INTRODUCTION

The excess of reactive oxygen species (ROS) might induce oxidative stress that can contribute to degenerative diseases [1,2]. Previous research has shown that phenolic and flavonoid content could be correlated to their antioxidant activities [3-7]. Consumption of fruits and vegetables is effective in preventing negative effects 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 plant extracts. Plants included pomelo (Citrus maxima) contained phenolic and flavonoid compounds that can act as antioxidant and determination of 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, and beta-carotene were purchased from Sigma-Aldrich (MO, 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 grind 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
DPPH scavenging activity and phosphomolybdenum capacity

The IC\textsubscript{50} of DPPH scavenging activities and EC\textsubscript{50} of phosphomolybdenum capacities in various organs extracts of pomelo as determined by DPPH and phosphomolybdenum assays were shown in Figs. 1 and 2. IC\textsubscript{50} of DPPH scavenging activities of each extract were compared to IC\textsubscript{50} of ascorbic acid and EC\textsubscript{50} of phosphomolybdenum capacity compared to EC\textsubscript{50} of alpha-tocopherol as standard. The lowest value of IC\textsubscript{50} or EC\textsubscript{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−0.01, R\textsuperscript{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 cortex 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\textsuperscript{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\textsuperscript{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\textsubscript{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\textsubscript{50} phosphomolybdenum capacities (Table 2).

RESULTS

The 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\textsubscript{50} of phosphomolybdenum capacity of each extract can be determined using its calibration curve.

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
Table 1: TPC, TFC, and TCC in different organs of pomelo

| Sample   | TPC (g GAE/100 g) | TFC (g QE/100 g) | TCC (g BE/100 g) |
|----------|-------------------|------------------|------------------|
| LEA1     | 4.29              | 8.02             | 24.09            |
| LEA2     | 5.57              | 21.28            | 11.83            |
| LEA3     | 2.99              | 7.3              | 1.74             |
| COR1     | 4.24              | 6.25             | 6.98             |
| COR2     | 1.48              | 9.18             | 2.89             |
| COR3     | 1.37              | 1.52             | 0.07             |
| PEE1     | 2.62              | 3.31             | 5.83             |
| PEE2     | 3.78              | 5.69             | 1.92             |
| PEE3     | 6.88              | 1.17             | 0.39             |

LEA: Leaves of pomelo, COR: Cortex of pomelo, PEE: Peels of pomelo, 1: n-hexane extract, 2: Ethyl acetate extract, 3: Ethanolic extract, TPC: Total phenolic content, TFC: Total flavonoid content, TCC: Total carotenoid content, GAE: Gallic acid equivalent, QE: Quercetin equivalent, BE: Beta-carotene equivalent

Table 2: Pearson’s correlation coefficient of total phenolic, flavonoid, and carotenoid content in different organs of pomelo with their IC50 of DPPH scavenging activities and EC50 of phosphomolybdenum capacities

| Antioxidant parameter | Pearson’s correlation coefficient (r) |
|-----------------------|--------------------------------------|
|                       | TPC        | TFC        | TCC        | IC50 DPPH LEA | IC50 DPPH COR | IC50 DPPH PEE |
| IC50 DPPH LEA         | -0.736**   | -0.964**   | 0.264ns    | -0.659**      | -0.589ns      | 0.372ns       |
| IC50 DPPH COR         | 0.997**    | 0.071ns    | 0.886**    | 0.937**       | -0.795*       | -0.802**      |
| IC50 DPPH PEE         | -0.659*    | -0.100ns   | 0.937**    | -0.589ns      | -0.795*       | -0.802**      |
| EC50 phosphomolybd LEA| -0.892**   | -0.589ns   | -0.795*    | 0.372ns       | -0.438ns      | -0.375ns      |
| EC50 phosphomolybd COR| 0.407ns    | -0.922**   | -0.802**   | -0.671*       |              |              |
| EC50 phosphomolybd PEE| 0.939**    | -0.872**   | -0.671*    |              |              |              |

IC50: DPPH scavenging activity, EC50: phosphomolybd: EC50 phosphomolybdenum capacity, LEA: Leaves of pomelo, COR: Cortex of pomelo, PEE: Peels of pomelo, ns: Not significant, *Significant at p<0.05, **Significant at p<0.01. DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC50: Inhibitory concentration 50%, EC50: Exhbitory concentration 50%

DISCUSSION

The previous studies [14,15] expressed that pomelo (C. maxima) had antioxidant capacity. 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 (V) – 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 695 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 IC50 of DPPH of all the organs extracts of pomelo in the range of 0.68-122.06 µg/ml and only the n-hexane peels extract had IC50 of DPPH more than 50 µg/ml which was 122.06 µg/ml (Fig. 1). The lowest IC50 means showed the highest antioxidant activity. Sample which had IC50 or EC50 lower than 50 µg/ml was a very strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was a medium antioxidant, whereas a weak antioxidant with IC50 or EC50 >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 IC50 of DPPH was presented by ethyl acetate cortex extract of pomelo (0.68 µg/ml), meanwhile ascorbic acid as standard had IC50 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 peel extract of Citrus sinensis from three different sites (Kintamani, Jember, and Banyuwangi) were 2.25, 8.84, 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 IC50 of DPPH 44.96 µg/ml.

The previous studies revealed that IC50 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 IC50 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 C. hystrix, Citrus aurantifolia, C. maxima, Citrus reticulata, and Citrus medica had IC50 of DPPH scavenging activities 74.0, 736, 730, 1070, and 1753 µg/ml, respectively. It was contrary to the previous research which showed that IC50 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, 442, 25.27, and 17.38 µg/ml, respectively [14].

The previous study demonstrated that EC50 of FRAP capacity of ethanolic leaves extracts 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 EC50 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 FeSO4/g, and 58 µmol TE/g, respectively) compared to its medium fruit.
large fruit, flower, leaves, and branch. A study by Pichaiyongvongdee 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 seeds extract of pomelo cultivate Kao Paen gave the highest percentage DPPH scavenging activity (85.34%) while using FRAP assay ethanolic seeds extract of pomelo cultivate Thong Dee exposed the highest antioxidant capacity (2431.3 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 12.06 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 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 6.88 g GAE/100 g, respectively. It was similar to the previous study [14] regarding citrus leaves extracts, which expressed 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 tambun pomelo showed higher TPC (406.65 mg GAE/100 g) compared to its peel and pulp extracts of pink tambun pomelo [27]. Research by Hayat [31] exhibited that TPC in methanolic peel extract of C. reticulata using the microwave-assisted extraction was 17.5 g 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 Pichaiyongvongdee [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 Ghafar [30] showed that TPC in fruit juice of C. hystrix, C. aurantifolia, Citrus Microcarpa, and C. sinensis were 22.25, 10.67, 8.77, and 2.99 mg QE/100 ml juice, whereas TFC in methanolic extracts of peel and pulp of white tambun pomelo and pink tambun pomelo were 356.95, 13.06 and 228.06, 13.20 mg QE/100 g, respectively [27]. Previous research demonstrated that TPC in ethanolic leaves extract of C. hystrix (3.0 g QE/100 g) was higher than its peel and stem extracts 1.3 and 0.9 g QE/100 g [26]. Study by Ghasemi [25] stated that TPC in methanol peel extracts of C. sinensis var. Washington Navel were 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 extracts 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 [26]. It means increasing in TFC, TPC, and TCC would increase antioxidant activities, which was expressed by lower IC₅₀ of DPPH scavenging activity and 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 TFC, 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 TFC 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 TPC and TFC of pomelo pulp and peel had negative and significant correlation with their antioxidant activity using FRAP assay, which was exposed in mmole 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. aurantifolia 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, quinoline, and quinone. In Table 1, it can be seen that TPC in ethanolic peel extract of pomelo (PEE3) 6.88 g GAE/100 g was higher than TPC in ethanolic leaves extract of pomelo (LEA3) 2.99 g GAE/100 g, but LEA3 had high antioxidant activity which showed by lower IC$_{50}$ of DPPH (18.93 μg/ml) than IC$_{50}$ 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 diOH 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 diOH 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 carotenoids have 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]. Beta-carotene 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 g BE/100 g was higher than TCC in n-hexane cortex extract of pomelo (COR1) 6.98 g BE/100 g; however, IC$_{50}$ of DPPH COR1 (12.03 μg/ml) was lower than IC$_{50}$ of DPPH LEA1 (22.82 μg/ml). Increasing in antioxidant activity which is shown by lower IC$_{50}$ of DPPH scavenging activity will be given by increasing in lipophilicity of carotenoids [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, EC$_{50}$ of phosphomolybdenum LEA1 (21.65±1.5 μg/l) was similar to EC$_{50}$ of phosphomolybdenum PEE2 (21.21±0.9 μg/l). 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 IC$_{50}$ of DPPH and EC$_{50}$ 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 IC$_{50}$ 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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