

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

Elfi Susanti VH^{1*}, Suryadi Budi Utomo¹, Yandi Syukri², Tri Redjeki¹

¹Department of Chemistry,
PMIPA FKIP, Universitas
Sebelas Maret. Jl. Ir Sutami
36 A Surakarta
Indonesia, 57126

²Departement of Pharmacy,
FMIPA, Universitas Islam
Indonesia Jl. Kaliurang KM
14,5 Sleman Yogyakarta
55584

Submitted: 06-01-2012

Revised: 12-01-2012

Accepted: 28-01-2012

*Corresponding author
Elfi Susanti VH
+62-8121523622
E-mail:
bunda3f@gmail.com

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

Flavonoid are polyphenolic compound 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, anti-bacterial, 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, peroxy radicals, hydroxyl radicals and peroxy nitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress, *ie.* 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 anti-oxidants 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 vining, 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 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 betacyanins extraction (Phebe *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 phytoalbumins 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, methanolic, n-hexane, hydrochloric acid, sulphuric acid, phenolic, anhydrous acetic acid, silica gel GF254 plates, and magnesium, were purchased from E-Merck. 2,2'-Diphenyl-1-picrylhydrazyl (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 (Egwaikhide *et al.*, 2007). Test for flavonoids; 1-2 mL methanolic 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 metanol of dragon fruit at different concentrations (10, 30, 50 and 70 ppm) was mixed with 500 μ L 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 $I\% = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$ where A_{sample} is the absorbance of the sample, and A_{blank} is the absorbance of blank solution (containing all reagents except the test sample). IC_{50} 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 IC_{50} 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: acetic acid: 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 showed that number 1 spot positive to contain flavonoids. This analysis was also supported by the phytochemical screening. The screening showed presence of triterpenoid, alkaloid, flavonoid, saponin, and showed the absence of sterol and tannin.

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 polyrhizus*) using DPPH method, showed that the effective concentration (EC_{50}) 2,90 mM vitamin C equivalents/g dried extract.

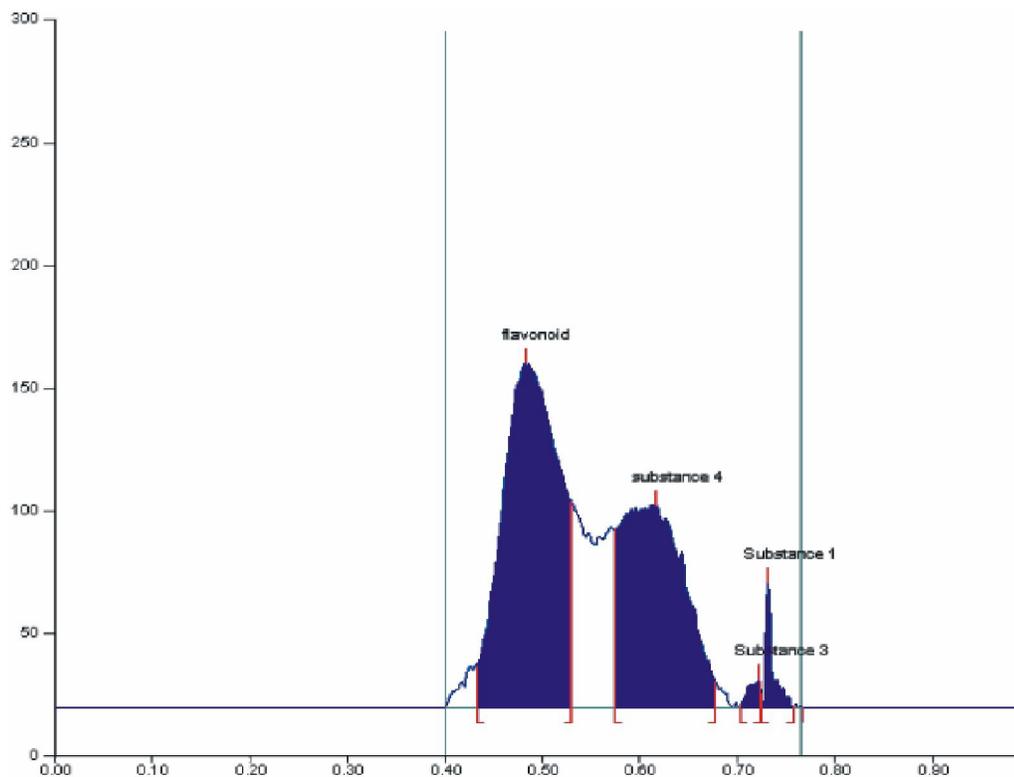


Figure 1. Chromatogram pattern of dragon fruit extract.

In this study IC_{50} for methanolic extract of white dragon fruit (*Hylocereus undatus*) 193 $\mu\text{g}/\text{mL}$. This means that the methanolic extract of white dragon fruit at a concentration of 193 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$ was able to inhibit 50% 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. IC_{50} values of methanolic extract of the white dragon fruit is much greater than the IC_{50} value of ascorbic acid that is equal to 4,3 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 A, 2010). The Folin-Ciocalteu 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 $\mu\text{g}/\text{g}$ total polyphenolic; *Ananas comosus* (Pineapple) with 150 $\mu\text{g}/\text{g}$; *Carica papaya* (Papaya) with 260 $\mu\text{g}/\text{g}$; tomatoes with 350 $\mu\text{g}/\text{g}$; cherries with 670 $\mu\text{g}/\text{g}$ and blueberries with 3180 $\mu\text{g}/\text{g}$. 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 flavanoid 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.

ACKNOWLEDGEMENTS

The authors are thankful to DP2M Dikti and head of LPPM UNS, Dean of FKIP UNS, Head of Chemistry Study Program and Chemistry Laboratory PMIPA FKIP UNS.

REFERENCES

- Belsare, D.P., Pal, S.C., Kazi, A.A., Kankate, R.S., and Vanjari, S.S., 2010, Evaluation of Antioxidant Activity of Chalcones and Flavonoids. *Int. J. ChemTech. Res.*, 2(2), 1080-1089.
- Egwaikhide, P.A., Okeniyi, S.O., and Gimba, C.E., 2007, Screening for Anti-Microbial Activity and Phytochemical Constituents of some Nigerian Medicinal Plants, *Advan. in Biol. Res.* 1(5-6), 155-158
- Ghafar, M.F.A., Prasad, K.N., Weng, K.K., and Ismail, A., 2010, Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species, *African J. Biotech.*, 9(3), 326-330
- Gill, N.S., Bajwa, J., Sharma, P., Dhiman, K., Sood, S., and Sharma, P.D. 2011, Evaluation of Antioxidant and Antiulcer Activity of Traditionally Ocnsumed Cucumis melo Seeds, *J. Pharmacol. Toxicol.*, 6(1), 82-89
- Meda, A.L., Lamien, C.E., Compaoré, M.M.Y., Meda, R.N.T., Kiendrebeogo, M., and Zeba, B., 2008, Polyphenolic Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso, *Molecules*, 13, 581-594
- Nijveldt, R.J., Nood, E., Hoorn, D.E.C., Boelens, P.G., Norren, K., and Leeuwen, P.A.M., 2001, Flavonoids: a Review of Probable Mechanisms of action and Potential Applications. *Am. J. Clin. Nutr.*, 74, 418-425
- Patil, C.B., Mahajan, S.K., and Katti, S.A., 2009, Chalcone: A Versatile Molecule, *J. Pharm. Sci & Res.*, 1(3), 11-22
- Phebe, D., Chew, M.K., Suraini, A.A., Lai, O.M., and Janna, O.A., 2009, Red-fleshed pitaya (*Hylocereus polyrhizus*) fruit colour and betacyanin content depend on maturity, *Inter. Food Res. J.*, 16, 233-242
- Rebecca, O.P.S., Boyce, A.N., and Chandran, S., 2010, Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*), *African J. Biotech.*, 9(10),1450-1454
- Sandhar, H.K., Kumar, B., Prasher, S., Tiwari, P., Salhan, M., and Sharma, P., 2011, A Review of Phytochemistry and Pharmacology of Flavonoids, *Int Pharm Sci.*, 1(1), 25-41
- Wu, L., Hsu, H., Chen, Y., Chiu, C., Lin, Y., and Ho, J.A., 2006, Antioxidant and antiproliferative Activities of Red Pitaya. *Food Chem.*, 95, 319-327