Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

TOTAL ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT AND RADICAL SCAVENGING ACTIVITY BOTH FLESH AND PEEL OF RED PITAYA, WHITE PITAYA AND PAPAYA

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Received: 8 April 2011, Revised and Accepted: 18 Sep 2011

ABSTRACT

Red pitaya is known to have a number of nutritional benefits, including cholesterol-lowering effects, protection against diabetes and cancer. The objectives of this study was to evaluate total antioxidant capacity, total phenolic content and radical scavenging activity of both flesh and peel of red pitaya, white pitaya and papaya of the hexane, dichloromethane (DCM), ethyl acetate (EA) and methanol extracts. Based on the results obtained, the tested fruit extracts showed strong antioxidant activity or differential capacity to inhibit lipid peroxidation by FTC and TBA method which is indicated by their low absorbance values. At a given concentration, the relatively higher activity was recorded in the extracts of red pitaya flesh followed red pitaya peel, white pitaya flesh, white pitaya peel, papaya flesh and papaya peel, surpassing the activity of standard commercial antioxidant, α-tocopherol and BHT. Red pitaya flesh showed low radical scavenging activity as compared to BHA but greater than BHT and α-tocopherol, showing that red pitaya flesh contained high amount of radical scavenging compounds. Red pitaya flesh also showed the highest reductive activity as compared with white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel. The fruit flesh extracts activities were lower than α-tocopherol, BHA and BHT standards and these differences were statistically very significant ($p<0.05$). Superoxide radical scavenging activity of those samples followed the order: BHA>BHT>α-tocopherol>red pitaya flesh> white pitaya flesh>papaya flesh>red pitaya peel> papaya peel>white pitaya peel. Red pitaya flesh showed low radical scavenging activity as compared to BHA but greater than BHT and α-tocopherol, showing that red pitaya flesh contained high amount of radical scavenging compounds.

Keywords: Antioxidant activity, TBA, FTC, DPPH, Phenolic compound and chelating activity.

INTRODUCTION

The red pitaya (*Hylocereus sp*.) have recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products but also for their nutritional content[1]. Red pitaya is believed native to Southern Mexico, the pacific side of Guatemala and Costa Rica and El- Salvador derive from climbing epiphytes belonging to the *Cactaceaca* family[2]. Red pitaya possesses large scales instead of spines and its pulp only contains small digestible seeds[3]. They are currently being grown commercially in Taiwan, Nicaragua, Colombia, Vietnam, Israel, and Australia and now in Malaysia. In cacti, the most important fruit pigments are the betacyanins and betaxanthins[1]. Betalains, composed of red-violet betacyanin and yellow betaxanthins, are water-soluble pigments that provide colours in flowers and fruits. The known betacyanin pigments of *Hylocereus polyrhizus* flesh are betanin, phyllocactin and recently discovered betacyanin and hylocerenin[1,4].

Efforts have been made to study the chemistry of betalains in *H.polyrhizus* [1,2] and nutrition composition, antioxidant composition and health potential of red pitaya fruit on hypercholesterolemia and diabetes mellitus in reducing risk factors for cardiovascular disease[5,6]. Wu *et al.*,[7] also indicated that the flesh and peel of red pitaya, both rich in polyphenols (good sources of antioxidants) and show positive effect to inhibit the growth of melanoma cells. Previous study also showed that red pitaya rich in micronutrients and flavonoids, antioxidant vitamins and radical scavenging activity[8]. However there is little information available on the previous research that has been carried out to show the potential of source of antioxidants to improve human health from red pitaya fruit. The objectives of this study was to evaluate total phenolics content, antioxidant capacity and scavenging activity of the hexane, dimethyl sulphite (DMS), ethyl acetate (EA) and methanol extracts peel and flesh of red pitaya, white pitaya and papaya.

MATERIALS & METHODS

Extraction Procedure

The method of Abdul et al.,[9] with slightly modification was used. Red pitaya, white pitaya and papaya fruits were obtained from a local organic plantation in Setiu, Terengganu, Malaysia. The fruits were extracted with different solvents in the order of increasing polarity (hexane, DMS, EA and methanol). 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 hexane for 7 days with occasional shaking and the process was repeated three times. The residue was air dried overnight and used for next solvent extraction (DMS) as per the above procedure and the same procedure was repeated for next two other solvents (EA and Methanol). Finally, all the extracts for each solvent were filtered through Whatman ® No. 41 filter paper (pore size 20-25 µm) and was then concentrated under reduced pressure at 40^0C and store at -20^0C until were used for the analysis.

Determination of Total Phenolic Compounds

Total phenolic content of the red pitaya, white pitaya and papaya extracts was determined using standard method of Folin-ciocalteu reagent introduced by Kahkonen et al.,[10] and Gulcin et al.,[11] with slightly modification using gallic acid as a standard phenolic compound. A total of 100 µL of extract solution (0.10 – 0.50 mg/mL) was added in test tube than 0.75 mL of Folin-ciocalteu reagent and left the mixture under room temperature for 5 minutes. After 5 minutes 0.75 mL natrium carbonate (60 g/L) was added and the mixture was allowed to stand at room temperature for 90 minutes. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Secoman, France). The concentration of total phenolic compounds in the extracts determined as microgram of gallic acid equivalent by using equitation that was obtained from standard gallic acid graph.

Antioxidant Assay

The antioxidant activities of red pitaya, white pitaya and papaya extracts were measured using ferric thiocyanate (FTC) and thiobarbituric acid (TBA). The FTC method was used to measure the amount of peroxide at the beginning of peroxidation while TBA method was used to measure free radicals present after peroxide oxidation.

Ferric Thiocyanate Method

The standard methods proposed by Kikuzaki and Nakatani[12] with slightly modification was use for this study. A mixture of 4.0 mg of sample extract in 4.0 mL of absolute ethanol, 4.1 mL of 2.52%

linolenic acid in absolute ethanol, 8.0 mL of 0.05 M phosphate buffer (pH 7.0), and 3.9 mL of distilled water was placed in test tube with a screw cap and then placed in dark oven at 40^0C. To 0.1 mL of this solution were added 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely 3 minutes after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 532 nm every 24 hours until one day after the absorbance of the control reached its maximum. BHA, BHT and α-tocopherol were used as positive controls and mixture without sample extract was used as the negative control.

Thiobarbituric Acid (TBA) Method

The combination method Kikuzaki and Nakatani[12] and Z.M. Zin et al.[13] with slightly modification were followed. Approximately 1 mL of sample solution from FTC method was added with 2.0 mL of 20% trichloroacetic acid (TCA) and 2.0 mL of 0.67% thiobarbituric acid (TBA) in the test tube. The mixture was placed in water bath (95^0C) for 10 minutes. After cooling the mixture was centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance on the final day of the FTC method. BHA, BHT and α-tocopherol were used as positive controls and mixture without sample extract was used as the negative control.

Metal Chelating Activity

The chelating of ferrous ions by the sample extract was estimated by method introduced by Dinis et al.[15]. A total of 1 mL of solution extracts was added to solution of 2 mM $FeCl_2$ (0.05 mL). Approximately 0.2 mL of 5 mM ferrozine was added to initiated in the previous mixture; the mixture was shaken vigorously and left standing at room temperature for 10 minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine – Fe^{2+} complex formation was given below formula:

$$\% \text{ Inhibition} = (A_0\text{-}A_1/A_0) \text{ x}100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample extracts and standards (BHA, BHT and α-tocopherol). The control contains $FeCL_2$ and ferrozine, complex formation molecules.

Superoxide Anion Scavenging Activity

Measurement of superoxide anion scavenging activity of sample extract was based on the proposed method by Liu et al.[16] with slightly modification by Oktay et al.[17]. The superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 µM) solution, 1 mL of NADH (78 µM) solution and sample extracts in water were mixed. The reaction started by adding 1 mL of PMS solution (10 µM) to the mixture, the reaction mixture was incubated at 25^0C for 5 minutes and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. BHA, BHT and α-tocopherol were used as standards and L-Ascorbic acid was used as a control. Decrease absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0\text{-}A_1) / A_0] \text{ x}100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of sample extracts and standard.

Free Radical Scavenging Activity

The free radical scavenging activity of the sample extract was measured in accordance to the standard method Shimada et al.[18] with slightly modification. A total of 10 mg extracts were dissolved in 1.0 mL methanol and the solution added to a 1.0 mL DPPH· solution at room temperature. The absorbance at 517 nm was measured utilizing UV-1601 Shimadzu spectrophotometer. The results were expressed as percentage of reduction of the initial DPPH· absorption by test samples as follows:

$$\text{DPPH scavenging effect (\%)} = 100 - [(A_0\text{-}A_1/A_0) \text{ x}100]$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of twenty independent samples and ten replicates. Statistical analysis was performed with single factor and one way ANOVA to identify the significant difference on antioxidant activity, reductive potential activity, metal chelating activity, superoxide anion scavenging activity, free radical scavenging activity and total phenolic content of the hexane, dimethyl sulphite (DMS), ethyl acetate (EA) and methanol extracts peel and flesh of red pitaya, white pitaya and papaya.

RESULTS AND DISCUSSION

Extract Yield and Total Phenolic Content

The total phenolics contents of the flesh and peel of red pitaya, white pitaya and papaya in hexane, dimethyl sulphite (DMS), ethyl acetate (EA) and methanol extracts was measured accordingly to the Folin-Ciocalteu method. The Folin-Ciocalteu reagent determines total phenols (and other easily oxidized substances), producing a blue colour by reducing yellow hetero polyphosphomolybdate-tungstate anions. This method gives a general measure of phenolics content, as it is not completely specific for phenolics compounds and not all phenolics compounds exhibit the same level of activity in the assay. Phenolics were extracted from red pitaya flesh and peel, white pitaya flesh and peel and papaya flesh and peel with 100% hexane, DMS, EA and methanol.

The total phenolics content of fruit extracts was determined by extrapolation from the calibration curve calibration curve ($Y=19.29x + 0.347$; $R^2=0.988$) prepared from gallic acid concentrations and expressed in milligrams of gallic acid (GAEs). The amount of phenolics compounds in methanol extracts was determined from regression equitation of and values were expressed in gallic acid equivalences (GAEs).

According to the **Table 1**, the weight of the crude extracts obtained was in the range of 8.05 g to 22.65 g for methanol extract. Red pitaya flesh indicates the highest percent yields (22.65 %); follow by white pitaya flesh (19.62 %), papaya flesh (18.01 %), red pitaya peel (10.65 %), white pitaya peel (9.41 %) and papaya peel (8.05 %) in methanol extract. The amount of phenolics compounds in methanol extracts varied from 3.45 to 16.70 mg/100 g GAEs of crude extract. It was found that red pitaya flesh showed the highest phenolics concentration of 16.70 mg/g of GAEs followed by white pitaya flesh (11.02 mg/g of GAEs), red pitaya peel (7.63 mg/g of GAEs), papaya flesh (7.24 mg/g of GAEs), white pitaya peel (5.74 mg/g of GAEs) and papaya peel (3.45 mg/g of GAEs). It was observed that reaction mixture with both flesh and peel of red pitaya extracts were dark blue in colour that visually indicated high phenolics content as compare with other samples.

The results also showed crude obtained for DMS extracts was in the range of 0.19 g to 4.02 g. Red pitaya flesh indicates the highest percent yields (11.49 %); follow by white pitaya flesh (7.23 %), papaya flesh (3.80 %), red pitaya peel (2.66 %), white pitaya peel (0.69 %) and papaya peel (0.54 %). The amount of phenolics compounds in DMS extract also varied from 0.36 to 2.04 mg/100 g GAEs of crude extract. It was found that red pitaya flesh showed the highest phenolics concentration of 2.04 mg/g of GAEs followed by white pitaya flesh (1.02 mg/g of GAEs), papaya flesh (0.92 mg/g of GAEs), red pitaya peel (0.48 mg/g of GAEs), white pitaya peel (0.38 mg/g of GAEs) and papaya peel (0.36 mg/g of GAEs).

Hexane extracts showed the total crude obtained in the range of 1.09 g to 3.52 g. Red pitaya flesh still indicates the highest percent yields (10.06 %); follow by white pitaya flesh (7.23 %), papaya flesh (5.80 %), red pitaya peel (3.51 %), white pitaya peel (3.26 %) and papaya peel (3.11 %). The amount of phenolics compounds in hexane extract also varied from 0.02 to 0.18 mg/100 g GAEs of crude extra. It was found that red pitaya flesh showed the highest phenolics concentration of 0.18 mg/g of GAEs followed by white pitaya flesh

(0.10 mg/g of GAEs), red pitaya peel (0.06 mg/g of GAEs), papaya flesh (0.04 mg/g of GAEs), white pitaya peel (0.44 mg/g of GAEs) and papaya peel (0.02 mg/g of GAEs).

The ethyl acetate extract (EA) showed the lowest percent extraction yield and phenolics concentration as compare with others solvent of extractions. The EA extracts showed the total crude obtained in the range of 0.09 g to 1.52 g. Red pitaya flesh also indicates the highest percent yields (4.34 %); follow by white pitaya flesh (3.23 %), papaya flesh (2.94 %), red pitaya peel (0.66 %), white pitaya peel (0.40 %) and papaya peel (0.26 %). The amount of phenolics compounds in hexane extract also varied from 0.02 to 0.09 mg/100 g GAEs of crude extra. It's also found that red pitaya flesh showed the highest phenolics concentration of 0.09 mg/g of GAEs followed by white pitaya flesh (0.08 mg/g of GAEs), red pitaya peel (0.05 mg/g of GAEs), papaya flesh (0.03 mg/g of GAEs), white pitaya peel (0.06 mg/g of GAEs) and papaya peel (0.02 mg/g of GAEs).

Table 1: Percentages of yields and total phenolics of hexane, dichloromethane (DCM), ethyl acetate (EA) and methanol extraction peel and flesh of red pitaya, white pitaya and papaya.

	Extracts	Wt. of starting material (g)	Weight of dry extract (g)	Yield (%)	Total phenolic (mg/100 g G.A.E)
Methanolic	RPF	100	22.65^{d}	22.65 ± 0.56^{d}	16.7001 ± 0.56^{d}
	WPF	100	19.62^{c}	19.62 ± 3.12^{c}	11.0217 ± 1.39^{c}
	PPF	100	18.01^{c}	18.01 ± 3.19^{c}	07.2357 ± 1.32^{b}
	RPP	100	10.65^{b}	10.65 ± 1.08^{b}	07.6308 ± 1.89^{b}
	WPP	100	9.41^{a}	09.41 ± 1.92^{a}	05.7378 ± 1.23^{a}
	PPP	100	8.05^{a}	08.05 ± 1.93^{a}	03.4497 ± 1.08^{a}
	RPF	35	3.52^{c}	10.06 ± 1.79^{d}	00.1777 ± 0.009^{e}
Hexane	WPF	35	2.53^{b}	07.23 ± 0.12^{c}	00.1016 ± 0.012^{d}
	PPF	35	2.03^{b}	05.80 ± 1.09^{b}	00.0414 ± 0.009^{a}
	RPP	35	1.23^{a}	03.51 ± 1.83^{a}	00.0615 ± 0.003^{c}
	WPP	35	1.14^{a}	03.26 ± 1.13^{a}	00.0439 ± 0.001^{b}
	PPP	35	1.09^{a}	03.11 ± 0.12^{a}	00.0214 ± 0.002^{a}
	RPF	35	1.52^{d}	04.34 ± 1.79^{e}	00.0904 ± 0.001^{c}
Ethyl Acetate	WPF	35	1.13^{c}	03.23 ± 0.12^{d}	00.0893 ± 0.000^{c}
	PPF	35	1.03^{c}	02.94 ± 1.09^{d}	00.0390 ± 0.001^{a}
	RPP	35	0.23^{b}	00.66 ± 0.00^{c}	00.0565 ± 0.000^{b}
	WPP	35	0.14^{a}	00.40 ± 0.00^{b}	00.0562 ± 0.000^{b}
	PPP	35	0.09^{a}	00.26 ± 0.00^{a}	00.0239 ± 0.000^{a}
	RPF	35	4.02^{d}	11.49 ± 0.92^{d}	02.0360 ± 0.092^{e}
Dimethyl Sulfite	WPF	35	2.53^{c}	07.23 ± 0.10^{c}	01.0160 ± 0.045^{d}
	PPF	35	1.33^{b}	03.80 ± 0.98^{b}	00.9247 ± 0.003^{c}
	RPP	35	0.93^{b}	02.66 ± 0.10^{b}	00.4760 ± 0.001^{b}
	WPP	35	0.24^{a}	00.69 ± 0.00^{a}	00.3754 ± 0.000^{a}
	PPP	35	0.19^{a}	00.54 ± 0.00^{a}	00.3598 ± 0.002^{a}

**Each mean represents analyses of 20 independent samples and ten replicates*

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

^{123}Variation in the following numbers between methods of extraction indicates significance of difference by Duncan's test at 5% level (p<0.05).

RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel; MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.

In this study, the dark blue colour produced in the reaction mixture indicated that both flesh and peel of red pitaya extractions contained high phenolic compounds. The flesh and peel of white pitaya and papaya only producing light blue colour solutions that indicated that fruits contain low phenolics content as compare with red pitaya for every solvent of extractions has been used (hexane, DMS, EA and methanol). The colour difference between red pitaya and white pitaya was strongly suggested to be the explanation. The red purple of red pitaya may indicate the presence of higher phenolics compounds and betalain than white pitaya and papaya. Betalains, composed of red violet betacyanins and yellow betaxanthins, are water-soluble pigments that provided colours in flowers and fruits[7]. It was shown that edible flesh of red pitaya, white pitaya and papaya had higher total phenolics content as compared to inedible peels portion. For white pitaya, that is white in colour, there is might be less non-betalainic phenolics compounds, and no or less betalains explaining low phenolics content measured in its flesh. Higher level of phenol content in both flesh and peel of red pitaya may lie on the structure of fruit itself. Beside the possible presence of different kinds of antioxidant compounds, it was postulated that the fruit size of white pitaya used in present study might be among the factors, as white pitaya was bigger that red pitaya that was more rounded.

Antioxidant Activity

The results in **Figure 1** showed the antioxidant activity of of the hexane, dimethyl sulfite (DMS), ethyl acetate (EA) and methanol extracts peel and flesh of red pitaya, white pitaya and papaya were measured using ferric thiocyanate method (FTC). From the figure also showed that all samples were oxidized when stored for seven days at 40-45°C. Initially the methanol fruit extracts had showed have the highest antioxidative activity for fruit extracts. A comparison between sample extracts with BHA, BHT and α-tocopherol showed has a significant difference ($p<0.05$) in total antioxidant activity compared to the control. After seven days, it had been shown that all samples effectively inhibit linoleic acid oxidation.

The percentage of inhibition of linoleic acid was in order are red pitaya flesh (70.85 ± 1.05%) followed by red pitaya peel (62.82 ± 0.15%), white pitaya flesh (60.82 ± 1.19%), papaya peel (55.37 ± 1.94%), papaya flesh (54.16 ± 0.99%) and white pitaya peel (50.95 ± 1.43%). The values of antioxidant activity for are BHA (71.09 ± 0.52%), α-tocopherol (62.19 ± 1.55%) and BHT (61.67 ± 1.23%). The methanol extracts of both flesh and peel of red pitaya had showed negligible antioxidant activities and were not significantly ($p<0.05$) different with the control and standard (BHA). It is interesting to note that methanol extracts of both flesh and peel of red pitaya exhibited higher activity than the white pitaya and papaya extracts.

The second highest in antioxidative activity was showed by DMS extraction fruit samples, the antioxidant activity was significantly highest in red pitaya flesh with the value of 52.12 ± 1.79% followed by white pitaya flesh (45.47 ± 1.01%), papaya flesh (41.76 ± 1.07%), red pitaya peel (42.78 ± 1.04%), papaya peel (39.25 ± 1.49%) and

white pitaya peel (32.83 ± 1.05%) respectively. For the standards, the values of antioxidant activity are 69.97 ± 3.05% (BHA), 60.22 ± 0.45% (α-tocopherol) and 61.59 ± 1.35% (BHT). The DMS extracts of both flesh and peel of red pitaya had also showed negligible antioxidant activities and significantly lower ($p<0.05$) as compared with the standards (BHA, BHT and α-tocopherol). It was showed that the DMS extracts of both flesh and peel of red pitaya exhibited higher activity than white pitaya and papaya extracts.

The antioxidant activity for hexane extraction fruit samples was found high in red pitaya flesh (49.89 ± 1.10%) followed by white pitaya flesh (45.05 ± 1.28%), papaya flesh (42.97 ± 0.16%), red pitaya peel (38.23 ± 0.11%), white pitaya peel (31.05 ± 0.96%) and papaya peel (29.86 ± 1.08%). For the standards, the values of antioxidant activity are BHA (69.37 ± 1.58%), α-tocopherol (60.11 ± 2.02%) and BHT (59.53 ± 0.35%). The hexane extracts of both flesh of red pitaya and white pitaya showed negligible antioxidant activities respectively. The antioxidative activity of ethyl acetate extract fruit samples, the red pitaya peel was found show high in value of antioxidant activity (25.74 ± 2.86%) followed by red pitaya flesh (21.34 ± 1.89%), white pitaya flesh (19.91 ± 0.09%), papaya flesh (16.43 ± 1.05%), papaya peel (16.69 ± 0.39%) and white pitaya peel (15.39 ± 0.38%) but lower when comparable with BHA (70.89 ± 1.58%), α-tocopherol (61.33 ± 2.02%) and BHT (60.13 ± 0.24%).

Based on the results obtained, ethyl acetate extraction has showed the lowest antioxidative activity respectively when compared with methanol, hexane and DMS extracts. It is highly possible that several compounds of different polarity may contribute to the antioxidative activity of flesh and peel (red pitaya, white pitaya and papaya). Methanol extracts may include phenolics and hydrox-phenolic compound with acids, alcohols, sugars or glycosides, it was reported that[28], have a strong antioxidant activity of fruits of *Ficus deltiodea* var *angustifolia sp.* in FTC test with percent of inhibition range from 90.70% to 97.78% respectively and the methanol extract showed higher antioxidant activity as compared with DMS, hexane and ethyl acetate extract after seven days of incubation.

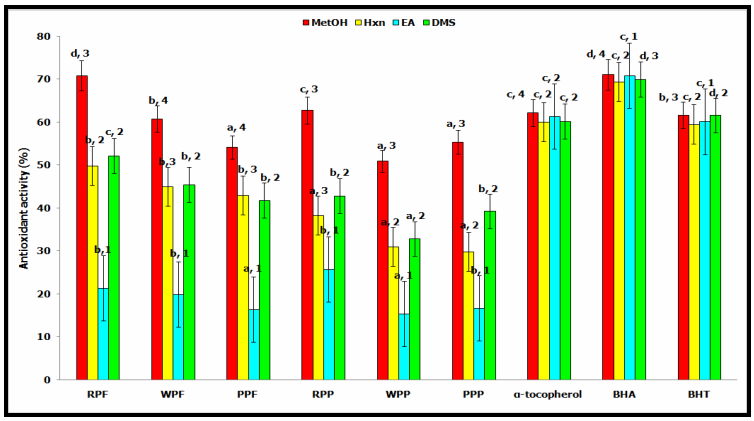

Fig. 1: Total antioxidant activity of different solvent extractions for RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT using FTC analysis.

**Each mean represents analyses of 20 independent samples and ten replicates.*

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

[123]Variation in the following numbers between methods of extraction indicates significance of difference by Duncan's test at 5% level ($p<0.05$);RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT:Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.

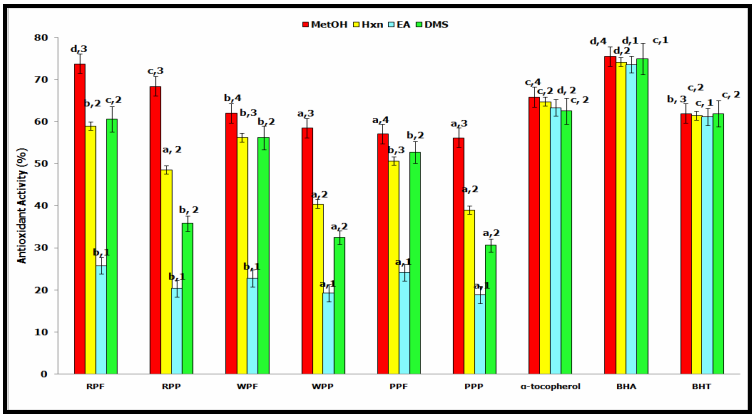

Fig. 2: Total antioxidant activity of different solvent extractions for RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT using TBA analysis.

**Each mean represents analyses of 20 independent samples and ten replicates*

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

[123]Variation in the following numbers between methods of extraction indicates significance of difference by Duncan's test at 5% level ($p<0.05$); RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT:Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.

Figure 2 showed the total antioxidant activity of the TBA method in both flesh and peel of red pitaya, white pitaya and papaya. Results were also obtained for the antioxidant activity of the methanol, DMS, hexane and ethyl acetate extracts. The methanol fruit extracts showed that there is a significant difference ($p<0.05$) in total antioxidant activity compared with BHA, BHT and α-tocopherol by using TBA method (Figure 3.3). The antioxidant activity was highest in value ($p<0.05$) for red pitaya flesh (73.81 ± 6.45%) followed by red pitaya peel (68.45 ± 9.12%), white pitaya flesh (62.02 ± 1.23%), white pitaya peel (58.49 ± 1.94%), papaya flesh (57.10 ± 3.91%) and papaya peel (56.23 ± 3.94%). For the standards, the values of antioxidant activity are BHA (75.53 ± 1.05%), α-tocopherol (65.89 ± 6.45%) and BHT (62.00 ± 2.95%).

The DMS extraction samples showed the highest antioxidant activity in red pitaya flesh (58.71 ± 1.06%) followed by white pitaya flesh (46.87 ± 1.81%), red pitaya peel (45.18 ± 2.04%), papaya flesh (42.78 ± 1.04%), papaya peel (40.22 ± 2.99%) and white pitaya peel (39.03 ± 1.75%). For the standards, the values of antioxidant activity are 74.89 ± 2.35% (BHA), 62.19 ± 2.05% (α-tocopherol) and 61.89 ± 1.55% (BHT). The obtained result for hexane extraction samples, antioxidant activity was significant highest in red pitaya flesh (58.93 ± 2.10%) followed by white pitaya flesh (56.25 ± 2.58%), papaya flesh (50.67 ± 1.27%), white pitaya peel (40.48 ± 0.96%), red pitaya peel (40.06 ± 1.12%) and papaya peel (38.96 ± 1.78%). For the standards, the values of antioxidant activity are BHA (74.23 ± 1.02%), α-tocopherol (64.81 ± 1.75%) and BHT (61.45 ± 2.91%). The ethyl acetate extraction samples were show significant difference in antioxidant activity. The highest antioxidant activity was showed by red pitaya flesh with the values of 20.12 ± 2.09%, followed by red pitaya peel (20.04 ± 0.28%), white pitaya flesh (18.93 ± 1.59%), papaya peel (16.43 ± 1.05%), papaya peel (16.99 ± 1.42%) and white pitaya peel (14.31 ± 1.08%). Whereas, the values of antioxidant activity for BHA (73.58 ± 1.58%), α-tocopherol (63.25 ± 1.95%) and BHT (61.16 ± 1.35%).

Based on the results obtained, the tested fruit extracts showed strong antioxidant activity or differential capacity to inhibit lipid peroxidation by FTC and TBA method which is indicated by their low absorbance values. The FTC method measures the amount of peroxide produced during the initial stages of lipid peroxidation. At a given concentration, the relatively higher activity was recorded in the extracts of red pitaya flesh followed red pitaya peel, white pitaya flesh, white pitaya peel, papaya flesh and papaya peel, surpassing the activity of standard commercial antioxidant, α-tocopherol and BHT. In general, the antioxidant by TBA method is higher than that of FTC method. This might suggest that the amount of peroxide in the initial stage of lipid per oxidation is less than the amount of peroxide in the secondary stage. Furthermore, the secondary product is much more stable for a period of time[12]. This antioxidant assays, showed the reduction in peroxide level at the concentrations investigated may indicated the ability of the fruit extracts to minimize oxidative damage to some vital tissues in the body[20].

Metal Chelating Activity

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator. In this assay, the methanol, DMS, hexane and ethyl acetate extracts for both flesh and peel of red pitaya, white pitaya, papaya and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkonyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[24].

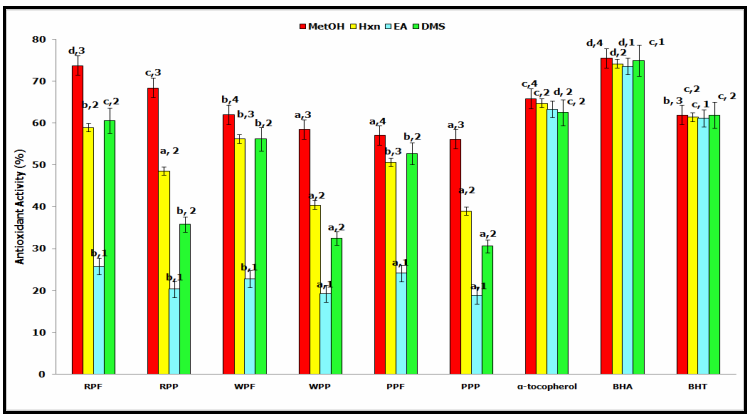

Fig. 3: Metal chelating effect of different solvent extractions for RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT.

**Each mean represents analyses of 20 independent samples and ten replicates*

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

[123]Variation in the following numbers between methods of extraction indicates significance of difference by Duncan's test at 5% level ($p<0.05$);RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT:Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.

As shown in **Figure 3**, the formation of Fe^{2+} to ferrozine complex is not complete in the presence of the methanol, DMS, hexane and ethyl acetate extracts of both flesh and peel of red pitaya, white pitaya and papaya. Indicating that, the methanol, DMS, hexane and ethyl acetate extracts of both flesh and peel of red pitaya, white pitaya and papaya chelate the iron. The absorbance of Fe^{2+} to ferrozine complex was linearly decreased dose dependently (from 20 to 100 ug/mL). The percentage of metal chelating capacity of 100 ug/mL concentration of methanol extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel, papaya peel, BHA, BHT and α-tocopherol were found as 76.38 ± 0.06%, 60.14 ± 3.06%, 43.97 ± 1.17%, 43.34 ± 1.26%, 32.59 ± 0.62%, 31.04 ± 1.90%, 69.97 ± 1.02%, 71.67 ± 6.92% and 62.79 ± 1.00% respectively. However, there was statistically significant different between 100 ug/mL of methanol extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel, and same concentration of BHA, BHT and α-tocopherol ($p<0.05$). The metal scavenging effect of methanol extract samples and standards decreased in the order of red pitaya flesh>BHA>BHT>α-tocopherol>white pitaya flesh>papaya flesh>red pitaya peel>white pitaya peel>papaya peel.

The percentage of metal chelating capacity at 100 ug/mL concentration of DMS extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel, papaya peel, BHA, BHT and α-tocopherol were found as 53.94 ± 0.09%, 47.87 ± 2.06%, 49.14 ± 0.56%, 40.32 ± 2.29%, 29.58 ± 0.63%, 26.87 ± 0.26%, 60.89 ± 1.09%, 60.44 ± 0.34% and 50.34 ± 0.61% respectively. The metal scavenging effect of DMS extract samples and standards decreased in the order of BHA>BHT>α-tocopherol>red pitaya flesh>papaya flesh>white pitaya flesh>red pitaya peel>white pitaya peel>papaya peel.

The percentage of metal chelating capacity at 100 ug/mL concentration of hexane extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel, papaya peel, BHA, BHT and α-tocopherol were found as 48.71 ± 1.49%, 27.10 ± 1.01%, 29.04 ± 1.05%, 22.58 ± 2.09%, 27.21 ± 0.96%, 15.97 ± 1.17%, 64.21 ± 1.66%, 63.96 ± 0.65% and 56.41 ± 1.35% respectively. The metal scavenging effect of DMS extract samples and standards decreased in the order of BHA>BHT>α-tocopherol>red pitaya flesh>papaya flesh>white pitaya peel>white pitaya flesh>red pitaya peel>papaya peel.

The percentage of metal chelating capacity at 100 ug/mL concentration of ethyl acetate extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel, papaya peel, BHA, BHT and α-tocopherol were found as 23.96 ± 1.45%, 17.24 ± 1.34%, 19.36 ± 0.21%, 12.33 ± 1.06%, 07.63 ± 0.11%, 05.79 ± 0.07%, 64.67 ± 0.30%, 62.98 ± 2.01% and 59.56 ± 0.05% respectively. The metal scavenging effect of DMS extract samples and standards decreased in the order of BHA>BHT>α-tocopherol>red pitaya flesh>papaya flesh>white pitaya flesh>red pitaya peel>white pitaya peel>papaya peel.

The difference between methanol, DMS, hexane and ethyl acetate extracts of both flesh and peel of red pitaya, white pitaya, papaya and the control was statistically significant ($p<0.05$). Metal chelating capacity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation[25]. It was reported that chelating agents, who form α-bond with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[26]. The data obtained from Figure 3 reveal that both of flesh and peel of red pitaya, white pitaya and papaya extracts demonstrated a marked capacity for ion binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity[11].

Superoxide Anion Scavenging Activity

Superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Figure 4 shows the percentage inhibition of superoxide radical generation by 100 ug/mL concentration of methanol, DMS, hexane and ethyl acetate extracts for both flesh and peel of red pitaya, white pitaya and papaya, and comparison with same concentration of BHA, BHT and α-tocopherol. For methanol extract, red pitaya flesh have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity with the percentage inhibition value of 79.52 ± 1.69% respectively followed by white pitaya flesh (63.45 ± 1.87%), papaya flesh (45.23 ± 2.63%), red pitaya peel (41.32 ± 0.23%), white pitaya peel (31.35 ± 0.41%) and papaya peel (30.21 ± 1.34.%). The percentage inhibition value by standard substances is 65.61 ± 1.75% (α-tocopherol), 73.87 ± 1.48% (BHT) and 75.38 ± 3.01% (BHA). Superoxide radical scavenging activity of those samples followed the order: red pitaya flesh>BHA>BHT> α-tocopherol>white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel.

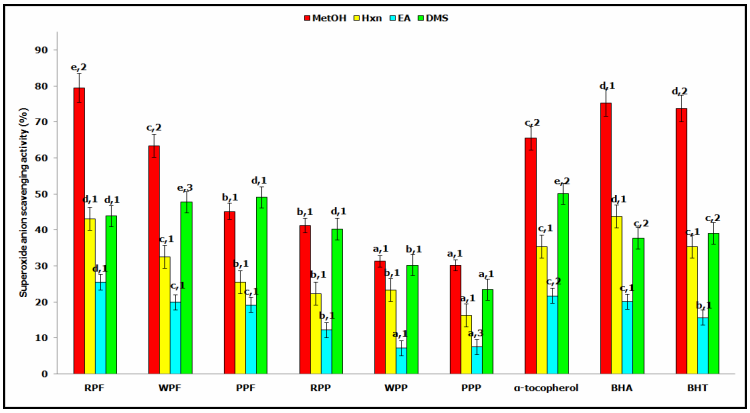

Fig. 4: Superoxide anion radical scavenging activity of 100 ug/mL concentration of methanol, DMS, hexane and ethyl acetate solvent extractions for RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT.

**Each mean represents analyses of 20 independent samples and ten replicates*

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

[123]Variation in the following numbers between methods of extraction indicates significance of difference by Duncan's test at 5% level ($p<0.05$);RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT:Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.

For DMS extract, papaya flesh have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity with the percentage inhibition value of 49.14 ± 0.56% respectively followed by white pitaya flesh (47.78 ± 0.17%), red pitaya flesh (43.95 ± 0.32%), red pitaya peel (40.38 ± 0.03%), white pitaya peel (30.32 ± 0.56%) and papaya peel (23.49 ± 1.09%). The percentage inhibition value by standard substances is 50.15 ± 1.20% (α-tocopherol), 60.14 ± 0.60% (BHT) and 61.78 ± 0.10% (BHA). Superoxide radical scavenging activity of those samples followed the order: BHA>BHT> α-tocopherol>papaya flesh>white pitaya flesh>red pitaya flesh, red pitaya peel, white pitaya peel and papaya peel. For hexane extract, red pitaya flesh have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity with the percentage inhibition value of 43.09 ± 0.69% respectively followed by white pitaya flesh (32.54 ± 3.29%), papaya flesh (25.56 ± 1.28%), white pitaya peel (23.45 ± 0.03%), red pitaya peel (22.42 ± 2.09%) and papaya peel (16.35 ± 0.32%). The percentage inhibition value by standard substances is 55.45 ± 0.23% (α-tocopherol), 65.45 ± 0.95% (BHT) and 65.78 ± 1.88% (BHA). Superoxide radical scavenging

activity of those samples followed the order: BHA>BHT>α-tocopherol>red pitaya flesh> white pitaya flesh>papaya flesh, white pitaya peel> red pitaya peel>papaya peel.

For ethyl acetate extract, red pitaya flesh also have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity with the percentage inhibition value of 25.59 ± 1.68% respectively followed by white pitaya flesh (19.98 ± 1.24%), papaya flesh (19.23 ± 1.02%), red pitaya peel (12.27 ± 1.69%), papaya peel (07.56 ± 0.02%) and white pitaya peel (07.24 ± 0.15%). The percentage inhibition value by standard substances is 59.76 ± 0.16% (α-tocopherol), 60.74 ± 0.76% (BHT) and 61.12 ± 0.78% (BHA). Superoxide radical scavenging activity of those samples followed the order: BHA>BHT>α-tocopherol>red pitaya flesh> white pitaya flesh>papaya flesh>red pitaya peel> papaya peel>white pitaya peel. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated. It also has the ability to change to other harmful reactive oxygen species and free radicals within the living cells. The fruits extracts has been found to have significant superoxide radical scavenging activity, which ultimately adds to its antioxidant potential. The scavenging activity of this radical by methanol, DMS, hexane and ethyl acetate extracts for both flesh and peel of red pitaya, white pitaya and papaya compared with α-tocopherol, BHA and BHT (standards) suggests that fruits is also a potent scavenger of superoxide radical like the standard compounds.

Free Radical Scavenging Activity

Figure 5 illustrates a significant ($p<0.05$) decrease the concentration of DPPH radical due to the scavenging ability of each concentration of methanol fruits extracts and standards. Methanol extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel was showed stronger DPPH scavenging activity rather than DMS, hexane and ethyl acetate extracts and this difference was found significantly statistically ($p<0.05$). The DPPH scavenging effect of methanol fruits extracts and standards on the DPPH radical decreased in the order of BHA (87.34 ± 1.47%)> RPF (83.19 ± 0.06%)> BHT (73.48 ± 1.06%)> α-tocopherol (59.00 ± 1.85%)> WPF (87.34 ± 1.47%)> PPF (87.34 ± 1.47%)> RPP (87.34 ± 1.47%)> WPP (87.34 ± 1.47%)> PPP (87.34 ± 1.47%) at the concentration 60 ug/mL respectively.

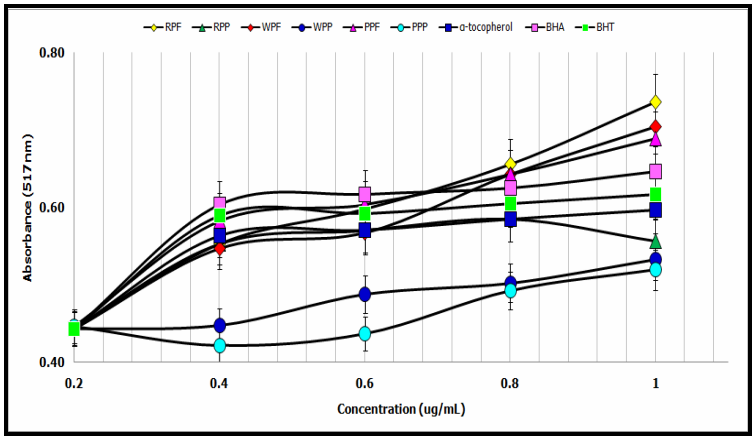

Fig. 5: Free radical scavenging activity of methanol extract of RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT by 1,1-diphenyl-2-picrylhydrazyl radicals.

**Each mean represents analyses of 20 independent samples and ten replicates*; *RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.*

These results indicated that both flesh and peel of fruits methanol extract have a noticeable effect on scavenging free radical. In the present study, all samples demonstrated purple bleaching reaction at increasing concentrations, showing the presence of compounds responsible as free radical scavengers which reduced the initial DPPH concentration. Very little absorbance changes occurred for papaya peel indicating the presence of very low radical scavenging compounds. Red pitaya flesh showed low radical scavenging activity as compared to BHA but greater than BHT and α-tocopherol, showing that red pitaya flesh contained high amount of radical scavenging compounds.

Figure 6 illustrates a significant ($p<0.05$) decrease the concentration of DPPH radical due to the scavenging ability of each concentration of DMS fruits extracts and standards. DMS extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel was showed stronger DPPH scavenging activity rather than hexane and ethyl acetate extracts and this difference was found significantly statistically ($p<0.05$). The DPPH scavenging effect of DMS fruits extracts and standards on the DPPH radical decreased in the order of BHA (63.12 ± 1.00%)> BHT (60.73 ± 1.02%)> α-tocopherol (55.24 ± 1.03%)> RPF (53.32 ± 0.06%)> RPP (42.53 ± 0.56%)> WPF (41.25 ± 1.09%)> PPF (40.17 ± 1.62%)> WPP (30.34 ± 0.29%)> PPP (26.68 ± 1.05%) at the concentration 60 ug/mL respectively.

In present study, all samples demonstrated purple bleaching reaction at increasing concentrations, showing the presence of compounds responsible as free radical scavengers which reduced the initial DPPH concentration. Very little absorbance changes occurred for papaya peel indicating the presence of very low radical scavenging compounds. These results indicated that both flesh and peel of fruits DMS extract have a noticeable effect on scavenging free radical. Red pitaya flesh showed low radical scavenging activity as compared to BHA but greater than BHT and α-tocopherol, showing that red pitaya flesh contained high amount of radical scavenging compounds.

Figure 7 illustrates a significant ($p<0.05$) decrease the concentration of DPPH radical due to the scavenging ability of each concentration of hexane fruits extracts and standards. Hexane extract of red pitaya flesh, white pitaya flesh, papaya flesh, red pitaya peel, white pitaya peel and papaya peel was showed stronger DPPH scavenging activity rather than ethyl acetate extracts and this difference was found significantly statistically ($p<0.05$). The DPPH scavenging effect of hexane fruits extracts and standards on the DPPH radical decreased in the order of BHA (61.45 ± 0.01%)>BHT (60.78 ± 0.56%)>α-tocopherol (59.12 ± 1.59%)>RPF (43.34 ± 1.63%)>WPF (37.33 ± 1.23%)>PPF(29.43 ± 2.13%)>RPP (22.27 ± 1.38%)>WPP (20.21 ± 1.39%)>PPP (16.89 ± 1.23%) at the concentration 60 ug/mL respectively

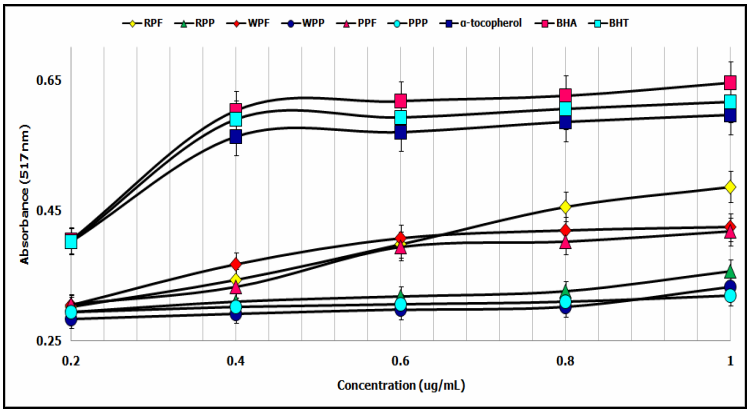

Fig. 6: Free radical scavenging activity of DMS extract of RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT by 1,1-diphenyl-2-picrylhydrazyl radicals.

**Each mean represents analyses of 20 independent samples and ten replicates; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.*

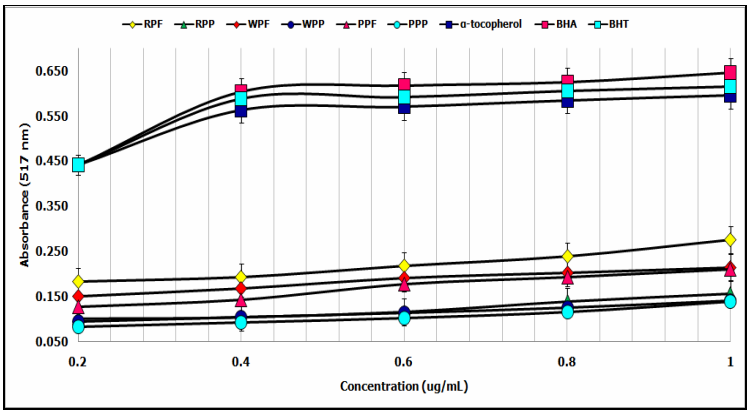

Fig. 7: Free radical scavenging activity of hexane extract of RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT by 1,1-diphenyl-2-picrylhydrazyl radicals.

**Each mean represents analyses of 20 independent samples and ten replicates; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.*

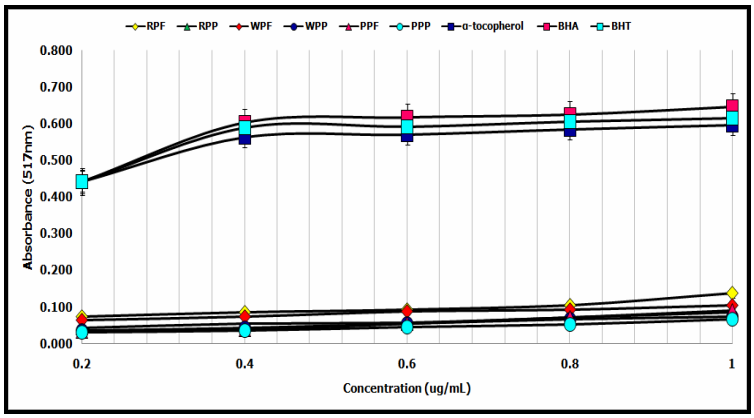

Fig. 8: Free radical scavenging activity of ethyl acetate extract of RPF, WPF, PPF, RPP, WPP, PPP, α-tocopherol, BHA and BHT by 1,1-diphenyl-2-picrylhydrazyl radicals.

**Each mean represents analyses of 20 independent samples and ten replicates; RPF: Red pitaya flesh, WPF: White pitaya flesh, PPF: Papaya flesh, RPP: Red pitaya peel, WPP: White pitaya peel, PPP: Papaya peel, α-tocopherol: alpha-tocopherol, BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, MetOH: Methanol extraction, Hxn: Hexane extraction, EA: Ethyl acetate extraction and DMS: Dimethyl sulfite extraction.*

Figure 8 illustrates a significant ($p<0.05$) decrease the concentration of DPPH radical due to the scavenging ability of each concentration of ethyl acetate fruits extracts and standards. The DPPH scavenging effect of ethyl acetate fruits extracts and standards on the DPPH radical decreased in the order of BHA (61.09 ± 0.05%)> BHT (60.27 ± 0.08%)> α-tocopherol (59.13 ± 0.09%)> RPF (29.95 ± 0.59%)> WPF (23.85 ± 0.15%)> PPF (19.47 ± 1.98%)> RPP (12.03 ± 0.09%)> WPP (07.25 ± 1.39%)> PPP (06.56 ± 0.09%) at the concentration 60 ug/mL respectively. In this study, all samples demonstrated purple bleaching reaction at increasing concentrations, showing the presence of compounds responsible as free radical scavengers which reduced the initial DPPH concentration. Very little absorbance changes occurred for papaya peel indicating the presence of very low radical scavenging compounds. Red pitaya flesh showed low radical scavenging activity as compared to BHA but greater than BHT and α-tocopherol, showing that red pitaya flesh contained high amount of radical scavenging compounds. In present study, all samples demonstrated purple bleaching reaction at increasing concentrations, showing the presence of compounds responsible as free radical scavengers which reduced the initial DPPH concentration. Very little absorbance changes occurred for papaya peel indicating the presence of very low radical scavenging compounds. Red pitaya flesh showed low radical scavenging activity as compared to BHA but greater than BHT and α-tocopherol, showing that red pitaya flesh contained high amount of radical scavenging compounds.

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators[27]. In comparison the methanol fruits extract showed the higher radical scavenging activity as compared with DMS, hexane and ethyl acetate fruits extract. The present results supported the previous findings whereby high primary antioxidant activity was found in methanol fruits flesh and peel extract as compare with DMS, hexane and ethyl acetate. Lim *et al.,*[28] was found that the methanol extract of guava peel and flesh showed the higher scavenging activity as compared with chloroform and hexane extracts. Li *et al.,*[29] also found that methanol extract of pomegranate peel and flesh showed higher antioxidant activity as compared with hexane and water extracts.

CONCLUSION

Total phenolics content was determined using Folin-Ciocalteu reagent and phenolics concentration equivalents of gallic acid were estimated. Gallic acid being the most important polyphenol in natural products was used to determine the phenolics for both flesh and peel of red pitaya, white pitaya and papaya. The results revealed that the methanol extract of both flesh and peel of red pitaya, white pitaya and papaya which are found having the significantly higher total phenolics content in the range of 3.45 to 16.70 mg/g GAE when compared with DMS (range from 0.36 mg/g to 2.04 mg/g GAE), hexane (range from 0.02 mg/g to 0.18 mg/g GAE) and EA (range from 0.02 mg/g to 0.09 mg/g GAE) extracts. The flesh and peel of red pitaya showed higher phenolic content than white pitaya and papaya. Even though red pitaya and white pitaya are from the *Hylocereus* species but the colour difference between flesh and peel was suggested to the explanation[30].

The red colour of red pitaya flesh may indicate the presence of higher phenolic compounds and betalains. Betalains, composed of red violet betacyanins and yellow betaxanthins, are water soluble pigments that provide colours in flowers and fruits[7]. Esquivel *et al.,*[32] found out that betalains were responsible for the major antioxidant capacity of purple *Hylocereus* juices evaluated, while non-betalainic phenolic compounds contributed only to a minor extent. In contrast for flesh of white pitaya that is white in colour, there might be less non-betalainic phenolic compounds and no or less betalains explaining low phenolic content measured in its peel, the same explanation should be used for the very low of phenolic content present in flesh and peel of papaya as compared with red pitaya and white pitaya. Antioxidant activity of methanol extract of both flesh and peel of red pitaya, white pitaya and papaya which are found showed strong antioxidant activity or differential capacity to inhibit lipid peroxidation (LPO) by FTC and TBA method which is indicated by their low absorbance values. Based on the results obtained, ethyl acetate extraction has showed the lowest antioxidative activity respectively when compared with methanol, hexane and DMS extracts. It is highly possible that several compounds of different polarity may contribute to the antioxidative activity of flesh and peel (red pitaya, white pitaya and papaya). Methanol extracts may include phenolics and hydrox-phenolic compound with acids, alcohols, sugars or glycosides.

Sharipah Ruzaina *et al.,*[19] was reported that have a strong antioxidant activity of fruits of *Ficus deltiodea* var *angustifolia sp.* in FTC test with percent of inhibition range from 90.70% to 97.78% respectively and the methanol extract showed higher antioxidant activity as compared with DMS, hexane and ethyl acetate extract after seven days of incubation. The antioxidant activities also increased, as concentration of the plant samples increased. The correlation analysis between total phenolics content and the FTC analysis was positive ($r=0.61$). These phenolics compounds may donate hydrogen and can terminate the free radical reaction chain by changing it to stable compounds[31]. Previous study done by Maznah *et al.,*[33] also reported that the organic extract samples had the highest antioxidant activity compared with tocopherol and BHT, the same finding also have been reported[34, 35]. Noriham *et al.,*[36] reported that the percentage of inhibition of *polygonum minus* and others herbs was increased, as concentration of the plant samples increased. Even though the percentage inhibition for *Murraya koenigil* was the highest, the result was not statistically different ($p<0.05$) from *polygonum minus* and all the others herbs extracts except for the control[34].

Metal chelating capacity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation[25]. It was reported that chelating agents, who form α-bond with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[26]. The data obtained from Figure 3.8 reveal that both of flesh and peel of red pitaya, white pitaya and papaya extracts demonstrated a marked capacity for ion binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity[11]. Superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated. It also has the ability to change to other harmful reactive oxygen species and free radicals within the living cells. The fruits extracts has been found to have significant superoxide radical scavenging activity, which ultimately adds to its antioxidant potential. The scavenging activity of this radical by methanol, DMS, hexane and ethyl acetate extracts for both flesh and peel of red pitaya, white pitaya and papaya compared with α-tocopherol, BHA and BHT (standards) suggests that fruits is also a potent scavenger of superoxide radical like the standard compounds.

DPPH assay reaction depends on the ability of the samples to scavenge free radicals which is visually noticeable as the colour change from purple to yellow due to hydrogen donating ability[37]. The more rapid the absorbance decrease, the more potent the primary antioxidant activity[38]. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators[27]. In comparison the methanol fruits extract showed the higher radical scavenging activity as compared with DMS, hexane and ethyl acetate fruits extract. The present results supported the previous findings whereby high primary antioxidant activity was found in methanol fruits flesh and peel extract as compare with DMS, hexane and ethyl acetate. The various antioxidant mechanisms of both flesh and peel of red pitaya, white pitaya and papaya in different extracts may be attributed to strong hydrogen donating ability, a metal chelating activity and their effectiveness as good scavengers of superoxide and free radicals. In addition, phenolic compounds appear to be responsible for the antioxidant activity of methanol, DMS, hexane and ethyl acetate extracts of flesh and peel of red pitaya, white pitaya and papaya. However, the components responsible for the antioxidative activity of all sample extracts are currently unclear. On the basis of the results of this study, it is clear that flesh and peel of red pitaya, white

pitaya and papaya extracts have powerful antioxidant activity against various antioxidant systems in vitro, moreover the red pitaya, white pitaya and papaya can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceuticals application. From these results, it can be concluded that red pitaya content high in antioxidant properties. Nevertheless, this study is useful as a step towards further work on the generation of antioxidant properties content database in fruits.

ACKNOWLEDGEMENTS

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, Mr. Hazlan Harun for their assist from Faculty of Food Technology, UniSZA, and all staff at Teaching Laboratory 1, Faculty of Agriculture and Biotechnology, UniSZA.

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