PS2-PS-3

ปริมาณฟีนอลิก ฤทธิ์ต้านออกซิเดชันและฤทธิ์ยับยั้งการทำงานของเอนไซม์เมทริกซ์เมทัลโลโปรตีนเนส-2 และ -9 ของสารสกัดดอกไม้ไทย

พลอยขวัญ กาญจนสุรัตน์, บังอร ศรีพานิชกุลชัย*

บทคัดย่อ

ปริมาณฟีนอลิก ฤทธิ์ต้านออกซิเดชันและฤทธิ์ยับยั้งการทำงานของเอนไซม์เมทริกซ์เมทัลโลโปรตีนเนส-2 และ -9 ของสารสกัดดอกไม้ไทย

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บทนำ: การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อหาปริมาณฟีนอลิก ฤทธิ์ต้านออกซิเดชันและตรวจสอบการยับยั้งเอนไซม์เมทริกซ์เมทัลโลโปรตีนเนส -2 และ -9 จากเซลล์ไฟโบรบลาสต์ของสารสกัดดอกตะแบก (*Lagerstroemia floribunda*) และดอกอินทนิล (*Lagerstroemia speciosa*) **วัสดุและวิธีการทดลอง:** เตรียมสารสกัดดอกตะแบกและอินทนิลด้วย 50% เอทานอล เพื่อศึกษาปริมาณฟีนอลิกและฤทธิ์การต้านออกซิเดชันด้วยวิธี DPPH และ FRAP และทดสอบฤทธิ์การยับยั้งการทำงานของเอนไซม์เอ็มเอ็มพี -2 และ -9 โดยวิธีเจลาตินไซโมกราฟี **ผลการทดลอง:** ปริมาณฟีนอลิกของสารสกัดดอกตะแบกมีค่าเท่ากับ 418.50±39.69 และดอกอินทนิลมีค่าเท่ากับ 636.74±44.14 มิลลิกรัมกรดแกลลิกต่อกรัมสารสกัด มีฤทธิ์ต้านออกซิเดชันสูง IC_{50} เท่ากับ 4.31±0.10 และ 3.89±0.12 ไมโครกรัมต่อมิลลิลิตร และค่า FRAP เท่ากับ 1.21±0.28 และ 1.79±0.26 มิลลิกรัม Fe^{2+} ต่อมิลลิกรัมสารสกัด และยังสามารถยับยั้งการทำงานของเอ็มเอ็มพี-2 และ เอ็มเอ็มพี-9 โดยตะแบกมีค่าการยับยั้งที่ 50% เท่ากับ 953.07 และ 771.07 พีพีเอ็ม ส่วนสารสกัดอินทนิลมีค่าการยับยั้งเท่ากับ 634.60 และ 548.40 พีพีเอ็ม ตามลำดับ **สรุปผลการทดลอง:** พบว่าสารสกัดของดอกตะแบกและอินทนิลมีความสามารถในการยับยั้งการทำงานของเอนไซม์เมทริกซ์เมทัลโลโปรตีนเนส-2 และ -9 และมีปริมาณของฟีนอลิกสูงรวมทั้งมีฤทธิ์ในการต้านออกซิเดชันได้ดี พืชทั้งสองชนิดนี้จึงเหมาะแก่การนำไปใช้ประโยชน์ต่อผลิตภัณฑ์ในการต้านอนุมูลอิสระและต้านการชรา

คำสำคัญ: เมทริกซ์เมทัลโลโปรตีนเนส, เจลาตินไซโมกราฟี, ฟีนอลิก, ฤทธิ์การต้านออกซิเดชัน, ตะแบก, อินทนิล

***ติดต่อผู้นิพนธ์:** โทร 043-203146, E-mail: bungorn@kku.ac.th

Abstract

Total phenolic contents, antioxidative activity and inhibitory on effect matrix metalloproteinase-2 and -9 of ethanolic extract from Thai flowers

Ploykwan Kranjanasurat, Bungorn Sripanidkulchai*

Introduction: The present study aimed to investigate the total phenolic content, antioxidant activity and MMP-2 and -9 inhibitory effect in fibroblast cell of ethanolic extracts from flowers of *Lagerstroemia floribunda* and *Lagerstroemia speciosa*. **Materials and Methods:** The powders of Thai flowers were extracted by 50% ethanol then the total phenolic contents were determind by the Folin-Ciocalteu method. The antioxidant activity including 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging and ferric reducing power (FRAP) were examined. The inhibitory effect on fibroblast MMP-2 and MMP-9 was

measured by gelatin zymography. **Results:** The extract of *L. floribunda* and *L. speciosa* gave total phenolic content at 418.50±39.69 and 636.74±44.14 mg gallic acid equivalent (GAE)/g of the extracts. They show the high antioxidative activities with IC_{50} values of 4.31±0.10 and 3.89±0.12 µg/ml and FRAP values at 1.21±0.28 and 1.79±0.26 mg Fe^{2+}/mg. Moreover, the flower extracts inhibited MMP-2 and MMP-9 activities 50% inhibition concentrations on MMP-2 and MMP-9 of *L. floribunda* were 953.07 and 771.07 ppm and 634.60 and 548.40 ppm of *L. speciosa*. **Conclusion:** The results of this study indicated that ethanolic extract of *L. floribunda* and *L. speciosa* have high inhibitory effect on MMP-2 and MMP-9 with high antioxidative activity. Therefore, these Thai flowers may be good sources for natural antioxidant products for anti-aging agent.

Keywords: Matrix metalloproteinase, Gelatin zymography, Phenolic, antioxidative activity, *Lagerstroemia floribunda*, *Lagerstroemia speciosa*

***Corresponding author**: Tel. 043-203146, E-mail: bungorn@kku.ac.th

Introduction

Ultraviolet (UV) radiation is damage to the human skin and causes its wrinkle and aging (Kim et al., 2011). It induces reactive oxygen species (ROS) and up regulating matrix metalloproteinases (MMPs), which are responsible for the turnover and degradation of extracellular matrix proteins (Silva et al., 2011) and give rise to the collagen destruction in skin. Recently, local plants such as *Emblica officinalis* (fruit) and Ocimum sanctum (leaves) were reported to have antioxidative activity and inhibit MMP activity (Adil et al., 2010; Kim et al., 2010). Thailand has several local plants that having antioxidative activities. Therefore, *L. floribunda* and *L. speciosa* were selected to investigate for their total phenolic contents, antioxidative activity and inhibitory effect against gelatinase enzyme MMP-2 and MMP-9.

Methods

Plant materials

The fresh flowers of *L. floribunda* (LF) and *L. speciosa* (LS) are locally called Ta-bag and Inthanin, respectively, were collected from Khon Kaen and Ubon Ratchathani provinces, Thailand, during April-May 2012. Plant materials were dried in an oven at 45°C and powdered in a mechanical grinder.

Preparation of the plant ethanolic extracts

The powders of plant materials were separately extracted by macerating in 50% ethanol at room temperature for 5 days. After filtration, the extracts were concentrated using rotary evaporator at 40°C. Then freeze dried and stored in airtight bottles at -20°C for further use.

Determination of total phenolic content

The total phenolic content in the extracts was measured by the Folin-Ciocalteu method (Singleton et al., 1999). Gallic acid was used for constructing the standard curve and the absorbance of the blue color was read at 725 nm. The results were expressed as mg of gallic acid equivalent (GAE) per g of dried extract.

Determination of antioxidant activity by DPPH radical scavenging

Various concentrations of extract were dissolved in water. Then the sample (0.5 ml) was added with 0.9 ml of methanol and 0.1 ml of 1mM DPPH solution. The absorbance at 515 nm was determined after standing at room temperature for 15 min. The % Inhibition was calculated using the following equation: % Inhibition = $[(A_c - A_s)/A_c] \times 100$ where Ac = absorbance of control and As = absorbance of sample. The inhibitory concentration to scavenge 50% free radicals (IC_{50}) value was calculated from equation of line range by plotting of concentration

versus % inhibition. Lower IC_{50} value indicated higher free radical scavenging activity. Ascorbic acid (Vit. C) and α-tocopherol (Vit. E) were used as the standard antioxidants.

The Ferric reducing antioxidant power (FRAP) assay

FRAP assay is based on the action of electron-donating. FRAP reagent was freshly prepared by mixing 300 mM acetic buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and 20mM ferric chloride at ratio of 10:1:1 (v/v/v). The extracts were dissolved in water. Then the samples (6 µl) were mixed with 18 µl of distilled water and 180 µl of FRAP reagent. After standing for exactly 4 min, the absorbance was determined at 600 nm by a microplate reader. A ferrous sulfate was use as a standard. The results were expressed as mg Fe^{2+} per mg dried extract.

Cell culture

Normal Human Dermal fibroblast (NHDF) cells were maintained in Fibroblast Basal Medium (FBM) supplemented with 5 µg/ml insulin, 1 ng/ml basic human fibroblast growth factor, 2% fetal bovine serum (FBS), antibiotics (gentamycin 50 µg/ml, amphoterici-B 50 mg/ml) (Lonza Inc.) at 37°C in humidified 5% CO_2 atmosphere. After cell confluence > 90%, the medium was changed to DMEM without serum for 24h and harvested by centrifugation at 1200 x g for 5 min. Culture supernatants containing MMP-2 (72 KDa) and MMP-9 (92 KDa) were collected and stored at -80°C. NHDF cells from passages 5-8 were used for the experiments.

Zymography

Gelatin was used to detect MMP-2 and MMP-9 activities in the culture medium. Briefly, the extract samples were mixed with medium and incubated at 4°C for 1 h. Then loading buffer containing 10% SDS without 2-mercaptoethanol was added and loaded on 7.5% gelatin zymography at 150 Volts for 1.5 h. After electrophoresis, the gels were washed in 2.5% Triton X-100 for 1h at room temperature and then incubated in developing buffer (50 mM Tris-HCL; pH 7.5, 200 mM NaCl and 5 mM $CaCl_2$) at 37°C for 20 h. The gels were stained with Coomassie brilliant blue R-250 and destained in the mixture of 8% acetic acid and 4% methanol until visualization of clear bands.

Results

The total phenolic contents of ethanolic extracts of LF and LS were 418.50±39.69 and 636.74±44.14 mgGAE/g, respectively (Table 1). LS and LF showed good DPPH radical scavenging activities (IC_{50} = 3.89 ±0.12, 4.31±0.10 µg/ml, respectively) and had comparable antioxidant activities with Vit. C and Vit. E. In addition, they had high FRAP values.

As shown in Fig. 1, the LF and LS extracts inhibited MMP-2 and MMP-9 in dose dependent manner. The IC_{50} values for MMP-2 activities were 953.07 (LF) and 634.60 (LS) ppm whereas there for MMP-9 were 771.07 (LF) and 548.40 (LS) ppm.

Table 1 Total phenolic contents and antioxidant activities of ethanolic extracts of *L. floribunda* and *L. speciosa* flowers as determined by DPPH and FRAP assays.

Sample	Total phenolic[a] (mgGAE/g)	DPPH[a] (µg/ml)	FRAP[a] (mgFe^{2+}/mg)
LF	418.50±39.69	4.31±0.10	1.21±0.28
LS	636.74±44.14	3.89±0.12	1.79±0.26
Ascorbic acid (Vit. C)[b]	N.D.	5.30±0.43	N.D.
α-tocopherol (Vit. E)[b]	N.D.	6.52±0.13	N.D.

[a] Values are mean ± S.D. (n=3)

[b] N.D.; Not detected.

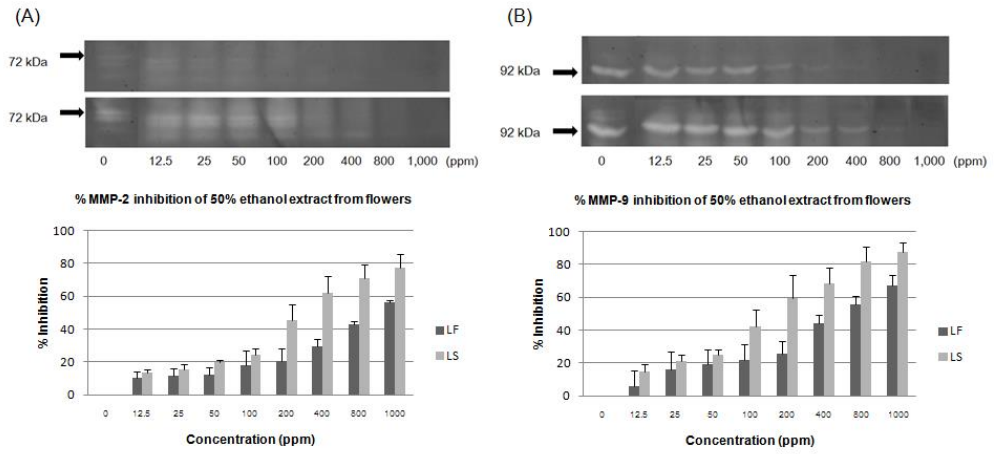

Fig. 1 Gelatin zymography of ethanolic extracts of LS and LF on MMP-2 (72 kDa), (A) and MMP-9 (92 kDa) (B).

Conclusion

The results of this study indicated that 50% ethanolic extracts of LF and LS have high antioxidant activity and strong inhibitory effect on fibroblast of MMP-2 and MMP-9 of. Therefore, these Thai flowers may be potential to further development as anti-aging agent.

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