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Characterization of Antioxidant Activities in Red Dragon Fruit (*Hylocereus polyrhizus*) Pulp Water-based Extract



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ARTICLE INFO	ABSTRACT
Article history: Received 9 April 2019 Received in revised form 12 July 2019 Accepted 3 August 2019 Available online 26 September 2019	The red dragon fruit, Hylocereus polyrhizus, locally known as Buah Naga has become increasingly popular among consumers and growers worldwide due to its antioxidative properties. The present study was conducted to examine the total phenolic content, total flavonoid content and antioxidant activities of water extract from fruit pulp of the species. The antioxidant activities were measured by DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2-2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), FRAP (Ferric Ion Antioxidant Power) and Phosphomolybdate assays. The total phenolic and flavonoid contents of pulp were recorded at 32.9 ± 0.92 mg GAE/100 mL and 2.26 ± 0.14 mg QE/100 mL of juice, respectively. The antioxidant or free radical scavenging activity, measured by the DPPH and ABTS protocols were 73.38 ± 2.24 % and 92.66 ± 0.22 %, respectively. The antioxidant capacity, determined by FRAP and Phosphomolybdate assays were 132.17 ± 3.74 µmol Fe ²⁺ /100 ml and 28.94 ± 0.83 mg AAE/100 mL of juice, respectively. The total phenolic and total flavonoid content in water extracts of pulp showed positively high correlations with DPPH, ABTS, FRAP and Phosphomolybdate assays. The results suggest that water, which is biodegradable and non-toxic, can be utilized to effectively extract active compounds in red dragon fruit pulp.
<i>Keywords:</i> Antioxidants capacity; dilution factor; red dragon fruit; water extract.	Copyright © 2019 PENERBIT AKADEMIA BARU - All rights reserved

1. Introduction

Intake of fresh fruits and vegetables is important for protection against actions of free radicals [1], which are super reactive [2] and can lead to cell damage and homeostatic disruption in human [3]. Generally, there is a vast range of antioxidant compounds in fruits and vegetables which possess the power to scavenge free radicals and hinder peroxidation [1, 2]. Natural antioxidants are more desirable and preferred to protect against disorders generated by free radicals [4]. Flavonoids, a class of polyphenols, are usually are the main contributors of antioxidants [2, 5].

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There are several *in-vitro* antioxidant procedures that can be utilized to estimate antioxidant properties and capacity of fresh fruits and vegetables [6] including DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), FRAP (Ferric Reducing Antioxidant Power) and others [5]. ABTS and DPPH radical scavenging capacity assays, FRAP and TP assays are in the category of assays that involve electron transfer (ET). ET-based mechanism measures the ability of an antioxidant to reduce an oxidant, which changes color when reduced, and the degree of color change is related to the concentration of the antioxidant [6]. Application of DPPH and ABTS procedures in the measurement of antioxidant activity of polyphenolics and colorants are popular and broadly used because of their simplicity, shorter reaction time and can be applied in both aqueous and organic solvent systems [7].

The Red Dragon Fruit or Red Pitaya, *Hylocereus polyrhizus* [8, 9], is a perennial climbing vine cactus species [10]. The oval-shape of the fruit is remarkably decorative, with a bright red-purple skin, studded with green scales or bracts [11-13]. The fruit does resemble a dragon and causing locals to call it as *Buah naga* or *Buah Mata Naga* [14]. The fruit, with its scaly structured peels, has a delicate and sweet red-purple color flesh, embedded with numerous small black seeds [12]. The species comes in a number of varieties, namely *H. undatus* (white pulp with bright red skin), *H. polyrhizus* (red pulp with bright red skin) and *Selenicereus megalanthus* (white pulp with yellow skin) [10-12]. The species originates from the tropical forest regions of Mexico and Central and South America [15, 16] and recently has been widely grown in Malaysia, Thailand, Vietnam, Australia, Taiwan and some other parts of the world [11, 17].

Dragon fruit has successfully garnered attention from many countries in the world due to its exotic aesthetic characteristics [17] high economic potential [16], live well in the dry tropical climate conditions and can withstand temperatures of up to 40°C [18]. The fruit is rich in bioactive compounds and nutrients such as Vitamin A, B1, B2, B3, C, protein, fat, carbohydrate, crude fiber, flavonoid, thiamin, niacin, pyridoxine, kobalamin, glucose, phenolic, betacyanins, polyphenol, carotene, phosphorus, iron and phytoalbumin, which possess strong antiradical properties [10, 12, 16, 17].

Several studies have been conducted on antioxidant properties and capacities of the red dragon fruits (peel and pulp) using various solvents such as ethanol, methanol, acetone and hexane [2, 10, 16, 17, 19, 20].

Water has been said to be unsuitable for non-polar compound extraction, while large number of antioxidant compounds in plants such as flavonoids and polyphenols are polar and soluble in water. The present study was undertaken to evaluate the antioxidant activities and capacities of water extracts from *H. polyrhizus* pulp.

2. Materials and Methods

2.1 Materials

Freshly harvested Red Dragon Fruit (*H. polyrhizus*) were purchased from a local farm in Sepang, Selangor, Malaysia.

2.2 Extraction of Dragon Fruit Juice

The fruit was initially washed to get rid of any adhering impurities on the fruit surfaces. The fruit was subsequently peeled manually splitting the flesh from the skin. The flesh was cut into smaller pieces using a kitchen knife, and subsequently placed in a juice extractor (Tefal ZC255B Juice Extractor



Infiny) to draw out the juice from the flesh. The resulting juice was collected, kept in a sealed polyethylene bag and stored at -80 °C until further analysis.

2.3 Clarification of Dragon Fruit Juice

Thawed red dragon fruit juices were filtered using a handheld kitchen sieve to remove mucilage content [21]. The filtrate was then centrifuged (Eppendorf 5810 R) at 10000 x g for 10 minutes at 4°C to remove insoluble particles. The supernatant was collected from the centrifuge and was diluted with distilled water at 1:1 (dilution factor of 2), 1:2 (dilution factor of 3), 1:3 (dilution factor of 4) and 1:4 (dilution factor of 5) (v/v) ratios prior to further analyses.

2.4 Antioxidant Properties and Capacities 2.4.1 Total phenolic content

The total phenolic content in *H. polyrhizus* pulp extract was tested using the Folin-Ciocalteau method based on the method modified by [22]. 100 μ L of diluted extract was mixed with 50 μ L Folin-Ciocalteau reagent (after diluted with 7.9 mL of distilled water). After 4 minutes, 1.5 mL of 7.5 (w/v) % of sodium carbonate (NaCO₃) was added to the mixture. The mixture was incubated in a dark room at room temperature for 2 hours. After incubation process, 200 μ L of mixture was transferred to microtiter plate and was read at 765nm using a UV-VIS microplate reader. Gallic acid at various concentrations of 0.03 - 0.5 mg/mL were prepared by dissolving it in ethanol. The results were expressed as mg of gallic acid equivalents (GAE) per 100 mL of juice. All samples and readings were measured in triplicates.

2.4.2 Total flavonoid content

Total flavonoid content in the extract was determined using spectrophotometric method [23] with minor modifications. 100 μ L of diluted extract and 2% AlCl₃ (2g of AlCl₃ diluted in 100 mL of distilled water) were mixed and set aside to incubated at room temperature for 15 minutes. The absorbance was measured spectrophotometrically at 415 nm using the UV-Vis Microplate reader. The same procedure was repeated for the standard solution of quercetin (0.125 mg/mL – 0.000196 mg/mL) to obtain the calibration curve. Flavonoids contents in the extracts were expressed in terms of quercetin equivalent, QE (mg of quercetin/100 mL of juice).

2.4.3 DPPH radical scavenging activity

The DPPH free radical scavenging activity was carried out according to the method described by [21] with slight modifications. 1mg of 2,2-Diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$) was dissolved in ethanol (EtOH) and made up to 10 mL in a beaker. Next, 50 µL of the diluted extract was reacted with 150 ethanolic solutions of DPPH in a 96-well microtiter plate. The mixture was allowed to stand in the dark at room temperature for 30 minutes. Absorbance was measured spectrophotometrically at 515 nm using a UV-Vis Microplate reader. Distilled water was used as blank and DPPH solution without test sample was used as control. All analyses were performed in triplicates. The DPPH radical scavenging activity in terms of percentage was calculated according to the following Eq. (1).

DPPH scavenging activity (%) =
$$\frac{A_B - A_E}{A_B} \ge 100$$

(1)



where,

A_{Blank} = Absorbance reading of controlA_{Extract} = Absorbance reading of the sample

2.4.4 ABTS radical scavenging assay

The antioxidant activity of *H. polyrhizus* was tested using technique reported by [24] with some modifications [4]. ABTS^{+•} (2-2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) stock solution was made by dissolving 30 mg of ABTS in 7.8 mL 0f 2.45 mM potassium peroxodisulphate ($K_2O_8S_2$). The solution was kept in the dark at room temperature for 12-16 h, and then diluted with ethanol (EtOH) to an absorbance of 0.70 (±0.02) at 734 nm. Next, 50 µL of the diluted extract was mixed with 1950 µL ABTS^{+•} working solution. The absorbance for the mixture was measured at 734 nm using a UV-Vis Spectrophotometer at room temperature after 1, 4 and 6 minutes of incubation. All measurements were performed in triplicates. The antioxidant activity of the exctract was determined using the following formula (2).

Inhibition (%) ABTS Absorbance =
$$\frac{Acontrol - Atest}{Acontrol} \times 100$$
 (2)

The control was prepared by adding 50 μ L of distilled water in place of the sample.

2.4.5 Ferric ion Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant potential (FRAP) of the extract of *H. polyrhizus* pulp was tested according to the technique proposed by [25] with modifications. The working FRAP solution was prepared daily by mixing acetate buffer (300 mM, pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM of HCl and 20 mM FeCl3.6H₂O solution. FRAP reagent was prepared by mixing 100 mL acetate buffer, 10 mL TPTZ solution and 10 mL ferric solution (FeCl3.6H₂O) in a ratio of 10:1:1 at the time of use. Next, 100 µL of extract was mixed with 1.8 mL of FRAP reagent. The reduction of colorless ferric complex (Fe³⁺-tripyridyltriazine) to a blue-colored ferrous complex (Fe²⁺-tripyridyltriazine) was measured spectrophotometrically at 593 nm using a UV-Vis microplate reader. A standard curve was prepared using various concentrations of Ferrous sulphate solution (0.1 mM to 1.0 mM). The results were reported as µmol Fe²⁺ equivalents per 100 mL of juice.

2.4.6 Phosphomolybdate assay

To determine the total antioxidant capacity of *H. polyrhizus,* samples of extracts were run as per phosphomolybdate assay proposed by [26] and described by [4] with slight modifications. 300 μ L extract was mixed with 3 mL phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a beaker. The beaker was covered with aluminium foil and incubated in a water bath at 95 °C for 90 min. The temperature of the mixtures were decreased to room temperature and the absorbance was recorded at 765 nm, against a blank of distilled water. The standard curve was constructed using concentration of ascorbic acid prepared in distilled water ranging from 0.25 mg/mL to 0.0125 mg/mL and the linear range was used for calculation. The analysis was done in triplicates for the standard and extracts.



2.5 Statistical Analysis

Data were presented as means \pm standard deviation (SD). One-way analysis of variance (ANOVA, SAS 9.4, SAS Institute Inc, Cary, NC, USA) was used to assess the difference in means among different samples dilution factors (p≤0.01). Pearson's correlation analysis (Microsoft Excel 2016, Microsoft Corporation, Redmond, WA, USA) was used for evaluating the correlation between the total phenolic/flavonoid contents and the total antioxidant activity and capacity values of water extract of pulp of *H. polyrhizus*.

3. Results and Discussions

3.1 Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content (TPC) of water extracts of pulp of H. polyrhizus was determined in terms of mg of Gallic Acid Equivalents per 100 mL of juice (mg GAE/100 mL of juice). TPC of the extract was computed from calibration curve of gallic acid, that was determined to be y=0.0004x + 0.1628 $(R^2=0.992)$, whereby y = absorbance value at wavelength of 765nm and x= amount of total phenolic compounds in mg per 100 mL of the juice. It was observed that the reaction of the mixture with water extract of pulp of H. polyrhizus were dark blue in color indicating high phenolic content. During reaction phenol lose an H⁺ ion, producing a phenolate ion which reduced Folin-Ciocalteu reagent [4]. From the experiments, the most concentrated extract with a dilution factor of 2, contained the highest amount of total phenolic 32.9 ± 0.92 mg of GAE in 100 mL of fruit juice (Figure 1) and (Table 1). According to [10], the total phenolic content in fruit (peel and pulp) and pulp of H. polyrhizus was found at 15.92 ± 1.28 mg GAE/100 g and 24.22 ± 0.95 mg GAE/100 g, respectively which was relatively lower than the results obtained from the present experiment. The difference in total phenolic content could be due to differences in sample preparation, environmental growth variations and/or differences in maturation stage of the fruits. In addition, phenolic compounds are water soluble [27] and contain polar phenolic hydroxyl group/s [4] thus provide high concentration of these compounds in water extracts of pulp of *H. polyrhizus*.

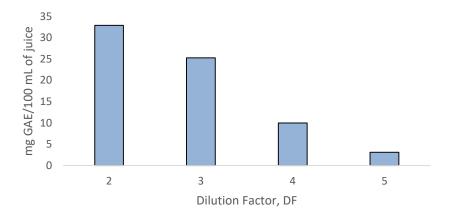


Fig. 1. Total Phenolic Content (TPC) of different dilution factors of water extract of pulp of *H. polyrhizus* expressed as mg GAE/100 mL of juice

The concentration of flavonoids in water extracts of the pulp of *H. polyrhizus* was determined using spectrophotometric method with aluminium trichloride. The flavonoid content was expressed in terms of quercetin equivalent (the standard curve equation: y = 0.0188x + 0.1343, $R^2 = 0.9974$), mg



of QE/ 100 mL of juice. From (Figure 2) and (Table 1), the highest concentration of flavonoids in water extracts from *H. polyrhizus* pulp was 2.26 ± 0.14 mg of QE/100 mL of juice at a dilution factor of 2, which was lower when compared with results obtained by [7] that used acetone as solvents during sample preparation. High phenolic content in the most concentrated extract, of dilution factor of 2 led to higher total flavonoid content in the present study.

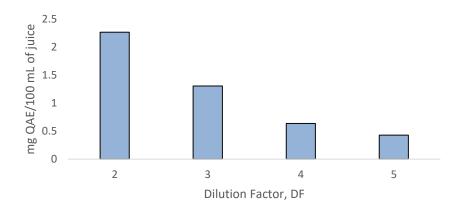


Fig. 2. Total Flavonoid Content (TFC) of different dilution factors of water extract of pulp of *H. polyrhizus* expressed as mg QAE/100 mL of juice

3.2 DPPH Assay

The DPPH radical scavenging activity assay, which analyzes the ability of a sample to donate hydrogen (H) to DPPH radical, is broadly used to determine antioxidant activities [10]. Ascorbic acid that acted as positive control acted immediately with DPPH solution by giving the earliest color change from dark purple of the DPPH solution to light yellow. During the reaction, antioxidants or reducing agents such as ascorbic acid, α -tocopherol and polyphenols delocalise the spare electron in DPPH molecule [35], causing the color to fade [28]. In the present study, water extracts from pulp of red dragon fruit showed no obvious purple bleaching effect during visual observation, even though there were decreases in the absorbance readings. The highest radical scavenging activity of water extract of pulp of *H. polyrhizus* was 73.38 ± 2.24% at a dilution factor of 2 (Figure 3) and (Table 1). Higher concentration of extract resulted higher antioxidant activity. Similar results also were reported by [10, 29]. According to [4] and [30], flavonoids and polyphenols which are antioxidant compounds in plants are polar and readily soluble in water thus have the capability to quench DPPH free radicals.



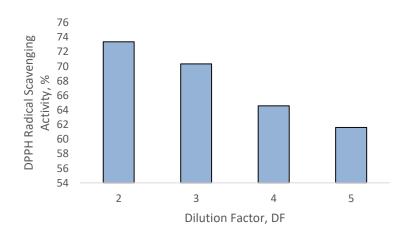
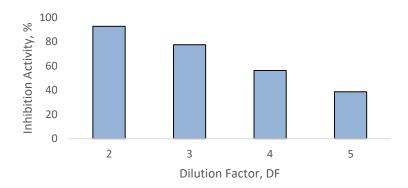
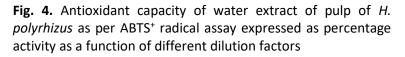


Fig. 3. Percent free radical scavenging activities of different dilution factors of water extract of pulp of *H. polyrhizus* as per DPPH assay

3.3 ABTS Assay

The ABTS assay was developed based on a decolourisation technique. Dark blue ABTS⁺ turns light blue or colourless when mixed with antioxidants, which can be measured spectrophotometrically at 734 nm [31]. Free radical scavenging capacities of various dilution factors of water extract of pulp of *H. polyrhizus* ranged from 38.69 % to 92.76 % (Table 1). In reaction mixture, extract at dilution factor 2 (most concentrate) gave the earliest color change from blue of the ABTS solution to light blue. Dilution factor 2 had the highest ABTS capacity (92.63 ± 0.22%), while dilution factor 5 had the lowest ABTS capacity at 38.69 ± 0.98 % (Figure 4) and (Table 1).





3.4 Ferric Reducing Antioxidant Potential (FRAP)

The antioxidant capacity of the extract was evaluated using FRAP assay. In this assay, ferrous sulphate solution was used as standard and the standard curve equation was determined to be y=0.4941x + 0.2601, $R^2 = 0.99$. In the presence of antioxidant, ferric ions are reduced to ferrous ions. As a result, blue-coloured ferrous tripyridydyltriazine complex (Fe²⁺ -TPTZ) at pH 3.6 is formed [4]. The FRAP value is expressed as µmol of Fe (II) equivalent per 100 mL of juice. From (Figure 5) and (Table 1), water extract of pulp of *H. polyrhizus* at dilution factor 2 displays the highest antioxidant



capacity at 132.68 \pm 3.74 μ mol Fe2⁺/100 mL of juice, while at dilution factor 5 (the lowest concentration) had the lowest FRAP value of 86.13 \pm 21.5 μ mol Fe2⁺/100 mL of juice.

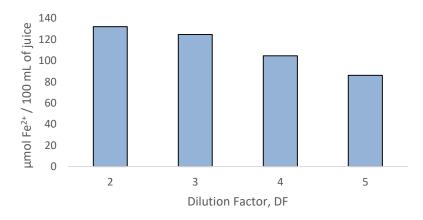


Fig. 5. Ferric reducing antioxidant potential (FRAP) values of different dilution factors of water extract of pulp of *H. polyrhizus* in terms of ferrous sulphate

3.5 Total Antioxidant Capacity as per Phosphomolybdate Assay

The phosphomolybdate assay, which involves an electron transfer mechanism and can be evaluated spectrophotometrically at 765 nm was used to determine the total antioxidant capacity (TAC) of water extract of pulp of *H. polyrhizus*. When there is antioxidant presence, molybdenum (VI) is reduced to molybdenum (V) forming a green phosphomolybdate(V) complex [4]. In this assay, ascorbic acid was used to develop a standard calibration curve (standard curve equation: y = 0.0075x - 0.0728, $R^2 = 0.9941$). From the results (Figure 6) and (Table 1), dilution factor 2 which was the most concentrated had the strongest antioxidant capacity (28.94 ± 0.83 mg AAE/100 mL of juice) while dilution factor 5 had the weakest capacity (7.81 ± 0.72 mg AAE/100 mL of juice).

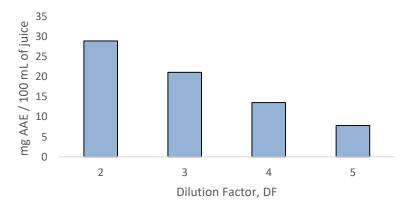


Fig. 6. Total antioxidant capacity of different dilution factors of water extract of pulp of *H. polyrhizus* following phosphomolybdate assay expressed as mg/100 mL of juice of ascorbic acid equivalents (AAE)



	Summar	Summary of antioxidant activities and capacities of water extract pulp of <i>H. polyrhizus</i>							
	Dilution	Total Phenolic	Total Flavonoid	DPPH Radical	ABTS Radical	FRAP (µmol	PA (mg		
	Factor	Content, (mg	Content, (mg	Scavenging	Scavenging	Fe2 ⁺ /100 mL	AAE/100 mL		
		GAE/100 mL	QE/100 mL of	Activity (%)	Capacity (%)	of juice)	of juice)		
		of juice)	juice)						
	2	32.90 ± 0.92 ^a	2.26 ± 0.14^{a}	73.38 ± 2.24 ^a	92.66 ± 0.22 ^a	132.17 ± 3.74 ^a	28.94 ± 0.83 ^a		
	3	25.27 ± 1.60 ^b	1.31 ± 0.36 ^b	70.35 ± 0.80 ^a	77.44 ± 0.41 ^b	124.71 ± 1.52 ^a	21.09 ± 0.99 ^b		
	4	9.97 ± 0.64 ^c	0.63 ± 0.05 ^c	64.59 ± 1.00 ^b	56.17 ± 2.30 ^c	104.67 ± 9.31 ^b	13.53 ± 1.06 ^c		
	5	3.11 ± 0.62 ^d	0.43 ± 0.18 ^c	61.61 ± 0.64 ^b	38.69 ± 0.98 ^d	86.13 ± 21.55 ^b	7.81 ± 0.72 ^d		
	R ²	0.994	0.934	0.938	0.997	0.698	0.989		
1			1		L D (0.01				

Table 1

Table 2

Note: Means with the same letter are not significantly different at P≤0.01 using LSD (Least Significance Different)

3.6 Correlation between Total Phenolic Content, Total Flavonoid Content and Antioxidant Assays

According to Fidrianny and Sahar [32], Pearson's correlation was positively high if $0.68 \le r \le 0.98$. Correlations between water extract of pulp of H. polyrhizus obtained from all assays (Table 2) show that TPC and TFC were positively high (0.693 $\leq r \leq 0.981$). The positive and high correlations between TPC and DPPH (r=0.963), TPC and ABTS (r=0.911), TPC and PA (r=0.981), TPC and FRAP (r=0.831) (Table 2), show that phenolic compounds contained in the water extract played a major role in contributing to the antioxidant capacity of H. polyrhizus [2,7,9,10,33] and very potent antioxidants [4,6]. According to [7], phenolic content of extracts from both pulp and peels of H. polyrhizus was found to be linearly correlated with antioxidant capacity due to the types of polyphenols contained in them. Hydroxyl groups (-OH) or other hydrogen-donating groups (=NH, -SH) in the molecular structure increase the antioxidant activity.

In comparison, the correlation between TFC and DPPH (r=0.890), TFC and ABTS (r=0.795), TFC and PA (r=0.942), TFC and FRAP (r=0.693) (Table 2), suggest that besides flavonoid, a type of phenolic compounds, betalains (pigment composed of red-violet betacyanin and yellow betaxanthins) were the major contributor for the antioxidant capacity of purple H. polyrhizus [33]. Several studies reported that high polyphenols content increased the ability to inhibit free radical deleterious effects [2, 4, 6, 7, 17, 32, 34]. A significant correlation between the content of phenolic compounds in plants and antioxidant activity displayed by the plants has been observed in other studies [6, 36]. According to [6] which studied water extracts of 30 different selected plants currently used in industries such as fragrance, cosmetic and food flavouring applications using DPPH, ABTS and FRAP noted that the correlation coefficients of Folin-Ciocalteu method and antioxidant potential were between 0.906 ≤ r \leq 0.939. This result establishes the fact that water can be a nutritionally relevant extraction solvent.

Trait ^a	DPPH	ABTS	TPC	TFC	PA	
ABTS	0.876					
ТРС	0.963	0.911				
TFC	0.890	0.795	0.915			
PA	0.959	0.907	0.981	0.942		
FRAP	0.794	0.885	0.831	0.693	0.821	

Pearson's correlation coefficients of antioxidants activities and capacities, total phenolic content and total

^a TPC= Total Phenolic Content, TFC = Total Flavonoid Content, DPPH = 2,2-Diphenyl-1-picrylhydrazyl assay, ABTS = 2-2'azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) assay, FRAP = Ferric Ion Antioxidant Power assay, PA = Phosphomolybdate assay



4. Conclusions

The present study reveals that water extract of pulp of *H. polyrhizus* possesses considerable antioxidant activities and capacities. There was a strong positive correlation between phenolics and antioxidant activities in the water extract of pulp. The fruit also exhibits significant source of antioxidants, making it a very valuable crop. Extraction of beneficial bioactive compounds in plant using water as a solvent has a promising future in the pharmaceutical and nutraceutical industries as water is natural, biodegradable and non-toxic.

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