INTRODUCTION

Phenolic compounds are produced by the plant as a defense mechanism against different stress condition. Phenolic compounds are commonly used as a subject in many researchers. Phenolic compounds included flavonoid compounds have various effects such as antioxidant activity, antibacterial activity, antidiabetic, and hepatoprotector [1-4]. The excessive of free radical in oxidative stress condition can be inhibited by antioxidant, which related to many degenerative diseases. Fruits and vegetables are a natural antioxidant, because they contain phenolic and flavonoid compounds which have the antioxidant capacity [5].

Some methods have been used to observe antioxidant activity in many plants extracts such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) methods [6]. The previous researchers presented that DPPH, ABTS, and FRAP methods can be performed to observe the antioxidant activity of fruits, vegetables, and food [5-8].

Previous studies demonstrated that red dragon fruit contained major flavonoid compounds such as quercetin and catechin [9] and betalain compounds such as lutein and zeaxanthin [10]. Phenolic compounds such as quercetin and catechin [9] and betalain compounds such as lutein and zeaxanthin [10] can act as high antioxidant. Previous studies [11,12] expressed that dragon fruit (Hylocereus sp.) had antioxidant capacity. Super red dragon fruit (Hylocereus costaricensis) is one species of dragon fruit. Peel and stem of super red dragon fruit (STE) are the waste products of super dragon fruit. There was no research regarding the antioxidant activity of different parts of super red dragon fruit (H. costaricensis) which were stem, peel, and flesh extracted using increasing polarity solvents (n-hexane, ethyl acetate, and ethanol) and tested by DPPH and ABTS assays.

The goals of this research were to observe antioxidant potential in various polarity extracts (n-hexane, ethyl acetate, and ethanol) from different parts of super red dragon fruit grown in West Java-Indonesia using DPPH and ABTS assays, and correlations of total phenolic and flavonoid content with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH, ABTS diammonium salt, gallic acid, and quercetin were purchased from Sigma-Aldrich (MO, USA), different parts of super red dragon fruit (H. costaricensis) which were stem named as STE, peel as PEE, and flesh as FLE were collected from Bogor, West Java-Indonesia, were thoroughly washed with tap water, sorted while wet, cut, dried, and grinded into powder.

Preparation of sample

Different parts of super red dragon fruit (H. costaricensis) were categorized as a very strong antioxidant by DPPH and ABTS assays, total phenolic content (TPC) using Folin–Ciocalteu reagent, flavonoid content by Chang’s method.

Results:

Inhibitory concentration 50% (IC50) of DPPH scavenging activity of all of the extracts in the range of 2.69 μg/ml was ~94.17 μg/mL. The ethyl acetate peel extract of super red dragon fruit expressed the highest TPC (4.56 g GAE/100 g) and the highest total flavonoid content (12.63 g QE/100 g). TPC in flesh extract of super red dragon fruit had a negative and significant correlation with their IC50 of ABTS. The IC50 of DPPH and IC50 of ABTS of flesh extract of super red dragon fruit showed positive and significant correlation.

Conclusion:

All different parts extracts of super red dragon fruit (except n-hexane flesh extract) were categorized as a very strong antioxidant by DPPH method. Phenolic compounds in flesh extract of super red dragon fruit were the major contributor in antioxidant activities by ABTS method. DPPH and ABTS showed linear results in antioxidant activities of super red dragon fruit flesh extract.

Keywords: Antioxidant, Super red, Dragon fruit, Hylocereus costaricensis.
Antioxidant activity by DPPH assay

Antioxidant activity by DPPH assay was conducted using a modification of Blois's method [17]. 2 ml of various concentration of each extract were added into 2 ml DPPH solution 50 μg/ml. After 30 min incubation, absorbance was determined at wavelength 515 nm by UV-Vis spectrophotometer Beckman Coulter DU 720. Methanol was used as a blank, DPPH solution 50 μg/ml as control and ascorbic acid as standard. Analysis was conducted in triplicate for standard and each extract. Antioxidant activity was observed by calculating the percentage of reduction of DPPH absorbance. Inhibitory concentration 50% (IC\textsubscript{50}) of DPPH scavenging activity of each extract can be determined using its calibration curve.

Antioxidant activity by ABTS assay

ABTS solution was prepared using a modification of Li et al. [14]. Each solution of ABTS diammonium salt 7.6 mm and potassium persulfate 2.5 mm were prepared in Aq. dest and left in dark room for 12 h. The two solutions were mixed with 30 min incubation, left in the refrigerator for 24 h, and then diluted in ethanol. 2 ml of various concentration of each extract was added into 2 ml ABTS solution 50 μg/ml. The absorbance was read at wavelength 734 nm using UV-Vis spectrophotometer Beckman Coulter DU 720. Ethanol (95%) was used as a blank, ascorbic acid as standard, and ABTS solution 50 μg/ml as a control. Analysis was performed in triplicate for standard and each extract. Antioxidant capacity of each extract by ABTS method was evaluated by observing percentage of antioxidant activity using reduction of ABTS absorbance. IC\textsubscript{50} of ABTS scavenging activity of each extract can be determined using its calibration curve.

Total phenolic content (TPC)

Determination of TPC used Folon–Ciocalteu reagent [15]. The absorbance was determined at wavelength 575 nm. Analysis was performed in triplicate for each extract. Standard solution of gallic acid (50-160 μg/ml) was used to evaluate a calibration curve. TPC was expressed as gallic acid equivalent per 100 g extract (g GAE/100 g).

Total flavonoid content (TFC)

Chang's method [16] with minor modification was used to evaluate TFC. The absorbance was observed at wavelength 415 nm. Analysis was conducted in triplicate for each extract. Quercetin solution 50-125 μg/ml was used to obtain a calibration curve. TFC was demonstrated as quercetin equivalent per 100 g extract (g QE/100 g).

Statistical analysis

Each sample analysis was performed in triplicate. All of the presented results are means ± standard deviation of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at p<0.05 and post hoc Tukey procedure was performed with SPSS 16 for Windows. Correlation between the total flavonoid and phenolic content and antioxidant activities and the correlation between two antioxidant testing methods were performed using the Pearson’s method.

RESULTS AND DISCUSSION

The activities and phytochemical content among the extracts can be compared if the density among the extracts were similar. One extract with high density may give the higher activity and the higher phytochemical content than low-density extract. Therefore, all extracts (nine extracts) which were used in the present study should prepare in similar density. The density of extract did not expose in 100% concentrated extract, due to its difficulty to determine the density of concentrated extract using pycnometer, hence the density of the extracts was presented as density 1% extract (Table 1).

In vitro, the antioxidant activity can be categorized base on type of reaction, which are single electron transfer (SET) based assay and hydrogen atom transfer (HAT) based assay [17]. SET is based on the ability of an antioxidant to transfer one electron to reduce oxidant, meanwhile HAT based on the ability of an antioxidant to quench radical by hydrogen donation [18]. The degree of color change (either increase or decrease of absorbance of the probe at a given wavelength) is depended to the concentration of antioxidant in the sample [17]. SET and HAT mechanisms almost always occur together: Ionization potential (ΔIP), bond dissociation energy (BDE), redox potential, pH, and solvent will influence the predominant mechanism will be appeared [17,18]. Compound with ΔIP > -45 kcal/mol is predominantly by SET mechanism, while compound with ΔBDE of -10 kcal/mol and ΔIP < -36 kcal/mol by HAT mechanism [18].

DPPH is free radical and show absorption at wavelength 516 nm. Antioxidant will transfer the hydrogen to DPPH to scavenge the free radical and DPPH will stable. DPPH in methanol give the purple color and color of DPPH solution will be changed to yellow when free radicals are scavenged by antioxidant [14]. The ability of an antioxidant to scavenge the free radical DPPH correlates with decreasing in absorbance of DPPH. IC\textsubscript{50} of DPPH scavenging activity is a concentration of sample or standard that can inhibit 50% of DPPH radical activity. The highest antioxidant activity will be shown by the lowest IC\textsubscript{50}. IC\textsubscript{50} was used to observe the antioxidant activity of the sample and compared to standard.

2.2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) is not soluble in water and a polar solvent. Hence for determining antioxidant activity by ABTS assay should use ABTS diammonium salt. ABTS method is also called as Trolox equivalent antioxidant capacity (TEAC) method. TEAC is Trolox equivalent antioxidant capacity, which antioxidant activity exposed as Trolox equivalent. Sample which had higher antioxidant will give higher Trolox equivalent value. ABTS assay has been modified, and antioxidant activities did not revealed as Trolox equivalent, but presented as IC\textsubscript{50} of ABTS scavenging activity and compared to ascorbic acid standard.

The free radical of ABTS will be formed after ABTS reacting with potassium persulfate, and it will give blue color and the maximum absorbance at 734 nm. Antioxidant will scavenge the free radical, and the ability of antioxidant can be exhibited by decreasing in absorbance of the free radical ABTS.

IC\textsubscript{50} of DPPH and IC\textsubscript{50} of ABTS scavenging activities in different parts extracts of super red dragon fruit were demonstrated in Figs. 1 and 2. The lowest value of IC\textsubscript{50} means the highest antioxidant activity. Sample which had an IC\textsubscript{50} < 50 μg/ml was a very strong antioxidant, 50-100 μg/ml strong antioxidant, and 101-150 μg/ml medium antioxidant, while a weak antioxidant with IC\textsubscript{50} > 150 μg/ml [13].

Antioxidant activity by DPPH assay

Antioxidant activity in different parts extracts of super red dragon fruit by DPPH and ABTS assays were carried out by determining IC\textsubscript{50} of DPPH and IC\textsubscript{50} of ABTS scavenging activities. IC\textsubscript{50} of DPPH and IC\textsubscript{50} of ABTS scavenging activities of each extract were compared to IC\textsubscript{50} DPPH and IC\textsubscript{50} ABTS of ascorbic acid standard.

Nurliyana [12] studied regarding antioxidant activities of peels and pulps of white dragon fruit (Hylocereus undatus) and red dragon fruit (Hylocereus polyrhizus). The result showed IC\textsubscript{50} of DPPH scavenging activity of 70% ethanol peels extract of red dragon fruit (300 μg/ml) was lower than...
Fig. 1: Inhibitory concentration 50% of 2,2-diphenyl-1-picrylhydrazyl in parts extracts of super red dragon fruit (n=3)

![Image](image1)

Fig. 2: Inhibitory concentration 50% of 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) in parts extracts of super red dragon fruit (n=3)

![Image](image2)

Antioxidant activities can be stated by percentage of DPPH scavenging activity and compared to the percentage of DPPH scavenging activity of ascorbic acid. The percentage of DPPH scavenging activity can present the real antioxidant activity because the higher concentration of sample does not always give the higher percentage of DPPH scavenging activity. The linear result will be seen in some concentration only. This condition can be occurred in extract or sample which contained more than one compound. The extract consisted of many compounds, and not all of compounds have antioxidant activities. Some of them may act as an antagonist of antioxidant activities. The compounds will act as an antagonist of antioxidant activities if their minimum effective concentration has been reached. Therefore, this reason can explain why the extract with lower concentration can give higher activity than higher concentration.

**Antioxidant activity by ABTS assay**

The previous study [11] represented that 50% ethanolic pulp extract of white dragon fruit (H. undatus) had the highest antioxidant activity by ferrous ion chelating activity, followed by fruit (peel and pulp) extract of red dragon fruit (H. polyrhizus), fruit (peel and pulp) extract of white dragon fruit (H. undatus), and pulp extract of red dragon fruit (H. polyrhizus). It was different with the present study which exposed that ethanolic peel extract of super red dragon fruit (H. costaricensis) gave the highest antioxidant activity by ABTS method with IC_{50} of ABTS scavenging activity (1.55 μg/ml) compared to the other parts extracts, meanwhile IC_{50} of ascorbic acid was 0.52 μg/ml. Hence, it can be concluded that antioxidant activity of ethanolic peel extract of super red dragon fruit was around one-third of antioxidant activity of ascorbic acid, by ABTS method.

**TPC**

The TPC among the various extracts were demonstrated in term of gallic acid equivalent using the linear regression equation y=0.004x+0.055, R^2=0.997. The TPC in various extracts from different parts of super red dragon fruit gave different result in the range of 0.82-4.56 g GAE/100 g. PEE2 extract (ethyl acetate peels extract of super red dragon fruit) showed the highest TPC (4.56 g GAE/100 g) (Fig. 3).

The TPC might contribute to antioxidant activity [15,24]. Cinnamic acid had higher antioxidant capacity than phenylacetic acid and benzoic acid [25]. Flavonoids, tannins, and phenolic acids are included in phenolic groups. Ortho and para hydroxyl substitution have the strongest antioxidant capacity [26].

Research by Choo and Yong [11] stated that 50% ethanolic pulp extract of red dragon fruit (H. polyrhizus) had the highest TPC 24.22 mg GAE/100 g compared to fruit (peel and pulp) extract of red dragon fruit (15.93 mg GAE/100 g), fruit (peel and pulp) extract of white dragon fruit (H. undatus) (20.14 mg GAE/100 g), and pulp of white dragon fruit (28.65 mg GAE/100 g). Previous research [12] stated that 70% ethanolic peels extract of white dragon fruit (H. undatus) showed the highest TPC (36.12 mg GAE/100 g) compared to peels extract of red dragon fruit (H. polyrhizus) extract (28.16 mg GAE/100 g), pulp extracts of red dragon fruit (19.72 mg GAE/100 g), and pulp extracts of white dragon fruit (3.75 mg GAE/100 g). It was similar to the present study which presented that ethanolic peels extract of super red dragon fruit had the highest TPC (4.56 g GAE/100 g) compared to its stem extract (4.01 g GAE/100 g) and flesh extract (1.05 g GAE/100 g). Previous study revealed that ethanolic fruit extract of white dragon fruit (H. undatus) gave the highest TPC (179.35 mg GAE/l) compared to its distilled water extract and methanolic extract [21]. Research by De Mello et al. [10] presented that 80% acetone peels extract of white dragon fruit (moisture of 90.23 g/100 g) had TPC 40.68 mg GAE/100 g. The frozen filtrate of red dragon fruit (H. polyrhizus) pulp juice after precipitating with 96% ethanol, gave TPC 17.22 mg GAE/g [19] and seed extract of red dragon fruit (H. polyrhizus) had TPC 13.56 mg/g dry weight sample [9].

The previous study stated that betalin content in 80% acetone extract of white dragon fruit (H. undatus) peels extract was 101.04 mg betanin equivalent/100 g [10].

Antioxidant activities can be stated by percentage of DPPH scavenging activity and compared to the percentage of DPPH scavenging activity of ascorbic acid as standard. The percentage of DPPH scavenging activity of ascorbic acid did not achieve 100% because there was still residual yellow color in solution after antioxidant giving hydrogen atom to DPPH [22,23]. The percentage of DPPH scavenging activity cannot present the real antioxidant activity because the higher concentration of sample does not always give the higher percentage of DPPH scavenging activity. The linear result will be seen in some concentration only. This condition can be occurred in extract or sample which contained more than one compound.
TPC in ethyl acetate peel extract of super red dragon fruit (PEE2) 4.56 g GAE/100 g was higher than ethanolic peel extract of super red dragon fruit (PFE3) 1.87 g GAE/100 g, however IC_{50} DPPH of PEE2 (17.12 μg/ml) was similar to IC_{50} DPPH of PEE3 (16.45 μg/ml). The similar result in ABTS assay, PEE3 which had lower TPC than PEE2, gave the lower IC_{50} of ABTS (1.55 μg/ml) than PEE2 (8.01 μg/ml). It can be supposed that many phenolic compounds in PEE2 had low antioxidant activities and many phenolic compounds in PEE3 with high antioxidant activities.

**TFC**

The TFC among three parts extracts of super red dragon fruit were exposed in term of quercetin equivalent using the linear regression equation y=-0.006x+0.098, R^{2}=0.996. TPC in parts super red dragon fruit extracts were varied from 0.70 to 12.63 g QE/100 g. The highest TFC (12.63 g QE/100 g) was presented by ethyl acetate peels extract of super red dragon fruit (PEE2) (Fig. 4).

The frozen filtrate of pulp juice of red dragon fruit and after precipitating with 96% ethanol showed TFC 0.23 g catechin/100 g [19]. It was similar to the present study which expressed that TFC in ethanolic flesh extract of super red dragon fruit (0.82 g GAE/100 g) and lower than its stem (2.66 g GAE/100 g). Research by Adnan et al. [9] exhibited that there were two major compounds of flavonoid group were catechin 3.60 mg/g and quercetin 1.31 mg/g dry weight seed extract of red dragon fruit (H. polyrhizus). Flavonoid may have antioxidant effect as hydrogen donating compound, metal chelating ion, single oxygen transfer, and singlet oxygen quencher [27]. Hydrogen donating and metal chelating is related to ortho di-OH structure in ring B, C-2-C-3 double bond and o xo group at C-4 [27]. Flavonoid had higher antioxidant capacity than phenolic acid [25]. Flavonoid would give higher antioxidant capacity if flavonoid had ortho di-OH in C 3',4', -OH in C3, oxo function in C4, double bond at C2-C3, and di-OH in C3 an o xo function in C4 which also contribute to the high antioxidant activity. The flavonoid compound might be quercetin which has ortho di-OH in ring B and stated as the major flavonoid in red dragon fruit by Adnan et al. (2011).

**Pearson’s correlation coefficient**

Coefficient of Pearson correlation was significantly negative if -0.61 ≤ r ≤ -0.97 and significantly positive if 0.61 ≤ r ≤ 0.97 [6]. The lowest IC_{50} of DPPH and IC_{50} of ABTS scavenging activities will give the highest antioxidant activity. Increasing in TFC and TPC can influence increasing in antioxidant activities, which was exposed by lower IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. Therefore, the good correlation between TPC and TFC with IC_{50} of DPPH or IC_{50} of ABTS was significantly negative correlation [28].

TFC in flesh extract of super red dragon fruit had a significant and negative correlation with their IC_{50} of ABTS scavenging activities.
H. costaricensis

8. Arya N, Prakash OM, Verma AK, Vivekanand, Pant AK. Variation in antioxidant potential of Curcuma longa L. collected from different ecological niches of western Himalayan region. Int J Pharm Pharm Sci 2015;7(7):85-90.

9. Adnan L, Osman, A, Hamid AA. Antioxidant activity of different extracts of red pitaya (Hylocereus polyrhizus) seed. Int J Food Prop 2011;14(6):1171-81.

10. De Mello FR, Bernardo C, Dias CO, Gonzaga L, Amante ER, Fett R, et al. Antioxidant properties, quantification and stability of betalains from pitaya (Hylocereus undatus) peel. Ciência Rural 2015;45(2):323-8.

11. Choo WS, Yong WK. Antioxidant properties of two species of Hylocereus fruits. Adv Appl Sci Res 2011;2(3):418-25.

12. Nurliyana R, Zahir IS, Suleiman KM, Aisyah MR, Rahim KK. Antioxidant study of pulps and peels of dragon fruits: A comparative study. Int Food Res J 2010;17:367-75.

13. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958;181:1199-2000.

14. Li XC, Wang ZX, Chen DF, Chen SZ Antioxidant activity and mechanism of protocatechuic acid in vitro. J Funct Foods Health Dis 2011;1(7):232-44.

15. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. Afr J Biotechnol 2006;5(11):1142-5.

16. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-92.

17. Apak R, Gorinstein S, Böhm VK, Schaich MK. Methods of measurement and evaluation of natural antioxidant capacity/activity: UAPAC technical report. Pure Appl Chem 2013:85:957-98.

18. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005;53(10):4290-302.

19. Fidrianny I, Johan Y, Sukrasno. Antioxidant activities of different parts of red dragon fruit (Hylocereus polyrhizus). Afr J Biotechnol 2010;9(10):1930-4.

20. Luo H, Cai Y, Peng Z, Liu T, Yang S. Chemical composition and in vitro evaluation of the cytotoxic and antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel. Chem Cent J 2014;8(1):1.

21. Halimoon N, Hasan MH. Determination and evaluation of ant oxidative activity in red dragon fruit (Hylocereus undatus) and green kiwi fruit (Actinidia delicosa). Am J Appl Sci 2010;7(11):1432-8.

22. Barreira JC, Ferreira IC, Oliveira MB, Pereira JA. Effects of different phenols extraction conditions on antioxidant activity of almond (Prunus dulcis) fruits. J Food Biochem 2009;33(6):765-76.

23. Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004;85(2):231-7.

24. Mongkolpis P, Pongpapikit P, Sae-Lee N, Sithithaworn W. Radical scavenging activity and total phenolic content of medicinal plants used in primary health care. SWU J Pharm Sci 2004;9(1):32-5.

25. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002;13(10):572-584.

26. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol 2003;41(6):753-8.

27. Amic D, Davidovic-Amic D, Beslo D, Rastija V, Lucic B, Trinajstic N. SAR and QSAR of the antioxidant activity of flavonoids. Curr Med Chem 2007;14(7):827-45.

28. Fidrianny I, Johan Y, Sukrasno. Antioxidant activities of different polarity extracts from three organs of makrut lime (Citrus hystrix DC) and correlation with total flavonoid, phenolic, carotenoid content. Asian J Pharm Clin Res 2015;8(4):239-43.