ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Suppl. 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

ANTIOXIDANT ACTIVITIES FROM VARIOUS EXTRACTS OF DIFFERENT PARTS OF KELAKAI (*STENOCHLAENA PALUSTRIS*) GROWN IN CENTRAL KALIMANTAN - INDONESIA

SITI KUSMARDIYANI, GRACE NOVITA, IRDA FIDRIANNY*

Pharmaceutical Biology Research Group, School of Pharmacy, Bandung Institute of Technology, Indonesia.
Email: irdafidrianny@gmail.com

Received: 23 June 2016, Revised and Accepted: 11 July 2016

ABSTRACT

Objectives: The aims of this research were to determine antioxidant activity from various extracts of different parts of kelakai (*Stenochlaena palustris* [Burm.f.] Bedd) using two antioxidant testing methods, which were 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), and correlation of total phenolic contents (TPC), total flavonoid contents (TFC), and total carotenoid contents (TCC) with their inhibitory concentration 50% (IC_{50}) of DPPH and exhibitory concentration 50% (EC_{50}) of FRAP.

Methods: Sample was extracted by reflux using different polarity solvents. The extracts were evaporated using vacuum rotary evaporator. Antioxidant activities were tested using DPPH and FRAP assays, determination of TPC, TFC, and TCC was carried out by ultraviolet-visible spectrophotometry, and correlation with their IC_{50} of DPPH and EC_{50} of FRAP capacities was analyzed by Pearson's method.

Results: Ethanolic root extract of kelakai (*S. palustris*) had the lowest IC_{50} of DPPH scavenging activity 0.8 µg/ml and the lowest EC_{50} of FRAP capacity 5.4 µg/ml. Ethanolic kelakai root extract demonstrated the highest phenolic content, ethyl acetate young leaves extract had the highest flavonoid content, and the highest carotenoid content was given by n-hexane root extract. There was significantly negative correlation between TPC in root extract of kelakai with their IC_{50} of DPPH and EC_{50} of FRAP.

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.

 DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9s2.13630

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 glycosides 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 8435. 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 determined by calculating the percentage of reduction of DPPH absorbance [15]. Inhibitory concentration 50% (IC_{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_{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 seen 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 TFC. 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 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 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_{50} of DPPH scavenging activities and EC_{50}. IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities of each extract were compared to IC_{50} or EC_{50} ascorbic acid as standard. The lowest value of IC_{50} or EC_{50} means the highest antioxidant activity.

TPC in kelakai extracts

TPC among different part extracts of kelakai was represented in term of GAE using the standard curve equation $y=0.005x-0.016$, $R^2=0.987$. 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.

TFC in kelakai extracts

TFC among different part extracts of kelakai were exhibited in term of QE using the standard curve equation $y=0.006x+0.029$, $R^2=0.998$. TFC 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).

TCC in kelakai extracts

TCC in different part extracts of kelakai were demonstrated in term of beta carotene equivalent using the standard curve equation $y=0.012x-0.178$, $R^2=0.981$. 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_{50} of DPPH scavenging activities, EC_{50} of FRAP capacities

TPC in YLs and root extracts of kelakai had significant and negative correlation with their IC_{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_{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

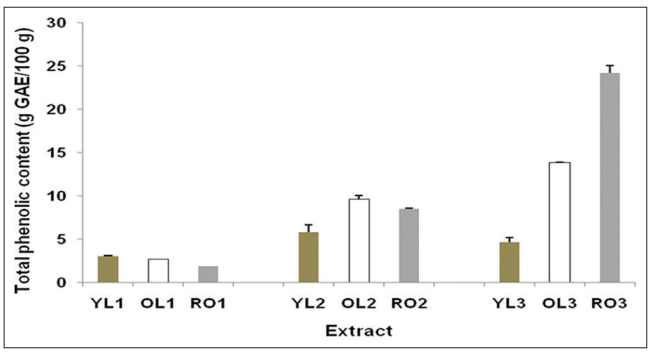

Fig.1: Total phenolic content in kelakai extracts, n=3

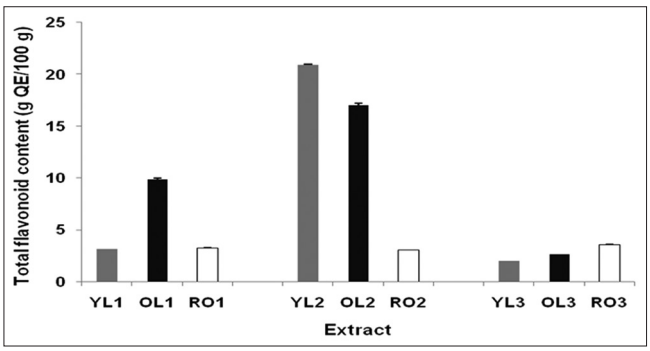

Fig. 2: Total flavonoid content in kelakai extracts, n=3

Table 1: Pearson's correlation coefficient of TPC, flavonoid, carotenoid content in different parts extracts of kelakai parts with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities

Antioxidant parameter	Pearson's correlation coefficient (r)					
	TPC	TFC	TCC	EC_{50} FRAP YL	EC_{50} FRAP OL	EC_{50} FRAP RO
IC_{50} DPPH YL	−0.695*	−0.992**	−0.991**	0.879**		
IC_{50} DPPH OL	0.858**	0.105 ns	−0.129 ns		−0.888**	
IC_{50} DPPH RO	−0.818**	−0.320 ns	0.980**			0.996**
EC_{50} FRAP YL	−0.328 ns	−0.863**	−0.916**			
EC_{50} FRAP OL	−0.991**	0.312 ns	0.527 ns			
EC_{50} FRAP RO	−0.839**	−0.345 ns	0.989**			

*Significant at p<0.05, **Significant at p<0.01. IC_{50} DPPH=IC_{50} DPPH scavenging activity, EC_{50} FRAP=EC_{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_{50}: Exhibitory concentration 50%, FRAP: Ferric reducing antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: Inhibitory concentration 50%

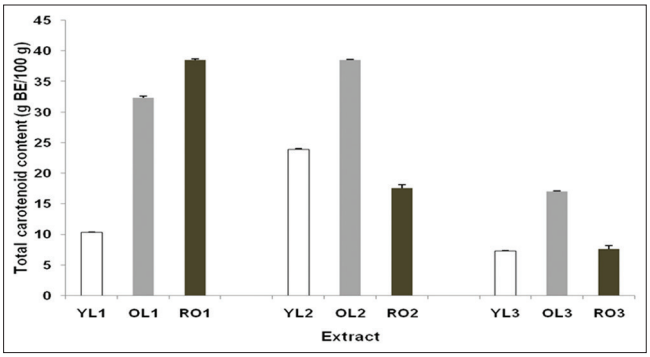

Fig. 3: Total carotenoid content in kelakai extracts, n=3

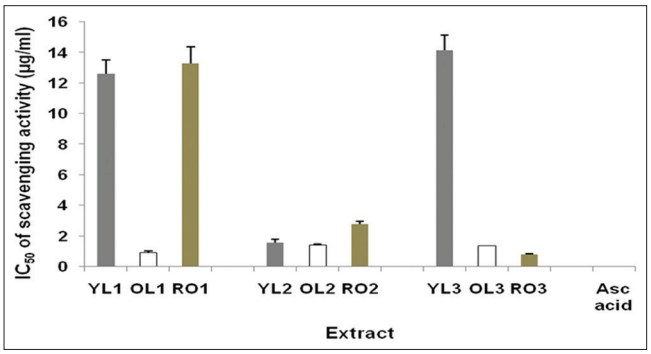

Fig. 4: Inhibitory concentration 50% of 2,2-diphenyl-1-picrylhydrazyl in different extracts of kelakai parts, n=3

pH 3.6. Reduction potential of Fe (III)/Fe (II) is 0.77 V. Antioxidant will reduce Fe (III) to Fe (II) if it has reduction potential lower than 0.77 V. Complex of Fe (II) - TPTZ shows blue and gave 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_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in different parts of kelakai can be seen in Figs. 4 and 5. The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in different parts extracts of kelakai were compared to IC_{50} or EC_{50} of ascorbic acid standard. The lowest value of IC_{50} or EC_{50} means the highest antioxidant activity. Sample, which had an IC_{50} or EC_{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_{50} or EC_{50} >150 µg/ml [14].

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

Moreover, antioxidant activities can be expressed by the percentage of DPPH scavenging activity, by reacting DPPH 50 µg/ml and sample 50 µg/ml. The result was compared to the percentage of DPPH scavenging activity of ascorbic acid, by adding DPPH 50 µg/ml and ascorbic acid 50 µg/ml. The value of the percentage of DPPH scavenging activity of ascorbic acid did not achieve 100% because there was still residual yellow in solution after giving hydrogen atom to DPPH by antioxidant in sample extract [22,23]. The percentage of DPPH scavenging activity could not present the real antioxidant activities. Antioxidant activity can be stated in percentage of DPPH scavenging activity, which was performed normally by adding extract with one concentration 50 µg/ml only to DPPH solution 50 µg/ml (volume 1:1). If extract 50 µg/ml can scavenge DPPH 50 µg/ml 57%, its not means extract 60 µg/ml will always scavenge DPPH more than 57%. The extract 60 µg/ml will present the percentage of DPPH may be >57% or lower than 57%. It is due to extract consisted

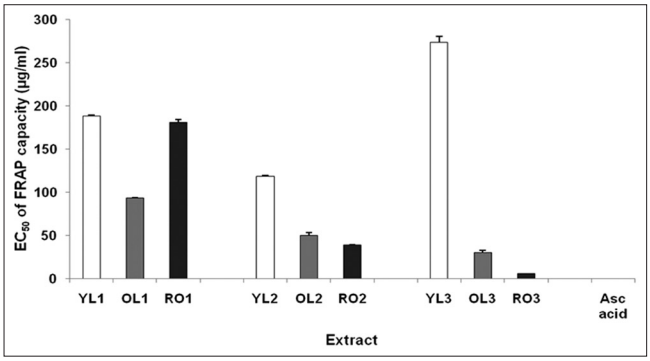

Fig. 5: Exhibitory concentration 50% of ferric reducing antioxidant power capacities in different extracts of kelakai parts, n=3

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 DPPH 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 DPPH lower than 57%. Antioxidant activity can be stated as IC_{50}. For calculating IC_{50} of DPPH should be used many concentrations of extract which shown linear decreasing in absorbance of DPPH. 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_{50} of DPPH can be calculated. Based on the explanation above, it can be seen that the percentage of DPPH scavenging activities will not represent the real antioxidant activities; however, the real of antioxidant activities will be exposed by IC_{50} of DPPH value.

Antioxidant capacities by FRAP assay were exposed by EC_{50} of FRAP capacity. EC_{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 EC_{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 [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 EC_{50} of FRAP (5.4 µg/ml), whereas EC_{50} of FRAP capacity of ascorbic acid was 3.36 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 Chear *et al*. [13] 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).

TFC in methanolic extract of mature frond of kelakai (50.356 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 TFC 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 TFC in water extract of mature sterile frond of kelakai (5.805 g catechin equivalent [CE]/100 g) was higher than TFC in water extract of young fertile frond, young sterile frond, and mature fertile frond of kelakai (5.721, 4.659, and 1.895 g CE/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 $-0.61 \leq r \leq -0.97$ and significantly positive if $0.61 \leq r \leq 0.97$ [11]. The highest antioxidant activity was expressed by the lowest IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity. It means increasing in TFC, TPC, and TCC caused increasing in antioxidant activities, which was stated by lower IC_{50} of DPPH scavenging activity and or EC_{50} of FRAP capacity. Therefore, the good correlation between TPC, TFC, and TCC with IC_{50} of DPPH or EC_{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 EC_{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 denoted 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 EC_{50} of FRAP ($r=0.879$; $r=0.996$, $p<0.01$, respectively). 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. Cinamic 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 Chear *et al*. [13] reported that mature sterile frond of *S. palustris* contained kaempferol glycosides. In Fig. 2, it can be seen TFC in n-hexane root extract of kelakai (RO1) 3.23 g QE/100 g was similar to TFC in ethanolic root extract (RO3) 3.55 g/QE/100 g, but RO3 had higher antioxidant activity which stated by lower IC_{50} of DPPH of RO3 (0.8 µg/ml) than RO1 (13.3 µg/ml). Flavonoids, which soluble in n-hexane, are high methoxylated flavonoid, high acetylated flavonoid, flavan, and flavanon. It can be suggested that many flavonoid compounds in RO1 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 oxo function at C-4, or without free OH at C-3. Meanwhile, many flavonoid compounds in RO3 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 higher 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 (RO1) 38.49 g

BE/100 g; however, IC_{50} of DPPH of YL1 (12.63 µg/ml) was similar to IC_{50} of DPPH of RO1 (13.3 µg/ml). Increasing in lipophilicity of carotenoid would increase antioxidant activity which was revealed by lower IC_{50} 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. TFC in n-hexane root extract of kelakai (RO1) 3.23 g QE/100 g was similar to TFC in ethanolic root extract (RO3) 3.55 g QE/100 g, while EC_{50} of FRAP of RO3 5.4 µg/ml which was categorized as very strong antioxidant, was lower than EC_{50} 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 IC_{50} of DPPH scavenging activities and EC_{50} 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 IC_{50} of DPPH scavenging activities and EC_{50} 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.

REFERENCES

1. Cheel J, Theoduloz C, Rodríguez J, Schmeda-Hirschmann G. Free radical scavengers and antioxidants from lemongrass (*Cymbopogon citratus* (dc.) Stapf.). J Agric Food Chem 2005;53(7):2511-7.
2. Zielinski AA, Haminiuk CW, Alberti A, Nogueira A, Demiate IM, Granato D. A comparative study of the phenolic compounds and the *in vitro* antioxidant activity of different Brazilian teas using multivariate statistical techniques. Food Res Int 2014;60:246-54.
3. Sebei K, Gnouma A, Herchi W, Sakouhi F, Boukhchina S. Lipids, proteins, phenolic composition, antioxidant and antibacterial activities of seeds of peanuts (*Arachis hypogaea* L) cultivated in Tunisia. Biol Res 2013;46(3):257-63.
4. Mokbel MS, Hashinaga F. Antibacterial and antioxidant activities of banana (*Musa*, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 2005;1(3):125-31.
5. 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.
6. Gramza-Michalowska A, Czlapka-Matyasik M. Evaluation of the antiradical potential of fruit and vegetable snacks. Acta Sci Pol Technol Aliment 2011;10(1):63-72.
7. Mongkolsilp S, Pongbupakit I, Sae-Lee N, Sitthithaworn 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.
8. Xu BJ, Chang SK. Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) As affected by processing methods. J Food Sci 2008;73(2):H19-27.
9. Mashkor IM. Phenolic content and antioxidant activity of fenugreek seeds extract. Int J Pharmacogn Phytochem Res 2014;6(4):841-4.
10. Fidrianny I, Windyaswari AS, Wirasutisna KR. Antioxidant capacities of various leaves extract from five colors varieties of sweet potatoes tubers using ABTS, DPPH assays and correlation with total flavonoid, phenolic, carotenoid content. Res J Med Plant 2013;7(3):130-40.
11. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compost Anal 2006;19(6):669-75.
12. Pellegrini N, Serafini M, Colombi B, Rio DD, Salvatore S. Total antioxidant capacity of plant food, beverages and oils consumed in Italy assessed by three different *in vitro* assays. J Am Coll Nutr 2003;133(9):2812-9.
13. Chear NJ, Khaw KY, Murugaiyah V, Lai CS. Cholinesterase inhibitory activity and chemical constituents of *Stenochlaena palustris* fronds at two different stages of maturity. J Food Drug Anal 2016;24:358-66.
14. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958;181(4617):1199-2000.
15. Bedawey AA. Characteristics of antioxidant isolated from some plants sources. Shibin El-Kom, Cairo: Shibin El-Kom; 2010. p. 1-11.
16. Benzi IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239(1):70-6.
17. 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.
18. 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;10:178-82.
19. Chai TT, Panirchellvum E, Ong HC, Wong FC. Phenolic contents and antioxidant properties of *Stenochlaena palustris*, an edible medicinal fern. Bot Stud 2012;53:439-46.
20. Suhartono E, Viani E, Rahmadhan MA, Gultom IS, Rakhman MF, Indrawardhana D. Total flavonoid and antioxidant activity of some selected medicinal plants in South Kalimantan of Indonesian. APCBEE Procedia 2012;4:235-9.
21. Li XC, Wang XZ, Chen DF, Chen SZ. Antioxidant activity and mechanism of protochatechuic acid *in vitro*. J Funct Food Health Dis 2011;1(7):232-44.
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):763-76.
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. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009;2(5):270-8.
25. Kondratyuk T, Pezzuto J. Natural product polyphenols of relevance to human health. Pharm Biol 2004;42(1):46-63.
26. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002;13(10):572-84.
27. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol 2003;41(6):753-8.
28. Foote CS. Free Radicals in Biology. 3rd ed. New York: Academic Press; 1976.
29. Charles DJ. Antioxidant properties of spices shells and other. London: John Willey; 2013.
30. Kobayashi M, Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. Biotech Lett 1999;21(4):265-9.
31. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. Methods Enzymol 2000;319:226-41.