The study of antioxidant capacity in various parts of *Areca catechu* L.

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Abstract

Areca catechu L. has traditionally been used as herbal medicines in many contries. Its shoot is edible. Its seed is chewed as areca quid and used as medicines. The seeds contain alkaloids, tannins, polyphenols, sugars and lipids. The seed has anthelmintic, antifungal, antibacterial, anti-inflammatory and antioxidant activities. The objectives of this study were to find the antioxidant activities in various ages of seeds and various plant parts of areca related to their % tannin and total phenols contents and to find out that which kind of solvent giving the highest antioxidant activities by TEAC method. Results showed that the water and methanol extracts of the seeds in various ages presented higher % tannin and total phenols than the other parts of areca tree extracts. The methanol extracts of the seeds in various ages gave higher antioxidant activities than the other parts (leaves, crownshafts, fruit shells (4 and 8 months), root and adventitious root) of areca tree extracts. The sequence of antioxidant activities of methanol seeds extracts from high to low were 4, 8, 6, 3, 2 and 1-month seed extract. The 82.05% antioxidant activities accessed from phenolic compounds in areca, and arecoline indicated no antioxidant activity.

Keywords: Antioxidant acivity, *Areca catechu* L., TEAC, Tannins, Total phenols

Introduction

Areca nut (*Areca catechu* L., Palmaceae) is one of popular traditional herbal medicines used in Thailand. The activities of areca seed are anthelmintic, antifungal, antibacterial, anti-inflammatory, antioxidant (Anjali & Rao, 1995), insecticide, and lavicidal. The areca seed extract could inhibit enzyme elastase (Lee & Choi, 1999), act as parasympathomimetic (Anwar et al., 2004) on muscarinic receptor and at high dose on nicotinic receptor, increase smooth muscle tone, dilate blood vessel, decreased blood pressure and increase secretion (saliva and sweat). The seed contains 50-60% sugars, 15% lipid (glyceride of lauric, myristic and oleic acid), 15% condensed tannins (phlobatannin, catechin), polyphenolics (NPF-86IA, NPF-86IB, NPF-86A and NPF-86B) (Reijiro et al., 1988) and 0.2-0.5% alkaloids (arecoline, arecaidine, guvacine and guvacoline).

Fresh seeds are used to heal foot sores. Dry seeds are used in animals as taenicide especially in horse. The chewing areca quid is older generations social lifestyle in lots of countries such as Thailand, Malaysia, China, India, Srilanka, Taiwan and so on. Areca quid causes strong dental health, reduces teeth decay and discolors teeth and mouth but it also implicates as carcinogen (Stich & Stich, 1982) that causes oral mucosa lesions (Avon, 2004) and oral cancer (Zain, 2001).

However, in Taiwan, a lot of young generations are chewing areca quid for stimulating (Lin et al., 2004) but the mechanism of psychostimulant effect of areca quid still not know. People in Taiwan believe that chewing areca quid made from young areca nut bring elixir of life. Although areca nut has incident of causing oral cancer, a lot of literatures showed that areca nut had strong

antioxidant activity (Ohsugi et al., 1999; Lee et al., 2003). This paradoxical truth may answer by the different ages of areca nut. This research would like to find out the benefit of other useless part of areca tree. Then the aims of this study were 1) to find the antioxidant activities in various ages of seeds and various plant parts of areca, and 2) to find out that which extracting solvents give extracts that have the highest antioxidant activities.

Materials and methods

Plant

The leaves, crownshafts (leaf sheaths), seeds (1, 2, 3, 4, 6, and 8-months), fruit shells (4 and 8-months) (fibrous skin of fruit or excocarp, mesocarp and endocarp), root and adventitious root of *Areca catechu* L. were collected from orchard at Nakhon-Pathom province by the researchers of Faculty of Pharmacy, Silpakorn University, in October of 2004. The voucher specimens were deposited in the Department of Pharmacognosy, Silpakorn University in Nakhon-Pathom, Thailand.

Chemicals

ABTS2- , 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate), was obtained as sulfonic acid from Sigma (St.Louis, USA). Trolox, (+/-)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%, was purchased from Aldrich(Steinheim, Germany). Arecoline hydrobromide and potassium permanganate ($KMnO_4$) was purchased from Sigma. Potassium persulfate was obtained from Asia Pacific Specialty Chemicals Limited. Sodium chloride, sodium carbonate, sulfuric acid and gelatin were purchased from Ajax Finechem (Seven Hills, Australia). Indigo carmine was purchased from Himedia. Folin-Ciocalteu reagent was purchased from CarLo Erba Reagenti (Milano, Italy). Absolute ethanol, methanol, methylene chloride, petroleum ether, ethyl acetate were purchased from Merck (Darmstadt, Germany). Bidistilled water was produced by our laboratory.

Preparation of extracts

Each of 1,000 g (10 mesh) powder of leaves, crownshafts, 1, 2, 3, 4, 6, and 8-month seeds, 4 and 8-month fruit shell, root and adventitious root of *Areca catechu* L. were macerated in sequence of 2,000 ml petroleum ether, methylene chloride, ethyl acetate, methanol and bidistilled water, respectively. They were shaken at room temperature for 1 day. Each extracts was evaporated under vacuum until dried. The % yield of each crude extract was calculated. All crude extracts were kept in refrigerator at 4^oC.

Quantitative of tannins (Kumazawa et al., 2002)

Crude seed extracts were dissolved in bidistilled water and then filtrated to get clear solution. The 10-20 ml clear solution (equal to tannins 0.01 g) was added to 25 ml indigo carmine solution [indigo carmine 1 g dissloved in 1 L acidic water, (50 ml concentrate H_2SO_4 dissloved and

adjusted to 1 L with water)] and then diluted this solution with 500-700 ml water to get clear solution. This solution was titrated with 0.04 N $KMnO_4$ solution till the solution became pale yellow (end point). The used volume in ml of $KMnO_4$ solution (A) was recorded.

The 60 ml of clear solution was added to 25 ml gelatin solution. Then filter aid was added, and the mixture was shaked for 15 min and filtrated. Filtrate 50 ml was added to 20 ml indigo carmine solution and then diluted this solution with 500-750 ml water. The solution was titrated with 0.04 N $KMnO_4$ solution untill the solution became pale yellow (end point). The used volume in ml of $KMnO_4$ solution (B) was recorded.

The amount of tannin that was oxidized with $KMnO_4$ was calculated from the equation as follow.

$$\% \text{ Tannin (as gallotannic acid)} = \frac{(A - B) \times 100 \times (\text{g tannin/ml } KMnO_4)}{\text{ml of sample solution}}$$

When A = Total tannin like materials, B = Non tannin materials, A - B = True tannins
Titer: 1 ml of 0.1 N $KMnO_4$ = 0.0042 g of tannin (as gallotannic acid)

Quantitative of total polyphenols (Kumazawa et al., 2002)

The 0.5 ml (10 μg/ml) crude extract solution was mixed with 0.5 ml Folin-Ciocalteu reagent and 0.5 ml 10 % Na_2CO_3. The mixture was throughly shaked and placed at room temperature for 1 hr. Then the absorbance was measured at 760 nm.

The calibration curve of polyphenol was prepared by using gallic acid as standard in concentration range 2-8 mg/L The total polyphenols was calculated in g of gallic acid/ 100 g of crude extract.

Preparation of $ABTS^{\cdot+}$ solution (Re et al., 1999)

An $ABTS^{\cdot+}$ solution was prepared by mixing equal volumn of 7 mM $ABTS^{2-}$ in water with 4.9 mM potassium persulfate in water. The solution was protected from light and stored at room temperature for 12-16 hr. $ABTS^{\cdot+}$ formation was checked its absorbance (A) at 734 nm. The absorbance of $ABTS^{\cdot+}$ was equilibrated to 0.7 ($\pm$0.02) by diluting with water at room temperature.

Preparation of calibration curve

Trolox was used as standard substance. The calibration curve in concentration range between 0-17.27 x 10^{-3} mg was prepared. The experiments were done in quadruplicate in each concentration.

For establishing calibration curve, standard solution (50 μl) was mixed with of $ABTS^{\cdot+}$ solution (3 ml). Absorbances were measured at 734 nm at 6 min, giving A trolox or A compound in equation (1). Absorbance of solvent was prepared by mixing absolute ethanol (50 μl) with $ABTS^{\cdot-}$ solution (3 ml) and monitored its absorbance at 6 min, giving A solvent.

Calculation of antioxidant capacity

The % inhibition was calculated as equation (1). The calibration curve was plotted between %inhibition at t = 6 min and concentration of trolox. The regression coefficient (r^2) was calculated from the curve.

$$\% \text{ inhibition T} = \frac{(\text{A solvent} - \text{A compound}) \times 100}{\text{A solvent}} \quad (1)$$

When % inhibition T = % inhibition of $ABTS^{\cdot+}$ absorbance that was inhibited by trolox (T).

Preparation of samples for measuring antioxidant (all extracts)

All experiments were done in quadruplicate in each concentration. All of these substances were diluted with absoluted ethanol. Their final concentration rage of samples per 50 μl are presented in Tables 1 and 2.

Determination the absorbances of samples

All experiments were done in quadruplicate. Sample solution 50 μl was mixed with $ABTS^{\cdot+}$ solution 3 ml. Absorbances were measured at 734 nm at 6 min, giving and A sample or A compound in equation (1). Absorbance of solvent was prepared by mixing absolute ethanol 50 μl with $ABTS^{\cdot+}$ solution 3 ml and monitored its absorbance at 6 min, giving A solvent.

The curve of each sample was plotted between % inhibition of absorbance at t = 6 min and concentration of each sample.

Calculation for TEAC

$$\text{TEAC} = \frac{\% \text{ inhibition of sample}}{\% \text{ inhibition of trolox}}$$

TEAC (trolox equivalent antioxidant capacity) is the ratio of % inhibition of sample to % inhibition of trolox at the same concentration of sample and trolox.

Apparatus

UV-vis spectrophotometer (Agilent 8453E UV-Visible Spectroscopy System) was used.

Results

The calculated % yields of extracts from dry weight of various part of areca plants in various solvents are shown in Table 3. The % yields of methanol extracts of seed in various ages of areca are shown in Table 4. The amount of areca extracts from methanol and water were higher than the other solvents. These indicated that substances in areca were quite polar, since a lot of polar compounds were dissloved by polar solvents. The 4-month seed gave the highest % yield of methanol and water extracts. The methanol and water extracts of seed also gave the higher % tannin and total phenol (calculated as gallic acid) than the other parts of plant extract (Table 5). From the

water extract of seed, the sequence of % tannins in extracts from high to low were 6, 1, 2, 3, 4 and 8-month seeds and the sequence of total phenol in extracts from high to low were 6, 4, 2, 8, 1 and 3-month seeds. From the methanol extract of seed, the sequence of % tannins in extracts from high to low were 8, 6, 1, 2, 3 and 4-month seeds and the sequence of total phenol in extracts from high to low were 8, 6, 4, 2, 3 and 1-month seeds.

Table 1 The concentration of various parts of areca plant extracts.

Type of samples and their extracted solvents	Concentration range (μg/50 μl)
Leaves	
Petroleum ether	0 – 400
Methylene chloride	0 – 50
Ethylacetate	0 – 400
Methanol	0 – 50
Water	0 – 400
Crownshafts	
Petroleum ether	0 – 2500
Methylene chloride	0 – 2500
Ethylacetate	0 – 200
Methanol	0 – 500
Water	0 – 4000
4-month seed	
Petroleum ether	0 – 500
Methylene chloride	0 – 500
Ethylacetate	0 – 250
Methanol	0 – 20
Water	0 – 50
8-month seed	
Petroleum ether	0 – 500
Methylene chloride	0 – 500
Ethylacetate	0 – 15
Methanol	0 – 20
Water	0 – 1
4-month fruit shell	
Petroleum ether	0 – 250
Methylene chloride	0 – 250
4-month fruit shell	0 – 200
Ethylacetate	0 – 500
Methanol	0 – 500
Water	
8-month fruit shell	0 – 250
Petroleum ether	0 – 500
Methylene chloride	0 – 500
Ethylacetate	0 – 2500
Methanol	0 – 2500
Water	
Root	0 – 50
Petroleum ether	0 – 50
Methylene chloride	0 – 50
Ethylacetate	0 – 50
Methanol	0 – 125
Water	
Adventitious root	0 – 250
Petroleum ether	0 – 250
Methylene chloride	0 – 500
Ethylacetate	0 – 50
Methanol	0 – 50
Water	

Table 2 The concentration of various ages of areca seed extracts.

Type of seed extracts (by Methanol) and arecoline	Concentraion range (μg/50 μl)
1-month seed	0 – 20
2-month seed	0 – 20
3-month seed	0 – 20
4-month seed	0 – 20
6-month seed	0 – 20
8-month seed	0 – 20
arecoline HBr	10 – 500

Table 3 The % yield of sample extracts.

Type of samples and their extracted solvents	% Yield of extracts
Leaves	
Petroleum ether	1.56
Methylene chloride	1.35
Ethylacetate	0.65
Methanol	6.69
Water	11.58
Crownshafts	
Petroleum ether	0.21
Methylene chloride	0.25
Ethylacetate	0.22
Methanol	0.62
Water	2.15
4-month seeds	
Petroleum ether	1.97
Methylene chloride	0.68
Ethylacetate	0.32
Methanol	23.41
Water	23.35
8-month seeds	
Petroleum ether	2.29
Methylene chloride	1.46
Ethylacetate	0.59
Methanol	13.77
Water	9.25
4-month fruit shells	
Petroleum ether	0.43

*calculated from dry weight

Table 4 The % yield of seed extracts.

Type of seed extracts (by methanol)	% Yield of extracts
1-month seeds	21.85
2-month seeds	20.59
3-month seeds	16.10
4-month seeds	23.41
6-month seeds	18.88
8-month seeds	13.77

Table 5 The % tannin and total phenols of various parts of areca plant extracts.

Type of extracts	% Tannin		Total phenols [g/100 g crude extract as gallic acid, (%SD)]	
	Water extract	Methanol extract	Water extract	Methanol extract
Crownshafts	0.90	0.16	1.59 (0.07)	1.43 (0.11)
1-month seeds	53.18	52.00	37.74 (2.54)	36.37 (1.61)
2-month seeds	48.20	51.99	38.91 (0.94)	39.15 (1.18)
3-month seeds	43.14	47.84	36.16 (1.20)	38.51 (1.46)
4-month seeds	42.41	47.72	42.06 (2.99)	45.12 (2.04)
6-month seeds	63.42	54.18	42.53 (6.80)	45.32 (2.06)
8-month seeds	31.56	62.70	38.59 (0.47)	50.76 (3.07)
4-month fruit shells	<0.01	0.35	1.17 (0.09)	1.08 (0.06)
8-month fruit shells	0.26	<0.01	1.60 (0.12)	1.42 (0.11)
Roots	11.52	19.41	6.25 (0.08)	21.56 (0.56)
Adventitious roots	3.33	8.93	4.15 (0.31)	11.43 (2.36)

The standard curve of % inhibition of trolox is shown in Figure 1. The slope, intercept and r^2 of % inhibition equations from TEAC method of all areca extracts are shown in Tables 6 and 7 and curves are shown in Figure 2. The slopes of all equations have $p< 0.01$. Most of their r^2 values are in 0.7500 - 0.9990. The IC_{50} values and antioxidant activities of trolox and various parts of all areca extracts are shown in Tables 8 and 9. The seed extracts from methanol showed higher antioxidant activities than the other part of plant from all solvents extracts (Table 8). The order of antioxidant activities of methanol extracts of areca seed from high to low were 4, 8, 6, 3, 2 and 1-month extracts. The % inhibition curve of various ages of seed extracts was presented in Figure 3. The methanol extract of 4-month seed had the highest antioxidant activity (Table 9). Its water extract had low antioxidant acitvity and its petroleum ether, ethylacetate extracts had very low antioxidant activities, while its methylene chloride extract had too low activity to calculate. In 8-month seed, its methanol extract had high antioxidant activity while its water extract had moderate activity and its ethylacetate extract had low activity. The petroleum ether and methylene chloride extracts of young seed had too low activity to calculate. For arecoline HBr, the alkaloid that contained in areca seed, its antioxidant activity was very low, which could not be calculated.

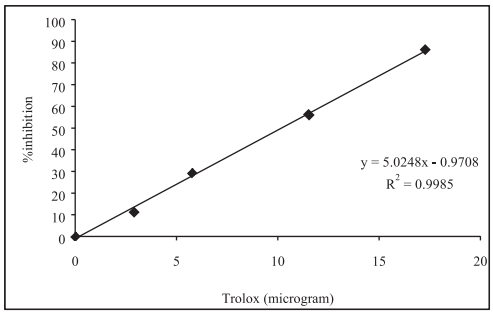

Figure 1 The standard % inhibition curve of trolox.

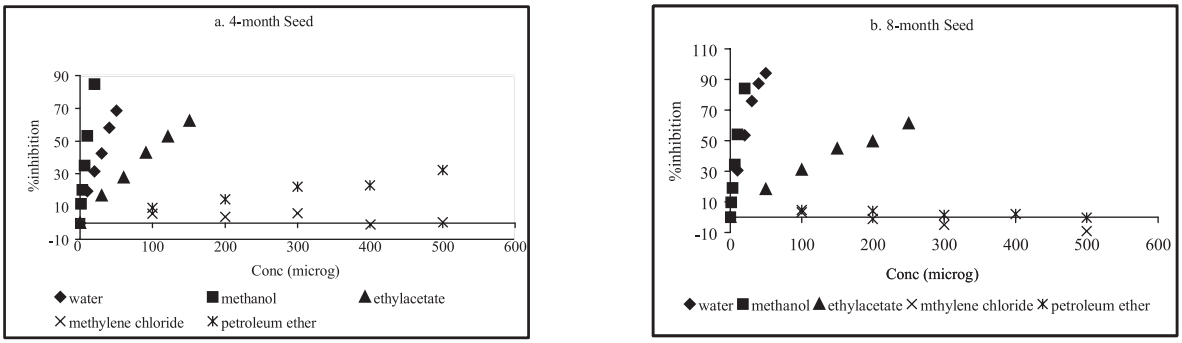

Figure 2 The % inhibition curve of the crude extracts obtained from a. 4-month seed and b. 8-month seed

All solvent extracts of leaf, crownshaft, 4-month fruit shell, 8-month fruit shell, root and adventitious root had very low % yield of extracts and antioxidant activities, except the methanol extract of root and water extract of adventitious root had moderate and low antioxidant activities, respectively.

Discussion

The methanol and water seed extracts had high percentage of tannins and total phenols. This is the reason why their methanol and water extracts had high to moderate antioxidant activities. From this study, the methanol extracts had higher antioxidant activities than water extract. This may due to the diversity and complexity of phenolic compounds in areca seed and methanol is a suitable solvent for extraction. Although the methanol extracts of 6 and 8-month seeds had % tannin and total phenols higher than those of 4-month seeds, the antioxidant activities of them were little lower than 4-month seed extract. This meant that % tannin and total phenols were not only factor of antioxidant activity. This could be confirmed by the correlation between TEAC (y) and total phenolic content (x) of areca which had a correlation coefficient of $r^2 = 0.8205$ (Figure 4). The r^2 suggested that 82.05 % of the antioxidant activity of areca accessed from phenolic compounds. However arecoline, the major alkaloid in areca seed, was not contributed to the antioxidant activity. The activities may also came from the other compounds that present in the seed, such as volatile oil and so on, in which caused 17.95 % of antioxidant activities. The percentage of tannins of root was higher than those of crownshafts, 4 and 8- month fruit shells and adventitious roots, then the methanol extract of roots showed moderate antioxidant activity, while the others had lower activities.

Table 6 The slope, intercept and r^2 of % inhibition equations from TEAC method of various parts of areca plant extracts.

Type of substances and extracts	Equations		
	slope [a]	intercept	r^2
Trolox	5.0248	-0.9708	0.9985
Leaves			
Petroleum ether	0.1143	6.8533	0.9120
Methylene chloride	0.2383	3.0028	0.8043
Ethylacetate	0.1098	4.7698	0.9494
Methanol	0.2836	3.0309	0.8653
Water	0.0609	-3.0673	0.9376
Crownshafts			
Petroleum ether	0.0743	2.4001	0.3155
Methylene chloride	0.1032	1.8755	0.5382
Ethylacetate	0.2724	2.5818	0.9813
Methanol	0.1156	4.3837	0.9685
Water	0.0192	9.3646	0.9385
4-month seeds			
Petroleum ether	0.0602	1.8104	0.9697
Methylene chloride	*	*	*
Ethylacetate	4.1355	2.593	0.9914
Methanol	4.1019	6.869	0.9776
Water	1.3418	3.1021	0.9921
8-month seeds			
Petroleum ether	*	*	*
Methylene chloride	*	*	*
8-month seeds			
Ethylacetate	0.2359	4.5084	0.9743
Methanol	4.1279	6.1069	0.9757
Water	1.8983	9.4706	0.9507
4-month fruit shells			
Petroleum ether	0.0512	1.2894	0.3155
Methylene chloride	*	*	*
Ethylacetate	0.038	3.8457	0.8847
Methanol	0.0201	7.0637	0.9406
Water	0.0155	4.5366	0.9457
8-month fruit shells			
Petroleum ether	0.1702	2.2845	0.7504
Methylene chloride	0.0536	3.0706	0.8565
Ethylacetate	0.0972	-1.7502	0.9487
Methanol	0.0709	-0.4363	0.9903
Water	0.0599	-1.1476	0.9703
Roots			
Petroleum ether	0.0764	3.1614	0.2466
Methylene chloride	0.0948	3.1707	0.3469
Ethylacetate	0.9973	4.0618	0.9773
Methanol	1.835	11.205	0.953
Water	0.3957	0.6218	0.9973
Adventitious roots			
Petroleum ether	0.0793	-0.2573	0.956
Methylene chloride	0.0322	-0.6055	0.8208
Ethylacetate	0.1412	4.285	0.9828
Methanol	0.4043	3.5267	0.8820
Water	1.1743	5.7329	0.9700

Note.

[a] The slope of all equations have P-value less than 0.01.

* The slopes, intercepts and r^2 cannot be calculated, since their % inhibition were too low.

Table 7 The slope, intercept and r^2 of % inhibition equations from TEAC method of seed extracts.

Type of seed extracts and arecoline	Equations		
	slope [a]	intercept	r^2
1-month seeds	3.6348	1.0175	0.9917
2-month seeds	3.6719	1.4838	0.9852
3-month seeds	3.9359	2.3602	0.9839
4-month seeds	4.1019	6.869	0.9776
6-month seeds	3.9281	4.4743	0.9777
8-month seeds	4.1279	6.1069	0.9757
arecoline HBr	-0.0006[b]	2.932	0.1711

Note.
[a] The slope of all equations have p-value less than 0.01.
[b] Its slope have *P*-value more than 0.05.

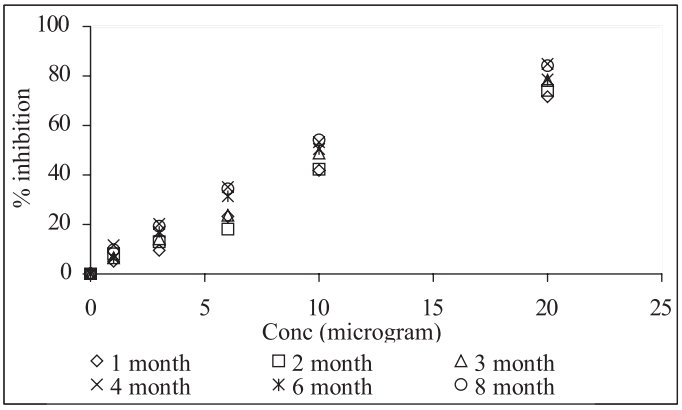

Figure 3 The % inhibition curve of the 1, 2, 3, 4, 6 and 8-month seed extracts.

Conclusions

Areca is the important plant in Asia both in cultural role and traditional medicine. Its nut is used as betel quid in many countries. Its seed is claimed as stimulant and taenicide. Many of incidents show that areca seed is carcinogen which cause buccal cancer. The carcinogenic effect comes from *N*-nitrosamine that occur during chewing areca seed. However, from this study shows that areca seed has strong radical scavengers activities that could be considered as natural antioxidant for medicinal uses, while the other parts of areca give low activities. Although, the incident of causing oral cancer must be kept in mind and used it with awareness.

Acknowledgements

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Table 8 The IC_{50} values and antioxidant activities of trolox and various parts of areca plant extracts.

Type of substances and extracts	IC_{50} (μg)	Antioxidant activity (equivalent to trolox) TEAC
Trolox	10.14385	1
Leaves		
Petroleum ether	377.4864	0.1623
Methylene chloride	197.2186	0.1093
Ethylacetate	411.9326	0.1191
Methanol	165.6174	0.1191
Water	871.3842	*
Crownshafts		
Petroleum ether	640.6447	0.0638
Methylene chloride	466.3227	0.0590
Ethylacetate	174.0756	0.1077
Methanol	394.6047	0.1124
Water	2116.4271	0.1939
4-month seeds		
Petroleum ether	800.4917	0.0490
Methylene chloride	*	*
Ethylacetate	114.6204	0.1366
Methanol	10.5149	0.9718
Water	34.9515	0.3352
8-month seeds		
Petroleum ether	*	*
Methylene chloride	*	*
Ethylacetate	192.8427	0.1394
Methanol	10.6333	0.9616
8-month seeds		
Water	21.3504	0.5774
4-month fruit shells		
Petroleum ether	951.3789	0.0366
Methylene chloride	*	*
Ethylacetate	1214.5868	0.0858
Methanol	2136.1343	0.1474
Water	2933.1226	0.0952
8-month fruit shells		
Petroleum ether	280.3496	0.0809
Methylene chloride	438.4855	0.0582
Ethylacetate	306.4518	0.0720
Methanol	711.3724	0.0055
Water	853.8831	0.0005
Roots		
Petroleum ether	613.0707	0.0797
Methylene chloride	493.9800	0.0836
Ethylacetate	46.0626	0.2848
Methanol	21.1417	0.5998
Water	124.7870	0.0929
Adventitious roots		
Petroleum ether	633.7617	0.0109
Methylene chloride	1571.5994	0.0004
Ethylacetate	323.7606	0.1156
Methanol	114.9476	0.1536
Water	37.6966	0.3546

Note.

*Their IC_{50} and TEAC values were too small.

Table 9 The IC_{50} values and antioxidant activities of various ages of seed extracts.

Type of seed extracts and arecoline	IC_{50} (μg)	Antioxidant activity (equivalent to trolox) TEAC
1-month seeds	13.47598	0.7583
2-month seeds	13.21283	0.7753
3-month seeds	12.10392	0.8466
4-month seeds	10.51488	0.9718
6-month seeds	12.05928	0.8879
8-month seeds	10.63328	0.9616
arecoline HBr	-78446.7	*

Note.
*Their IC_{50} and TEAC values were too small.

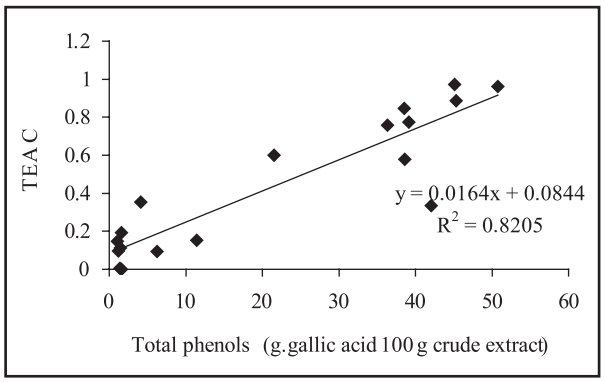

Figure 4 Linear correlation of Trolox equivalent antioxidant capacity (TEAC) versus total phenols of areca seed.

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