

# Anti-inflammatory and Antioxidant Activities of Extracts from *Musa sapientum* Peel

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**Background:** Many parts of *Musa sapientum* Linn. (Musaceae) are used in Thai traditional medicine as drugs, food supplements and cosmetics. The banana peel is used as an astringent in foot care, the unripe fruit is used to treat diarrhea and, the ripe fruit is used as tonic.

**Objective:** To evaluate anti-inflammatory and antioxidant activities of banana peel extracts obtained from different extraction methods and to determine their total phenolic content.

**Material and Method:** Four extraction methods were used to extract unripe and ripe peels. Nitric oxide inhibitory and DPPH scavenging assays were used to evaluate anti-inflammatory and antioxidant activities, respectively. Folin-Ciocalteu's reagent was used to determine total phenolic content.

**Results:** The water extract of fresh ripe peel exhibited the most potent NO inhibitory activity ( $IC_{50} = 6.68 \pm 0.34 \mu\text{g/ml}$ ), but apparently exhibited no antioxidant activity. The decoction extract of fresh unripe peel exhibited strong antioxidant activity as well as had the highest total phenolic compound. The antioxidant activity exhibited a correlation with the total phenolic content.

**Conclusion:** This study supports the use of *Musa sapientum* peel in Thai Traditional Medicine for treatment of inflammatory-related diseases.

**Keywords:** *Musa sapientum* Linn., Anti-inflammatory, Antioxidant, Total phenolic content

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Inflammation is normally a localized, protective response to trauma or microbial invasion that destroys, dilutes, or walls off the injurious agent and the injured tissue<sup>(1)</sup>. Nitric oxide (NO) is a potent mediator causing inflammation in many organs and also a free radical involved in various physiological and pathological processes such as vasodilatation, nonspecific host defenses and acute and chronic inflammation in organ system. NO synthesized in high amounts by activated inflammatory cells possesses cytotoxic properties for killing bacteria, virus or tumor cells. Although it is involved in host defense mechanisms, it also damages tissue causing acute and chronic inflammation<sup>(1)</sup>.

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) include both radical and non-radical molecules with unpaired orbital electrons derived from nitrogen, such as nitric oxide and oxygen, such as peroxy radical. RNS and ROS play important roles in killing foreign organisms and in acute inflammation but

their over-production may cause tissue damage and vascular leakage in septicemia, rheumatoid arthritis and inflammatory disease<sup>(2)</sup>.

*Musa sapientum* Linn. (Musaceae) or banana is mainly used in Indian folk medicine for the treatment of diabetes mellitus and in Thai Traditional Medicine for the treatment of diarrhea, constipation, allergy and foot wounds. The water extract of dried unripe peel was reported to have anti-allergic activity against antigen-induced  $\beta$ -hexosaminidase release as a marker of degranulation in RBL-2H3 cells with an  $IC_{50}$  value of  $62.0 \pm 1.0 \mu\text{g/ml}$ <sup>(3)</sup>. The 95% EtOH extract of unripe peel showed the antioxidant activity on DPPH assay, with an  $IC_{50}$  of  $0.031 \pm 0.004 \text{ mg/ml}$  and on ABTS assay, with an TEAC of  $1.80 \pm 0.038 \text{ mM/mg}$ <sup>(4)</sup>. However, the antioxidant activity of banana peel obtained by other extraction methods has not been reported. Moreover, anti-inflammatory activity, particularly nitric oxide inhibitory effect, has not yet been documented. Thus, the present study aimed to investigate anti-inflammatory and antioxidant activities of *Musa sapientum* peel, which were extracted by different methods and also to determine total phenolic content of extracts from *Musa sapientum* peel. Furthermore, the correlation between total phenolic content and

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antioxidant activity of *Musa sapientum* peel extracts was also studied. These results should promote waste product from banana peel to be used as health products.

## **Material and Method**

### **Reagents**

#### **Animal cell lines and Reagents**

RAW 264.7 murine macrophage leukemia cell lines were established and kindly provided by Assoc. Prof. Dr. Supinya Tewtrakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. RPMI Medium 1640 (RPMI 1640) powder with L-glutamine, Fetal Bovine Serum (FBS), Penicillin-Streptomycin (P/S), trypsin-EDTA and trypan blue were purchased from Gibco, USA. Phosphate Buffer Saline (PBS) was from Amresco (USA); sodium bicarbonate was from BDH; England, lipopolysaccharide (LPS, from *Escherichia coli*) and 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were from Sigma. DPPH and BHT were from Fluka, and other chemicals were from Sigma and Merck.

#### **Plant material and extraction**

The fruit of *Musa sapientum* Linn. was collected from Nakornrachasima province in Thailand on May 2010. The fresh unripe and ripe peels were cut separately into small pieces and divided into two parts. One part of each peel type was dried as a dried sample by hot air oven at 50°C and crushed into fine powder. The other part was used as a fresh sample. Fresh and dried ripe and unripe peels were extracted by four methods such as maceration in 95% Ethanol, 50% Ethanol, decoction and soaking in water. For two maceration methods, the fresh and dried parts of each banana peel type were extracted with maceration in 95% Ethanol or 50% Ethanol for three days at room temperature, filtrated and concentrated by rotary evaporator. The obtained semisolid extracts were kept in a refrigerator at -20°C for further use. For a decoction method, the fresh and dried parts of banana were boiled for 15 minutes, filtrated and dried by lyophilizer. For a method of soaking in water, the fresh and dried parts of banana were soaked in water at room temperature, for 24 hours, filtrated and dried by lyophilizer to obtain water extract.

#### **Assay for NO inhibitory effect and cytotoxicity test in RAW 264.7 cells**

Inhibitory effect on NO production by Mouse

Macrophage Leukemia-like RAW 264.7 cells was evaluated using a modified method, as previously reported<sup>(5)</sup>. RAW 264.7 cell line was cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml) and streptomycin (100 µg/ml). The cells were seeded in 96-well plate (cell concentration  $1 \times 10^5$  cells/well) and incubated in CO<sub>2</sub> incubator at 37°C for 1 hour. 100 µl/well of RPMI medium containing 5 µg/ml of LPS was added into control and sample wells, whereas only RPMI medium was added into a blank well. 100 µl per well of different sample concentrations (1-100 µg/ml) was added into sample wells and their corresponding blank sample wells. Then cells were incubated at 37°C for 48 hours. Supernatant (100 µl) was added in another 96-well plate followed by the addition of 100 µl per well of Griess reagent. The color was detected at a wavelength of 570 nm.

Cytotoxicity was also determined using the MTT method. After 48 hours incubation with the test samples, MTT solution (10 µl, 5 mg/ml in PBS) was added to the wells and incubated at 37°C for 2 hours. The medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells. The formazan solution was measured with a microplate reader at 570 nm. The test sample was considered to be cytotoxic when the optical density of the sample-treated group was less than 70-80% of that in the control (vehicle-treated) group. Indomethacin was used as positive controls. Inhibition (%) was calculated and IC<sub>50</sub> values were calculated from the Prism program.

#### **DPPH radical scavenging activity**

Antioxidant activity was determined using DPPH assay, according to the modified method of Yamasaki et al (1994)<sup>(6)</sup>. The ethanolic extracts were mixed with ethanol and the water extracts were mixed with water to prepare the tested solutions with different concentrations (1-100 µg/ml). DPPH was dissolved in absolute ethanol and mixed with a certain amount of the ethanolic and water tested solutions. The solution was adjusted to a final DPPH concentration of 100 µM. The mixture was left to stand for 30 min in the dark at room temperature. Then the decrease in absorbance due to DPPH was measured at 520 nm using a microplate reader. The antioxidant activity of each extract was expressed as EC<sub>50</sub> (µg/ml).

#### **Total phenolic content**

Total phenolic contents (TP) of the fruit extracts were determined using Folin-Ciocalteu's

method which was described by Miliauskus (2004)<sup>(7)</sup>. 20 µl of extract solution was mixed with 100 µl of Folin-Ciocalteu's reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 5 min at room temperature, 80 µl of (7.5% w/v) sodium carbonate solution was added. The solutions were mixed and allowed to stand for 30 minutes at room temperature. Then, the absorbance was measured at 765 nm, using a microplate reader. A calibration curve was prepared, using a standard solution of gallic acid (5, 10, 20, 40, 60, 80 and 100 µg/ml,  $r^2 = 0.9997$ ). Results were expressed on weight basis as mg gallic acid equivalents/g of sample.

## Results and Discussion

### The yield of extracts

Table 1 shows the percent yield of fresh and dried peel extracts. The highest (31.98%) and lowest (0.86%) yields of extraction were obtained from the dried ripe peel in decoction extract and fresh unripe in 95% EtOH, respectively.

### Anti-inflammatory activity by NO inhibitory effect and cytotoxicity test in RAW 264.7 cells

Water extract of fresh ripe peel exhibited the most potent NO-inhibitory activity with an  $IC_{50}$  value of  $6.68 \pm 0.34$  µg/ml, followed by 95% EtOH extract of dried unripe peel ( $IC_{50} = 36.62 \pm 3.68$  µg/ml) and 50% EtOH extract of dried ripe peel ( $IC_{50} = 54.69 \pm 1.71$  µg/ml), respectively (Table 1). The cytotoxic effects of all extracts were also determined using the MTT assay. All concentrations of banana peel extracts showed no cytotoxicity or more than 70% viable cells were detected except at a concentration of 100 µg/ml of water extract of fresh ripe peel. Interestingly, water extract of fresh ripe peel showed higher NO-inhibitory activity ( $IC_{50} = 6.68 \pm 0.34$  µg/ml) than indomethacin as positive control ( $IC_{50} = 20.32 \pm 3.68$  µg/ml). Therefore, such extract has a high potential as an anti-inflammatory agent.

### Antioxidant activity by DPPH radical-scavenging activity

The  $EC_{50}$  values of banana peel extracts are shown in Table 1. The 50% ethanolic extract of dried unripe peel displayed the highest antioxidant activity, with an  $EC_{50}$  of  $7.33 \pm 0.55$  µg/ml, followed by the decoction extract of fresh unripe peel and 95% ethanolic extract of dried unripe peel, with  $EC_{50}$  values of  $7.81 \pm 0.62$  and  $13.92 \pm 0.21$  µg/ml, respectively. In this study, 95% ethanolic extract of dried unripe peel exhibited better antioxidant activity than that in a previous

**Table 1.** Percent yield, anti-inflammatory activity by NO inhibitory effect,  $EC_{50}$  values of DPPH radical scavenging activity and total phenolic content of peel extracts from *Musa sapientum* Linn

Banana part used	Solvent extraction	% Yield	$IC_{50}$ of NO inhibitory activity ( $IC_{50} \pm SEM$ , µg/ml)		$EC_{50}$ values of DPPH radical scavenging activity ( $EC_{50} \pm SEM$ , µg/ml)		Total phenolic content (mg GAE/g)		Coefficient between total phenolic content and antioxidant activity	
			Fresh banana	Dried banana	Fresh banana	Dried banana	Fresh banana	Dried banana	Fresh banana	Dried banana
Ripe peel	95%EtOH	3.41	11.35	72.82 ± 3.04	> 100	61.23 ± 4.37	27.13 ± 0.71	55.48 ± 1.55	< 0.27	0.90
	50%EtOH	1.32	11.38	54.69 ± 1.71	> 100	32.08 ± 1.07	9.90 ± 1.62	52.34 ± 0.63	< 0.99	0.17
	Decoction	7.03	31.98	> 100	19.90 ± 1.74	27.55 ± 3.10	66.99 ± 1.31	45.91 ± 1.09	3.37	1.67
Unripe peel	Water	1.86	19.04	6.68 ± 0.34	> 100	77.51 ± 0.85	18.91 ± 0.03	26.56 ± 1.18	< 0.19	0.34
	95%EtOH	0.86	1.70	78.80 ± 1.54	36.62 ± 3.68	13.92 ± 0.21	38.23 ± 2.02	72.65 ± 2.96	< 0.38	5.21
	50%EtOH	3.16	8.81	74.67 ± 2.48	73.52 ± 2.41	7.33 ± 0.55	17.59 ± 2.35	49.97 ± 0.83	0.48	6.81
Indomethacin BHT	Decoction	2.39	22.33	> 100	> 100	> 100	117.68 ± 0.19	27.36 ± 0.71	15.06	< 0.27
	Water	0.69	8.19	> 100	20.32 ± 3.68	40.40 ± 2.92	44.19 ± 0.06	38.76 ± 0.48	0.44	0.88
11.05 ± 0.40										

report<sup>(4)</sup>. These results support that antioxidant agents are highly obtained from dried unripe peel using a maceration extraction. However, decoction of fresh of ripe and unripe peels are also used as natural antioxidant agents in health product.

#### **Total Phenolic content**

In addition to anti-inflammatory and antioxidant activities, total phenolic content of extracts from *Musa sapientum* peel, an active compound of antioxidant activity, was also determined.

The results of total phenolic content of banana peel extracts measured using Folin-Ciocalteu's method are shown in Table 1. The fresh banana extracts had their total phenolic contents in the range of 9.90 mg GAE/g to 117.68 mg GAE/g, while dried banana showed total phenolic contents in the range of 26.56 mg GAE/g to 72.65 mg GAE/g.

The fresh ripe and unripe banana by decoction showed the highest total phenolic content and these results were also related with the antioxidant activity. The antioxidant activity of decoction extract of dried unripe peel was dependent on polyphenol level. However, the coefficient between antioxidant values and total phenolic content showed in Table 1. The results showed that decoction method for unripe fresh banana also showed the highest value of relationship coefficient between total phenolic content and antioxidant capacity (15.06). The second value was maceration with 50% and 95% ethanol (6.81 and 5.21 respectively) of dried unripe banana peel. The results could explain that the boiling method with high temperature could extract phenolic compound from fresh unripe banana peel more than maceration and soaking in water and unripe banana peel will be source of phenolic content and antioxidant compound more than ripe banana peel. The boiling method can extract the tannin (as a phenolic group) from fresh banana peel more than ripe banana peel. The dried unripe peel was extracted by maceration method with 50% ethanol showed the highest antioxidant but the phenolic compound was extracted less than the boiling method. However, the results can concluded that unripe banana peel was a source antioxidant compound more than ripe banana peel.

#### **Conclusion**

The waste products especially fresh ripe and unripe banana peels obtained by a decoction method can be used as antioxidant agents. Also, these extracts had the highest total phenolic content and showed a

good correlation with their antioxidant activity. In conclusion, the ripe and unripe peels from fresh banana obtained from a decoction method should be further investigated for health product development. In anti-inflammatory study, the water extract of fresh ripe peel exhibited the most potent NO-inhibitory activity. However, this extract possessed low total phenolic content and low antioxidant activity. Accordingly, the present study supports the Thai Traditional Medicine use of *Musa sapientum* Linn. peel for treatment of inflammatory-related diseases.

#### **Acknowledgments**

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#### **Potential conflicts of interest**

None.

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## การศึกษาฤทธิ์ต้านการอักเสบและฤทธิ์ต้านอนุมูลอิสระของสารสกัดจากเปลือกกล้วยน้ำว้า

ปฐมพงษ์ เผือกกลี, ศรีโสภา เรืองหนู, อรุณพร อธิรัตน์

**ภูมิหลัง:** การแพทย์แผนไทยได้มีการใช้ส่วนต่างๆ ของกล้วยน้ำว้า (*Musa sapientum* Linn. อยู่ในวงศ์ Musaceae) เป็นยา, อาหารสุขภาพ และเครื่องสำอาง เช่น ผลดิบใช้รักษาอาการท้องเสีย ผลสุกใช้เป็นยาระบาย เปลือกผลใช้ในเครื่องสำอางเพื่อสมานแผลที่เท้า

**วัตถุประสงค์:** เพื่อประเมินฤทธิ์ต้านการอักเสบ ฤทธิ์ต้านอนุมูลอิสระ และวิเคราะห์หาปริมาณสารกลุ่มฟีนอลของเปลือกผลซึ่งถูกสกัดด้วยวิธีต่างๆ

**วัสดุและวิธีการ:** สกัดเปลือกผลดิบและสุกทั้งสดและแห้งด้วย 4 วิธี นำสารสกัดไปทดสอบการยับยั้งการหลั่งไนตริกออกไซด์และทดสอบวิธี DPPH assay เพื่อประเมินฤทธิ์ต้านการอักเสบและฤทธิ์ต้านอนุมูลอิสระ และทดสอบด้วย Folin-Ciocalteu's reagent เพื่อหาปริมาณสารกลุ่มฟีนอล

**ผลการศึกษา:** สารสกัดจากการแช่น้ำของเปลือกผลสุกแบบสดมีฤทธิ์ยับยั้งการหลั่งไนตริกออกไซด์ได้ดีที่สุด โดยมีค่า  $IC_{50}$  เท่ากับ  $6.68 \pm 0.34$  ไมโครกรัมต่อมิลลิลิตร ขณะที่ไม่มีฤทธิ์ต้านอนุมูลอิสระ สารสกัดชั้นน้ำของเปลือกผลดิบแบบสด มีฤทธิ์ต้านอนุมูลอิสระสูงที่สุดอีกทั้งยังมีปริมาณสารกลุ่มฟีนอลสูงที่สุด ฤทธิ์ต้านอนุมูลอิสระสัมพันธ์กับปริมาณสารกลุ่มฟีนอล

**สรุป:** การศึกษาครั้งนี้สนับสนุนการแพทย์แผนไทยในการใช้เปลือกผลของกล้วยน้ำว้า เพื่อรักษาโรคที่เกี่ยวข้องกับการอักเสบ

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