PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITIES IN DIFFERENT ORGANS OF POMELO (CITRUS MAXIMA [BURM.] MERR.) USING 2,2-DIPHENYL-1-PICRYLHYDRAZYL AND PHOSPHOMOLYBDENUM ASSAYS

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ABSTRACT

Objectives: The aims of this research were to determine antioxidant activity from various organs extract of pomelo using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdenum assays, total phenolic, flavonoid, and carotenoid content, correlation of total phenolic, flavonoid, and carotenoid content in various extracts of chayote with their inhibitory concentration 50% (IC50) of DPPH antioxidant activities and exhibitory concentration 50% (EC50) of phosphomolybdenum capacity, and correlation between two antioxidant assays.

Methods: Extraction was carried out by reflux using various polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and phosphomolybdenum assays, determination of total phenolic, flavonoid, and carotenoid content were conducted by UV-visible spectrophotometry and its correlation with IC50 of DPPH and EC50 of phosphomolybdenum was analyzed by Pearson’s method.

Results: The lowest IC50 of DPPH scavenging activity was shown by ethyl acetate cortex extract of pomelo (0.68 µg/ml), whereas the lowest EC50 of phosphomolybdenum capacity was given by ethyl acetate leaves extract of pomelo (101.36 µg/ml). Ethyl acetate cortex extract of pomelo had the highest total phenolic content and ethyl acetate leaves extract had the highest total flavonoid content (TFC). There was a negative and significant correlation between TFC in cortex and peel extracts of pomelo with their IC50 of DPPH.

Conclusions: All organs extracts of pomelo (except n-hexane peel extract) were classified as a very strong antioxidant by DPPH method. Flavonoid compounds in cortex and peel extract of pomelo were the major contributor in antioxidant activities by DPPH method. DPPH and phosphomolybdenum assays gave no linear results in antioxidant activities of leaves, cortex, and peel extract of pomelo.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Phosphomolybdenum, Pomelo, Three organs.

INTRODUCTION

The excess of reactive oxygen species (ROS) might induce oxidative stress that can contribute to degenerative diseases [1,2]. Previous research reported that phenolic and flavonoid content could be correlated to their antioxidant activities [3-7]. Consumption of fruits and vegetables effective to prevent negative effect of oxidative stress because they have phenolic, flavonoid, and carotenoid compounds [7,8]. Reactivity of ROS can be scavenged by antioxidant.

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) can be used to determine antioxidant activity of fruits, vegetables, and food [8-11]. Previous studies [9,12,13] reported that ABTS, DPPH, FRAP, CUPRAC, and phosphomolybdenum methods could be used to determine antioxidant activity in many plants extracts. Plants included pomelo (Citrus maxima) contained phenolic and flavonoid compounds that can act as antioxidant and determination its antioxidant activities had been performed by DPPH, FRAP, and CUPRAC assays [9,14-16].

The objectives of this research were to determine antioxidant activities of various polarities extracts (n-hexane, ethyl acetate, and ethanol) of different organs of pomelo (leaves, cortex, and peel) using DPPH and phosphomolybdenum assays and correlations of total phenolic, flavonoid, and carotenoid content with their antioxidant capacities.

MATERIALS AND METHODS

Materials
DPPH, sodium phosphate, ammonium molybdate, gallic acid, quercetin, beta-carotene were purchased from Sigma-Aldrich (M0, USA), three organs of pomelo. All other reagents were analytical grades.

Preparation of sample
Three organs of pomelo were collected from Subang-West Java, which were leaves namely as LEA, cortex as COR, peel as PEE, were thoroughly washed with tap water, sorted while wet, cut, dried, and grinded into powder.

Extraction
About 300 g of powdered samples were extracted by reflux using different polarity solvents. Extraction using n-hexane was repeated three times. The remaining residue was then extracted three times using ethyl acetate. Finally, the remaining residue was extracted three times using ethanol. Therefore, totally there were nine extracts: Three n-hexane extracts (namely LEA1, COR1, and PEE1), three ethyl acetate extracts (LEA2, COR2, and PEE2) and three ethanolic extracts (LEA3, COR3, and PEE3).

Inhibitory concentration 50% (IC50) of DPPH scavenging activity
Determination of DPPH scavenging activity was carried out using method from Blois [17] with minor modification. Various concentrations of each extract were pipetted into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction for creating a calibration curve. After 30 minutes incubation, the absorbance was observed at
wavelength 515 nm using a UV-Vis Spectrophotometer Hewlett Packard 8435. Methanol was used as a blank, DPPH solution in 50 μg/ml as control and ascorbic acid as standard. The analysis was done in triplicate for each extract and standard. Antioxidant activity was measured by determining the percentage of antioxidant activity using reduction of DPPH absorbance [18]. IC$_{50}$ of DPPH scavenging activity of each extract can be calculated using its calibration curve.

**Exhibitory concentration 50% (EC$_{50}$) of phosphomolybdenum capacity**

Determination of phosphomolybdenum capacity was performed by Prieto's method [19]. Various concentrations of each extract were pipetted into phosphomolybdenum reagent 50 μg/ml (volume 1:1) to initiate the reaction for figuring a calibration curve. Incubation was carried out at 95°C for 90 minutes and cooled 20 minutes; then, absorbance was observed at wavelength 695 nm using the UV-Vis Spectrophotometer Beckman Coulter DU 720. Aquadest was used as a blank, phosphomolybdenum solution 50 μg/ml which was incubated at 95°C for 90 minutes and cooled 20 minutes as control, whereas alpha-tocopherol incubated at 37°C for 90 minutes and cooled 20 minutes as standard. The analysis was conducted in triplicate for each extract and standard. Antioxidant capacity was evaluated based on increasing in phosphomolybdenum complex absorbance by calculating the percentage of antioxidant capacity. EC$_{50}$ of phosphomolybdenum capacity of each extract can be determined using its calibration curve.

**Total phenolic content (TPC) determination**

TPC determination was performed using Folin-Ciocalteu reagent [20]. The absorbance was measured at wavelength 765 nm. Standard solution of gallic acid (55-175 μg/ml) was used to obtain a calibration curve. The analysis was conducted in triplicate for each extract. TPC was represented as a percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

**Total flavonoid content (TFC) determination**

Determination of TFC was done using Chang et al. method [21] with minor modification. The absorbance was measured at wavelength 415 nm. Standard solution of quercetin (30-120 μg/ml) was used to obtain a calibration curve. The analysis was carried out in triplicate for each extract. The TFC was exposed as a percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

**Total carotenoid content (TCC) determination**

TCC determination was measured using modification method from Thaipong et al. [9]. Each extract was diluted in n-hexane. The analysis was performed in triplicate for each extract. Standard solution of beta-carotene (40-80 μg/ml) was used to obtain a calibration curve. The absorbance was read at wavelength 470 nm. The TCC was revealed as a percentage of total beta-carotene equivalent per 100 g extract (g BE/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 conducted with SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, and carotenoid content and antioxidant activities and the correlation between two antioxidant activity methods were performed using the Pearson’s method.

**RESULTS**

**DPPH scavenging activity and phosphomolybdenum capacity**

The IC$_{50}$ of DPPH scavenging activities and EC$_{50}$ of phosphomolybdenum capacities in various organs extracts of pomelo by DPPH and phosphomolybdenum assays were shown in Figs. 1 and 2. IC$_{50}$ of DPPH scavenging activities of each extract were compared to IC$_{50}$ ascorbic acid and EC$_{50}$ of phosphomolybdenum capacity compared to EC$_{50}$ of alpha-tocopherol as standard. The lowest value of IC$_{50}$ or EC$_{50}$ of means had the highest antioxidant activity.

**TPC, TFC, and TCC in various organs extracts of pomelo**

TPC among the various organs extracts was presented in term of GAE using the standard curve equation y=0.005x−0.016, R$^2=0.987$. The TPC in various organs extracts of pomelo denoted results varied from 2.62 to 14.8 g GAE/100 g. The highest phenolic content (14.8 g GAE/100 g) was given by ethyl acetate extract of pomelo (COR2) followed by ethanolic cortex extract of pomelo (COR3) 13.7 g GAE/100 g (Table 1).

TFC among the various organs extracts was stated in term of QE using the standard curve equation y=0.006x+0.029, R$^2=0.998$. The TFC in various organs extracts of pomelo had different results in the range of 1.17-21.28 g QE/100 g (Table 1). Ethyl acetate leaves extract of pomelo (LEA2) had the highest TFC (21.28 g QE/100 g), and the lowest was given by ethanol peels extract of pomelo (PEE3).

TCC among the various extracts was demonstrated in term of BE using the standard curve equation y=0.012x−0.178, R$^2=0.981$. The TCC in various organs extracts of pomelo showed results ranging of 0.07-24.09 g BE/100 g (Table 1). N-hexane leaves extract of pomelo (LEA1) gave the highest carotenoid content (24.09 g BE/100 g), whereas the lowest carotenoid shown by ethanolic cortex extract of pomelo (COR3).

Pearson’s correlation coefficient between TFC in different organs of pomelo and their antioxidant activities revealed that TPC in all of the extracts (LEA, COR, and PEE) had significantly negative correlation with their IC$_{50}$ of DPPH scavenging activities (r=−0.736, p<0.05; r=−0.997, p<0.01; r=−0.659, p<0.05, respectively). Meanwhile, TCC in all of sample had a negative and significant correlation with their EC$_{50}$ phosphomolybdenum capacities (Table 2).

**Fig. 1: Inhibitory concentration 50% of 2,2-diphenyl-1-picrylhydrazyl scavenging activities in various organs extracts of pomelo, n=3**

**Fig. 2: Exhibitory concentration 50% of phosphomolybdenum capacities in various organs extracts of pomelo, n=3**
DISCUSSION

The previous studies [14,15] expressed that pomelo (C. maxima) had antioxidant activity. There was no study regarding the antioxidant activity of various extracts (which were n-hexane, ethyl acetate, and ethanol) of different organs (leaves, cortex, and peels) of pomelo using DPPH and phosphomolybdenum assays. DPPH free radicals dissolve in methanol give absorption at wavelength 516 nm. Color of DPPH would be changed from purple to yellow when the free radicals were scavenged by antioxidant [22]. Phosphomolybdenum reagent is sodium phosphate which was combined with ammonium molybdate in sulfuric acid solution. Color in phosphomolybdenum method would be changed from colorless to green-blue. Reduction potential of Mo (VI)/Mo (V) is 0.43 V. Intensity of green-blue depends on amount of Mo (VI) that is reduced to Mo (V). Complex Mo (VI) – sodium phosphate gives green-blue and shows characteristic absorption at wavelength 695 nm [19]. The first step of reaction will be produced 12-molybdophosphoric acid (Mo VI) [23]. Antioxidant that has potential lower than 0.43 V will reduce 12-molybdophosphoric acid (Mo VI) to phosphomolybdenum (Mo V) because reduction potential of Mo VI/Mo V is 0.43 V. The absorbance of phosphomolybdenum will be measured at 1695 nm. Increasing in the amount of phosphomolybdenum which was produced will increase the intensity of green-blue and also increase the absorbance.

The present research stated that IC_{50} of DPPH all of the organs extracts of pomelo in the range of 0.68-122.06 µg/ml and only the n-hexane peels extract had IC_{50} of DPPH more than 50 µg/ml which was 122.06 µg/ml (Fig. 1). The lowest IC_{50} means showed the highest antioxidant activity. Sample which had IC_{50} or EC_{50} lower than 50 µg/ml was a strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was a medium antioxidant, whereas a weak antioxidant with IC_{50} or EC_{50}>150 µg/ml [17]. Based on the result, it can be concluded all of the organs extracts of pomelo (except n-hexane peels extract) can be classified as a very strong antioxidant by DPPH assay and potential as natural antioxidant sources.

The present study demonstrated that the lowest IC_{50} of DPPH was presented by ethyl acetate cortex extract of pomelo (0.68 µg/ml), meanwhile ascorbic acid as standard had IC_{50} of DPPH 0.14 µg/ml. It can be concluded that potency of ascorbic acid as antioxidant was around five times potency of ethyl acetate cortex extract of pomelo, by DPPH method. The previous research denoted that IC50 of DPPH ethanolic extract of Citrus sinensis from three different sites (Kintamani, Jember, and Banyuwangi) were 2.25, 8.84, and 17.94 µg/ml, respectively [24]. It was different from the present study which represented that ethanolic peel extract of pomelo (C. maxima) from Subang-West Java had IC_{50} of DPPH 44.96 µg/ml. The previous studies revealed that IC_{50} of DPPH scavenging activities of methanolic peel extract of Citrus limon, C. sinensis var. Sungin, C. sinensis var. Valencia, and C. sinensis var. Navel which was extracted using percolation had IC_{50} of DPPH 1.4, 1.7, 2.1, and 1.1 µg/ml [25]; ethanolic extract of leaves, peel, and stem of Citrus hystrix from Boyolali – Central Java, Indonesia were 16.6, 16.7, and 7.1 µg/ml respectively [26]. Research by Prasad [15] reported that ethanolic leaves extracts of Citrus hystrix, Citrus aurantifolia, C. maxima, Citrus reticulata, and Citrus medica had IC_{50} of DPPH scavenging activities 740, 736, 730, and 1753 µg/ml, respectively. It was contrary to the previous research which showed that IC_{50} of DPPH scavenging activities of ethanolic leaves extract of C. reticulata, C. maxima, C. limon, C. hystrix, and C. aurantifolia were 12.76, 263.49, 44.27, and 17.38 µg/ml, respectively [14].

The previous study demonstrated that EC_{50} of FRAP capacity of ethanolic leaves extract of C. reticulata, C. maxima, C. hystrix, and C. aurantifolia varied from 81.54 to 131.06 µg/ml [14], whereas the present study revealed that EC_{50} phosphomolybdenum capacity of ethanolic extract of leaves, cortex, and peel extracts of pomelo (C. maxima) were 278.31, 212.19, and 1243.13 µg/ml, respectively. Toh et al. [27] exposed that methanolic extracts of peel and pulp of white tumbon pomelo gave higher antioxidant activities by FRAP assays (1.01 and 0.42 mmol Fe(II), respectively) than pink tumbon pomelo (0.65 and 0.51 mmol Fe(II), respectively). The similar result was demonstrated by TEAC assay which showed white tumbon pomelo had higher antioxidant activity than pink tumbon pomelo. Previous research [28] stated that methanolic extract of small fruit of Citrus grandis "tomentosa" showed higher antioxidant activity by DPPH, FRAP, and ABTS methods (23 µmol trolox equivalent [TE]/g, 149 µmol FeSO_4/g, and 58 µmol TE/g, respectively) compared to its medium fruit,
large fruit, flower, leaves, and branch. A study by Pichayongvongdee et al. [29] reported that ethanolic extract of different parts of fruit (flavedo, albedo, segment membranes, and seeds) using seven cultivars of pomelo showed different results in antioxidant activities by DPPH and FRAP methods. Ethanolic seed extract of pomelo cultivar Kao Paen gave the highest percentage DPPH scavenging activity (85.34%) while using FRAP assay ethanolic seeds extract of pomelo cultivar Thong Dee exposed the highest antioxidant capacity (243.13 mg TE/100 ml).

The present research demonstrated that all organs extracts of pomelo (C. maxima) showed EC50 of phosphomolybdenum capacities varied from 101 to 2247 μg/ml. The ethyl acetate leaves extract of pomelo (LEA2) gave the lowest EC50 of phosphomolybdenum capacity 101 μg/ml while ascorbic acid had EC50 of phosphomolybdenum capacity 100.65 μg/ml. It can be concluded that potency of LEA2 as antioxidant was similar to ascorbic acid, using phosphomolybdenum assay. In the present study, the reagent of antioxidant and sample were prepared in the same volume 1:1.

The reagent of DPPH and reagent of 12-molybdophosphoric acid were prepared in concentration 50 µg/ml. The first method (DPPH method) showed that 50 µg/ml free radical of DPPH was enough for serving reaction completely and the sample could give lower IC50 of DPPH in varied from 0.68 to 122.06 µg/ml. While in the second method (phosphomolybdenum method) sample had high values of EC50 of phosphomolybdenum capacities in the range of 101-2247 μg/ml. The values of EC50 of phosphomolybdenum capacities were higher than their IC50 of DPPH. Based on the value of EC50 of phosphomolybdenum capacity, it can be supposed that the reagent 12-molybdophosphoric acid 50 µg/ml was not enough to react with all of antioxidants in the sample. Only a little antioxidant in sample reacted with the reagent, and there were still most of antioxidant in sample, which will oxidize again Mo V to Mo VI. This reaction will be repeated, between reduction from Mo VI to Mo V and then excessive of antioxidant in the sample will oxidize again Mo V to Mo VI. Based on the explanation above, it can be concluded that the high value of EC50 of phosphomolybdenum capacity because there was not enough amount of Mo VI (12-molybdophosphoric acid) and it means for completing reaction in phosphomolybdenum method it needed more than 50 µg/ml of 12-molybdophosphoric acid.

Antioxidant activity can be correlated with phenolic content [5,6,11]. The present study revealed that TPC in ethanolic extracts of leaves, cortex, and peel extracts of pomelo (C. maxima) were 2.99, 13.7, and 6.68 g GAE/100 g, respectively. It was similar to the previous study [14] regarding citrus leaves extracts, which showed that TPC in ethanolic leaves extract of C. reticulata, C. maxima, C. limon, C. hystrix, and C. aurantifolia from West Java-Indonesia were 5.30, 4.55, 3.31, 3.66, and 6.33 g GAE/100 g, respectively. It was contrary to the previous study which expressed that TPC in fruit juice of C. hystrix and C. aurantifolia, and C. sinensis were 490, 211, and 135 mg GAE/100 ml juice, respectively [30]. Previous research stated that TPC in methanolic peel extract of white tumban pomelo showed higher TPC (406.65 mg GAE/100 g) compared to its peel and pulp extracts of pink tumban pome [27]. Research by Hayat [31] exhibited that TPC in methanolic peel extract of C. reticulata using the microwave-assisted extraction was 17.5 mg GAE/100 g and TPC in methanolic peel extracts of C. sinensis and C. reticulata by ultrasound-assisted extraction method was 6.64 and 5.87 g GAE/100 g, respectively. The similar results were reported in previous studies which represented that TPC in ethanolic peel extract of C. sinensis var. Washington Navel, C. sinensis var. Sungin, C. sinensis var. Valencia were 16, 15.4, and 13.3 g GAE/100 g, respectively [25]; TPC in ethanolic peel extracts of C. sinensis from Kintamani, Jember, and Banyuwangi were 10.08, 8.85, and 9.54 g GAE/100 g, respectively [24], and methanolic peel extract of C. limon was 13.1 g GAE/100 g [25]. Research by Pichayongvongdee [29] presented that TPC in ethanolic seed extract of pomelo cultivar Thong Dee had the highest TPC (495 mg/100 g) among different parts of fruit from seven cultivars of pomelo, whereas Duan [28] figured that methanolic small fruit extract of C. grandis gave the highest TPC (105 mg GAE/g) compared to its medium fruit, large fruit, flower, leaves, and branch.

The previous research reported that TPC in ethanolic leaves extracts of C. reticulata, C. maxima, C. limon, C. hystrix, and C. aurantifolia were 5.44, 3.99, 3.97, 4.46, and 4.81 g QE/100 g, respectively [14], whereas the present study revealed that TPC in ethanolic extracts of cortex, peel, and leaves of pomelo (C. maxima) were 1.52, 1.17, and 7.3 g QE/100 g, respectively. A study by Ghasemi [25] stated that TPC in methanolic peel extracts of C. sinensis var. Washington Navel 2.3 g QE/100 g was higher than C. sinensis var. Sungin 0.21 g QE/100 g and C. sinensis var. Valencia 0.72 g QE/100 g, whereas TPC in ethanolic peel extract of C. sinensis from Jember (1.50 g QE/100 g) was higher than C. sinensis from Kintamani (1.22 g QE/100 g) and Banyuwangi (0.93 g QE/100 g) [24].

The present research revealed that ethanolic extract of peel, cortex, and leaves of C. maxima had TCC 0.39, 0.07, and 1.74 g BE/100 g, respectively. It was similar to the previous study which expressed that TCC in leaves extracts of C. reticulata, C. maxima, C. limon, C. hystrix, and C. aurantifolia from West Java-Indonesia were 0.56, 0.72, 0.19, 0.36, and 0.05 g BE/100 g, respectively [14]. The previous studies expressed that TCC in ethanolic peel extracts of C. sinensis from Kintamani, Jember, and Banyuwangi were 37, 21, and 22 mg BE/100 g, respectively [24], and TCC in all of the ethanolic peel, leaves, and stem extracts of C. hystrix from Boyolali, Central Java-Indonesia were 0.2 g BE/100 g extract [26].

Pearson’s correlation coefficient was significantly negative if -0.61 ≤ r ≤ -0.97 and significantly positive high if 0.61 ≤ r ≤ 0.97 [9]. The sample which had the lowest IC50 of DPPH scavenging activity and EC50 of phosphomolybdenum capacity gave the highest antioxidant activity. Therefore, significantly negative correlation will be shown in a good correlation between TPC, TFC, and TCC with IC50 of DPPH or EC50 of phosphomolybdenum. It means increasing in TFC, TPC, and TCC would increase antioxidant activities, which was expressed by lower IC50 of DPPH scavenging activity or EC50 of phosphomolybdenum capacity.

The significant and negative correlation between TPC in peel and cortex extracts of C. maxima and IC50 of DPPH can be seen in Table 2 (r = -0.659, p<0.05; r = -0.997, p<0.01). Based on the result, it can be suggested that phenolic compounds in peel and cortex of C. maxima were the major contributor in their antioxidant activity by DPPH assay. Previous studies demonstrated that TPC in C. aurantifolia leaves extracts, and its IC50 of DPPH scavenging activities had significant and negative correlation (r = -0.983, p<0.01) and also with its EC50 of FRAP capacities (r = -0.974, p<0.01) [14]. It can be concluded that phenolic compounds were the major contributor in antioxidant activities of C. aurantifolia leaves extract using DPPH, FRAP assays. There was significantly negative correlation also between TPC, TCC in leaves, peel and stem extracts of C. hystrix with their EC50 of CUPRAC capacities [26]. It was similar to the present research which exposed that TPC and TCC in cortex extract of C. maxima had a significant and negative correlation with its EC50 of phosphomolybdenum.

In previous research, the Pearson’s correlation was analyzed between TPC, TFC, and TCC with their percentage of DPPH scavenging activities [24]. Therefore, the good correlation between TPC, TFC, and TCC with the percentage of DPPH scavenging activity or percentage of FRAP capacity when there was a positive and high correlation. The previous result [24] stated that TPC in peel extracts of C. sinensis from Kintamani, Jember, and Banyuwangi were significantly positive
correlation with their percentage of DPPH scavenging activities. A study by Toh [27] reported that TFC and TFC of pomelo pulp and peel had negative and significant correlation with their antioxidant activity using FRAP assay, which was exposed in mmol Fe(II)/100 g, and significantly positive correlation with their antioxidant activity by TEAC assay, which presented in mmol TE/100 g. Ghafar [30] revealed that there was a positive and significant correlation between TPC in fruit juice of C. aurantiolia with its percentage of FRAP capacity, but there was no correlation with its percentage of DPPH scavenging activity.

Phenolic groups in plant included phenolic acid, flavonoid, tannins, quinone, and quinoline. In Table 1, it can be seen that TFC in ethanolic peel extract of pomelo (PEE3) 6.88 g GAE/100 g was higher than TFC in ethanolic leaves extract of pomelo (LEA3) 2.99 g GAE/100 g, but LEA3 had high antioxidant activity which showed by lower IC50 of DPPH (18.93 μg/ml) than IC50 of DPPH PEE3 (44.96 μg/ml). Hein [32] stated that cinnamic acid had higher antioxidant activity compared to benzoic acid and phyllylactic acid. It can be predicted that LEA3 contained many phenolic compounds which had high antioxidant activity. Meanwhile, PEE3 contained many phenolic compounds with low antioxidant activity.

TFC in ethanolic cortex extract of pomelo (COR3) 1.52 g QE/100 g was similar to TFC in ethanolic peel extract of pomelo (PEE3) 1.17 g QE/100 g; however, IC50 of DPPH COR3 (1.46 μg/ml) was lower than IC50 of DPPH PEE3 (44.96 μg/ml). Flavonoid compound which has OH in A ring and or B ring will be included in phenolic groups. Flavonoid had higher antioxidant activity than phenolic acid [32]. Flavonoid which had ortho dixH at C-3', C-4', OH at C-3, oxo function at C-4, double bond at C-2 - C-3 have high antioxidant activity. The dixH with ortho position at C-3' - C-4' had the highest influence to the antioxidant activity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides [32]. Based on the results above, it can be suggested that COR3 contained many flavonoid compounds that had OH at position above, which had high antioxidant activity whereas PEE3 contained many flavonoid compounds which had OH at C-5 or C-7 or only C-3' or only C-4'; or without double bond at C-2-C-3, or without OH at C-3, which had low antioxidant activity.

The previous study exposed that carotenoid has the antioxidant capacity by scavenging free radical [33]. It can scavenge free radical because it consists of many double bonds. Carotenoid would give higher scavenging radical activity if contain >7 double bonds [34]. Betacarotene can be used as standard because it has conjugation double bonds which have the ability to scavenge free radicals [35]. TCC in n-hexane leaves extract of pomelo (LEA1) 24.09% BE/100 g was higher than TCC in n-hexane cortex extract of pomelo (COR1) 6.98% BE/100 g; however, IC50 of DPPH COR1 (12.03 μg/ml) was lower than IC50 of DPPH LEA1 (22.62 μg/ml). Increasing in antioxidant activity which is shown by lower IC50 of DPPH scavenging activity will be given by increasing in lipophilic of carotenoid [36]. It can be predicted that many carotenoid compounds in COR1 had more than 7 double bonds, whereas many carotenoid compounds in LEA1 had maximum 7 double bonds.

TFC in n-hexane leaves extract of pomelo (LEA1) 8.02 g QE/100 g was higher than TFC in ethyl acetate peel extract of pomelo (PEE2) 5.69 g QE/100 g; however, EC50 of phosphomolybdenum LEA1 (21.65 μg/ml) was similar to EC50 of phosphomolybdenum PEE2 (21.21 μg/ml). Sample will act as antioxidant in phosphomolybdenum assay if sample had reduction potential lower than reduction potential of Mo (VI) to Mo (V) 0.43 V. Based on the results, it can be suggested that LEA1 and PEE2 contained the similar amount of flavonoid compounds which had reduction potential lower than 0.43 V.

Pearson's correlation was used to analyze the correlation between two antioxidant methods. The good correlation between two methods if there was a positive and significant correlation between one method to the other method. There was no correlation between IC50 of DPPH and IC50 of phosphomolybdenum in all of the pomelo organs extracts. It could be seen DPPH method gave no linear results with phosphomolybdenum method in antioxidant activity of pomelo organs extracts.

CONCLUSION

Various antioxidant assays could give different results. Therefore, the antioxidant activity of sample should be determined by different assays in parallel. All of the pomelo organs extracts (except n-hexane peel extract of pomelo) can be classified as very strong antioxidant by DPPH method. TPC in cortex and peel extracts of pomelo had a significant and negative correlation with their IC50 of DPPH scavenging activities. Antioxidant activity of pomelo cortex and peel extracts by DPPH method can be estimated using its TPC data. DPPH and phosphomolybdenum assays showed no linear results for antioxidant activity in all of the pomelo organs extracts. Leaves, cortex, and peel of C. maxima may be exploited as sources of natural antioxidant.

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