PHYTOCHEMICAL SCREENING AND ANALYSIS POLYPHENOLIC ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF WHITE DRAGON FRUIT (Hylocereus undatus)

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ABSTRACT

White dragon fruit is a well known and widely used herbal medicine, especially in Asia, which contains several interesting bioactive constituents and possesses health promoting properties. The aim of this study was to analyze for the bioactive compounds, evaluate total phenolic contents and antioxidant capacities of methanolic extract of white dragon fruit. The antioxidant activity was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay. Total phenolic content were determined by Folin-Ciocalteu method. Phytochemical screening of the white dragon fruit showed the presence of triterpenoid, alkaloid, flavonoid and saponin. The extract exhibited strong antioxidant activity with IC50 of 193 µg/mL, and total phenolic content of 246 µg/mL in 1 Kg dry extract.

Key words: antioxidant activity, total phenolic, DPPH, white dragon fruit

INTRODUCTION

Flavonoids are polyphenolic compounds that are widely distributed in fruits and vegetables. They possess a large range of structure, according to chemical structure into flavones, isoflavones, flavonones, flavonols, anthocyanidin and chalcone. Flavonoids have been reported to exert wide range of biological activities, such as anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic antitumour (Sandhar et al., 2011). An important effect of flavonoids is their capacity to act as antioxidants (Nijveldt et al., 2001). Antioxidants are compounds which capable of preventing and even counteracting the damage caused in human tissue by the normal effect of physiological oxidation (Belsare et al., 2010). Antioxidants could protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress, i.e. increased reactive oxygen species (ROS) production, has been linked to asthma, cancer, cataracts, diabetes, gastrointestinal inflammatory diseases, liver disease, aging, atherosclerosis, ischemic injury, and neurodegenerative diseases (Parkinson’s and Alzheimer’s) (Patil et al. 2009). Flavonoids as antioxidants may help provide protection against these diseases. At present most of the antioxidants used for this are manufactured synthetically. Several synthetic antioxidants are commercially accessible but have been reported to be toxic, therefore, it is very significant to find and develop a new, safe and cheap antioxidants of natural origin. One of the high-potential natural antioxidants is white dragon fruit (Hylocereus undatus).

White dragon fruit, golden outside and white inside, a fantastic new edible fruit plant obtained from Thailand. The Dragon Fruit is a vine, terrestrial with fleshy stems. Flowers are elaborate and bloom only at night. Dragon fruit is rich in vitamins and helps the digestive process due to its fiber, prevents colon cancer and diabetes, neutralize toxic substances such as heavy metal, and helps to reduce cholesterol production.
levels and high blood pressure. The red-fleshed varieties contain lycopene, which is a natural antioxidant known to fight cancer, heart disease, and lower blood pressure. Red-fleshed pitaya fruit is a potential fruit for betacarotins extraction (Pche et al., 2009). Regularly consuming the dragon fruit can help against asthma and cough. Dragon fruit is rich in fiber, Vitamin C and minerals. Dragon fruit is also rich in phytochemicals which are highly valued for their antioxidant properties. Antioxidants prevent the formation of cancer-causing free radicals. In Taiwan, diabetics use the fruit as a food substitute for rice and as a source of dietary fiber. Wu et al., (2006) investigated that the flesh and peel of red dragon fruits were both rich of polyphenols and were good sources of antioxidants.

White dragon fruit is a type of cactus plants that still do not have complete reference information, both in terms of phytochemical and pharmacology in order to be optimally used as a form of alternative medicine. Utilization of these plants as traditional medicine is based on empirical evidence so there is a need to find a scientific basis about utilities and types of bioactive compounds in dragon fruit with the use of research approaches to chemistry and modern biology.

The objectives of the present study are phytochemical screening and polyphenolic antioxidant activity of methanolic extract of white dragon fruit. The present study aimed to promote the contribution of white dragon fruit in public health campaigns to encourage the daily consumption of white dragon fruit, through phytochemical screening, evaluation of the total phenolic contents, and the antioxidant capacities.

**METHODOLOGY**

**Materials**

All chemicals and solvents, such as acetone, butanol, chloroform, ethyl acetate, ethanol, methanol, n-hexane, hydrochloric acid, sulphuric acid, phenolic, anhydrous acetic acid, silica gel GF254 plates, and magnesium, were purchase from E-Merck, 2,2'-Diphenyl-1-pycrylhydrazyl (DPPH), Folin-Ciocalteu reagent, and gallic acid, were purchased from Sigma. All other reagents, such as Lieberman-Burchard reagent, Dragendorff reagent, sodium carbonate, acetic acid, and ascorbic acid, from local sources were of analytical grade.

**PROCEDURE**

**Extraction and Isolation of Compounds**

The fruit pulp of white dragon fruit were cleaned, dried, coarsely and extracted with ethanol using maceration technique for 24 h at room temperature. The extracts were filtered by filter paper Whatman No. 42 (125mm). The extract was evaporated and concentrated under reduced pressure using rotary evaporator with the water bath set at 50°C. The crude extracts were further used for the next investigation.

**Phytochemical Screening**

The crude extract were analyzed for the presence of alkaloids, flavonoids, saponins, steroids, tannins and terpenoids using standard procedures of analysis (Egwaldshide et al., 2007). Test for flavonoids: 1-2 mL methanic acid was added to a portion of filtrate of the extract. Magnesium metal and concentrated hydrochloric acid (4-5 drops) was added. A red or orange colouration indicates the presence of flavonoids.

Test for terpenoids and Sterol; To each 0.5 g of the extract was added 0.5 mL of chloroform. 0.5 mL Anhydrous acetic acid was added. Then, concentrated sulphuric acid (1-2 mL) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids. A blue-green of the interface indicates the presence of sterol. Test for alkaloids; 0.5 g of extract was diluted to 10 mL with acid alcohol, boiled and filtered. To 5 mL of the filtrate was added 2 mL of dilute ammonia. 5 mL of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer’s reagent was added to one portion and Dragendorff’s reagent to the other. The formation of a cream (with Mayer’s reagent) or reddish brown precipitate (with Dragendorff’s reagent) was regarded as positive for the presence of alkaloids. Test for tannins; About 0.5 g of the extract was boiled in 1-2 mL of water in a test tube and then filtered.
A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration. Test for saponins; To 0.5 g of extract was added 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth.

Determination of antioxidant activity

Antioxidant activity was determined using DPPH radical-scavenging assay. The DPPH free radical scavenging capability of dragon fruit extract was determined according to the method described with slight modifications (Ghafar et al., 2010). An aliquot of 0.5 mL of extract methanol of dragon fruit at different concentrations (10, 30, 50 and 70 ppm) was mixed with 500 μL of 1 mM DPPH (dissolved in ethanol until 5 mL). The mixture was vigorously shaken and left to stand at room temperature for 30 min in a dark room. Absorbance was read at 515 nm using UV-vis spectrophotometer. Ascorbic acid was used as standard. Inhibition of DPPH radical scavenging activity in percent (I%) was calculated according to the equation of I% = [(Ablank - Asample)/ Ablank] × 100 where Asample is the absorbance of the sample, and Ablank is the absorbance of blank solution (containing all reagents except the test sample). IC₅₀ value was determined from the plotted graph of scavenging activity against the concentrations of the dragon fruit samples, which is defined as total antioxidant necessary to decrease the initial DPPH radical by 50%. Triplicate measurements were carried out and IC₅₀ was calculated based on the percentage of DPPH radicals scavenged.

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method as described by Meda et al., (2008). 0.5 mL of extract was added to deionized water (7.5 mL) and Folin-Ciocalteu phenolic reagents (0.5 mL). After 5 minutes, 20% sodium carbonate (1.5 mL) was added to the mixture. After being kept in 40°C for 20 minutes, the absorbance was measured at 760 nm using a spectrophotometer against a water blank. A standard calibration curve was plotted using gallic acid (0, 40, 80, 120, 160, dan 200 mg/L). Amounts of TPC were calculated for sample using gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) g/g of dry plant matter. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Extraction and Phytochemical Screening

Extraction from 1 Kg dragon fruit dried in 2 L methanolic for 24 h, yielded 1 L filtrate and obtained 320 grams crude extract after evaporated. TLC scanner analysis with mobile phase butanol: acetone: water (4:1:5) showed the existence of four spots of chromatograms (Figure 1) with Rf 0.48, 0.62, 0.72, and 0.73. Results of the examination with spectrophotometer is shown that number 1 spot positive to contain flavonoids. This analysis was also supported by the phytochemical screening. The screening showed presence of tannin, alkaloid, flavonoid, saponin, and showed the absence of sterol and tanin.

Determination of Antioxidant Activity

Measurement of antioxidant activity of dragon fruit is very important because knowing the quality and how much antioxidant activity contained in the dragon fruit can be used as a standard when the fruit will be used as herbal medicine for health. DPPH method was chosen because this method is rapid, simple and inexpensive to measure antioxidant capacity that involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds that acts as free radical scavengers or hydrogen donors, and evaluates antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. The red dragon fruit peel fulfilled its promise to inhibit the growth of melanoma cells. Rebecca et al., (2010) determined antioxidant activity for red dragon fruit (Hylocereus polyrhizhus) using DPPH method, showed that the effective concentration (EC₅₀) 2.90 mM vitamin C equivalents/g dried extract.
In this study IC₅₀ for methanolic extract of white dragon fruit (Hylocereus undatus) was found to be 193 µg/mL. This means that the methanolic extract of white dragon fruit at a concentration of 193 µg/mL have the ability to inhibit free radical DPPH by 50%. Based on these results it can be said that the methanolic extract of white dragon fruit is potential to inhibit free radical DPPH, because at concentrations less than 200 µg/mL was able to inhibit 50% of DPPH free radicals. The ability of flavonoids in arresting free radicals due to the hydroxy group on the molecule where the presence of free hydroxy groups in flavonoids. IC₅₀ values of methanolic extract of the white dragon fruit is much greater than the IC₅₀ value of ascorbic acid that is equal to 4.5 g/mL.

**Determination of Total Phenolic Content**

Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds. This activity is believed to be mainly due to their redox properties, which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Ghasemzadeh, 2010). The Polin-Gioceilta method was used because it is a generally preferred analytical method for determination of total polyphenolic using gallic acid as a standard. Rebecca et al., (2010) described that based on research of Lako et al., (2007, 2008), common fruits with significant content of total polyphenolic include: Musa sp. (Banana) with 110 µg/g total polyphenolic, Ananas comosus (Pineapple) with 150 µg/g; Carica papaya (Papaya) with 260 µg/g; tomatoes with 350 µg/g; cherries with 670 µg/g and blueberries with 3180 µg/g. In this study, result shows a total phenolic content of 246 mg/L in 1 Kg of dry white dragon fruit extract.

**CONCLUSION**

The present study indicated that white dragon fruit is rich in flavonoids and exhibit strong antioxidant activity in the DPPH methods. The antioxidant activities well correlated to flavonoid content content of flavonoid compounds. It can be concluded that, white dragon fruit, which are consumed as a vegetable, can be used as an accessible source of natural antioxidants with consequent health benefits.

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