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Determination of Polyphenols Ingredients Present in Areca Husk and Its Antioxidant Activity

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Abstract This paper was developed for the simultaneous determination of betel nut shell in a variety of phenolic compounds by high performance capillary electrophoresis method, analyzed the separation effect of different concentrations and pH values of buffer on 10 analytes, finally determined the best electrolytic buffer was 0.1 mol/L, pH 9.0 borate buffer, UV detection wavelength 280 nm, separation voltage 20 kV. The method is simple and fast, within 20 min 10 kinds of phenolic materials can be completely separated, the detection limit is 0.5~4.5 mg/L. In addition, this article further determines of the betel nut shell polyphenol antioxidant activity, by using three evaluation of the antioxidant capacity of index, namely: the DPPH free radical scavenging ability, reduction ability as well as ABTS free radical scavenging ability.

Key words Areca husk; Capillary electrophoresis; Polyphenols; Antioxidant activity
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槟榔壳多酚组分及抗氧化活性的测定

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摘　要　建立测定槟榔壳中多种酚类物质的高效毛细管电泳方法，分析不同浓度和不同 pH 值硼酸缓冲液对 10 种标准品的分离效果，最后确定最佳缓冲液为 0.1 mol/L，pH 9.0 的硼酸缓冲液，紫外检测波长为 280 nm，分离电压为 20 kV。方法简便快速，能在 20 min 之内将 10 种酚类物质完全分离开，检测限为 0.5~4.5 mg/L。此外，进一步测定了槟榔壳多酚的抗氧化活性，选用 3 个评价抗氧化能力的指标，即：对 DPPH 自由基的清除能力、对还原能力以及对 ABTS 自由基的清除能力。

关键词　槟榔壳；毛细管电泳；多酚；抗氧化活性
中图分类号　TQ461　　**文献标识码**　A

1 Introduction

The research on the polyphenols of areca is made as there is an interest in its antioxidant activities and anti-aging effects[1-4]. Areca nut contains polysaccharides, minerals, crude fiber, alkaloids and some phenolics[5]. The phenolics in areca nut are catechin dimers, leucocyanidin dimers and leucopelargonidin[6-7]. The shoot of areca is edible, the seed is usually made into areca quid and *Areca catechu* L. in many countries have traditionally been used as herbal medicines.

Methods which described in many papers[8-10] to assay the content and ingredients of polyphenols in some samples based on liquid chromatographic technique, whereas capillary electrophoresis (CE) can be used as an alternative to High-Performance Liquid Chromatography (HPLC) in the determination of polyphenols. The method to determine phenolic compounds using CE technique have been developed by several authors[11-12], but none of them have determined polyphenols in areca husk. In this work, areca husks were first analyzed for their phenolic compounds. The main objective of this work was determining and separating the ingredients of polyphenols presented in areca husk in an optimum condition by CE method and evaluating the polyphenol antioxidant activities so as to provide a theoretical basis for betel nut industry.

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2 Experiments

2.1 Chemicals

Standard solutions were prepared in 18 mΩ ultra-purified water from a Millipore Milli-Q integral water purification system. A 0.15 mol/L H_3BO_3 solution at pH 9.0 was used as a buffer solution. The standard solutions of the following polyphenols were prepared: (+)-catechin, (-)-epicatechin, gallic acid, coumaric acid, chlorogenic acid, caffeic acid, rutin, keampferol and naringenin from Sigma Chemical Company. A stock standard solution of 1 000 mg/L was prepared in methanol and stored at 4 ℃ in dark conditions and working standard solutions were prepared by diluting the stock standard solutions with methanol. Solutions of 0.1 and 0.5 mol/L sodium hydroxide were used for conditioning the capillary and adjusting the pH of the buffer solution. The reagents which were used for detecting the antioxidant activity of areca husk extract as follow: 2, 2-diphenyl-1-picryl-hydrazil(DPPH), potassium ferricyanide, trichloroacetic acid, ferric chloride, butylated hydroxytoluene (BHT) and 3-ethylbenzothiazoline-6-sulfonic acid from Sigma Chemical Co.

2.2 Apparatus

A P/ACE MDQ capillary electrophoresis unit equipped with a UV detector and fused-silica capillary (50 μm in diameter and 57 cm in length) from Beckman Co. was used to separate and determine the ingredients of polyphenols presented in areca husk extract. The extraction of the polyphenols from areca husk was carried out by using ultrasound-assisted method and was performed in an ultrasonic cleaning bath. The extract solution was filtered through a vacuum filter and was c oncentrated by a rotavapor. Before the sample was analyzed by CE unit, it was filtered through the 0.45 μm filter membrane. The determination of total phenolic content and the antioxidant activity of areca husk extract was carried out by using an ultraviolet-visible spectrophotometer (UV-1 200).

2.3 Sample preparation

The areca fruits were bought locally from the research site of Coconut Research Institute of Chinese Academy of Tropical Agricultural Science. Firstly, the fruits were cut into several pieces and were dried through the drying cabinet at 50 ℃. The extraction of the polyphenols was carried out by using ultrasound-assisted method with 41%(*V/V*) ethanol to increase the solubility of the desired compounds and improve the mass transformation. The ratio of material to liquid was 1: 20 and the extraction was conducted at 54 ℃ for 38 min in ultrasonic cleaning bath and then cooled down slowly at room temperature. The solvent of the eluent was removed through the rotavapor at 40 ℃ after the elute solution filtered by a vacuum filter. The residue was dissolved again by methanol and transferred into a 50 mL flask and this solution was stocked as a crud sample. When detected the ingredients of samples by CE unit, the crud sample must be filtered with an organic filter membrane (0.45 μm) and then injected into the sample bottle of CE unit, but for the detection of antioxidant activity there was no necessary to filter with organic filter membrane.

2.4 The condition of CE

The operating condition of CE unit as follow: the running buffer was the solution of 0.1 mol/L H_3BO_3 and pH 9.0 (pH value was adjusted with sodium hydroxide). The applied voltage was 20 kV, the average current was 97.0 μA, the temperature was 20 ℃ and the selected wavelength was 280 nm. Sample was injected by hydrodynamic injection for 5 s. The separation was carried out from the positive to the negative electrode. In order to maintain the capillary under good working conditions, its surface was regenerated once a day by consecutive washing with methanol (5 min), followed by ultrapure water (5 min) and then prepared 0.5 mol/L sodium hydroxide (5 min) freshly, 0.1 mol/L sodium hydroxide (5 min) and finally fresh buffer (5 min). In order to optimize the migration time and the peak shape reproducibility, the capillary was flushed between analyses with 0.1 mol/L sodium hydroxide (3 min), ultrapure water (3 min) and fresh buffer (5 min). When used under the conditions mentioned above, the capillary showed good performance for about three months without losing its initial efficiency.

2.5 Determination of total phenolic content of areca husk extract

The total phenolic content of areca husk extract was determined with the Folin-Ciocalteu method [13]. Briefly, 0.1 mL methanol solution as a sample solution at different concentration was mixed with 2.0 mL of 2%(*W/V*) sodium carbonate and reacted for 2 min,

then added to 0.1 mL Folin-Ciocalteu reagent which freshly prepared in our laboratory. Finally, the solution was brought up to 10 mL by adding distilled water. After 30 min of reaction at ambient temperature, the absorbance was evaluated at 720 nm. Gallic acid was used as a standard solution; the total phenolic content of extract was expressed as a gallic acid equivalent value. Data were reported as *MEANS* ±*SD* for at least three replications.

2.6 Determination of DPPH radical scavenging activity of areca husk extracts

The free radical scavenging activity of areca husk extracts was measured by 2, 2-diphenyl-1-picryl-hydrazil (DPPH) according to the method adopted by Siddhuraju P[14], 0.1 mL methanol solution of sample at different concentration was mixed with 1.4 mL of DPPH solution (in 95% ethanol) and then added 95% ethanol to 3 mL and incubated at room temperature for 30 min in dark. DPPH solution alone prepared as the control. Sample blank was prepared by replacing the DPPH solution with 95% ethanol. The absorbance of the mixture was measured at 517 nm after incubation and the scavenging activity was calculated as follow:

$$Scavenging/\% = [1-(A_{sample}-A_{sample\ blank})/A_{control}]\times 100 \quad (1)$$

A_{sample}-The absorbance of the sample

$A_{sample\ blank}$-The absorbance of the sample blank

$A_{control}$-The absorbance of the control

2.7 Reducing power

The reducing power was determined according to the improved method adopted by Ylmazeram [15]. 0.2 mL of the different concentration of areca husk extracts was mixed with 1 mL of sodium phosphate buffer (0.2 mol/L, pH6.6) and 1 mL of 1% potassium ferricyanide, then the mixture was incubated at 50 ℃ for 20 min. After 1 mL of 10% trichloroacetic acid was added, the mixture was centrifuged at 4 000 r/min for 10 min. The upper layer (2 mL) was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride solution, and the absorbance was read at 700 nm in a UV spectrophotometer. The higher absorbance indicated a higher reducing power. BHT (butylated hydroxytoluene) was used as comparison.

2.8 Antioxidant activity by the 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation assay

The total antioxidant activity of areca husk extracts was measured by the ABTS$^+$ radical cation decolourization assay[16]. ABTS was dissolved in water to a 7 mmol/L concentration. ABTS radical cation (ABTS$^+$) was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate and allowing the mixture to remain in the dark at room temperature for 12-16 h. The reaction of ABTS$^+$ with antioxidants was determined spectrophotometrically at 734 nm.

Prior to assay, the ABTS$^+$ solution was diluted with ethanol (1 : 89 *V/V*) to reach a final absorbance of the control of (0.7±0.02) at 734 nm at 30 ℃. Stock solutions of areca husk extracts were prepared in methanol. After the addition of 3.0 mL of diluted ABTS$^+$ solution till up to 0.5 mL of antioxidant compounds (ultimate concentration 0.111~0.555 mg/mL), the mixture was taken to 30 ℃ exactly for 30 min after the initial mixing. Appropriate solvent blanks and ABTS$^+$ solution alone as the control were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition of the blank at 734 nm was calculated.

$$Scavenging/\% = [1-(A_{sample}-A_{sample\ blank})/A_{control}]\times 100 \quad (2)$$

A_{sample}-The absorbance of the sample

$A_{sample\ blank}$-The absorbance of the sample blank

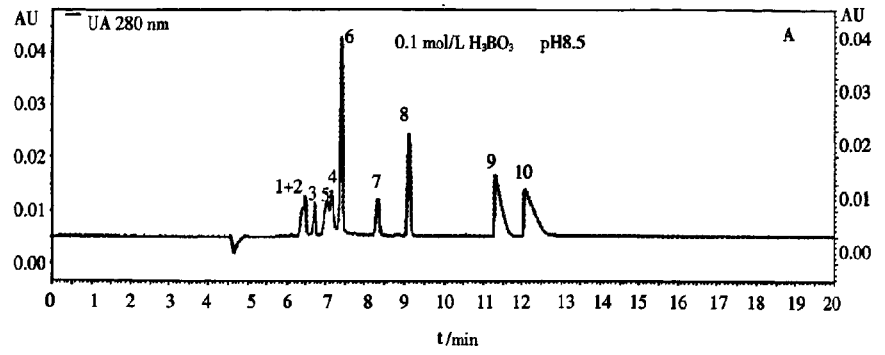
$A_{control}$-The absorbance of the control

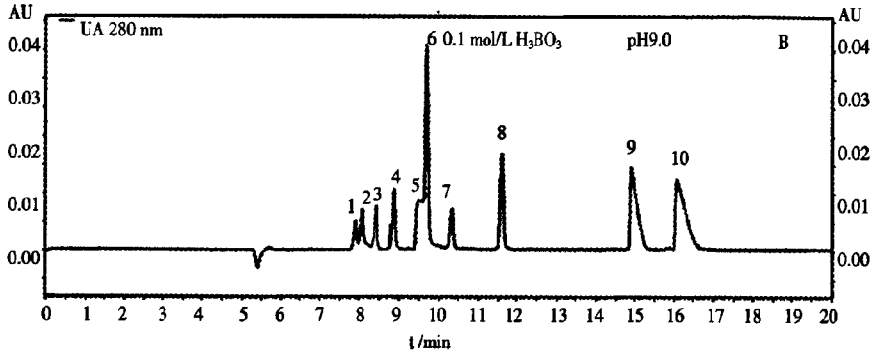
3 Results and discussion

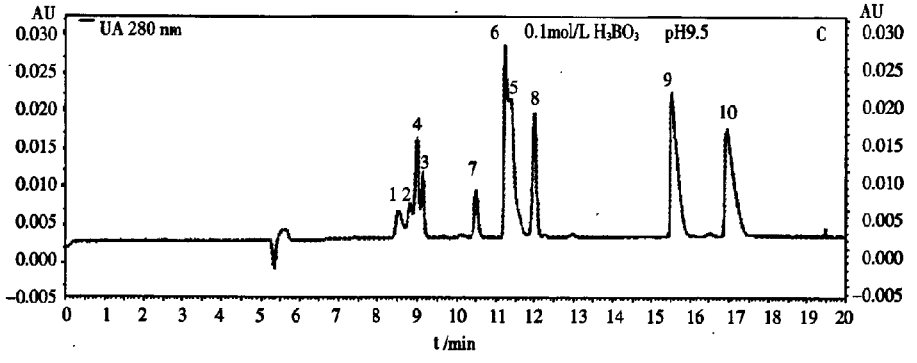
3.1 Separation conditions of polyphenol compounds

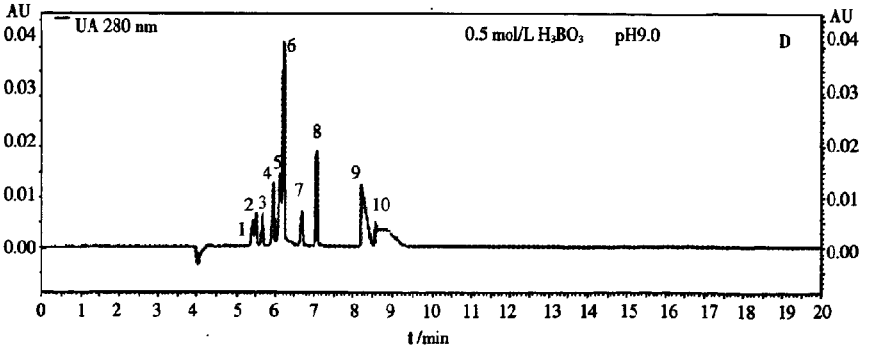
The CE method was mainly designed for the separation and quantification of the main polyphenols presented in *Areca catecgu* L., such as (-)-epicatechin, (+)-catechin, coumaric acid and chlorogenic acid, which were separated and could be identified and quantified using the appropriate standards. The following 10 analytes were chosen, such as (-)-epicatechin, (+)-catechin, keampferol, rutin, naringenin, ferulic acid, chlorogenic acid, coumaric acid, caffeic acid and gallic acid for optimization objective. The optimization of both chemical variables and instrumental variables were carried out.

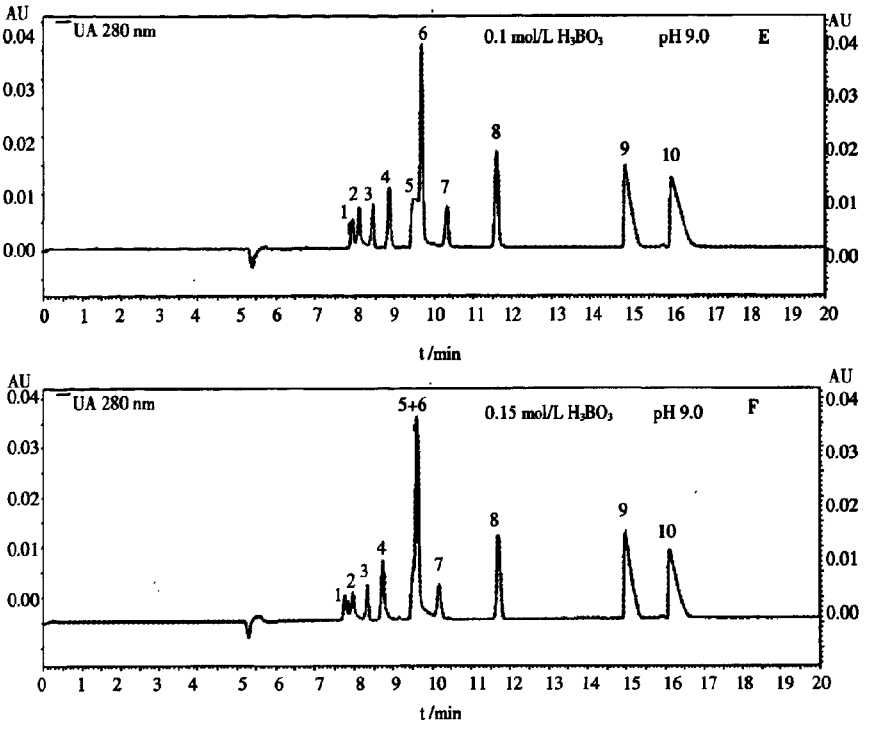
The separation effect of different pH (in the range of 8.5 to 9.5) of H_3BO_3 solutions at a certain concentration were analyzed [see Fig. 1 (A, B, C)], and different concentration (in the range of 0.05 ~0.15 mol/L) of solutions at a appropriate pH were also analyzed [see


AU
UA 280 nm
0.1 mol/L H_3BO_3　pH8.5
A
t/min


AU
UA 280 nm
0.1 mol/L H_3BO_3　pH9.0
B
t/min


AU
UA 280 nm
0.1mol/L H_3BO_3　pH9.5
C
t/min


AU
UA 280 nm
0.5 mol/L H_3BO_3　pH9.0
D
t/min



(A, B, C) is resulted from that the the separation effect of buffer at 9.0 pH is the best; (D, E, F) demonstrated that the the separation effect of 0.1 mol/L buffer is the best. Peaks of electropherogram of ten standard phenolic compounds as follow, 1=(-)epicatechin; 2=(+)catechin; 3=naringenin; 4= rutin; 5=keampferol; 6= ferulic acid; 7=chlorogenic acid; 8=coumaric acid; 9=caffeic acid; 10=gallic acid.

Fig. 1 Electrophorogram of 10 analytes

Fig.1 (D, E, F)]. Finally the separation effect of 0.1 mol/L H_3BO_3 solutions at pH 9.0 were the best (see Fig.1). The total time taken for the whole analysis was less than 20 min and the wavelength is 280 nm.

Finally, the instrumental variables were optimized. The voltage, temperature and wavelength were considered. By increasing the voltage (up to 30 kV) and the temperature, the time of migration was reduced, but high voltage may lead to significant loss of resolution and peak efficiencies, and high temperature may result in poorer sensitivity. The value of voltage and temperature finally chosen are 20 kV, 20 ℃. A UV detector was used to determine the maximum of absorbance of these compounds. All of them presented a maximum of absorbance at 280 nm.

3.2 The performance of CE method

The calibration graphs were produced from results obtained by injecting standard solutions in the range 100~300 μg/mL. Each point of the calibration graph corresponded to the mean value obtained from three independent area measurements. The limit of detection (LOD) was calculated from the blank value plus 3 times its standard deviation, whereas the limit of quantification (LOQ) was calculated from the blank value plus 10 times its standard deviation. The corresponding regression equation and other characteristic parameters for the determination of the phenolic compounds are shown in Table 1.

Twenty replicate analysis were performed on the standard solutions (100 μg/mL for each compound), in order to evaluate the precision of the method for every compound to be determined. In all cases, the value of the relative standard deviation for the absorbance was less than 6%.

The standard deviations of residuals and curve-fitting level were obtained by analysis of variance during the validation of the calibration model.

3.3 Analysis of areca husk extract

In order to avoid some components that we do not considered, the extraction of analytes was achieved by using ethanol solution (41%). Fig .2 shows the electrophorogram of the sample where the different peaks can

Table 1 Linear regression equation and limit of detection of 10 analytes

analytes	$y=ax+b$	r	R^2	*RSD*/% (*AU*)	*LOD*/(μg/mL)	*LOQ*/(μg/mL)
(−)epicatechin	$y=19\,793x-893.6$	0.997	0.995 4	3.0	4.415 2	14.72
(+)catechin	$y=20\,081x+10\,592$	0.999	0.997 5	6.0	3.301 1	11.00
naringenin	$y=18\,477x+7\,303$	0.997	0.993 2	5.2	3.038 6	10.13
rutin	$y=40\,238x-7\,575.9$	0.995	0.990 1	2.3	1.773 5	5.91
keampferol	$y=94\,464x+69\,596$	0.999	0.999 5	4.1	2.188 4	7.29
ferulic acid	$y=81\,491x+61\,595$	0.997	0.994 9	2.5	0.587 5	1.96
chlorogenic acid	$y=17\,957x+11\,186$	0.997	0.993 3	3.2	2.733 6	9.11
coumaric acid	$y=57\,524x+5.933\,3$	0.994	0.988 0	2.2	1.219 7	4.07
caffeic acid	$y=83\,988x+44\,708$	0.994	0.988 9	1.4	1.180 3	3.93
gallic acid	$y=108\,129x+25\,055$	0.997	0.994 9	2.5	1.566 2	5.22

Note: a=intercept; b=slope; r=correlation coefficient; R^2=curve-fitting level obtained by analysis of variance for the validation of the model; RSD=relative standard deviation; LOD=limit of detection; LOQ=limit of quantification; LOD and LOQ in μg/mL. (Buffer: 0.1 mol/L H3BO3 at pH 9.0, 20 kV; 20 ℃; 5 s pressure injection;280 nm.)

be clearly identified. The migration time obtained for the standard solutions and for the areca husk samples were different due to matrix effects. In order to identify these peaks the addition standard method was used.

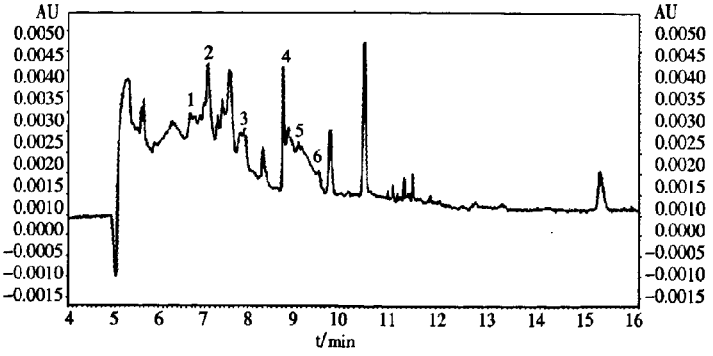
Spiked areca husk sample were prepared in order to evaluate the accuracy of the method. Excellent recoveries were obtained(see Table 2). In all cases, the T-test was applied for the slopes of the calibration graphs and showed no significative statistical differences. Consequently there is no evidence of systematic error affecting the determination of these analytes in areca husk by the proposed method. Recoveries and final concentrations found by using the standard addition method are shown in Table 2. Some polyphenolic compounds, such as gallic acid, caffeic acid, coumaric acid and keampferol are not found in the sample. In any case, the peaks corresponding to keampferol and ferulic acid almost overlapped.

3.4 The total phenol content of areca husk extract

The total phenol content of areca husk extract was expressed as the gallic acid equivalent value. The unit of total phenol content was defined as the concentration of gallic acid having the equivalent antioxidant activity expressed as areca husk extract solution. The standard curve of gallic acidis showed as Fig.3 According to the standard curve the total phenol content of areca husk extract was calculated 1.11 mg/mL.

3.5 DPPH radical scavenging activity

The f ree radical scavenging activity was determined by a stable radical 2, 2-diphenyl-1-picryl-hydrazil (DPPH). The reduction capability of DPPH induced by antioxidants was assayed by the decrease in absorbance at 517 nm. Fig.4 shows the free radical scavenging activity of the sample with BHT as control standard. For areca husk extracts and BHT, the DPPH radical scavenging activity increased with increasing



Peaks: 1= (-)-epicatechin; 2= (+)-catechin; 3= keampferol; 4= naringenin; 5= ferulic acid; 6=chlorogenic acid.

Fig. 2 Electrophorogram of the areca husk sample

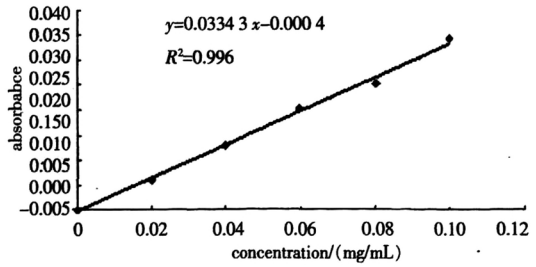
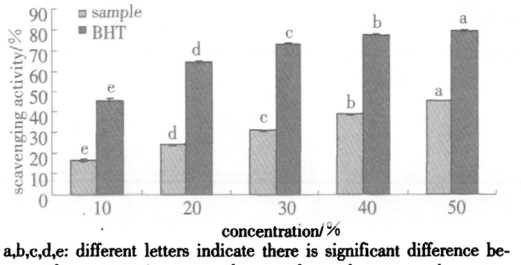


Fig. 3 Standard curve of gallic acid



a,b,c,d,e: different letters indicate there is significant difference between each concentration group, the same letters between each concentration indicate there is no significant difference. (the meaning of letters in the following graphs is the same as this.

Fig. 4 DPPH radical scavenging activity of areca husk extract and BHT

Table 2 The result of average recovery

analytes	concentration added/(μg/mL)	concentration measured/(μg/mL)	recovery/%
(-) epicatechin	100	109.47	109.5
	150	159.26	106.2
	200	191.15	95.5
(+) -catechin	100	104.19	104.2
	150	138.74	92.5
	200	212.96	101.0
keampferol	100	98.26	98.3
	150	147.22	98.1
	200	200.3	100.0
rutin	100	101.77	101.8
	150	151.3	101.0
	200	203.43	101.7
naringenin	100	106.54	106.5
	150	156.2	104.1
	200	190.26	95.1
ferulic acid	100	111.0	111.0
	150	143.43	95.6
	200	196.7	98.3
chlorogenic acid	100	113.4	113.4
	150	157.6	105.1
	200	213.47	106.7
coumaric acid	100	110.62	110.6
	150	152.9	101.9
	200	208.78	104.4
caffeic acid	100	92.24	92.2
	150	158.7	105.8
	200	194.6	97.3
gallic acid	100	87.0	87.0
	150	136.0	90.7
	200	203.0	101.5

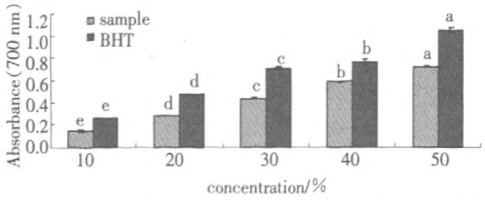


Fig. 5 reducing power of areca husk extract and BHT

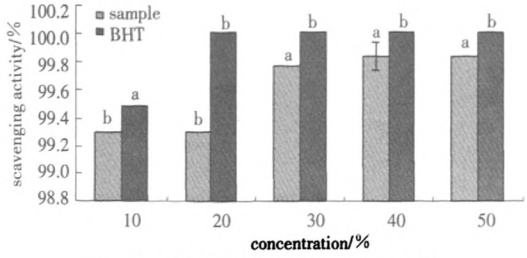


Fig. 6 ABTS radical scavenging activity of areca husk extract and BHT

concentration and there was significant difference between each concentration although the DPPH radical scavenging activities of areca husk extract were weaker than those of BHT.

3.6 Reducing power

The activity of antioxidant has been reported concomitant with the reducing power. Fig. 5 shows the reducing power of areca husk extracts with BHT as control. The higher absorbance indicates stronger reducing power. The reducing power of the areca husk extracts increased with increasing concentration and there was significant difference between each concentration. The areca husk extracts performed a high reducing power although weaker than that of BHT.

3.7 ABTS radical scavenging activity

The sample and BHT showed very high scavenging activity towards ABTS radical in a dose dependent manner. Even at a very low concentration of 10%, nearly 100% scavenging activity is observed and at the concentration of 20% BHT shows 100% scavenging activity. The areca husk extracts performed a weaker scavenging activity than that of BHT and there is no significant difference among concentrations of 30%, 40% and 50%(see Fig. 6).

4 Conclusions

CE is proved to be a suitable analytic separation technique for the identification and quantification of polyphenols in areca husk. The method developed allows polyphenols to be determined at low levels with detection limits between 0.5 ~4.5 mg/L. Moreover, it should be noted that the specific advantage of this method are based on the fact that pre-treatment of the sample is required, apart from filtration and a suitable dilution when it necessary. Linearity, recovery, precision and sensitivity are highly satisfactory. At the same time, through the experiment we also prove that there exist high antioxidant activities in the areca husk extracts because of its high total phenolic content.

References

[1] Wetwitayaklung P, Phaechamud T, Limmatvapirat C, *et al*. The study of antioxidant capacity in various parts of *Araca catechu* L[J]. Naresuan University Journal. 2006, 14(1): 1-14.

[2] Lei D, Chan C P, Wang Y J, *et al*. Antioxidative and antiplatelet effects of aqueous inflorescence Piper betel extract[J]. Agriculture and Food Chemistry Journal. 2003, 51(7): 2 083-2 088.

[3] Wang C K, Lee W H. Separation, characteristics and biological activities of phenolics in areca fruit[J]. Journal of Agriculture and Food Chemistry. 1996, 44(8): 2 014-2 019.

[4] Lee K K, Cho J J, Choi J D. Anti-elastase and anti-hyaluronidase of phenolic substance from *Areca catechu* as a new anti-aging agent[J]. International Journal of Cosmetic Science, 2001,23(6): 341-346.

[5] Mujumder A M, Kapadi A.H, Pendse G S. Chemistry and pharmacology of betel nut *Areca catechu* Linn[J]. Jounal of Plant. Crops 1979(7): 69-92.

[6] Mathew A G, Parpia H A B, Govindarajan V S. The nature of the complex proanthocyanidins[J]. Phytochemistry, 1969, 8(8): 1 543-1 547.

[7] Nonaka G, Hsu F, Nishioka I. Structures of dimeric, trimeric and tetrameric procyanidins from *Areca catechu* L[J]. Journal of the Chemical Society, Chemical Communications, 1981(15): 781-783.

[8] Wu Q L, Yang Y H, Simon J E. Qualitative and Quantitative HPLC/MS determination of proanthocyanidins in Areca Nut(*Areca catechu*)[J]. Chemistry & Biodiversity, 2007, 4(12): 2 817-2 826.

[9] He H M, He J. Determining tannic acid content of areca flower by reversed-phase HPLC[J]. Chinese Journal of Modern Applied Pharmacy, 1996, 13(1): 19-21.

[10] Alekseeva M A, Eller K I Arzamastsev A P. Determining polyphenolic components of common hop by reversed-phase HPLC[J]. Pharmaceutical Chemistry Journal, 2004, 38(12): 39-41.

[11] Lourdes A, María T T, Angel R, *et al*. Determination of trans－resveratrol and other polyphenols in wines by a continuous flow sample clean－up system followed by capillary electrophoresis separation[J]. Analytica Chimica Acta, 1998, 359(1): 27-38.

[12] Arce L, Ríos A, Valcárcel M. Determination of anti－carcinogenic polyphenols present in green tea using capillary electrophoresis coupled to a flow injection system[J]. Chromatography Journal, 1998, 827(1): 113-120.

[13] Yuan V Y, Bone D E, Carrington F. Antioxidant of activity of dulse (*Palmaria palmate*)extract evaluated in vitro [J]. Food Chemistry, 2005, 91(3): 485-494.

[14] Siddhuraju P. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *TamarIndus indica* seed coat[J]. LWT-Food Science and Technology, 2007, 40(6): 982-990.

[15] Ylmazer M, Stevens J F, Buhler D R. In vitro glucuronidation of xanthohumol, a favonoid in hop and beer, by rat and human liver microsomes[J]. FEBS Letters, 2001, 491(3): 252-256.

[16] Re R, Pellegrini N, Proteggente A, *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay[J]. Free Radical Biology and Medicine, 1999, 26(9/10): 1 231-1 237.

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