องค์ประกอบทางเคมีและฤทธิ์ต้านอนุมูลอิสระจากเปลือกเงาะ

Chemical Constituents and Antioxidant Activity from Rambutan Peels

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บทคัดย่อ

จากงานวิจัยที่ได้ศึกษามาอย่างต่อเนื่องในการสกัดสารจากเปลือกเงาะด้วย 95% เอทานอล และทำการแยกให้บริสุทธิ์ด้วยเทคนิคคอลัมน์โครมาโทกราฟีสามารถแยกสารได้ทั้งหมด 9 fractions และทำการทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH โดยพบว่าใน fraction ที่ RM 6 RM 7 RM 8 และ RM 9 มีค่า IC50 ดีที่สุดคือ 0.70 1.93 1.06 และ 0.78 ppm ตามลำดับ ในขณะที่วิตามินซี และ วิตามินอี มีค่า IC50 เท่ากับ 0.64 และ 0.71 ppm ตามลำดับ และจากการศึกษาทางพฤษเคมีของสารสกัดจากเปลือกเงาะที่มีฤทธิ์ต้านอนุมูลอิสระที่ดีที่สุดนั้นสามารถแยกสารบริสุทธิ์ที่เคยมีรายงานมาแล้วได้ทั้ง 3 ชนิดคือ 3,4-dihydroxybenzoic acid (1), 3,4-dihydroxybenzaldehyde (2) และ ascorbic acid (3) โครงสร้างของสารบริสุทธิ์ที่แยกได้ทั้งหมด ทำการศึกษาโดยใช้เทคนิคทางสเปกโทรสโกปีร่วมกันคือ 1H-NMR 13C-NMR 2D-NMR IR และ GC/MS

คำสำคัญ: *ฤทธิ์ต้านอนุมูลอิสระ เปลือกเงาะ วิธี DPPH*

Abstract

Our research focused on the extraction of rambutan peels with 95% ethanol and purified by column chromatography to obtain 9 fractions which were evaluated for the antioxidant activity against DPPH. We found that the fraction numbers RM 6, RM 7, RM 8 and RM 9 had the highest antioxidant activity against DPPH in IC_{50} 0.07, 0.03, 0.09 and 0.01 ppm, respectively, while vitamin C and E showed IC_{50}, 1.52 and 0.59 ppm, respectively. Phytochemical study of rambutan peels showed that the highest antioxidant activity led to the isolation of three compounds. Three pure compounds were (1) 3,4-dihydroxybenzoic acid, (2) 3,4-dihydroxybenzaldehyde and (3) ascorbic acid. The structures of all these isolates were determined by extensive spectroscopic studies, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, IR and GC/MS.

Keywords: *antioxidant activity, rambutan peel, DPPH method*

1. Introduction

Cancer is a major cause of mortality worldwide and cancer indents rapidly increase from year to year. In 2000, there were 10.4 million new cancer cases and it was expected that this number wouldl be doubled in 2030. Recent studies have shown strong evidence that biological reactive oxygen species such as hydroxyl radicals ($OH^{\cdot}$) and superoxide an ion ($O_2^{\cdot-}$) were involved in the development of cancer. Compounds with high reactive oxygen species reduction activity were likely able to prevent cancer incidence (Hursting et al, 1999). Fruits and vegetables were the major source of natural antioxidants and contain various kinds of antioxidant compounds such as vitamin C, vitamin E, carotenoids, lutein and lycopene (Cai et al., 2004). However, phenolics compounds or polyphenols were secondary plant metabolites, which wrre better scavengers of free radicals than vitamin C and vitamin E (Palanisamy et al., 2008). Thailand, as a tropical country, shows on amazing diversity of plants species. Some of them have been long used as traditional medicines. Many of them were reported to have various desirable activities (Tachakittirungrod et al., 2007).

2. Objectives

Rambutan (*Nephelium lappaceum* L.) is one variety of the attractive tropical fruits in South-East Asia. The popular cultivars of rambutan in Thailand are Rongrien and Seechompoo. Recently, antioxidant and phenolic characteristics of this fruit has been found to be of increasing interest (Gorinstein et al, 1999). This research was aimed at the screening test for the antioxidant activity of peels or seeds of Thai fruits, major component elucidation for the best antioxidant activity and nourishing cream production.

3. Materials and Method

The pulverized dried of rambutan peels were macerated 3 times for hexane, dichloromethane and 95% ethanol at room temperature for 3 day per time. Crude extracts were measured the antioxidant activity according to DPPH method, using vitamin C and E as a standard. The antioxidant activities of 12 samples; three crude extracts and nine fractions, pre-purified sample from 95% ethanol, were measured in term of radical-scavenging ability according to DPPH method. Briefly, an aliquot of sample extract at various concentrations was added to a DPPH solution, and the absorbance at 515 nm was measured until the reaction reached the plateau. A calibration curve at 515 nm was made with DPPH to calculate the remaining DPPH concentration in the reaction medium. The parameter IC_{50}, which reflects the depletion of DPPH free radical to 50%, was expresses in terms of mg sample equivalent g^{-1} DPPH in the reaction medium. The best antioxidant activity of crude extract was pre-purifying by column chromatography on silica gel and the antioxidant nourishing cream production.

4. Results and Discussion

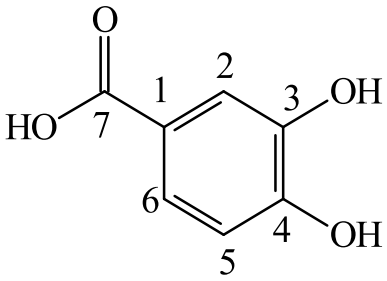
The antioxidant activity of hexane, dichloromethane and 95% ethanol extract of rambutan peels were evaluated for the antioxidant activity using free radical

scavenging (DPPH method). It showed that 95% ethanol extract had the highest antioxidant activity. 95% Ethanol extraction was pre-purify and separated 9 fractions. We found that fraction number 5 has the highest antioxidant activity with closely to vitamin C as shown in Table 1. Phytochemical study of rambutan peels that showed the highest antioxidant activity led to the isolation of three compounds. Three pure compounds were 3,4-dihydroxybenzoic acid (1), 3,4-dihydroxybenzaldehyde (2) and ascorbic acid (3) as shown in Figure 1. The structures of all these isolates were determined by extensive spectroscopic studies, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, IR and GC/MS.

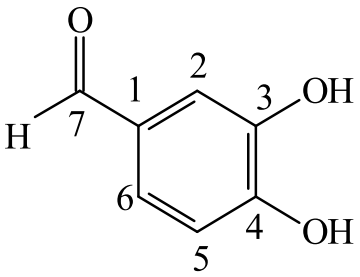
Table 1 %Inhibition and IC_{50} values of rambutan peels extract and pre-purify fractions of 95% ethanol extract

Sample name	% Inhibition (Mean ± SD) *			IC_{50} (μg/ml)
	1 (μg/ml)	10 (μg/ml)	100 (μg/ml)	
Crude n-hexane	43.93 ± 0.002	45.36 ±0.002	65.91 ±0.001	10.13
Crude CH_2Cl_2	44.06 ± 0.002	44.94 ±0.000	53.16 ±0.001	59.14
Crude 95% ethanol	52.78 ± 0.002	77.62 ±0.002	97.93 ±0.002	0.90
Fraction1	38.76 ± 0.001	42.39 ± 0.000	55.27 ± 0.001	75.43
Fraction2	28.68 ± 0.001	34.07 ± 0.002	55.98 ± 0.001	80.74
Fraction3	39.93 ± 0.002	44.73 ± 0.001	52.22 ±0.001	78.40
Fraction4	39.46 ± 0.001	40.16 ± 0.001	57.26 ± 0.000	79.89
Fraction5	58.75 ± 0.002	86.47 ± 0.002	97.93 ± 0.002	0.70
Fraction6	49.52 ± 0.002	83.47 ± 0.002	98.57 ± 0.002	1.93
Fraction7	44.67 ± 0.002	52.78 ± 0.003	92.98 ± 0.002	1.32
Fraction8	48.35 ± 0.002	70.60 ± 0.002	98.44 ± 0.002	1.06
Fraction9	52.90 ± 0.003	90.9 ± 0.000	98.05 ± 0.001	0.78
VitaminC	60.45 ± 0.002	85.96 ± 0.002	98.70 ± 0.001	0.64
VitaminE	54.47 ± 0.001	96.76 ± 0.002	98.57 ± 0.002	0.71

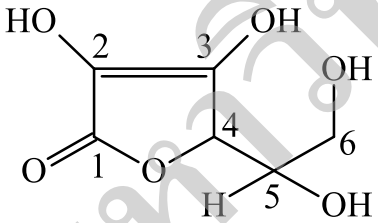
*Triplicate Analyses



3,4-dihydroxybenzoic acid (1)



3,4-dihydroxybenzaldehyde (2)



ascorbic acid (3)

Figure 1. Structures of compounds isolated from rambutan peels

Figure 2 showed ^{1}H-NMR of fraction number 5 from 95% ethanol rambutan peels extract. Ethanol extract was pre-purify using column chromatography and separated 9 fractions. We found that pre-purify compounds from rambutan peels extraction from fraction number 5, 6, 8 and 9 have the highest antioxidant activity in DPPH assay with IC_{50} values of 0.67, 0.93, 1.06 and 0.78 μg/ml, respectively, which were closed to vitamin C and E (IC_{50}, 0.63 and 0.70 μg/ml, respectively). From the phytochemical study of rambutan peels that showed the highest antioxidant activity led to the isolation of three compounds. Three pure compounds are 3,4-dihydroxybenzoic acid (1), 3,4-dihydroxy benzaldehyde (2) and ascorbic acid (3). And ^{1}H-NMR of three pure compounds showed in Figure 3.

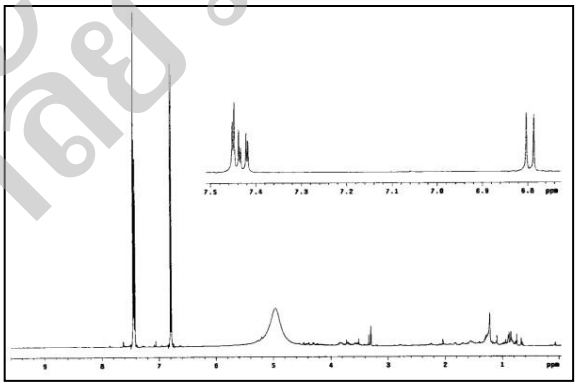
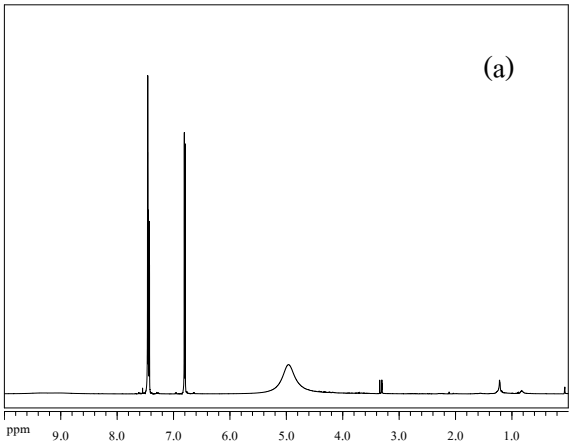


Figure 2 ^{1}H-NMR spectrum of fraction 5 from pre-purify 95% ethanol extract (Sample was dissolved in CD_3OD solvent for NMR analysis)



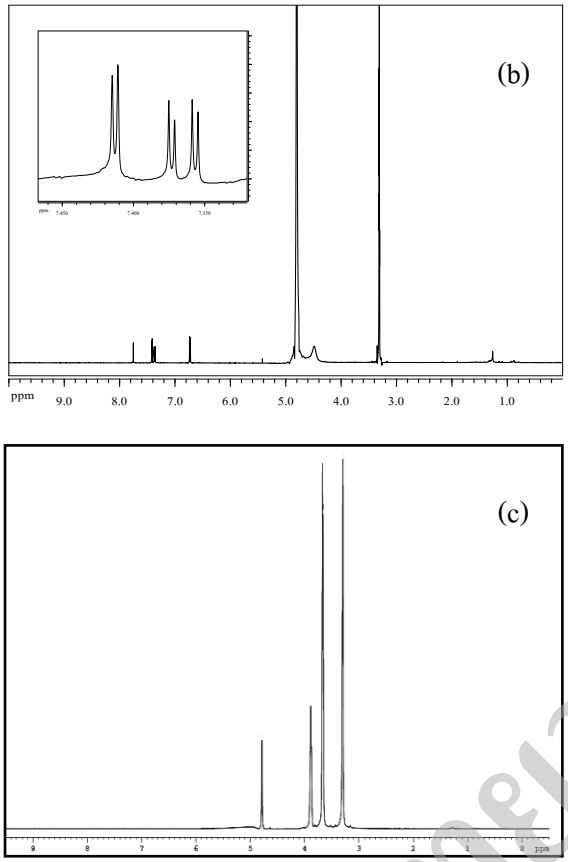


Figure 3 ^{1}H-NMR spectrum of (a) 3,4-dihydroxybenzoic acid, (b) 3,4-dihydroxy benzaldehyde and (c) ascorbic acid (Sample were dissolved in $CDCl_3$and CD_3OD solvent for NMR analysis)

5. Conclusion

In this investigation of hexane, dichloromethane and 95% ethanol, we found that 95% ethanol extract of rambutan peels showed the highest antioxidant activity while it's pre-purification of fraction number 5 to 9 had more antioxidant activity than 95% ethanol extract. Phytochemical study of rambutan peels that showed the highest antioxidant activity led to the isolation of three compounds. Three pure compounds are 3,4-dihyroxybenzoic acid, 3,4-dihydroxy benzaldehyde and ascorbic acid.

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