

Effect of Temperature and Duration Time on Polyphenols Extract of *Areca catechu* Linn. Seeds

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ABSTRACT

Objective: The study aimed to explore the effect of temperature and duration time on polyphenols content from extracts of areca seeds (*Areca catechu* Linn.). **Methods:** The extractions were performed at 3 different temperatures (60, 80 and 100 °C) and 4 different duration times (15, 30, 45 and 60 min). **Results:** It was found that temperature and duration time of extraction had effect on % yield (% w/w) of crude extract and the quantity (% w/w) of total polyphenols content by areca seed powder. The % yield of crude extract increased when temperature and duration time of extraction increased. The optimal extraction condition to obtain the highest amount of the crude extract (9.65 ± 0.35%) was at 100 °C for 45 min. However, the optimal condition of extraction for obtaining the highest % w/w total polyphenols content was at 80 °C for 45 min. The highest % w/w total polyphenols content equivalent to catechin was 2.76 ± 0.19%. But this condition did not give the highest %w/w yield (4.33 ± 0.26%). The optimal condition for the highest crude extract (% w/w) resulted in more impurities than did the optimal condition for the highest total polyphenols content. **Conclusion:** Temperature and duration time had effect on total polyphenols extraction. The optimal condition to achieve the highest % w/w total polyphenols content was 80 °C for 45 min.

Keywords: polyphenol extract, areca seed, temperature, duration, % yield, polyphenol content

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Introduction

Maag soeng, Thai's name of areca nut or betel nut, is one of traditional herbal medicines officially appeared in Thai herbal pharmacopoeia. Its scientific name is *Areca catechu* Linn. classified in family Palmae. The therapeutic categories of areca seed include foot sore healing (fresh seeds), anthelmintics for taeniasis and ascariasis in domestic animals, and anti-diarrhea (dry seeds).¹⁻³ The chemical constituents of seed are sugars, lipids (glycerides of lauric, myristic, and oleic acids), 0.2 - 0.5% alkaloids with a major alkaloid arecoline and other minor alkaloids including arecaidine, guvacine and guvacoline, and polyphenols such as flavonols ((+)-catechin and (-)-epicatechin) and condensed tannins (leucoanthocyanidins).^{2,3}

Concerning pharmacodynamic roles of areca nut, the seed extract acts as enzyme elastase inhibitor, parasympatho-mimetic on muscarinic receptor, and at high

dose on nicotinic receptor, smooth muscle tone enhancer, blood vessel dilator resulting in lowering blood pressure, enhancing secretion (saliva and sweat)², antioxidant⁴, antidepressant property via MAO-A inhibition⁵, enhancing the healing of burn wounds⁶, and reducing absorption of intestinal free cholesterol.⁷ Moreover, the extract can be used as anti-aging component for cosmetics. It improves skin hydration, skin elasticity and skin wrinkles.^{8,9} The most active pharma-ceutical effect comes from catechin and oligomer of catechins, which are the main components of polyphenols in areca seeds. In order to receive high yield of areca seed extract, it requires the right temperature and proper amount of time of the extraction process. The aim of this study was to examine the effect of temperature and duration time on polyphenols extraction from areca seed powder.

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Materials and Methods

Plant materials

Seeds of areca (*Areca catechu* Linn.) used in this study were dried crude drug purchased from Jao-Kom-Per Co., Ltd. (Thai medicinal herbs store, 229-321 Jakkawat road, Bangkok). The seeds were identified and verified with organoleptic testing. The seeds were washed 3 times with distilled water and dried at 40 - 50 °C in an oven. The clean and dried seeds were ground with the cutting mill SM 100 (Retsch®, Germany). The areca seed powder was stored at room temperature in dark and dry place.

Chemical and reagents

Catechin reference standard, which is (+)-catechin hydrate (minimum 98%, CAS 88191-48-4, C₁₅H₁₄O₆, FW 290.3), was obtained from SIGMA-ALDRICH (USA). Double-distilled water was used throughout the study.

Preparation of Folin-Ciocalteu reagent

Folin-Ciocalteu reagent was prepared by following processes. 100 g sodium tungstate and 25 g sodium molybdate were dissolved in 800 ml water, and 50 ml 85% phosphoric acid and 100 ml concentrated hydrochloric acid were added. The solution was refluxed for 10 hr, then added with 150 g lithium sulfate, 50 ml water and 4 - 6 drops of bromine. The solution stood for 2 hr then was refluxed for 15 min to eliminate excess bromine. The solution was cooled to room temperature, filtrated and adjusted to a volume of 1,000 ml with water. The reagent was clear bright yellow solution. It has been suggested that the reagent be stored tightly capped at cool temperature (about 4 °C). If the color becomes green, the solution is expired.¹⁰ All reagents and solvents used in this preparation were analytical grade.

Extraction of polyphenols from areca seed powder

One hundred grams of areca seed powder was extracted in 1000 ml of distilled water by reflux. The magnetic stirrer with heating (Heidolph® model MR 3001, Germany) was used for controlling extraction conditions. The extraction was performed at three different temperatures (60,

80 and 100 °C), four different duration times (15, 30, 45 and 60 min), and under continuous stirring at 300 rpm. Each of the extracts was filtrated by filter paper (Whatman no.1) with vacuum suction pump. The filtered samples were partitioned with dichloromethane in the ratio of 1:1 by volume. Then the water phase was collected and the impurities associated with dichloromethane were discarded. The partition with dichloromethane was repeated for 3 times. After that the water phase was partitioned with ethyl acetate (1:1 by volume), which was also repeated for 3 times. The ethyl acetate extracts were collected and evaporated to dryness with vacuum rotary evaporator (Buchi Rotavapor® model R-210, Switzerland) before analysis. Three replications of each extraction condition were performed in this study.

Preparation of catechin standard curve

Catechin reference standard (100 mg) was accurately weighed and dissolved in 1,000 ml volumetric flask with 1,000 ml distilled water. Catechin reference standard solution was pipetted for 5, 10, 15, 20 and 25 ml and transferred to 100 ml volumetric flask each, and adjusted to a volume of 100 ml with distilled water. The final catechin reference standard concentrations were 5, 10, 15, 20 and 25 µg/ml, respectively, used for setting up a standard curve.

Analysis of polyphenols content

The crude extract (1,000 mg) was accurately weighed and dissolved with 10 ml distilled water in 10 ml volumetric flask. One milliliter of the crude extract solution was transferred to 1,000 ml volumetric flask and the volume was adjusted to 1,000 ml with distilled water. Twenty milliliters of diluted crude extract solution were further transferred to 100 ml volumetric flask and the volume was adjusted to 100 ml with distilled water. The final concentration of the crude extract solution was 20 µg/ml.

Quantitative analysis

Quantitative analysis of catechin was determined by Folin-Ciocalteu method.¹¹ Aliquots of samples and reference standards were mixed with Folin-Ciocalteu reagent and 10% sodium bicarbonate (1:1:1 by volume). After sonication at

room temperature for 60 min, the absorbance of the reaction mixture was measured at 760 nm against a distilled water blank on UV/VIS spectrophotometer (JASCO[®] model V-530, Japan). The V-500 for Windows[®] connected to PC was used for data analysis. Each reference standard concentration was done in triplicate. The relation curve between concentrations and absorbance was plotted. The linearity was evaluated by regression analysis. Each sample was measured for absorbance in triplicates. The total polyphenols contents of each sample were calculated from the linear regression equation and expressed as catechin equivalent ($\mu\text{g/ml}$). The results were calculated as %w/w of total polyphenols content by areca seed powder.

Results and Discussion

Each sample of areca seed powder was extracted by reflux varying in temperatures and duration times. All extract solutions were partitioned with dichloromethane for 3 times and with ethyl acetate for 3 times, respectively. The ethyl acetate extract solution was then dried with vacuum rotary evaporator. All extraction experiments with dried ethyl acetate crude extracts resulted in a yield of pale brownish semi-solid mass with a mild characteristic odor. The %w/w yields of the crude extracts by areca seed powder from each experiment were shown in the second column of Table 1.

Table 1 Yield of crude extracts by areca seed powder (% w/w), and total polyphenols by areca seed powder (% w/w) in various extraction conditions.

Extraction condition	Yield of crude extracts (% w/w)	Total polyphenols (% w/w)
60 °C 15 min.	1.38 ± 0.26	1.18 ± 0.23
60 °C 30 min.	1.97 ± 0.17	1.69 ± 0.16
60 °C 45 min.	2.39 ± 0.28	1.73 ± 0.22
60 °C 60 min.	3.07 ± 0.27	1.76 ± 0.17
80 °C 15 min.	2.68 ± 0.28	1.83 ± 0.20
80 °C 30 min.	3.36 ± 0.16	2.20 ± 0.12
80 °C 45 min.	4.33 ± 0.26	2.76 ± 0.19*
80 °C 60 min.	4.28 ± 0.28	2.70 ± 0.19*
100 °C 15 min.	3.67 ± 0.07	1.86 ± 0.05
100 °C 30 min.	4.55 ± 0.25	2.00 ± 0.07
100 °C 45 min.	9.65 ± 0.35*	2.11 ± 0.11
100 °C 60 min.	9.38 ± 0.38*	2.28 ± 0.14

* Significant difference ($P < 0.05$) when compared with all other conditions.

Good linear relationship between catechin reference standard concentration and corresponding absorbance with coefficient of determination (r^2) of 0.9978 was evident (Figure 1). By UV/VIS spectrophotometer and linear regression equation, total polyphenols content in each extract was determined based on catechin reference standard. The total polyphenols contents were expressed as % mean \pm standard deviation (SD) (w/w) by the areca seed powder, as shown in the third column of Table 1.

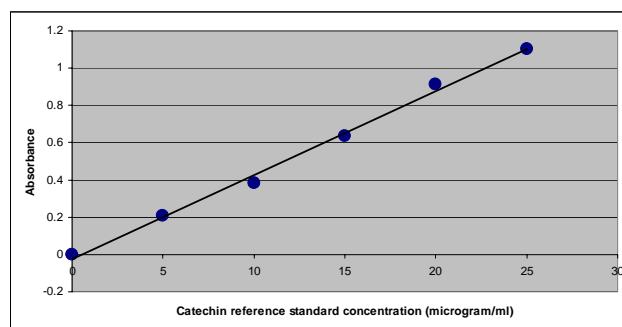


Figure 1 Calibration curve of catechin reference standard.

Note: Linear regression equation: $\text{Abs} = -0.0229 + 0.0450 \cdot \text{conc}$. Coefficient of determination (r^2) = 0.9978.

The temperature and duration time of extraction had a significant effect on the % w/w yield of crude extract by areca seed powder. The data shown in Table 1 indicated that the %w/w yield was increased relatively with temperature and duration time of extraction. The best condition of extraction was at 100 °C for 45 min. This condition gave the highest % w/w yield of the crude extract of areca seed powder ($9.65 \pm 0.35\%$) with significantly different ($P < 0.05$) from all other conditions. When duration time was increased to 60 min, a significant increase in % w/w yield was not found.

Crude extracts from each condition were investigated for % w/w total polyphenols content with Folin-Ciocalteu method and UV/VIS spectrophotometer. The % w/w total polyphenols content by areca seed powder was calculated and expressed as catechin equivalent ($\mu\text{g/ml}$) using the equation from the calibration curve shown in Figure 1. The data of % w/w total polyphenols content by powered areca seeds are shown in the third column of Table 1. We found that the best condition for the highest % w/w yield of crude extract did not give the highest total polyphenol content ($2.11 \pm 0.11\%$) by

powered areca seed. However, the best condition for the highest % w/w total polyphenols content ($2.76 \pm 0.19\%$) was 80°C in 45 min. This % w/w total polyphenols content was significantly different ($P < 0.05$) from other conditions, except the one from 80°C in 60 min. It indicated that the best condition for the highest % w/w yield did not give the highest total polyphenols content because the increased masses were from impurities other than polyphenols composition.

Although the 80°C 45 min condition did not give the highest yield of crude extract ($4.33 \pm 0.26\%$), it was the best condition to obtain the highest total polyphenols content ($2.76 \pm 0.19\%$) with impurities lower than that in the condition of 100°C and 45 min. The data indicated that some specific temperatures (60 and 80°C) with duration times of 15, 30, and 45 min, had certain effect on polyphenols extraction from areca seed powder. With temperature increased to 100°C and duration times of 45 and 60 min, we found that the % w/w yield of the crude extracts were higher (Table 1). But the total polyphenols content were not accordingly higher (Table 1). This suggested that impurities in the samples were increasingly extracted when temperature and duration time of extraction were increased.

Conclusions

The temperature and duration time of extraction had an effect on the amount of polyphenols and impurities extracted from areca seed powder. It could be concluded that the optimal condition offering the highest % w/w yield of the crude extract ($9.65 \pm 0.35\%$) was at 100°C in 45 min. The best condition for the highest % w/w total polyphenols content ($2.76 \pm 0.19\%$) from areca seed powder was at 80°C

for 45 min. However the highest % w/w yield of crude extract was not obtained in this condition.

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