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Chemical Compounds and Antioxidation Efficiency of *Livistona speciosa* Kurz. Seed Crude Extract

Prabnok J[1], Phasuk S[1], Takolpuckdee P[1]

[1] *Science Education Program, Facculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Klong Luang, Pathum Thani 12120, THAILAND*

Keywords: *Livistona speciosa* Kurz.; Antioxidant; Phenolic; Flavonoid; Tannin

Objectives: This research aimed 1) to study the amounts of total phenolic, total tannin and total flavonoid contents in *Livistona speciosa* Kurz. seed crude extracts, 2) to separate the chemical compounds of Livisosa speciosa Kurz. seed crude extracts via chromatography technique, and 3) to study the antioxidant efficiency of crude extract from *Livistona speciosa* Kurz. seeds.
Methods: *Livistona speciosa* Kurz. seed crude extract was obtained by maceration technique using ethanol as a solvent. The crude extract was analyzed by UV-Visble spectrophotometer for the total phenolic content, the total tannin content and the total flavonoid content. The chemical compounds from crude extract were separated by column chromatography. The antioxidant efficiency of *Livistona speciosa* Kurz. seed crude extract and its chemical compounds were tested using DPPH radical scavenging assay.
Results: The results showed that the total phenolic content was 2.35 mg of gallic acid/ 1g of sample, the total tannin content was 4.26 mg of tannic acid/ 1g of sample and the total flavonoid content was 39.27 mg of rutin/ 1g of sample. *Livistona* speciosa Kurz. seed crude extract was generally separated into 5 fractions. *Livistona* speciosa Kurz. seed crude extract showed antioxidant activity with EC_{50} as followed,13.50 x 10^{-2}, 2.59 x 10^{-2}, 17.73 x 10^{-2}, 4.09 x 10^{-2} and 28.43 x 10^{-2} mg/mL, respectively. While, EC_{50} of BHT was 0.83 mg/mL.
Conclusion: From this study, It was found that *Livistona* speciosa Kurz. seed crude extract contained the high amount of flavonoid contents. Moreover, the *Livistona* speciosa Kurz. seed crude extract and the separated compounds consisted good value for antioxidant activity. Therefore, the crude extract of *Livistona* speciosa Kurz. seed crude extract can be further developed for healthy product or cosmetics.

*Corresponding author: Science Education Program, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Klong Luang, Pathum Thani 12120, THAILAND ; Tel. +66(0)29093042; Fax. +66(0)29093041;
E-mail address: kungpop_1107@hotmail.com

Introduction

Livistona speciosa Kurz. was in the family of Aracaceae. This plant was a local herbal plant growing in Mountain area. *Livistona speciosa* Kurz. was also known as palm type with 25 metre in height. The leaves was fan-shaped with light green color. The ripe fruit was dark green color and the pulp can be used for cook. If was found that the core of the seed of *Livistona speciosa* Kurz. was red-purple which was known as Anthocyanin, a kind of flavonoid. Therefore, it could be assumed that *Livistona speciosa* Kurz. was an important source for antioxidants. Therefore, in this research, the anthors were focused on chemical compound and the efficiency of antioxidant of *Livistona speciosa* Kurz.

Figure 1 Kho (*Livistona speciosa* Kurz.)

Objectives

The aims of this reserch, the researchers were concentrated at 1) the study of the amounts of total phenolic, total tannin and total flavonoid contents in *Livistona speciosa* Kurz. seed crude extracts, 2) the separation of the chemical compounds of Livisosa speciosa Kurz. seed crude extracts via chromatography technique, and 3) the study of the antioxidant efficiency of crude extract from *Livistona speciosa* Kurz. seeds.

Methods
Fruits from Ton Kho and its crude extract preparation were prepared from the fresh *Livistona speciosa* Kurz. that was obtained from Phetchabun province, Thailand. Extraction of Ton Kho seeds were prepared by maceration method.

Crude extract preparation
1,000 g of *Livistona speciosa* Kurz. seed were accurately weighed and recorded. Then, it was transferred to a 2L tank and 95% ethanol was transferred to the tank and also used as the solvent for maceration. The ethanol solution was left to stand for 7 days. The macerated solution was then filtered and the solvent was removed to dryness by reduced pressure, and then stored at 5-10°C for further investigation. The % yields of the crude extracts was then calculated.

Total phenolic assay
Total phenol contents were confirmed and determined by Folin-Ciocalteau assay using gallic acid as the standard solution. Sample (10 mg of crude extract of *Livistona speciosa* Kurz. were dissolred with 99.9% ethanol and then the volume of the solution was adjusted to 5 mL) or standard solution (31.25, 62.50, 125.00, 250.00 and 500.00 mg/mL) 0.1 mL was added to a 25 mL test tube containing 8.4 mL of distilled water. Folin-Ciocalteau reagent 0.5 mL was added to the mixture and shaken for 5 min before adding 1 mL of 20%Na_2CO_3 solution. The mixture was shaken before incubated in the dark for 1 hour at room temperature. The absorbance was measured against the reagent blank at 760 nm.[1-2]

Total tannin assay
100 µL of each extracts with concentration of 1 mg/mL were mixed with 1,000 µL of 20% Na_2CO_3 then followed by leaving to stand for 3 min and then 500 µL of 50% Folin-Ciocalteau reagent were transferred to the mixture. After standing for 1 hour, the absorbance was measured at 760 nm using the UV-Visible spectrophotometer and total tannin contents were expressed and calculated as tannic acid equivalents. [1-2]

Total flavonoid assay
1 mL of 5 mg/mL crude extract solution of each species was transferred to a volumetric flask containing 4 mL Mill-Q water. At 0 min, 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3$ was then added. At 6 min, 2 mL of 1 M NaOH was transferred and the volume of volumetric flask was then adjusted to 10 mL with Mill-Q water. The absorbance was fixed at 510 nm using UV-visible spectrophotometer. The total flavonoid content was determined via calibration curve of rutin (0.05 to 1 mg/mL). Total flavonoid content was expressed and calculated as mg of rutin/g of sample. [1-2]

Chemical compounds analysis
Livistona speciosa Kurz. seed crude extract was characterized by UV-Visble spectrophotometry for total phenolic content, total tannin content and total flavonoid content .[3] The chemical compounds were separated by column chromatography using 40 g of *Livistona speciosa Kurz.* seed crude extract and 80 g of silica gel were dried and filled in 5 x 60 centimetre column with silica gel. Each 200 mL of mobile phase solution include hexane: acetone and acetone: methanol was sequentially added. Fractions were collected and identified by using TLC aluminium siliga gel (60 F_{254} Merck, layer thickness 0.25 mm) and grouped by focusing on the R_f value.

Antioxidant efficiency
The DPPH radical scavenging micro plate assay as explained was followed. Equal volumes of absolute ethanol solutions of the extract and 0.06 mM DPPH (2,2-diphenyl-1-picrylhydrazyl (Sigma, Germany) were mixed for 30 min, and absorbance measured at 517nm in UV-vis spectrophotometer. All samples were done and repeated with three times. The % scavenging activity of test samples was determined as follows:

$$\% \text{ Scavenging} = \frac{C-(A-B) \times 100}{C}$$

Where A, B and C represent the absorbance of DPPH in the reaction mixture, blank, and control, respectively. % Scavenging vs log concentration was plotted. By substituted y = 50, in the linear equation, the unknown value was identified. The antilog x was then converted to the EC_{50} (conc. of 50% scavenging) value. BHT and BHA were used as the reference standard.[4]

Results
Our experments showed that the phenolic contents from the crude extracts of *Livistona speciosa* Kurz. seed was 2.35 mg of gallic acid/ g extract, while the total tannin content was 4.26 mg of tannic acid/ g extract. In addition, the flavonoid content from *Livistona speciosa* Kurz. seed crude extract was 39.27 mg of rutin/g extract.

Table 1 Total phenolic, tannin and flavonoid contents of *Livistona speciosa Kurz*. seed crude extract.

Sample	Total Phenolic (mg of galic acid/ g extract)	Total Tannin (mg of tannic acid/ g extract)	Total Flavonoid (mg of rutin/ g extract)
Livistona speciosa Kurz. seed crude extract	2.35	4.26	39.27

Livistona speciosa Kurz. seed crude extract was separated by column chromatography and collected 130 fractions with 30 mL per fraction were collected. The fractions were combined to 5 parts based on R_f. The weights of part 1 to part 5 were 15.78, 5.63, 4.26,5.61 and 12.23 mg, respectively.

Table 2 Antioxidation efficiency of *Livistona speciosa* Kurz. seed crude extract.

Sample	concentration of sample (µg/mL)	% Scavenging	$EC_{50} \times 10^{-2}$ (mg/mL)
F1-74 (Group 1)	31.25	17.18	13.50
	62.50	35.14	
	125.00	62.73	
	250.00	85.26	
	500.00	93.53	
F75-80 (Group 2)	31.25	49.49	2.59
	62.50	65.58	
	125.00	82.10	
	250.00	90.08	
	500.00	95.85	
F81-88 (Group 3)	31.25	23.58	17.73
	62.50	33.81	
	125.00	50.95	
	250.00	74.40	
	500.00	93.47	
F89-99 (Group 4)	31.25	44.98	4.09
	62.50	50.46	
	125.00	80.84	
	250.00	92.15	
	500.00	93.97	
F100-130 (Group 5)	31.25	3.75	28.43
	62.50	9.44	
	125.00	19.84	
	250.00	51.05	
	500.00	87.31	

Livistona speciosa Kurz. seed crude extract.	31.25	14.97	12.91
	62.50	27.64	
	125.00	41.57	
	250.00	68.51	
	500.00	92.73	
BHT	31.25	62.68	0.83
	62.50	69.10	
	125.00	75.30	
	250.00	86.61	
	500.00	87.52	

The activity of antioxidant of *Livistona speciosa* Kurz. seed crude extract with 5 group via DPPH Radical Scavenging Assay was studied. The results found that *Livistona speciosa* Kurz. seed crude extract had antioxidant activity with EC_{50} = 12.91 x 10^{-2} mg/mL. The EC_{50} from fraction 1 to fraction 5 were 13.50 x 10^{-2} , 2.59 x 10^{-2}, 17.73 x 10^{-2} , 4.09 x 10^{-2} and 28.43 x 10^{-2} mg/mL, repectively while, the EC_{50} of standard BHT was EC_{50} = 0.83 x 10^{-2} mg/mL.

Discussion

Livistona speciosa Kurz. seed crude extract has ligh flavonoid content with 39.27 mg of rutin/ g extract. Moreover, it was also found that *Livistona speciosa* Kurz. seed crude extract has good antioxidant activity with EC_{50} = 12.91 x 10^{-2} mg/mL. Both high flavonoid content and good antioxidant activity *Livistona speciosa* Kurz. seed crude extract were obtained from the red-purple core inside the seed that consisted of anthocyanin (that can be generally found in some plant and fruit).[5-6] This compound can be used for human health. The results of this study were also confirmed that the higher flavonoid content, the ligher antioxidant activity can be detected.[3]

Conclusion

From this study, we found that *Livistona* speciosa Kurz. seed crude extract has ligh flavonoid content. In addition *Livistona* speciosa Kurz. seed crude extract and separated fraction consisted of good antioxidant activity. This, *Livistona* speciosa Kurz. seed crude extract has ability to pursue the product development for either health or cosmetic purposes.

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References

1. Lingkard, K, Singleton, V.L.Total phenal analysis:Automation and comparison with manual method, Am. J. Enol. Vitic.1977;28:49-55.
2. Liter, E. and Wilson, P.Measurement of total phenolics and ABTS assay for antioxidant activity,Crop Research Institute,Lincoln, New Zealand;2001
3. Chanudom, L.; Bhoopong,P.; Khwanchuea,R. & Tangpong, J. Antioxidant and antimicrobial activities of aqueous & ethanol crude extracts of 13 Thai traditional plants. Int.J.Curr.Microbiol.App.Sci.2014;3(1): 549-558.
4. Potduang,B.,Chongsiriroeg, C.,Benmart, Y.,Giwanon, G., Supatanakul, W. and Tanpanich, S.Biological activities of Schefflera leucantha. Afr.J.Trad.CAM.2007;2007:4(2):157-164
5. Ratthanatham, P, Laohakunjit, N. and Kerdchoechuen, O.Phenolic Compound, Anthocyanin and Antioxidant Activity of Germinated Colored Rice Agricultural Sci. J. 44(2)(Suppl.): 441-444 (2013)
6. Lee, Y.R., Woo, K.S., Kim, K.J., Son, J.R., Jeong, H.S., Antioxidant Activities of Ethanol Extracts from Germinated Specialty Rough Rice,Food Science and Biotechnology.2007;16: 765–770.