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Original Article

COMPARATIVE ANTIOXIDANT PROPERTIES OF METHANOLIC EXTRACT OF RED AND WHITE DRAGON FRUITS

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ABSTRACT

Objective: In the present study we are interested to carry out comparative antioxidant activity of red and white dragon fruits.

Methods: The methanolic extract of both dragon fruits were tested for antioxidant activity by using DPPH method.

Results: The results showed that the methanolic extract of red dragon fruits showed better antioxidant activity than methanolic extract of white dragon fruits.

Conclusion: The red dragon fruit is a good candidate for further investigation.

Keywords: Hylocereus undatus, Hylocereous polyrhizus, Pitaya, Dragonfruit, Antioxidant

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INTRODUCTION

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. The cell damage is caused by the deleterious effect of process mediated by free radicals in cell membranes; by reducing the susceptibility of tissues due to oxidative stress. The higher the antioxidant defences against the free radicals activity, the lower the oxidation stress and will prevent from cell damage [1, 2].

Antioxidants are found in many foods, including fruits and vegetables and they are also available as dietary supplements. Examples of antioxidants are Beta-carotene, Lutein, Lycopene, Selenium, Vitamin A, Vitamin C and Vitamin E.

Vegetables and fruits are rich sources of antioxidants. There is good evidence that a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors. Dragon fruit is one of the fruits that contains antioxidant properties [3].

Dragon fruit, also known as pitahaya or strawberry pear is the fruit of several cactus species, especially of the genus Hylocereus. Native to Mexico and Central and South America, these vine-like epiphytic cacti are also found in Taiwan and are also cultivated in Southeast Asian countries such as Malaysia, Vietnam, and the southeast coast of China. They are also found in Taiwan. The flesh, which is eaten raw, is mildly sweet and low in calories. The flavour is sometimes likened to that of the kiwifruit. The fruit may be converted into juice or wine; the flowers can be eaten or steeped as tea. Sesame seedsized seeds are embedded throughout the flesh. Although the tiny pitahaya seeds are eaten with the flesh, the seeds are indigestible. The red flesh variety is believed to be rich in antioxidants and has an exceptionally high content of soluble fiber [4], It is considered a good source of vitamin C. The scientific name of these fruits comes in three types all with leathery, slightly leafy skin:

1) Hylocereus undatus, white flesh with pink skin

2) Hylocereus polyrhizus, red flesh with pink skin

3) Selenicereus megalanthus, white flesh with yellow skin

But there is no studies reported so far that shows comparative antioxidant properties of methanolic extract of the flesh of red and white dragon fruits. Therefore, we decided to carry out detail antioxidants investigation on both type of dragon fruits to show the differences.

MATERIALS AND METHODS

Sample collection and identification

The fruits of red and white dragon fruits were collected from the local market Ipoh, Perak, Malaysia and identified.

Methanol extraction

The collected fruits were washed thoroughly in distilled water to remove contaminants; the peels were removed, then it was cut into small pieces and subjected to extraction (100 g) by maceration in 250 ml of pure methanol (100%) at room temperature with occasional shaking for seven days. The macerates were filtered, and the filtrate was dried at low temperature (40-50 °C) under vacuum. The extracts were stored in air-tight containers at 4 °C until further use.

Qualitative phytochemical screening

The methanolic extracts of white and red dragon fruits were tested for the following qualitative chemical tests for the identification of various phytoconstituents [5, 6].

Tests for alkaloids

1. **Dragendorff's test:** To the extract, 1 ml of Dragendorff's reagent was added. An orange-red precipitate indicates the presence of alkaloid.

2. **Wagner's test:** To the extract, Wagner's reagent was added. Reddish brown precipitate indicates the presence of alkaloid.

3. **Mayer's test:** To the extract, 1 or 2 ml of Mayer's reagent was added. A dull white precipitate indicates the presence of alkaloid.

4. **Hager's test:** To the extract, 3 ml of Hager's reagent was added. Yellow precipitate indicates the presence of alkaloid.

Tests for carbohydrates

1. **Molisch test:** To the extract, 1 ml of α -naphthol solution was added and concentrated sulfuric acid was added along the sides of test tube. Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates.

2. **Fehling's test:** To the extract, equal quantities of Fehling's solution A and B was added. Upon heating gently, a brick red precipitate indicates the presence of carbohydrates.

3. **Benedict's test:** To 5 ml of Benedict's reagent, 8 drops of the solution under test was added, mixed and the mixture was boiled vigorously for two minutes and cooled. A red precipitate indicates the presence of carbohydrates.

Tests for proteins

1. **Biuret test:** To the extract, 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulfate solutions were added. A violet color indicates the presence of proteins.

2. **Xanthoproteic test:** To the extract, 1 ml of concentrated nitric acid was added, a white precipitate formed, it was boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

3. Lead acetate test: To the extract, 1 ml of lead acetate solution was added. A white precipitate indicates the presence of proteins.

Test for amino acids

Ninhydrin test: Two drops of freshly prepared 0.2% ninhydrin reagent was added to the extract and heated. Development of blue colour indicates the presence of proteins, peptides or amino acids.

Tests for steroids and sterols

1. **Libermann Burchard test:** The test extract was dissolved in 2 ml of chloroform in a dry test tube. Ten drops of acetic anhydride and 2 drops of concentrated sulfuric acid were added. The solution becomes red, then blue and finally bluish green in colour indicating the presence of steroids.

2. Salkowaski test: The extract was dissolved in chloroform and an equal volume of concentrated sulfuric acid was added. Bluish red to cherry red colour is observed in chloroform layer, whereas the acid layer assumes marked green fluorescence indicating the presence of steroids.

Tests for glycosides

1. **Legal test:** The extract was dissolved in pyridine and sodium nitroprusside solution added to it and made alkaline. Pink red or red colour indicates the presence of glycosides.

2. **Baljet test:** To the extract, sodium picrate solution was added. Yellow to orange colour indicates the presence of glycosides.

3. **Borntrager's test:** Few ml of dilute sulfuric acid was added to the test solution. Boiled, filtered and extracted the filtrate with ether or chloroform. The organic layer was separated and treated with ammonia. Pink, red or violet colour indicates the presence of glycosides.

4. **Keller Killiani test:** Sample was dissolved in acetic acid containing a trace of ferric chloride and transferred to the surface of concentrated sulfuric acid. At the junction, the reddish brown colour was formed, which gradually becomes blue indicating the presence of glycosides.

Test for flavonoids

Shinoda test: To the extract, magnesium turnings were added, followed by the addition of concentrated hydrochloric acid. A red colour indicates the presence of flavonoids.

Tests for tannins

1. To the extract, ferric chloride was added. Dark blue or greenish black colour indicates the presence of tannins.

2. To the extract, potassium dichromate solution was added. A precipitate indicates the presence of tannins.

Test for triterpenoids

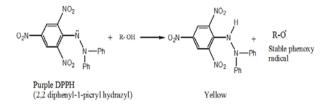
In the test tube, few tin granules were added and dissolved in 2 ml of thionyl chloride solution. Then, the test solution was added. Production of pink colour indicates the presence of triterpenoids.

Tests for fixed oils

1. **Spot test:** A small quantity of extract was pressed between two filter papers. Oil stains on paper indicate the presence of fixed oils.

2. **Saponification test:** To the extract, few drops of 0.5 N of alcoholic potassium hydroxide were added along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 h. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils.

In vitro antioxidant activity using DPPH method



The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor's changes to yellow in colour. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490 nm.

Reagents

2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 μ M): Accurately weighed 22 mg of DPPH and dissolved in 100 ml of methanol. From this stock solution, 18 ml was diluted to 100 ml with methanol to obtain 100 μ M DPPH solution.

Preparation of extract solutions

Accurately weighed 21 mg of each of the extracts and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 21 mg/ml concentration. These solutions were serially diluted separately to obtain the lower concentrations.

Preparation of standard solutions

Accurately weighed 10 mg each of ascorbic acid and rutin and dissolved in 0.95 ml of freshly distilled DMSO to get 10.5 mg/ml concentration. These solutions were serially diluted with DMSO to get the lower concentrations.

Procedure

To 200 μ l of DPPH solution, 10 μ l of each of the extract or standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 °C for 30 min and the absorbance of each solution was measured at 490 nm (Hwang *et al.*, 2001), using UV-VIS spectrophotometer. The concentration which is showed 50% inhibition (IC₅₀) will be calculated.



Fig. 1: Hylocereus undatus (white dragon fruit) and Hylocereus polyrhizus (red dragon fruit).

Table 1: Yields and nature of methanolic extract of red and white dragon fruits

Plant source	Nature of the extracts	Yield (%)
Red dragon fruits	Pinkish red semisolid	3.56
White dragon fruits	Yellowish white semisolid	3.92

S. No.	Chemical test	Methanolic extract of red dragon fruits	Methanolic extract of white dragon fruits
1	Alkaloids	-	-
2	Carbohydrates	+	+
3	Proteins	+	+
4	Amino Acids	+	+
5	Steroids and Sterols	+	+
6	Glycosides	+	+
7	Flavonoids	+	+
8	Tannins	-	-
9	Triterpenoids	-	-
10	Fixed oils	+	+

Table 3: DPPH radical scavenging activity of methanolic extracts of red and white dragon fruits

Concentration (µg/ml)	% of inhibition			
	Methanolic extract of red dragon fruits	Methanolic extract of white dragon fruits	Standard ascorbic acid	
1000	70.42	56.66		
500	46.29	26.86		
250	29.19	21.18		
125	10.04	15.94		
IC 50	>600	>800	58.24	

Values are mean n=3

RESULTS AND DISCUSSION

Based on the phytochemical screening carried out, both the extracts showed the presence of carbohydrates, protein, amino acids, steroids, glycosides flavonoids, fixed oils and absence of alkaloids, tannins and triterpenoids (table 1 and 2).

In the antioxidant activity, the methanolic extract of red dragon fruits at 1000, 500, 250 and 125 μ g/ml showed 70.42%, 46.29%, 29.19% and 10.04% of inhibition, respectively (table 3). The white dragon fruits at 1000, 500, 250 and 125 μ g/ml showed 56.66%, 26.86%, 21.18% and 15.94% inhibition, respectively.

The IC₅₀ value of red and white dragon fruits were>600 and<800 μ g/ml respectively. This showed the red dragon fruits showed better antioxidant activity than compare to the white dragon fruits. However, the standard ascorbic acid showed better antioxidant activity than both the extracts.

The results indicate that the methanol extract of red and white dragon fruit showed lower to moderate antioxidant activity. At the same time red dragon fruit showed better antioxidant activity than compare to the white dragon fruits. Therefore red dragon fruit is more effective than white dragon fruit. The results of this study showed that red dragon fruit contains more antioxidant properties and is more nutritional. This may be due to the presence of higher amount of phenolic compounds in the extract, which are responsible for the antioxidant properties. So, that's answer the question of why people preferred red dragon fruit more as it is sweeter. Therefore, red dragon fruit can be used to prevent anti-cancer more effectively as it contains more antioxidant properties that will overcome the effects of free radicals towards cells. In conclusion, these findings can form the basis for further studies to identify more detailed antioxidant properties that can be used as medicine to cure diseases.

CONFLICT OF INTERESTS

Declare none

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