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

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ABSTRACT
Red pitaya is a 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 Serratia marcescens. The methanol extract of 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. 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. 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. 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 diarrhoea 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. 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. Because of the possible toxicities of the synthetic antioxidants, Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed towards natural antioxidants. 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.

Red pitaya is a tropical fruit may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant. Red pitaya should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. 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 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 prolong the shelf-life of relevant food products. 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 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°C and store at -20°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°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 (ATC
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
bacteriological pathogens by the agar disc diffusion assay method
as described by the National Committee of Clinical Laboratory
Standards13. The bacterial strains were prepared from an overnight
broth cultures were diluted using sterile normal saline to give an
inoculum size about 10^8 cfu/mL. The density of bacterial
concentration of the cultures was standardized turbidometrically
to 500 000 – 1 000 000 colony forming units per mLfilter (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 was dissolved initially in dimethyl
sulfoxide (DMSO) diluted to a concentration of 100 µ/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
during the MBC determination. All tests were repeated for five
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 antioxidant compounds to counteract reactive
oxygen species (ROS) in order to survive21,22. 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 heart disease and cancers27,28. The level of the essential antioxidant compound in contrast to other
antioxidative defenses is determined mainly by their dietary
supply33.

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 benefits33. The phenolic compounds are
commonly found in both edible and non-edible plants and foods
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 donors and singlet oxygen quenchers29. 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 food30. 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 potential30,31. Microorganisms including Gram positive and Gram negative bacteria
have been recognized as the main causes of various human
infections32. Though effective antimicrobials have been developed
over the years, there has been increased development of
antimicrobial drug resistance to presently available antimicrobials32.

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 Klebsiella (19.50 ± 0.10 mm), Pseudomonas
aeruginosa (15.00 ± 0.50 mm), Salmonella enterica typhi (14.50 ± 0.90
mm), serratia marcescens (15.80 ± 0.04 mm) and Escherichia coli
(12.50 ± 0.90 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 showed any inhibition activity on
Listeria monocytogenes, Klebsiella, Pseudomonas aeruginosa and Salmonella enterica 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).

<table>
<thead>
<tr>
<th>Extract/Organisms</th>
<th>S. epidermidis</th>
<th>S. aureus</th>
<th>E. faecalis</th>
<th>L. monocytogenes</th>
<th>Klebsiella</th>
<th>P. aeruginosa</th>
<th>S. typhi</th>
<th>S. marcescens</th>
<th>S. flexneri</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF</td>
<td>15.0 ± 0.03a</td>
<td>19.0 ± 0.43a</td>
<td>18.5 ± 0.80a</td>
<td>16.0 ± 1.81a</td>
<td>15.0 ± 0.10a</td>
<td>15.0 ± 0.50a</td>
<td>14.5 ± 0.90a</td>
<td>15.8 ± 0.04a</td>
<td>12.5 ± 0.90a</td>
<td>14.5 ± 0.04a</td>
</tr>
<tr>
<td>WPF</td>
<td>10.0 ± 0.30a</td>
<td>9.00 ± 0.90a</td>
<td>11.0 ± 0.15a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>PPF</td>
<td>8.50 ± 0.99a</td>
<td>7.50 ± 0.10a</td>
<td>8.50 ± 0.80a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>PFP</td>
<td>9.00 ± 0.50a</td>
<td>10.0 ± 0.50a</td>
<td>8.50 ± 0.40a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>WPP</td>
<td>7.50 ± 0.70a</td>
<td>8.00 ± 0.30a</td>
<td>9.00 ± 0.90a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>PPP</td>
<td>15.0 ± 0.02a</td>
<td>16.0 ± 0.10a</td>
<td>18.0 ± 0.09a</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10.0 ± 0.01a</td>
<td>18.3 ± 0.70a</td>
<td>16.5 ± 0.70a</td>
<td>10.0 ± 0.01a</td>
<td>18.0 ± 0.07a</td>
<td>18.5 ± 0.51a</td>
<td>15.5 ± 0.27a</td>
<td>18.5 ± 0.30a</td>
<td>18.0 ± 0.07a</td>
<td></td>
</tr>
</tbody>
</table>

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

Inhibition zones are the mean including disc (6 mm) diameter; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, PFP: 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 enterica 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 m.m. 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 enterica 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).

<table>
<thead>
<tr>
<th>Samples</th>
<th>S. epidermidis</th>
<th>S. aureus</th>
<th>E. faecalis</th>
<th>L. monocytogenes</th>
<th>Klebsiella</th>
<th>P. aeruginosa</th>
<th>S. typhi</th>
<th>S. marcescens</th>
<th>S. flexneri</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WPF</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PPF</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PFP</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

Results are presented as the minimum inhibitory concentration (MIC) in (µg/mL).

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 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 unincell 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 S. marcescens, S. flexneri, E. coli, Listeria monocytogenes, 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 white pitaya extract on Klebsiella, Pseudomonas aeruginosa and Salmonella entrica typhi. The MBC values of methanol extract of papaya peel 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 faecalis (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 (incubated on agar plates).

<table>
<thead>
<tr>
<th>Samples</th>
<th>S. epidermidis</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>S. marcescens</th>
<th>S. flexneri</th>
<th>P. aeruginosa</th>
<th>S. typhi</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF</td>
<td>6.25</td>
<td>1.56</td>
<td>0.39</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WPF</td>
<td>6.25</td>
<td>1.56</td>
<td>0.39</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>PPF</td>
<td>6.25</td>
<td>1.56</td>
<td>0.39</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The values of S. epidermidis, S. aureus, E. coli, S. marcescens, S. flexneri, P. aeruginosa, S. typhi, and E. coli are indicated by +. The values of S. flexneri are indicated by -. The values of S. marcescens are indicated by -. The values of P. aeruginosa are indicated by -. The values of S. typhi are indicated by -. The values of E. coli are indicated by -. The values of S. epidermidis are indicated by -. The values of S. aureus are indicated by -. The values of E. coli are indicated by -.

Khalili et al.
Int J Pharm Pharm Sci, Vol 4, Suppl 3, 185-190
188
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 enterica 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 enterica 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 and antioxidant and antiproliferative activities of red pitaya. Red pitaya fruit flesh and peel rich in polyphenols and were good sources of antioxidants and fulfilled its promise to inhibit the growth of melanoma cells. All six extracts of the fruits tested showed varying degree of antibacterial activities against the test bacterial species. The antibacterial activity of the methanol extract of red pitaya flesh with two standard antibiotics (penicillin and gentamicin) (penicillin (0.25 mg/mL) and gentamicin (1 mg/mL)) 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 against 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. *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 enterica typhi* was the least susceptible except for red pitaya flesh extract.

The activity of this methanol red pitaya flesh against *E. coli* is interesting since *E. coli* strains have developed resistance to antimicrobial drugs commonly used in poultry production and even to front line antibiotics such as the fluoroquinolones. 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 Mizrahim 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 enterica 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, and Bourne and Roberts. Nonetheless, *Staphylococcus aureus* and *Staphylococcus epidermidis* was sensitive to all assayed extract. This justifies that red pitaya flesh and peel 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. and Vlie tinck et al., 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. 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 *Staphylococcus* 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. Therefore this fruits extracts may suitable as a bactericidal agent in the case of infections 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 against 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*.

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