TECHNICAL REPORT

STUDY CHEMICAL PROPERTIES OF RATTAN SHOOT FROM PLANTATION IN THAILAND

by

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Abstract

Edible rattan shoot was investigated for the total polyphenol content, total antioxidant activity, and chemical composition of polyphenols. Total polyphenol content was estimated with the Folin-Ciocalteau assay and % total polyphenols was 0.0540. Total antioxidant activity of the extract of rattan shoot was assessed by scavenging of the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical. The efficient concentration (EC_{50} value) of the ethanol extract containing lipid was 0.147 mg/ml and the EC_{50} value of the ethanol extract without lipid was 0.104 mg/ml. The EC_{50} value of the polyphenols isolated from the methanol extract of rattan shoot which was soaked in brine for one year was 0.588 mg/ml and 0.371 mg/ml for fresh rattan shoot. In consideration of the beneficial nutritional, physiological and health promoting effects of polyphenols rattan shoot extract could be regarded as potent antioxidant and radical scavenging active and functional food ingredient or dietary food supplement.

Keyword: Edible rattan shoot, Total polyphenols, Antioxidant activity, DPPH assay, Folin-Ciocalteau assay.

Introduction

Fresh edible rattan shoot is a traditional vegetable of North-Eastern part of Thailand and one of the most important components of the North-Isan diet. The rattan shoot which is used as vegetable is the soft edible part of the shoot after removing the hard covering part. The young rattan shoot is mostly used to make a curry called "Kang Om Vai". The study on rattan nutritive value for edible shoot has been reported (Planklamyong, 2002).

It is well known that polyphenols are responsible for the potential antioxidant activity and radical scavenging capacity of plant food. This is now increasing evidence to suggest that many age-related human diseases such as heart disease, cancer, immune system decline are the result of cellular damage by free radicals and antioxidants in nature, especially those that are present naturally in human diets could play an important role in such disease prevention (Kris-Etherton et.al., 2002).

In this study a first approach to the efficiency of polyphenols extraction from rattan shoot was attempted. The aim of this study is to investigate edible rattan shoot as a potential source of natural polyphenols for use as dietary or food antioxidants. Research in the total polyphenol content, antioxidant activity, and chemical compositions of rattan shoot

will help people to realize the useful nutrients from having it in their meals. It could be another choice of healthy food.

Materials and Methods

Plant materials

1. Fresh rattan shoot was gathered from Sakon Nakhon province. Collected plant material was cut and dried in hot air oven at 40 ºC and ground to a fine powder.

2. Rattan shoot in brine was kept in bottle for one year, drained, washed, cut and dried in hot air oven at 40 ºC and ground to a fine powder.

Chemicals and Reagents

All chemicals and reagents were analytical grade.

Spectrophotometer

The UV/visible spectra were obtained on a UV/visible spectrophotometer, Perkin Elmer Lambda 35 at Department of Chemistry, Kasetsart University, Bangkok, Thailand.

Methods

1. Preparation of ethanol extracts

The fine powder (4-8 g) of dried rattan shoot was extracted with ethanol in appropriate volume using soxhlet extraction apparatus. After extraction and evaporation of ethanol to dryness, the extracts were dissolved in water and lyophilized and kept refrigerated before antioxidant activity assay.

2. Preparation of methanol extracts

2.1 By marceration in methanol for a week

The fine powder (10-20 g) of dried rattan shoot was marcerated in methanol (1g/60 ml) for a week. The extract was filtered and evaporated under reduced pressure to dryness to give crude methanol extract.

2.2 By microwave- assisted extraction

The fine powder of dried rattan shoot (5-10 g) was marcerated in methanol (1g/20 ml) for 90 min. After that the sample was irradiated with microwaves for 30 s,the sample was cooled at room temperature and irradiated with microwaves for another 30 s.The procedure was repeated until the time for microwaves irradiation was 10 min. The extract was filtered and evaporated in vacuo to dryness to give crude methanol extract.

3. Isolation of polyphenols

3.1 From methanol extract

The methanol extract was dissolved with hot water in a ratio 1 g of the extract to 10 ml of hot water. Poured into separatory funnel and extracted with hexane (3 x 15 ml). The aqueous layer was extracted with ethyl acetate (3 x 20 ml). The hexane and ethyl acetate layers were kept for testing of other compounds. The aqueous layer was lyophilized and kept in the dark at 8^{o}C until tested and separation.

3.2 From ethanol extract

The ethanol extract was dissolved in hot water in a ratio 1 g of the extract to 10 ml of hot water and extracted with hexane (3 x 15 ml) to remove lipid. The aqueous layer was lyophilized and kept refrigerated until used for antioxidant activity assay.

4. Separation of polyphenols

Separation of polyphenols from methanol extract was conducted on a 70cm long x 2.5 cm i.d. Sephadex LH-20 column using methanol as eluent. The phenolic compounds were lyophilized and used for antioxidant activity assay.

5. Radical Scavenging Activity Assays

The free radical scavenging activity assay was conducted using the DPPH radical scavenging method (Molyneux.P.2004).All samples of the tested polyphenols were diluted with methanol. The bleaching of the DPPH from purple to yellow indicates amounts of the free radical after it was reduced by the antioxidants. For the analysis, 3 ml of various concentrations of the tested samples in methanol were added to 4 ml of a 0.004 % methanol solution of the DPPH. Using UV-spectrophotometer, the absorbance was read at λ = 515 nm after a 30 minutes incubation period at room temperature.

6. Total polyphenols by Folin-Ciocalteau assay

1. Standard calibration curve

The amount (%) of total polyphenols was estimated by standard calibration curve of tannic acid.

Tannic acid stock solution was prepared by dissolving 10 mg of dried tannic acid in distilled water and made up solution to 100 ml. To each tube of 1 ml of Folin-Ciocalteau phenol reagent and 5 ml of 20% Na_2CO_3 the aliquot of tannic acid stock solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml was added respectively and made up each solution to 50 ml with distilled water. The absorbance was read at 735 nm after a 20 min. incubation period at room temperature.

2. Folin-Ciocalteau assay for total polyphenol content

The fine powder of rattan shoot (4-7 g) was extracted with ethanol by soxhlet extraction apparatus. The ethanol extract was dissolved in 100 ml distilled water and diluted to 10 folds. Added 1 ml of this solution to 1 ml of Folin-Ciocalteau phenol reagent and 5 ml of 20% Na_2CO_3, then added distilled water to 50 ml. The absorbance was read at 735 nm after a 20 min. incubation period at room temperature.

Results and Discussion

Total polyphenol content and antioxidant activity assay from ethanol extracts.

The result of total polyphenol content of rattan shoot from ethanol extract using soxhlet extraction method by Folin-Ciocalteau assay was shown in Table 1 and percent yield of total polyphenols was about 0.054 and percent yield of the ethanol extracts were shown in Table 2. The yield of the crude extracts was about 31.7 %.

Table 1 *Total polyphenol content in rattan shoot from ethanol extract by Folin-Ciocalteau assay*

Test	Weight of rattan shoot (g)	%Total polyphenols
1	5.30	0.054
2	4.33	0.052
3	6.77	0.056
4	6.77	0.052
average	5.79	0.054

Table 2 *Yield of the ethanol extraction of rattan shoot via soxhlet extraction method*

Test	Weight of sample (g)	Weight of extract (g)	% yield
1	7.46	2.34	31.4
2	7.47	2.40	32.1
average	7.47	2.37	31.7

The crude ethanol extracts were subjected to the DPPH radical scavenging activity assay. Table 3 showed the concentration and the absorbance of sample 1 and 2. Figure 1 and 2 showed the relation ships

between the absorbances and concentrations of sample 1 and 2, respectively. The quantity of polyphenols caused 50 % loss of the DPPH activity, (EC_{50} value) of sample 1 and 2 were shown in Table 4. The EC_{50} was about 0.147 mg/ml.

Table 3 *Absorbance of sample 1 and 2 before extracting lipids at λ = 515 nm*

Concentration (ppm)	Sample 1 A (λ = 515.47 nm)	Sample2 A (λ = 515.34 nm)
0	0.7138	0.97414
100	0.30278	0.49974
200	0.11529	0.36431
300	0.046788	0.13001
400	0.036508	0.0539
500	0.034347	0.047496
600	0.03164	0.047924
700	-	0.046436
800	0.034202	0.049637
900	0.033757	0.048242
1000	0.036724	0.05158

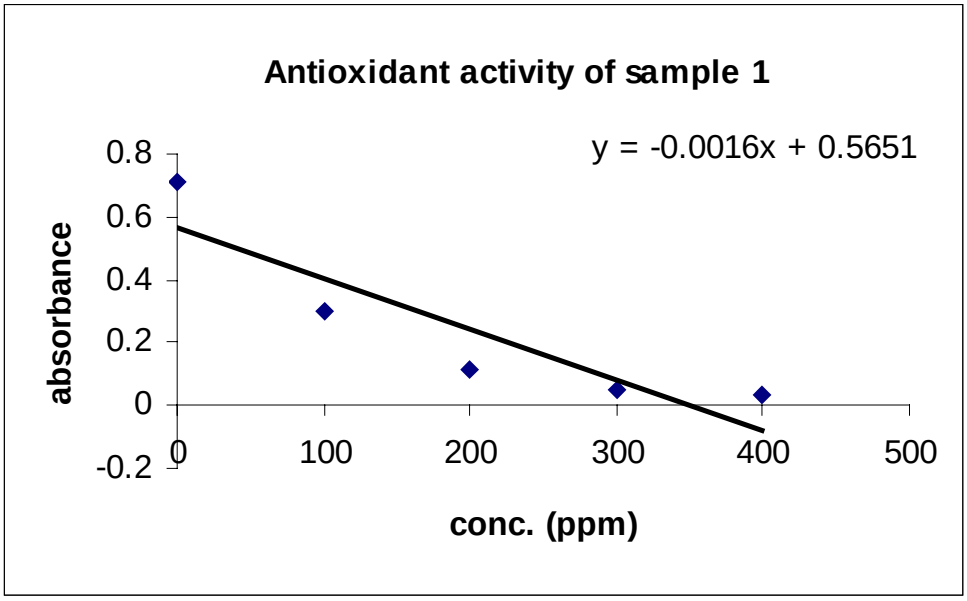

Figure 1 *Relationship between the absorbance and the concentration of sample 1*

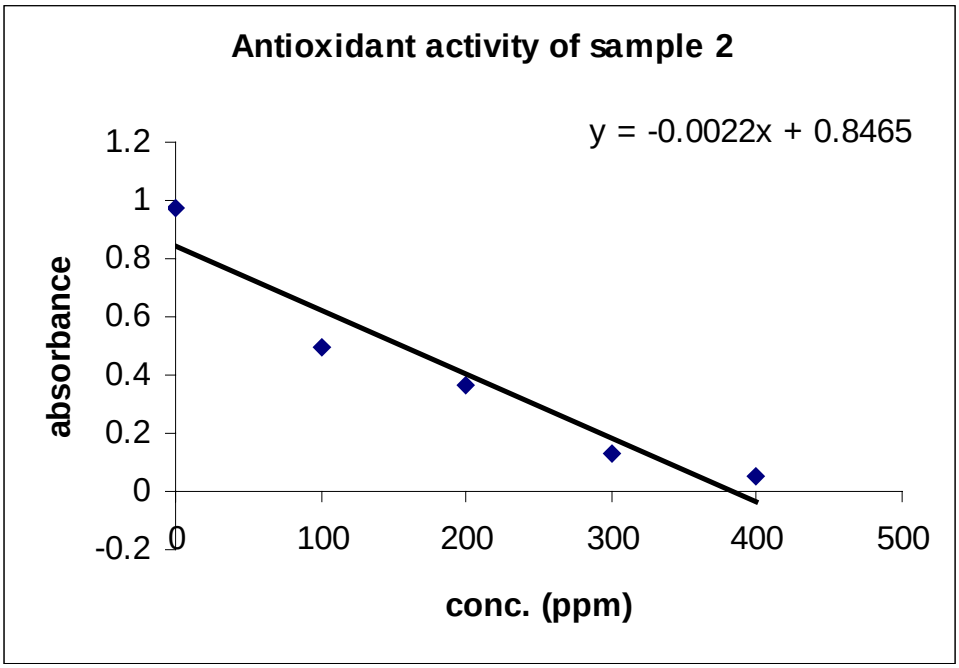

Figure 2 *Relationship between the absorbance and concentration of sample 2*

Table 4 *The efficient concentration of polyphenols (the quantity of polyphenols caused 50% loss of the DPPH activity, EC_{50}) from the ethanol extracts before lipid extraction*

sample	EC_{50} (ppm)	EC_{50} (mg/ml)
1	130.12	0.130
2	163.38	0.163
average	146.75	0.147

The crude ethanol extracts (sample 1 and 2) were extracted with hexane to remove lipid and subjected to antioxidant activity assay. Table 5 showed the yield of the ethanol extracts (sample 1 and 2 after lipid extraction). The yield was about 23.0 %. Table 6 showed the concentrations and absorbances of sample 1 and 2 after lipid extraction. Figure 3 and 4 showed the relationship between the absorbances and concentrations of sample 1 and 2 without lipid. The EC_{50} of the ethanol extracts without lipid (sample 1 and 2 without lipid) were shown in Table 7. It was found that the EC_{50} was reduced to 0.105 mg/ml from 0.147 mg/ml.

Table 5 *Yield of the ethanol extraction of rattan shoot after lipid extraction*

sample	Weight of sample (g)	Weight of hexane extract (g)	% yield
1	7.46	1.71	22.9
2	7.47	1.72	23.0
average	7.47	1.71	23.0

Table 6 *Absorbance of sample 1 and 2 after lipid extraction*

Concentration (ppm)	Sample 1 A (λ = 515.25)	Sample2 A (λ = 515.25)
0	0.64417	0.6666
25	0.55788	0.5892
50	0.47756	0.50007
75	0.34133	0.4277
100	0.31974	0.3415
150	0.17845	0.20169
200	0.05318	0.079833
300	0.035616	0.037024
400	0.035455	0.035869
500	0.03435	0.036132

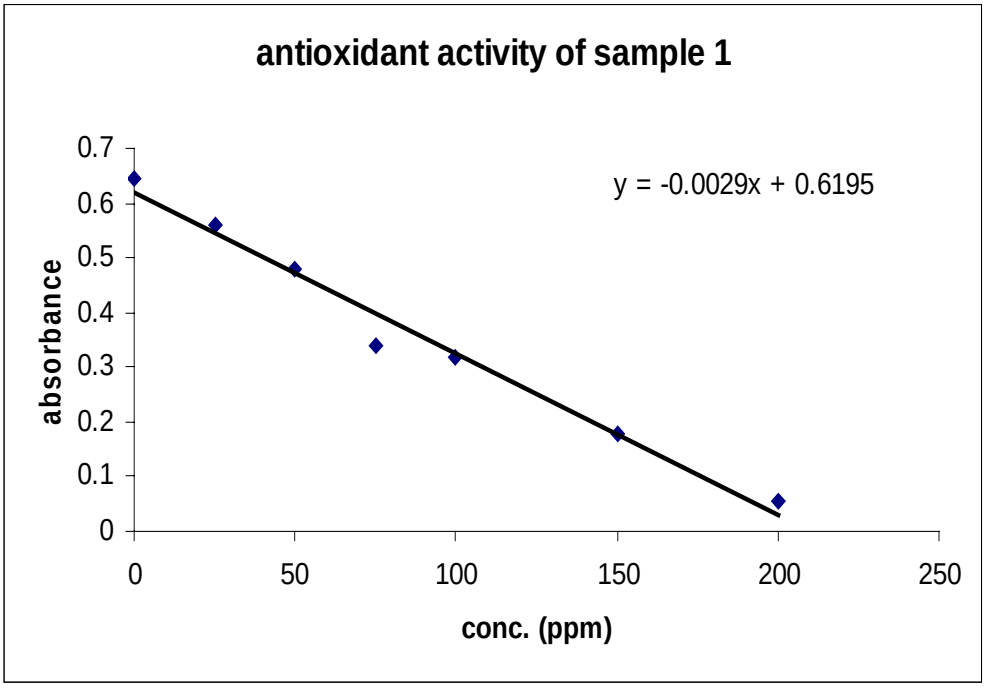

Figure 3 *Relationship between the absorbance and concentration of sample 1 without lipid*

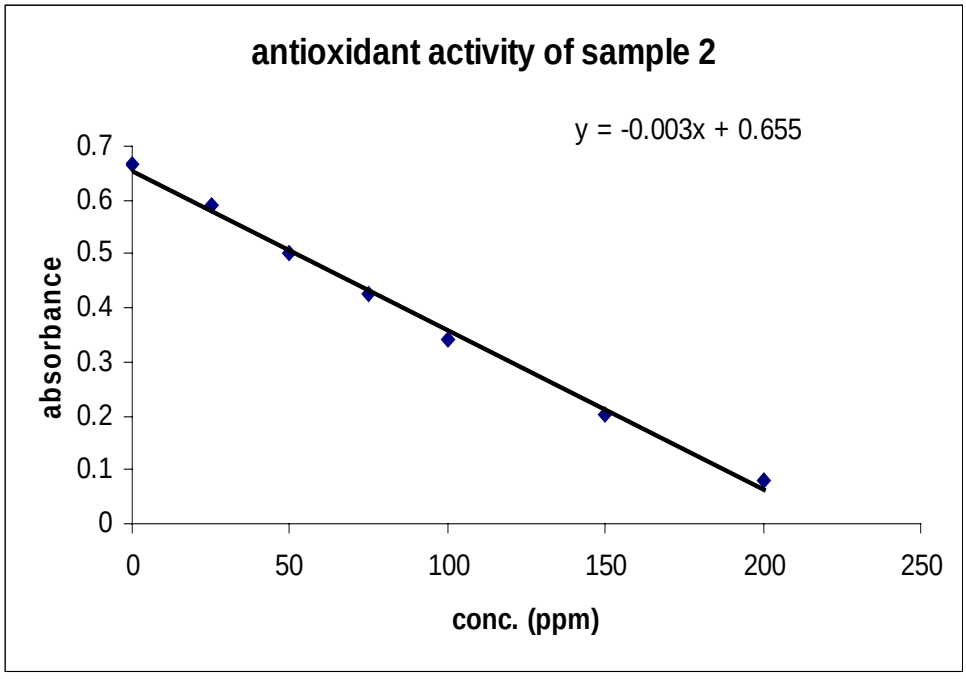

Figure 4 *Relationship between the absorbance and concentration of sample 2 without lipid*

Table 7 EC_{50} *of sample 1 and 2 without lipid*

sample	EC_{50} (ppm)	EC_{50} (mg/ml)
1	102.56	0.103
2	107.23	0.107
average	104.90	0.105

Separation of polyphenols from methanol extracts

The crude methanol extract of fresh rattan shoot and one year in brine were extracted with hexane and ethyl acetate. The yields of extracts were shown in Table 8. The aqueous extract after subjecting to column chromatography, each subfraction was monitored by thin layer chromatography. Subfractions with R_f of 0.42, 0.55 and 0.66 were combined and tested their antioxidant activity. Figure 5 and 6 showed the relationship between the absorbances and concentrations of polyphenols from fresh rattan shoot and rattan shoot one year in brine, respectively. Table 9 showed EC_{50} of polyphenols from rattan shoot (fresh and one year in brine). It was found that the antioxidant activity of polyphenols separated from methanol extracts of fresh rattan shoot was higher than soaking in brine for one year.

Table 8 Comparable yield of the extraction of the methanol extract of rattan shoot

Test	Methanol extract (%)		Aqueous extract (%)		Ethyl acetate extract (%)		Hexane extract (%)	
	fresh	1 year in brine	fresh	1 year in brine	fresh	1 year in brine	fresh	1 year in brine
1	20.69	40.34	12.34	9.40	0.35	0.73	7.15	0.49
2	20.58	31.40	11.76	12.60	0.34	1.81	6.90	3.81
average	20.63	35.87	12.05	11.00	0.69	1.27	7.03	2.15

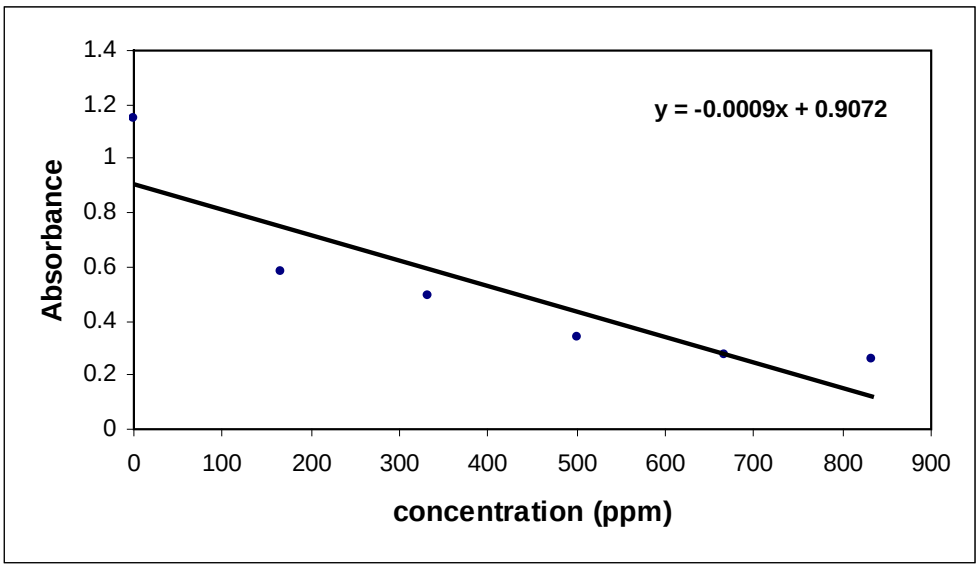

Figure 5 *Relationship between absorbance and concentration of polyphenols from fresh rattan shoot*

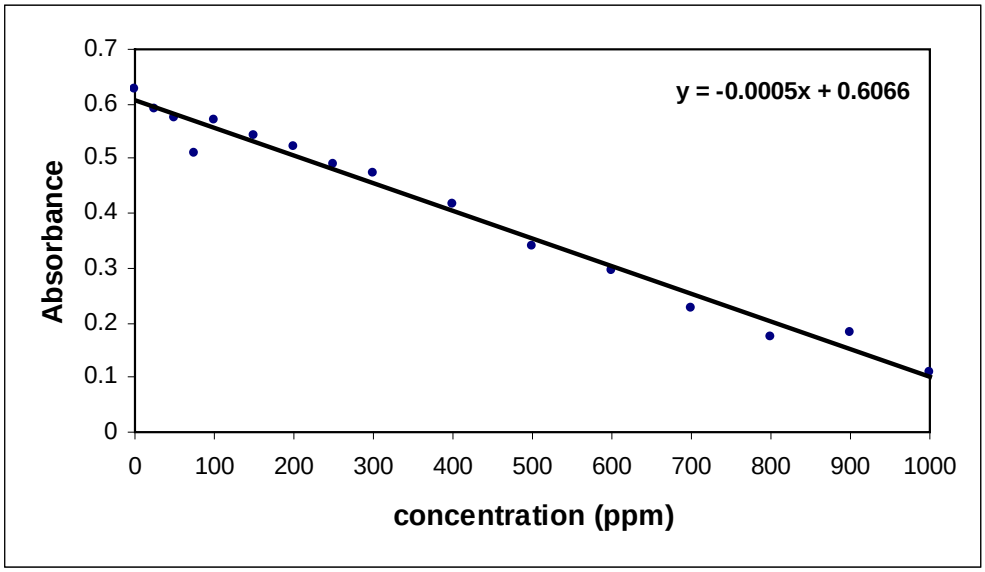

Figure 6 *Relationship between absorbance and concentration of polyphenols from rattan shoot one year in brine*

Table 9 *EC_{50} of polyphenols from rattan shoot (fresh and one year in brine)*

Polyphenols from rattan shoot	EC_{50} (ppm)	EC_{50} (mg/ml)
fresh	370.61	0.371
1 year in brine	588.02	0.588

It is assurance that the antioxidant activity of polyphenols from fresh rattan shoot is quite higher than rattan shoot one year in brine. Similarly, the EC_{50} value of fresh rattan shoot is quite less than rattan shoot one year in brine. Although the rattan shoot was soaked in brine for one year, the quantity of polyphenols was not decreased significantly. The aftermath of this research is revealed that the rattan shoot soaking in brine within one year can conserve and cherish the nutrient therefore this keeping in brine which containing the rattan shoot in the bottle is one of the conservation method for rattan shoot.

Conclusion

According to Folin-Ciocalteau, the total polyphenol contents of rattan shoot was quite high and the DPPH assay referred to the quite low EC_{50} which was revealed that the antioxidant activity was quite high as well. The antioxidant activity of polyphenols separated from methanol extracts of fresh rattan shoot was nearly twice as high as rattan shoot one year in brine. Edible rattan shoot is one of the source of the potent antioxidants in nature, even though rattan shoot soaked in brine for one year is also the source of the antioxidants.

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References

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval.S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., and Etherton, T.D., Bioactive Compounds in Foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine, 113*: *(9B) 71-88 (2002).*

Molyneux, P., "The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity"*Songklannakarin J. Sci.Tecnol.26, 2, 211-219,(2004).*

Plaklamyong, S., "Study on Rattan Shoots Processing" ITTO Project on PD24/00 Rev.1 (I) (2002).