on the growth of *Enterococcus faecalis* (8.50 ± 0.60 mm), *Staphylococcus epidermidis* (8.50 ± 0.90 mm) and *Staphylococcus aureus* (7.50 ± 0.10 mm) with the mean value of inhibition zones more than 10.00 mm.

Table 1: Zones of inhibition (mm) of methanol extract peel and flesh of red pitaya, white pitaya and papaya (100 mg/mL).

Extract/ Organisms	*S. epidermidis*	*S. aureus*	*E. faecalis*	*L. monocytogenes*	*Klebsiella*	*P. aeruginosa*	*S. typhi*	*S. marcescens*	*S. flexneri*	*E. coli*
RPF	15.0 ± 0.03^{c}	19.0 ± 0.43^{f}	18.5 ± 0.80^{e}	16.0 ± 1.84^{d}	19.5 ± 0.10^{a}	15.0 ± 0.50^{c}	14.5 ± 0.90^{b}	15.8 ± 0.04^{c}	12.5 ± 0.90^{a}	14.5 ± 0.04^{b}
WPF	10.0 ± 0.30^{c}	9.00 ± 0.90^{b}	11.0 ± 0.15^{d}	na	na	na	na	8.50 ± 0.10^{b}	10.5 ± 0.30^{c}	7.00 ± 0.70^{a}
PPF	8.50 ± 0.90^{b}	7.50 ± 0.10^{a}	8.50 ± 0.60^{b}	na	na	na	na	na	na	na
RPP	9.00 ± 0.50^{a}	10.0 ± 0.50^{b}	8.50 ± 0.20^{a}	na	na	na	na	na	na	na
WPP	7.50 ± 0.70^{a}	8.00 ± 0.30^{a}	9.00 ± 0.90^{b}	na	na	na	na	na	na	na
PPP	na	7.00 ± 0.10^{a}	na	na	na	na	na	na	na	na
Penicillin	15.0 ± 0.02^{a}	16.0 ± 0.12^{b}	18.0 ± 0.09^{c}	na	na	na	na	na	na	na
Gentamicin	10.0 ± 0.03^{a}	18.0 ± 0.07^{c}	na	18.5 ± 0.76^{c}	16.5 ± 0.76^{a}	10.0 ± 0.01^{a}	18.0 ± 0.07^{c}	18.5 ± 0.51^{c}	18.5 ± 0.27^{c}	15.5 ± 0.21^{b}

Results are means ± standard deviation of tenth replicate and repeated for five times to minimize test error; na=no activity; there was no inhibition found in negative control (DMSO).

[Abc] Variation in the following letters between samples indicates significance of difference by Duncan's test at 5% level (p<0.05).

Inhibition zones are the mean including disc (6 mm) diameter; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel.

The red pitaya peel and white pitaya peel extracts has showed similar pattern on antibacterial activity, both samples extract was showed a significant inhibitory effect against Gram positive bacteria; *Staphylococcus epidermidis (*9.00 ± 0.50 mm; 7.50 ± 0.50 mm), *Staphylococcus aureus (*10.00 ± 0.50 mm; 8.00 ± 0.30 mm) and *Enterococcus faecalis (*8.50 ± 0.20 mm; 9.00 ± 0.90 mm) and both samples are do not showed any inhibition activity on *Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa, S. marcescens, S. flexneri* and *Salmonella entrica typhi.* Papaya peel extract only showed significant inhibitory effects on the growth of *Staphylococcus aureus* with the mean of inhibition zone 7.00 ± 0.10 mm. the papaya peel extract did not showed any inhibition activity on *Staphylococcus epidermidis, Enterococcus faecalis, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa, S. marcescens, S. flexneri* and *Salmonella entrica typhi.* Red pitaya flesh extract give an inhibition effect on *Staphylococcus aureus* greater than antimicrobial activity by commercial antibiotic (Gentamicin). Overall results showed that there is a significant difference on inhibition activity between red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel extracts with standard antibiotics discs (penicillin and gentamicin).

Table 2: The minimum inhibitory concentrations of methanol extract peel and flesh of red pitaya, white pitaya and papaya (four-fold dilutions).

Samples	(mg/mL)	*S. epidermidis*	*S. aureus*	*E. faecalis*	*L. monocytogenes*	*Klebsiella*	*P. aeruginosa*	*S. typhi*	*S. marcescens*	*S. flexneri*	*E. coli*
RPF	100	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-	-	-
	1.56	-	-	+	+	-	+	-	-	-	+
	0.39	+	+	+	+	+	+	+	+	+	+
WPF	100	-	-	-	+	+	+	+	-	-	-
	25	-	-	-	+	+	+	+	-	-	-
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
PPF	100	-	-	-	+	+	+	+	+	+	+
	25	-	-	-	+	+	+	+	+	+	+
	6.25	-	-	-	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
RPP	100	-	-	-	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
WPP	100	-	-	-	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
PPP	100	+	-	+	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+

'+'= indicates bacterial growth; '-'= indicates no bacterial growth; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel and PPP: Papaya peels.

Minimum Inhibitory Concentration

The minimum inhibitory concentration expressed as the lowest extract concentration at which no visible growth in broth was observed. The MIC of methanol extract of both flesh and peel of red pitaya, white pitaya and papaya against four Gram positive bacteria and sixth Gram negative bacteria are shown in **Table 2**. The MIC values of methanol extract of red pitaya flesh on *Staphylococcus epidermidis (*1.56 mg/mL), *Staphylococcus aureus (*1.56 mg/mL)*, Enterococcus faecalis (*6.25 mg/mL)*, Listeria monocytogenes (*6.25 mg/mL)*, Klebsiella (*1.56 mg/mL)*, Pseudomonas aeruginosa (*6.25 mg/mL)*, S. marcescens (*1.56 mg/mL)*, S. flexneri (*1.56 mg/mL)*,Salmonella entrica typhi (*1.56 mg/mL) and *E.coli (*6.25 mg/mL)*.*

The MIC values of methanol extract of white pitaya flesh on *Staphylococcus epidermidis (*25.00 mg/mL), *Staphylococcus aureus (*25.00 mg/mL), *Enterococcus faecalis (*25.00 mg/mL)*, S. marcescens (*25.00 mg/mL)*, S. flexneri (*25.00 mg/mL)*,* and *E.coli (*25.00 mg/mL)*.* There was no MIC value for white pitaya extract on *Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.* The MIC values of methanol extract of papaya flesh on *Staphylococcus epidermidis (*6.25 mg/mL), *Staphylococcus aureus (6*.50 mg/mL) and *Enterococcus faecalis (*6.50 mg/mL)*.* There was no MIC value for papaya extract on *S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.* The MIC values of methanol extract of red pitaya peel on *Staphylococcus epidermidis (*25.00 mg/mL), *Staphylococcus aureus (*25.00 mg/mL) and *Enterococcus faecalis* (25.00 mg/mL)*.* There was no MIC value for red pitaya peel extract on *S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.*

The MIC values of methanol extract of white pitaya peel on *Staphylococcus epidermidis (*100 mg/mL), *Staphylococcus aureus (100* mg/mL) and *Enterococcus faecalis (*100 mg/mL)*.* There was no MIC value for white pitaya peel extract on *S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.* The MIC values of methanol extract of papaya peel only show positive on *Staphylococcus aureus (100* mg/mL). There was no MIC value for white pitaya peel extract on *Staphylococcus epidermidis, Enterococcus faecalis, S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.*

Minimum Bactericidal Concentration

The minimum bactericidal concentration recorded as the lowest extract concentration that exhibited capacity to kill 99.9 % of unicell inocula. The MBC of methanol extract of both flesh and peel of red pitaya, white pitaya and papaya against four Gram positive bacteria and sixth Gram negative bacteria are shown in **Table 3**. The MBC values of methanol extract of red pitaya flesh on *Staphylococcus epidermidis (*1.56 mg/mL), *Staphylococcus aureus (*6.25 mg/mL)*, Enterococcus faecalis (*6.25 mg/mL)*, Listeria monocytogenes (*6.25 mg/mL)*, Klebsiella (*6.25 mg/mL)*, Pseudomonas aeruginosa (*6.25 mg/mL)*, S. marcescens (*6.25 mg/mL)*, S. flexneri (*6.25 mg/mL)*,Salmonella entrica typhi (*6.25 mg/mL) and *E.coli (*6.25 mg/mL)*.*

The MBC values of methanol extract of white pitaya flesh on *Staphylococcus epidermidis (*6.25 mg/mL), *Staphylococcus aureus (*6.25 mg/mL), *Enterococcus faecalis (*6.25 mg/mL)*, Listeria monocytogenes (*6.25 mg/mL) *S. marcescens (*6.25 mg/mL)*, S. flexneri (*6.25 mg/mL), and *E.coli (*6.25 mg/mL)*.* There were no MBC values for white pitaya extract on *Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.* The MBC values of methanol extract of papaya flesh on *Staphylococcus epidermidis (*6.25 mg/mL), *Staphylococcus aureus (6*.50 mg/mL) and *Enterococcus faecalis (*6.50 mg/mL)*.* There were no MBC values for papaya extract on *S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.* The MBC values of methanol extract of red pitaya peel on *Staphylococcus epidermidis (*25.00 mg/mL), *Staphylococcus aureus (*25.00 mg/mL) and *Enterococcus faecali* (25.00 mg/mL)*.* There were no MBC values for red pitaya peel extract on *S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.*

Table 3: The minimum bactericidal concentrations of methanol extract flesh and peel of red pitaya, white pitaya and papaya (inoculated on agar plates).

Samples	(mg/mL)	*S. epidermidis*	*S. aureus*	*E. faecalis*	*L. monocytogenes*	*Klebsiella*	*P. aeruginosa*	*S. typhi*	*S. marcescens*	*S. flexneri*	*E. coli*
RPF	100	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-	-	-
	1.56	-	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
WPF	100	-	-	-	+	+	+	+	-	-	-
	25	-	-	-	+	+	+	+	-	-	-
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
PPF	100	-	-	-	+	+	+	+	+	+	+
	25	-	-	-	+	+	+	+	+	+	+
	6.25	-	-	-	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
RPP	100	-	-	-	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
WPP	100	-	-	-	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+
PPP	100	+	-	+	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+	+	+	+
	1.56	+	+	+	+	+	+	+	+	+	+
	0.39	+	+	+	+	+	+	+	+	+	+

'+'= indicates colonies growth; '-'= indicates no colonies growth; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel and PPP: Papaya peels.

The MBC values of methanol extract of white pitaya peel on *Staphylococcus epidermidis (*25.00 mg/mL), *Staphylococcus aureus (*25.00 mg/mL) and *Enterococcus faecalis (*25.00 mg/mL)*.* There were no MBC values for white pitaya peel extract on *S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.* The MBC values of methanol extract of papaya peel only show positive on *Staphylococcus aureus* (25.00 mg/mL). There were no MBC values for white pitaya peel extract on *Staphylococcus epidermidis, Enterococcus faecalis, S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi.*

DISCUSSION

The present study were pioneering report on the potential of red pitaya as antibacterial agent, previously efforts have been made to study the chemistry of betalains in red pitaya[33,34,35] and antioxidant and antiproliferative activities of red pitaya[36]. Red pitaya fruit flesh and peel rich in polyphenols and were good sources of antioxidants and fullfilled its promise to inhibit the growth of melanoma cells[36]. All sixth extracts of the fruits tested showed varying degree of antibacterial activities againts the test bacterial species. The antibacterial activitiy of the methanol extract of red pitaya flesh with that two standard antibiotics (penicillin and gentamicin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The methanol extract of the fruits flesh showed broad spectrum of activity againts all the Gram positive bacteria used in the study. This indicates that methanol extract has better antibacterial properties compared to the narrow spectrum of activity of crude extracts as reported by Geidam *et al*[37]. *Staphylococcus aureus* was found to be most susceptible to methanol extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel. *Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa* and *Salmonella entrica typhi* was the least susceptible except for red pitaya flesh extracts.

The activity of this methanol red pitaya flesh againts *E. coli* is interesting since *E.coli* strains have developed resistance to antimicrobial drugs commonly used in poultry production[38] and even to frontline antimicrobials, such as the fluoroquinolones[39,40]. Methanol extracts of both flesh and peel of red pitaya showed significantly higher antimicrobial activity compared to white pitaya and papaya, which certainly indicates that red pitaya extracts contain higher concentration of active antimicrobial agents. But it also could be attributable to the polarity nature of active antimicrobial agents. These may include alkaloids, glycosides, volatile oils or tannins, which are all found in more abundant in fruits of red pitaya, white pitaya and papaya. Previous study done by Wybraniec and Mizrahi[35] was found that red pitaya, recently drawn much attention not only because of their red-purple color and economic value as a food products, but also for their antioxidative activity from the betacyanin contents. The extracts of both flesh and peel of white pitaya and papaya were observed to be weakly active on *Staphylococcus epidermidis* and *Staphylococcus aureus,* did not show any inhibition activity on *Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa, S. marcescens, S. flexneri, Salmonella entrica typhi* and *E. coli.* This probably is due to dilution effect or chemical antagonism of the various constituents on each other, resulting in inactive or less active products, this was agreed and proposed by Hugo and Russell[41], and Bourne and Roberts[42]. Nonetheless, *Staphylococcus aureus* and *Staphylococcus epidermidis* was sensitive to all assayed extract. This justifies that red pitaya fruit peel and flesh can be used for treatment of skin infections.The greater resistance of Gram negative bacteria to plant and fruit extracts has been documented previously by Kudi *et al.* [43] and Vlietinck *et al.*[44]*,* and it was supported by this study results.

The observation are likely to be the result of the difference in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a barrier to many environmental substances including antibiotics[45]. The susceptibility of only Gram positive bacteria to the phenolics and anthocyanin extract was interesting as we may suggest that there could have been a possible interaction between these alkaloids and some constituents of the Gram negative cell wall composition. Therefore the phenolics extract could be explored as a narrow spectrum of phenolic antibacterial agent. The MIC values are remarkable for a crude extract as well as the MBC values. The results obtained shows that these *staphylococci* may be inhibited by a very low concentration of the extract while all studied concentrations had capacity to trigger lysis of the cells by activation of autolysis enzymes in the cell wall. This situation may be referred to as tolerance, a resistance mechanism, due to the small difference between the MIC and MBC[46]. Therefore this fruits extracts may suitable as a bactericidal agent in the case of infections of from non-MRSA.

CONCLUSION

This study has demosted of antimicrobial activity of both flesh and peel of red pitaya, white pitaya and papaya from methanol extraction againts Gram negative and Gram positive bacteria is an indication that the fruits extracts is a potential source for production of drugs with a broad spectrum of activity against bacteria (Gram positive and negative) and can be used in the treatment of infectious diseases causes by resistant microorganisms. From the present study we can conclude that the crude compound from both flesh and peel of red pitaya, white pitaya and papaya have great potential as antimicrobial compounds against microorganisms and can significantly inhibit the growth of *Staphylococcus epidermidis, Staphylococcus aureus* and *Listeria monocytogenes.*

ACKNOWLEDGEMENT

The authors would like to thank Universiti Sultan Zainal Abidin (UniSZA) for the financial aid and Faculty of Food Technology for providing the facilities. The authors also would like to acknowledge Mr. Roslan Arshad and Mr. Hazlan Harun for their assist from Faculty of Food Technology, UniSZA, Mr. Zarizal Suhaili, Mr. Noor Muzamil Mohamad, Madam Afnani Alwi and all staff at Teaching Laboratory 1, Faculty of Agriculture and Biotechnology, UniSZA.

REFERENCES

1. Anderson, J. W, Hanna, T. J (1999). Whole grains and protection against coronary heart disease: what are the active components and mechanisms?. Am. J. Clin. Nutr. 70: 307-308.
2. Chun D, Gall CM, Bi X, Lynch G (2001). Evidence that integrins contribute to multiple stages in the consolidation of long term potentiation. Neuroscience 105:815–829.
3. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM Jr, Musher DM, Niederman MS, Torres A, Whitney CG (2007). Infectious Diseases Society of America; American Thoracic Society Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin. Infect. Dis. 44(suppl2): S27-S72.
4. Lis-Balchin, S. G. Deans (1997). Bioactivity of selected plant essential oils against listeria monocytogenes. Journal of Applied Microbiology.Volume 82, Issue 6, pages 759–762.
5. Namiki, M., 1990. Antioxidants/antimutagenes in food. Crit. Rev. Food Sci. Nutr., 29: 273-300.
6. Zgoda JR, Porter JR (2001). A convenient microdilution method for screening natural products against bacteria and fungi. Pharm. Biol. 39: 221-225.
7. Ashoor, S.H. and knox, J.M. 1982. Determination of organic acids in foods by high-performance liquid chromatography. J. Chromatogr. 299, 288–292.
8. Mohd Adzim Khalili, R., Norhayati, A. H, Rokiah, M.Y., Asmah, R., Siti Muskinah, M. and Abdul Manaf, A. 2009. Hypocholesterolemic effect of red pitaya (Hylocereus sp.) on hypercholesterolemia induced rats. International Food Research Journal 16: 431-440.
9. Burt S. (2004): Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology, 94: 223–253.
10. Dorman H.J., Deans S.G. (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88: 308–316.

11. Adel S. Al-Zubairi, Ahmad Bustamam Abdul, Siddig Ibrahim Abdelwahab, Chew Yuan Peng, Syam Mohan, and Manal Mohamed Elhassan (2009). Eleucine indica Possesses Antioxidant, Antibacterial and Cytotoxic Properties. Evidence-Based Complementary and Alternative Medicine, Vol 2009.
12. National Committee for Clinical Laboratory Standard), 1999. Performance Standards for Antimicrobial Susceptibility Testing. 9th International Supplement.M100-S9, Wayne Pa.
13. Ramzi AA, Mothana RAA, Lindequist U (2005). Antimicrobial activity of some medicinal plants of the island soqotra. J. Ethnopharmacol. 96: 177-181.
14. Vollekova, A.D., Kostalova and R. Sochorova (2001). Isoquinoline Alkaloids from Mahonia aquifolium stem bark is active against Malassezia Sp. Folia Microbiol., 46: 107-111.
15. Usman, H., F.I. Abdulrahman and A.H. Ladan (2007). Phytochemical and Antimicrobial Evaluation of Tribulus terrestris L. (Zygophylaceae). Growing in Nigeria. Res. J. Bio. Sci. Medwell Journals, 2007 2(3): 244-247.
16. Srinivasan, D., Nathan, S. Suresh, T. and Perumalsamy, P. Z. (2001). Antimicrobial activities of certain Indian Medicinal plants in Folkloric Medicine. J. Ethnopharmacol. 74:217-220.
17. Smith-Palmer, A., Stewart, J., Fyfe, L., 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Letters in Applied Microbiology 26, 118–122.
18. Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS (2007). Antioxidative activities of water extracts of some Malaysian herbs. ASEAN Food J 14: 61-68.
19. Lu F, Foo LY (1995). Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, editors. Food antioxidants. New York: Marcel Dekker.
20. Beuchat, L.R., Golden, D.A., 1989. Antimicrobials occurring naturally in foods. Food Technology 43,134–142.
21. Russell, A.D., 1991. Mechanisms of bacterial-resistance to non-antibiotics —food-additives and food and pharmaceutical preservatives. Journal of Applied Bacteriology 71, 191–201.
22. Gülçin I, Oktay M, Kıreçci E, Küfrevio�lu I (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpella anisum L.) seed extracts. Food. Chem. 83: 371-382.
23. Arnous A, Makris DP, Kefalas P (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J. Agric. Food. Chem. 49: 5736-5742.
24. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free. Radical. Res. 23: 375-383.
25. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food. Chem. 47: 3954-3962.
26. Cai, Y.Z., Luo, Q., Sun, M., Corke, H., 2004.Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science 74, 2157–2184.
27. Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B. & Jacob, R. (2003) Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. J. Agric. Food Chem. 51:3273-3279.
28. Matasyoh, L.G., J.C. Matasyoh, F.N. Wachira, M.G. Kinyua, A.W.T. Muigai and T.K. Mukiama, 2008. Antimicrobial activity of essential oils of Ocimum gratissimum L. from different populations of Kenya. Afr. J. Trad. Complimen. Alternat. Med., 5: 187-193.
29. Stintzing, F.C., Kammerer, D., Schieber, A., Adama, H., Nacoulma, O.G., & Carle,R. (2004). Betacyanins and phenolic compounds from Amaranthusspinosus L. and Boerhaviaerecta L. Zeitschriftfu¨r Naturforschung/Biosciences,59c,1–8.
30. Wybraniec, S., Platzner, I., Geresh, S., Gottlieb, H.E., Haimberg, M. and Mogilnitzki, M. 2001. Betacyanin from vine cactus Hylocereus polyrhizus. Phytochemistry 58: 1209-1212.
31. Wybraniec, S. and Mizrahi, Y. 2002. Fruit fesh betacyanin pigments in Hylocereus Cacti. Journal of Agricultural and Food Chemistry 50: 6086–6089.
32. Wu, L. C., Hsu, H. W., Chen, Y. C., Chiu, C. C., Lin, Y. I. and Ho. J. A. 2006. Antioxidant and antiproliferative activities of red pitaya. Food Chemistry 95 (2): 319-327.
33. Geidam, Y.A., H. Usman, M.B. Abubakar, and B. Ibrahim. 2007. "Effects of Aqueous Leaf Extracts of Psidium guajava on Bacteria Isolated from the Navel of Day-old Chicks". Research Journal of Microbiology. 2(12):960-965.
34. Yang, H, J.G. Boersma, M. You, B.J. Buirchell and M.W. Sweetingham. 2004. Development and implementation of a sequence-specific PCR marker linked to a gene conferring resistance to Anthracnose disease in narrow-leafed lupin (Lupinus angustifolius L.). Molecular Breeding 14: 145-151.
35. Livermore DM, Struelens M, Amorim J, et al (2002) Multicentre evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests. J Antimicrob Chem other 49:289–300.
36. Angulo, F.J., K.R. Johnson, R.V. Tauxe, and M.L. Cohen, 2000: Origins and consequences of antimicrobial-resistant non typhoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microb. Drug Resist. 6, 77–83.
37. Hugo, W.B. and Russell, A.D. (1992) Types of antimicrobial agents. In Principles and Practice of Disinfection, Preservation and Sterilisation pp.7–88. ed. Russell, A.D., Hugo,W.B. and Ayliffe, G.A. J.Oxford, UK:Blackwell Scientific.
38. Dangoggo, S.M., S.S. Sadiq, U.Z. Faruq, L.G. Hassan, and S.B. Manga. 2005. "Preliminary Phytochemical and Antimicrobial Analysis of Securidaca longipedunculata". Proceedings of the Chemical Society of Nigeria (Supplement to Journal of Chemical Society of Nigeria). 2(2):510-514.
39. Kudi AC, Umoh JU, Eduvie LO, Gefu J (1999). Screening of some Nigerian Medicinal plants for antibacterial activity. J. Ethnopharmacol.,67: 225-228.
40. Vlietinck, A.J., Van Hoof, L., Totte, J., Lasure, A., vanden, Berghe, D., Rwangobo, P.C., Mvukiyuniwami, J. (1995): Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol. 46: 31-47.
41. Tortora, G., Funke, B., Case, C. 2001. Microbiology: An Introduction, 7th ed. Benjamin Cummings, CA p 19.
42. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. (1966): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.
43. Chopra, R.N., Nayar, S.L., Chopra, I.C. (1986): Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, CSIR Publications, New Delhi.
44. Brown. D. (1995): Encyclopaedia of Herbs and their Uses. Dorling Kindersley, D K Publishers, London, New York.
45. de Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I., Levenfors, J.J. (2005): Antifungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96: 461-469.
46. Janovska, D., Kubikova, K., Kokoska, L. (2003): Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. Czech. J. Food Sci. 21: 107-111.