INTRODUCTION

Phenolic compounds and flavonoids are commonly found in many plants, which have many effects such as antioxidant activity and antibacterial activity [1-4]. Phenolic, flavonoid, and carotenoid compounds might be having antioxidant activity [5]. Antioxidant has many benefits to prevent the excessive of free radical in oxidative stress which can cause many degenerative diseases. Consumption of fruits and vegetables can prevent negative effect of oxidative stress because they contain phenolic, flavonoid, and carotenoid compounds, which have antioxidant capacity [6]. Previous researches represented that total phenolic content (TPC) and total flavonoid content (TFC) could be correlated to their antioxidant activities [7-9]. Plants included sweet potatoes, guava, lemon grass, tea, and legumes contained phenolic and flavonoid compounds [1-3,10,11].

Ferric reducing antioxidant power (FRAP), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS)), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods could be used to observe antioxidant activity in many plants extracts [4,10,11]. The previous researches [3,8,11,12] revealed that DPPH, ABTS, and FRAP can be performed to determine antioxidant activity of fruits, vegetables, and food. Kelakai (Stenochlaena palustris) empirically used in Central Kalimantan for antiaging, contained many derivates of kaempferol flavonoids which can act as antioxidant [13].

The objectives of this research were to evaluate antioxidant activities in various polarity extracts (n-hexane, ethyl acetate, and ethanol) from different parts of kelakai grown in Central Kalimantan – Indonesia, using DPPH and FRAP assays and correlations of TPC, TFC, and total carotenoid content (TCC) with their antioxidant activities.

METHODS

Materials

DPPH, 2,4,6-tripyridyl-S-triazine (TPTZ), gallic acid, quercetin, and beta carotene were purchased from Sigma-Aldrich (MO, USA), different parts of kelakai (S. palustris). All of other reagents were analytical grades.

Preparation of sample

Different parts of kelakai (S. palustris), which were young leaves named as YL, old leaves as OL, and roots as RO, were collected from Palangkaraya, Central Kalimantan - Indonesia, 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. Hence, totally there were nine extracts: Three n-hexane extracts (namely, YL1, OL1, and RO1), three ethyl acetate extracts (YL2, OL2, and RO2), and three ethanolic extracts (YL3, OL3, and RO3).

Antioxidant activity by DPPH assay

Antioxidant activity by DPPH assay was performed using modified Blois’s method [14]. Various concentrations of each extract were pipetted into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was observed after 30 minutes incubation at wavelength 515 nm by ultraviolet-visible (UV-VIS) spectrophotometer Hewlett Packard 8455. Methanol was used as a blank, DPPH solution 50 µg/ml as control and ascorbic acid as standard.

Results

Conclusions:

All different extracts of kelakai parts were categorized as very strong antioxidants by DPPH method. Phenolic compounds in kelakai root extract were the major contributor in antioxidant activities by DPPH and FRAP methods. DPPH and FRAP showed linear results in antioxidant activities of root kelakai extract.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Ferric reducing antioxidant power, Stenochlaena palustris, Young leaves, Old leaves, Root.
acid as standard. Analysis was conducted in triplicate for standard and each extract. Antioxidant activity was determined by calculating the percentage of reduction of DPPH absorbance [15]. Inhibitory concentration 50% (IC\textsubscript{50}) of DPPH scavenging activity of each extract can be evaluated using its calibration curve.

**Antioxidant capacity by FRAP assay**

Preparation of FRAP solution was adopted from Benzi [16], which was prepared in acetate buffer pH 3.6. Each extract 50 µg/ml was added into FRAP solution 50 µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was measured at wavelength 593 nm using the UV-VIS spectrophotometer Hewlett Packard 8435. Acetate buffer was used as a blank, FRAP solution 50 µg/ml as control and ascorbic acid as standard. Analysis was performed in triplicate for standard and each extract. Antioxidant capacity was determined based on increasing in Fe(II)-TPTZ absorbance by calculating the percentage of antioxidant capacity [16]. Exhibitory concentration 50% (EC\textsubscript{50}) of FRAP capacity of each extract can be determined using its calibration curve.

**TPC**

TPC determination was carried out using Folin-Ciocalteu reagent [17]. The absorbance was observed at wavelength 765 nm. Analysis was performed in triplicate for each extract. Standard solution of gallic acid (55-175 µg/ml) was used to obtain a calibration curve. TPC was exposed as the percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

**TFC**

Chang's method [18] with minor modification was done in determining TPC. The absorbance was observed at wavelength 415 nm. Analysis was conducted in triplicate for each extract. Quercetin solution 30-120 µg/ml was used to obtain a calibration curve. TFC was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

**TCC**

TCC was performed by modified Thaipong's method [11]. Each extract was diluted in n-hexane [5]. The absorbance was measured at wavelength 470 nm. Analysis was conducted in triplicate for each extract. Beta carotene solution 40-80 µg/ml was used to obtain a calibration curve. TCC was presented as 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 mean ± 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 carried out with SPSS 16 for Windows. Correlation between the TPC, TFC, TCC, and antioxidant activities and correlation between two antioxidant activity methods were performed using the Pearson’s method.

**RESULTS**

**Antioxidant activity by DPPH and FRAP assays**

Antioxidant activity in different extracts of kelakai parts by DPPH and FRAP assays was performed by determining IC\textsubscript{50} of DPPH scavenging activities and EC\textsubscript{50} of FRAP capacities of each extract were compared to IC\textsubscript{50} or EC\textsubscript{50} ascorbic acid as standard. The lowest value of IC\textsubscript{50} or EC\textsubscript{50} means the highest antioxidant activity.

**TPC in kelakai extracts**

TPC among different part extracts of kelakai were represented in term of GAE using the standard curve equation y=0.005x−0.016, R\textsuperscript{2}=0.997. TPC in kelakai extracts had different results varied from 1.87 to 24.22 g GAE/100 g (Fig. 1). Ethanolic roots extract of kelakai (RO3) had the highest TPC (24.22 g GAE/100 g), and its n-hexane extract (RO1) gave the lowest TPC 1.87 g GAE/100 g.

**TCC in kelakai extracts**

TCC among different part extracts of kelakai were represented in term of QE using the standard curve equation y=0.006x+0.029, R\textsuperscript{2}=0.998. TCC in kelakai extracts were in the range of 1.99-20.93 g QE/100 g. The highest TFC (20.93 g QE/100 g) was denoted by ethyl acetate YLs extract of kelakai (YL2), and the lowest TFC given by its ethanolic extract (YL3) (Fig. 2).

**TFC in kelakai extracts**

TFC among different part extracts of kelakai were demonstrated in term of beta carotene equivalent using the standard curve equation y=0.012x−0.178, R\textsuperscript{2}=0.998. TCC in different extracts of kelakai parts ranged from 7.28 to 38.49 g BE/100 g. N-hexane root extract of kelakai (RO1) revealed the highest TCC (38.49 g BE/100 g), whereas the lowest carotenoid content (7.28 g BE/100 g) was shown by ethanolic YLs extract of kelakai (YL3) (Fig. 3).

**Correlations between TPC, TFC, TCC in kelakai extracts and IC\textsubscript{50} of DPPH scavenging activities, EC\textsubscript{50} of FRAP capacities**

TPC in YLs and root extracts of kelakai had significant and negative correlation with their EC\textsubscript{50} of DPPH scavenging activities (r=−0.695, p<0.05; r=−0.818, p<0.01, respectively), and TPC in OL and root extracts of kelakai gave negative and significant correlation with their EC\textsubscript{50} FRAP capacities (r=−0.991, r=−0.839, p<0.01, respectively) (Table 1).

**DISCUSSION**

The previous researches [19,20] revealed that kelakai (S. palustris) had antioxidant capacity. There was no research regarding the antioxidant activity of different parts of kelakai (S. palustris), which were YLs, OL, and root extracted using increasing polarity solvents (n-hexane, ethyl acetate, and ethanol) and tested by DPPH and FRAP assays. Antioxidant will transfer the hydrogen to DPPH and DPPH will stable. DPPH free radicals dissolved in methanol give absorption at wavelength 516 nm. Colors of DPPH would be changed from purple to yellow when the free radicals were scavenged by antioxidant [21]. FRAP reagent is ferric (III) chloride which was combined with TPTZ in acetate buffer.
Fig. 3: Total carotenoid content in kelakai extracts, n=3

Fig. 4: Inhibitory concentration 50% of 2,2-diphenyl-1-picyrlhydrazyl in different extracts of kelakai parts, n=3

Fig. 5: Exhibitory concentration 50% of ferric reducing antioxidant power capacities in different extracts of kelakai parts, n=3

pH 3.6. Reduction potential of Fe (II)/Fe (III) is 0.77 V. Antioxidant will reduce Fe (II) to Fe (II) if it has reduction potential lower than 0.77 V. Complex of Fe (II) TPTZ shows blue and give characteristic absorption at wavelength 593 nm. Intensity of blue depends on amount of Fe (III) which is reduced to Fe (II) and form complex with TPTZ. IC\textsubscript{50} of DPH scavenging activities and EC\textsubscript{50} of FRAP capacities in different parts of kelakai can be seen in Figs. 4 and 5. The IC\textsubscript{50} of DPH scavenging activities and EC\textsubscript{50} of FRAP capacities in different parts extracts of kelakai were compared to IC\textsubscript{50} or EC\textsubscript{50} of ascorbic acid standard. The lowest value of IC\textsubscript{50} or EC\textsubscript{50} means the highest antioxidant activity. Sample, which had an IC\textsubscript{50} or EC\textsubscript{50} lower than 50 µg/ml, was a very strong antioxidant, 50-100 µg/ml was a strong antioxidant, and 101-150 µg/ml was a medium antioxidant while a weak antioxidant with IC\textsubscript{50} or EC\textsubscript{50} >150 µg/ml [14].

In the present research, antioxidant activities by DPH method were represented by IC\textsubscript{50} of DPH. IC\textsubscript{50} means the concentration of extract (antioxidant sample) that can scavenge free radical DPHH 50% and figured by decreasing absorbance of DPHH after adding extract. IC\textsubscript{50} of DPHH can be calculated using regression linear equation of calibration curve of each extract.

Moreover, antioxidant activities can be expressed by the percentage of DPHH scavenging activity, by reacting DPHH 50 µg/ml and sample 50 µg/ml. The result was compared to the percentage of DPHH scavenging activity of ascorbic acid, by adding DPHH 50 µg/ml and ascorbic acid 50 µg/ml. The value of the percentage of DPHH scavenging activity of ascorbic acid did not achieve 100% because there was still residual yellow in solution after giving hydrogen atom to DPHH by antioxidant in sample extract [22,23]. The percentage of DPHH scavenging activity could not present the real antioxidant activities. Antioxidant activity can be stated in percentage of DPHH scavenging activity which was performed normally by adding extract with one concentration 50 µg/ml only to DPHH solution 50 µg/ml (volume 1:1). If extract 50 µg/ml can scavenge DPHH 50 µg/ml 57%, its not means extract 60 µg/ml will always scavenge DPHH more than 57%. The extract 60 µg/ml will present the percentage of DPHH may be >57% or lower than 57%. It is due to extract consisted of many compounds and not all compounds in extract have antioxidant activities, may be some of them act as antagonist of antioxidant. In extract 50 µg/ml, the compounds, which can act as antagonist antioxidant, have not achieved yet its effective concentration. Therefore, the antioxidant components can scavenge DPHH 57%. In extract 60 µg/ml, the antagonist of antioxidant components have achieved its effective concentration, and it will reduce the ability of antioxidant components, therefore, extract 60 µg/ml will give percentage of DPHH lower than 57%. Antioxidant activity can be stated as IC\textsubscript{50} of DPHH should be used many concentrations of extract which shown linear decreasing in absorbance of DPHH. Based on the results, the regression linear equation of calibration curve of each extract can be assessed. After determining regression linear equation, value of IC\textsubscript{50} of DPHH can be calculated. Based on the explanation above, it can be seen that the percentage of DPHH scavenging activities will not represent the real antioxidant activities; however, the real of antioxidant activities will be exposed by IC\textsubscript{50} of DPHH value.

Table 1: Pearson's correlation coefficient of TPC, flavonoid, carotenoid content in different parts extracts of kelakai parts with their IC\textsubscript{50} of DPHH scavenging activities and EC\textsubscript{50} of FRAP capacities

| Antioxidant parameter | Pearson's correlation coefficient (r) |
|-----------------------|--------------------------------------|
|                       | TPC       | TFC       | TCC       | EC\textsubscript{50} FRAP YL | EC\textsubscript{50} FRAP OL | EC\textsubscript{50} FRAP RO |
| IC\textsubscript{50} DPHH YL | -0.695*   | -0.992**  | -0.991**  | 0.879**                      | -0.888**                      |
| IC\textsubscript{50} DPHH RO  | 0.858**   | 0.105 ns  | 0.129 ns  |                           |                           |
| EC\textsubscript{50} FRAP YL  | -0.328 ns | -0.863**  | -0.916**  |                           |                           |
| EC\textsubscript{50} FRAP RO  | -0.839**  | -0.345 ns | 0.989**   |                           |                           |

*Significant at p<0.05, **Significant at p<0.01. IC\textsubscript{50} DPHH=IC\textsubscript{50} DPHH scavenging activity, EC\textsubscript{50} FRAP=EC\textsubscript{50} FRAP capacity, YL: Young leaves of kelakai, OL: Old leaves of kelakai, RO: Root of kelakai, TPC: Total phenolic content, TFC: Total flavonoid content, TCC: Total carotenoid content, NS: Not significant, EC\textsubscript{50}: Exhibitory concentration 50%, FRAP: Ferric reducing antioxidant power, DPHH: 2,2-diphenyl-1-picyrlhydrazyl, IC\textsubscript{50}: Inhibitory concentration 50%.
Antioxidant capacities by FRAP assay were exposed by $E_{50}$ of FRAP capacity. $E_{50}$ is concentration of antioxidant sample that can increase FRAP capacity 50% and stated by increasing absorbance of complex of Fe (II) – TPTZ after adding antioxidant sample. Regression linear equation of calibration curve of each extract can be used to determine $E_{50}$ of FRAP.

The present study exposed that all of different parts (YL, OL and root) extracts of kelakai (S. palustris) had $IC_{50}$ of DPPH varied from 0.8 to 14.13 µg/ml, and it means their antioxidant by DPPH assay can be classified as a very strong antioxidant because lower than 50 µg/ml. The highest antioxidant was exposed by ethanolic root extract of kelakai ($IC_{50}$ of DPPH 0.8 µg/ml), meanwhile $IC_{50}$ of DPPH ascorbic acid 0.14 µg/ml.

It can be conclude that antioxidant potency of ascorbic acid was around six-fold potency of ethanolic root extract of kelakai. Research by Chai et al. [19] reported that water extract of mature sterile frond of S. palustris 150 mg/ml showed the highest percentage of DPPH radical scavenging activity (95%) compared to young sterile frond, young fertile frond, and mature fertile frond, while Trolox 50 µg/ml can scavenge DPPH around 99%. The present study stated that ethanolic extract of YL, OL and root of kelakai (S. palustris) were 14.13, 1.38, and 0.8 µg/ml respectively.

A previous study revealed that percentage of hydroxyl radical scavenging activity of kelakai leaves (16.60%) was similar to ascorbic acid (16.43%). It was linear with its percentage hydrogen peroxide scavenging activity 60.10%, ascorbic acid 60.10%, its percentage of chelating effect on ferrous ions 27.64%, ascorbic acid 26.68%. Based on this result, antioxidant activity of kelakai leaves was similar to ascorbic acid using percentage of hydroxyl radical scavenging activity, percentage hydrogen peroxide scavenging activity, and percentage of chelating effect on ferrous ions [20]. The other research expressed that mature sterile frond of kelakai with concentration of 500 µg/ml had the highest FRAP value (72.36 mM Fe$^{2+}$ equivalents) compared to its young fertile frond (45.07 mM Fe$^{2+}$ equivalents), young sterile frond (41.92 mM Fe$^{2+}$ equivalents), and mature fertile frond (20.99 mM Fe$^{2+}$ equivalents) [19]. It was different from the present research which demonstrated that FRAP capacity of different parts of kelakai ranged from 5.4 to 273.34 µg/ml. The highest antioxidant capacity by FRAP assay was shown by ethanolic root extract of kelakai which had the lowest $E_{50}$ of FRAP (5.8 µg/ml), whereas $E_{50}$ of FRAP capacity of ascorbic acid was 3.56 µg/ml.

It can be concluded that antioxidant potency of ascorbic acid was 1.5-fold potency of ethanolic root extract of kelakai by FRAP assay.

TPC, TFC, and TCC might have the antioxidant capacity [5]. Antioxidant activity can be related with the TPC [24,25]. Previous research by Chai et al. [12] revealed that TPC in methanolic extract of young frond and mature frond of kelakai were 9.415 and 25.2 g GAE/100 g, respectively. TPC in water extracts of young sterile frond, mature sterile frond, young fertile frond, and mature fertile frond were 4.258, 5.169, 4.168, and 1.878 g GAE/100 g, respectively [19]. It was similar to the present study which demonstrated that ethanolic YL extract of kelakai gave TPC 4.67 g GAE/100 g but different from ethanolic OL and root extracts of kelakai (13.83 and 24.22 g GAE/100 g, respectively).

TPC in ethanolic extract of mature frond of kelakai (50.35 g QE/100 g) was higher than young frond (20.566 g QE/100 g) [13]. It was contrary to the present study which exposed that TPC in ethanolic extract of YLs, OLs, and root extract of kelakai were 1.99, 2.63, and 3.55 g QE/100 g, respectively, meanwhile TCC in ethanolic extract of YLs, OLs, and root extracts of kelakai were 7.28, 17.03, and 7.61 g BE/100 g, respectively. The previous research stated that TPC in water extract of mature sterile frond of kelakai (5.805 g catechin equivalent (GCE)/100 g) was higher than TPC in water extract of young fertile frond, young sterile frond, and mature fertile frond of kelakai (5.721, 4.659, and 1.895 g CEE/100 g, respectively). Study by Chai et al. [19] also expressed total anthocyanin content. The water extract of young sterile frond of kelakai contained the highest total anthocyanin content which was 51.32 mg cyanidin-3-glucoside equivalent (C3GE/100 g) compared to mature sterile frond (2.56 mg C3GE/100 g), young fertile frond 92.67 mg C3GE/100 g), and mature fertile frond (2.67 mg C3GE/100 g), meanwhile TFC in kelakai leaves extract was 14.5 µg QE/ml [20].

Coefficient of Pearson correlation was significantly negative if $r ≤ 0.61$ and $≤ 0.61$ and significantly positive if $0.61 ≤ r ≤ 0.97$ [11]. The highest antioxidant activity was expressed by the lowest $IC_{50}$ of DPPH scavenging activity and $E_{50}$ of FRAP capacity. It means increasing in TPC, TFC, and TCC caused increasing in antioxidant activities, which was stated by lower $IC_{50}$ of DPPH scavenging activity and or $E_{50}$ of FRAP capacity. Therefore, the good correlation between TPC, TFC, and TCC with $IC_{50}$ of DPPH or $E_{50}$ of FRAP was significant and negative correlation [5]. The present study showed that TPC in kelakai root extract had significant and negative correlation with their $IC_{50}$ of DPPH ($r=0.818, p=0.01$) and $E_{50}$ of FRAP ($r=-0.839, p=0.01$). Previous research [19] assessed the correlation between TPC and percentage of radical scavenging activities, also with FRAP values which were presented in mM Fe$^{2+}$ equivalent. Therefore, the good correlation was a positive and significant correlation. It means increasing in TPC will increase the percentage of radical scavenging activities and FRAP values. The research demonstrated that TPC in water extract of young sterile frond, mature sterile frond, young fertile frond, and mature fertile frond extract of kelakai showed significant and positive correlation with their percentage of radical scavenging activities (R$^2=0.968$, p$<0.05$) and FRAP values (R$^2=0.960$, p$<0.05$). The present study also exhibited that $IC_{50}$ of DPPH of YLs and root extracts of kelakai was positive and significant with their $E_{50}$ of FRAP ($r=0.879; p=0.006$).

It means antioxidant activity of YLs and root extracts of kelakai (S. palustris) were linear in DPPH and FRAP assays.

Flavonoids, tannins, and phenolic acids are included in phenolic groups. Cinnamic acid has higher antioxidant than benzoic acid [26]. Ortho- and para hydroxyl substitution have stronger antioxidant capacity [27]. TPC in ethyl acetate OL extract of kelakai (OL2) 9.62 g GAE/100 g was lower than TPC in ethanolic OL extract (OL3) 13.83 g GAE/100 g, whereas $IC_{50}$ of DPPH of OL2 1.43 µg/ml was similar to $IC_{50}$ of DPPH of OL3 1.38 µg/ml. It can be suggested that OL3 contained many phenolic compounds which had low antioxidant activity and only little amount with high antioxidant activity, whereas OL2 consisted of many phenolic compounds which had high antioxidant activity.

Flavonoid compound will be included in phenolic groups which have OH in A ring and or B ring. The flavonoid aglycones had higher antioxidant activity than flavonoid glycosides. Flavonoid will show high antioxidant activity if has ortho di-OH at C-3’-C4’, OH at C-3, oxo function at C-4, and double bond at C-2 and C-3. The ortho di-OH position at C-3’-C-4’ had the highest influence to antioxidant activity of flavonoid [26]. Study by Chai et al. [13] reported that mature sterile frond of S. palustris contained kaempferol glycosides. In Fig. 2, it can be seen TPC in n-hexane root extract of kelakai (R01) 3.2 g QE/100 g was similar to TPC in ethanolic root extract (R03) 3.55 g QE/100 g, but R03 had higher antioxidant activity which stated by lower $IC_{50}$ of DPPH of R03 (0.8 µg/ml) than R01 (13.3 µg/ml). Flavonoids, which soluble in n-hexane, are high methoxylated flavonoid, high acetylated flavonoid, flavan, and flavanone. It can be suggested that many flavonoid compounds in R01 were the flavonoid which had low antioxidant activity, with OH at position in C-3’ only, or OH at C-4 only, or flavanon, or without OH function at C-4, or without free OH at C-3. Meanwhile, many flavonoid compounds in R03 were flavonoid with high antioxidant activity. The kaempferol glycoside is soluble in ethanol, but its antioxidant activity is lower than quercetin glycoside because kaempferol glycoside does not contain OH at the highest influence antioxidant activity in flavonoid which is ortho di-OH at C-3’-C-4’.

Carotenoid compounds have the antioxidant capacity by scavenging free radical [28] and more double bonds in carotenoid will give scavenging free radical activity. Beta carotene was used as standard because it has conjugation double bonds which have ability to scavenge free radicals [29]. TCC in n-hexane YL extract of kelakai (YL1) 10.32 g BE/100 g was lower than TCC in n-hexane root extract (R01) 38.49 g
BE/100 g; however, IC50 of DPPH of YL1 (12.63 µg/ml) was similar to IC50 of DPPH of RO1 (13.3 µg/ml). Increasing in lipophilicity of carotenoid would increase antioxidant activity which was revealed by lower IC50 of DPPH scavenging activity [30]. Research by Beutner et al. [31] reported that a carotenoid had ±7 double bonds would give higher scavenging radical activity. Based on the results above, it can be predicted that many carotenoid compounds in YL1 had more than 7 double bonds which had high antioxidant activity, whereas in RO1 had maximum 7 double bonds.

In FRAP assay, a compound will act as antioxidant if it has reduction potential lower than 0.77 V because reduction potential of Fe(III)/Fe(II) is 0.77 V. A sample will have high antioxidant activity in FRAP assay if it contain many compound with reduction potential lower than 0.77 V. TPC in n-hexane root extract of kelakai (RO1) 3.23 g QE/100 g was similar to TPC in ethanolic root extract (RO3) 3.55 g QE/100 g, while EC50 of FRAP of RO3 5.4 µg/ml which was categorized as very strong antioxidant, was lower than EC50 of FRAP of RO1 180.58 µg/ml as weak antioxidant. It can be predicted that many flavonoid compounds in RO3 have reduction potential lower than 0.77 V; therefore, it reduced Fe(III) to Fe(II) then the Fe(II) formed blue complex with TPTZ and gave a blue, meanwhile many flavonoid compounds in RO1 have reduction potential >0.77 V.

CONCLUSION

Different methods in parallel should be used to evaluate antioxidant activity of samples, due to the possibility of different results given by various methods. All different extracts of kelakai (S. palustris) parts were very strong antioxidant using DPPH assay. TPC in root extracts of kelakai had significantly negative correlation with their IC50 of DPPH scavenging activities and EC50 of FRAP capacities. Phenolic compounds in root extracts of kelakai were the major contributor in their antioxidant activity by DPPH and FRAP methods. There was linear correlation between IC50 of DPPH scavenging activities and EC50 of FRAP capacities in YL and root extracts of kelakai. YL, OL and root of kelakai (S. palustris) have many benefits to prevent oxidative stress and potential as sources of natural antioxidant for further exploitation.

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