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Antioxidant activity of latex in Sago palm (*Metroxylon sagu* Rottb.)

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Abstract: Sago palm (*Metroxylon sagu* Rottb.) is one of many economically agricultural crops in Thailand. Sago palm is a very common native plant in the southern part of Thailand, especially in Nakhon Si Thammarat. The study aims to investigate the quantification of total phenolic content and *in-vitro* antioxidant activity of the ethanol extracts of sago latex in samples from Ron Phibun, Tha Sala and Phrom Khiri districts, Nakhon Si Thammarat province. Gallic acid was used as a standard to estimate total phenolic content. *In-vitro* antioxidant activity of the extracts was determined by using the DPPH assay. The ethanol extract of sago latex from Phrom Khiri showed high phenolic content (854.40 $\pm$ 0.40 mg GAE/100 g FW) and high antioxidant activity (IC_{50} = 18.09 $\pm$ 0.01 mg/L). In the present study, we found that the ethanol extracts of sago latex showed a good antioxidant activity.

Keywords: Sago palm (*Metroxylon sagu* Rottb.), Latex, Antioxidant activity, DPPH assay, Total phenolic content

Introduction

Plants are being used as a source of medicine since long. The properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent against several disease, no side effects and economic viability. Several compounds widely distributed in plants that reported to exert multiple biological effect, such as antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic[1-4]. Nowadays, natural plants have received much interesting in sources of biological active

substances including antioxidants. Many studies have been performed in some plants, fruits and vegetables because of the rich sources of antioxidants, including polyphenolic compounds, carotenoids, flavonoids, vitamin A, vitamin C and Vitamin E, which prevent free radical damage. Living cells may produce free radicals and reactive oxygen species byproducts by physiological and biochemical processes. Free radicals cause oxidative damage to lipids, proteins and DNA, leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans[5]. Sago palm (*Metroxylon sagu* Rottb.) is an economically agricultural crops in Thailand. Sago palm is a very common native plant in the southern part of Thailand such as Nakhon Si Thammarat, Phatthalung, Songkhla, Pattani, Yala and Narathiwas. In particular, Nakhon Si Thammarat province found that sago palm was very common in the districts of Ron Phibun, Tha Sala and Phrom Khiri. This plant stores a large amount of latex in its trunk, with a higher yield level than that of any other. To find out the best source of antioxidant, different solvents were used for extraction of sago latex samples collected from Ron Phibun, Tha Sala and Phrom Khiri District, Nakhon Si Thammarat Province.

Experimental

Chemicals

The organic solvents were used in the experiments for analytical grade and purchased from Merck, Thailand.

Plant sample

Latex samples of sago palm were collected from Ron Phibun, Tha Sala and Phrom Khiri District, Nakhon Si Thammarat Province.

Extraction procedure

The trunks of sago palm were washed several times with tap water and finally with distilled water to remove dust, then the samples were cut into pieces of about 30 cm. Sago latex seeped out and stored in the brown bottle cover tightly. 40 grams of sago latex were sequentially extracted for 72 h with different solvents hexane, chloroform, acetone and ethanol. The extracts obtained were filtered through filter paper Whatman No.1 and then evaporated to dryness by using a rotary evaporator. The crude extracts were kept at 4$\pm$ 2 °C for further used.

Quantification of the total phenolic contents

The concentration of the phenolics in the latex extracts was analyzed with the Folin Ciocalteau assay [6] and using gallic acid as a standard. The 0.125 mL of extracts and 1.25 mL of Folin Ciocalteau's reagent was added and then shaken. After 5 min, 1.25 mL of 7.5% Na_2CO_3 was added the mixture. The final volume was brought up to 3 mL by adding distilled water and then mixed. After 90 min of incubation in darkness at room temperature, then the absorbance was measured at 760 nm (UV-VIS). The results were presented as the gallic acid equivalent per 100 grams fresh weigh (mg GAE/ 100 g FW).

In-vitro antioxidant activity

The ability of the sago latex extracted to scavenge DPPH free radical was assayed by using the standard method[7]. Aliquots of various concentrations (6.25-100 mg/L) of the extract samples were determined with a methanolic solution having a final DPPH radical concentration of 2.5 mM. After an incubation of 30 min in darkness at room temperature. The absorbance at 516 nm was measured against a blank of pure 95% ethanol. Ascrobic acid was used as the standard compound. The percentage of DPPH inhibition was calculated from the following equation:

$$\% \text{ DPPH Inhibition} = (A_c - A_s)/ A_c \times 100$$

Where A_c and A_s are the absorbance of control and the sample, respectively.

Statistical analysis

Data were expressed on dry weight basis and were presented as mean ± SD. Data were subjected to statistical analysis using SPSS 14.0 package. Pearson's correlation was used to determine the correlation between antioxidant capacity and phenol content. Significance differences were set at $p<0.05$.

Results and Discussion

Percentage yield and total phenolic content

Comparison of the extracts of sago latex, the extraction yield was highest as ethanol, showing a percentage yield of 1.27 (Table 1). Therefore, the ethanol was selected for the extraction of sago latex to assay total phenolic content and antioxidant activity.

Table 1 Percentage yield of crude extracts of sago latex with each solvent hexane, chloroform, acetone and ethanol

Extracts	% yield
Hexane	0.20
Chloroform	0.27
Acetone	0.78
Ethanol	1.27

Total phenolic content

The amount of the total phenolic content ranged 703.40-854.40 gallic acid equivalents (GAE mg/g) of fresh weight of extract, respectively, which they were highest in samples from the Phrom Khiri district (Table 2). Phenolic compounds are secondary metabolites which play as antioxidants due to their ability to donate hydrogen, quench singlet oxygen and act as metal chelators[8]. They are found to be useful, such as an antimicrobial agent, a mitochondrial adhesion inhibitor and an anticancer agent[9].

Table 2 Concentrations of total phenolic content of sago latex from different sample locations

Sample locations (District)	Total phenolic contents (mg GAE/100 g FW)
Ron Phibun	703.40 $\pm$ 0.53
Tha Sala	803.73 $\pm$ 1.10
Phrom Khiri	854.40 $\pm$ 0.40

In-vitro antioxidant activity

The antioxidant activity of sago latex from different locations was presented in Table 3. It showed that the highest antioxidant activity with a lowest IC_{50} value (18.09 $\pm$ 0.01 mg/L) in samples from the Phrom Khiri district. The DPPH assay is a common spectrophotometry method to measure the activity of antioxidant. The advantage of this method is the

antioxidant activity is assayed at ambient temperature, so the risk of thermal degradation of the molecule tested is eliminated[10]. Free radical scavenging activity is well known mechanism which antioxidant inhibits lipid oxidation.

Table 3 Antioxidant activity of sago latex from different sample locations

Sample locations (District)	R^2	IC_{50} (mg/L)
Ron Phibun	0.9955	58.83 ± 0.02
Tha Sala	0.9958	88.43 ± 0.02
Phrom Khiri	0.9955	18.09 ± 0.01

Conclusion

The results of scavenging DPPH activity assay in this study indicated that the ethanol extract of sago latex was potently active in samples from the Phrom Khiri district. The data clearly exhibited that the ethanol extract of sago latex showed the highest phenolic compounds and a good antioxidant activity.

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