

ANTITYROSINASE AND ANTIBACTERIAL ACTIVITIES OF MANGOSTEEN PERICARP EXTRACT

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ABSTRACT: The objective of this study is to determine the tyrosinase inhibition and antibacterial activities against the pathogenic bacteria in the oral cavity of the mangosteen pericarp extract. The results showed that the mangosteen pericarp extract inhibited the tyrosinase enzyme at IC₅₀ = 67 ng/ml. Furthermore, the mangosteen pericarp extract also exhibited the antibacterial activities against the pathogenic bacteria in the oral cavity, *Streptococcus mutans* DMST18777, *Porphyromonas gingivalis* DMST2136, and *Streptococcus pyogenes* DMST17020 at minimal inhibitory concentration (MIC) of 0.01 mg/ml, and *Staphylococcus aureus* ATCC25923 at MIC of 0.1 mg/ml by agar dilution method.

Keywords: *Garcinia mangostana*, antityrosinase, antimicrobial, agar dilution method

INTRODUCTION: Mangosteen (*Garcinia mangostana* Linn, Guttiferae) is the plant mostly found in Asia, especially in Thailand. The pericarp of this plant has been used as a medicinal agent in Southeast Asia for the treatment of skin infections and wounds, inflammation, and diarrhea. Its pericarp contains a variety of xanthenes, such as α -, β -, γ -mangostins which have remarkable biological activities. The biological activities of xanthenes isolated from mangosteen include antioxidant, antitumoral, antibacterial, antiviral, antifungal, antiallergic, and anti-inflammatory properties. The antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Bacillus subtilis*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella thyphimurium*, *Proteus* sp., *Klebsiella* sp., *Escherichia coli*, *Enterococcus* spp., and vancomycin resistant *Enterococci* (VRE) of the mangosteen has been reported¹. However, the antityrosinase and antibacterial activities against the pathogenic bacteria in the oral cavity have not been reported. Thus, the purpose of this study is to determine the tyrosinase inhibition and antibacterial activities against the pathogenic bacteria in the oral cavity of the mangosteen pericarp extract.

MATERIALS AND METHODS:

Plant materials and extraction method

The pericarp of mangosteen was collected from Kambang district, Chanthaburi Province, Thailand in April 2006. Air dried and powdered of *G. mangostana* (0.5 kg) was thoroughly extracted with ethyl acetate at 50°C for 48 h. The combined extracts were concentrated in vacuo to give crude extract (41 g) which stored at room temperature.

Mangosteen pericarp extract-PVP complex

Due the solubilities of mangosteen pericarp extract in the water and buffer were very low, the complex between Polyvinylpyrrolidone MW 40,000 (PVP K30) (Fluka, USA) and the mangosteen pericarp extract was made. The complex between mangosteen pericarp extract and PVP K30 was performed by modifying the method as described earlier². In brief, the mangosteen pericarp extract was dissolved in methanol (Labscan, Thailand) and then slowly poured into the solution of PVP in methanol in the ratio 1:1. The organic solvent was completely removed under vacuum to yield the mangosteen pericarp extract-PVP complex (MPC).

HPLC Analysis for α -mangostin content

The α -mangostin content in the mangosteen pericarp extract was analyzed by HPLC. The mangosteen pericarp extract and MPC were dissolved in methanol (1 mg/ml). The sample was injected into a C-18 column (Hypersil BDS, 25 cm X 4.6 micron) (Thermo Scientific, England), using a Perkin Elmer HPLC system with a UV spectrophotometer detector. The mobile phase

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was run in isocratic fashion of 6% water in MeOH at flow rate of 1 ml/min. The α -mangostin was detected at wavelength of 319 nm³. The standard α -mangostin (>98 % purity) was dissolved in methanol to make a stock solution at concentration of 0.1 mg/ml. The stock solution was further diluted with methanol to make the standard concentrations of 20 μ g/ml, 40 μ g/ml, 60 μ g/ml, and 80 μ g/ml. The standard curve of α -mangostin was plotted between peak area ratio and concentration.

Antityrosinase Assay

Chemicals

DL-DOPA, mushroom tyrosinase (T3824-25KU) and kojic acid were purchased from Sigma (USA).

Inhibition of tyrosinase activity

Tyrosinase activity inhibition was determined by the method as described earlier⁴. In brief, MPC was dissolved in 95% ethanol to make the stock solutions at concentrations of 0.2, 0.02, 0.002, 0.0002, and 0.00002 mg/ml, respectively. The 96-well plate was prepared by applying 140 μ l of phosphate buffer pH 6.8, 20 μ l of tyrosinase (48 units/ml), 20 μ l of sample, and 20 μ l of 0.85 mM DL-DOPA to make the test concentrations of MPC at 0.01, 0.001, 0.0001, 0.00001, and 0.000001 mg/ml, respectively. After incubation for 10 minutes, the enzyme activity was determined by measuring the absorbance at 492 nm using the microplate reader (Anthos, Zenith 200rt) comparing to the control consisting of 95% ethanol instead of sample. Kojic acid (1 mg/ml) was used as positive control. Three independent experiments were performed and each experiment was run in triplicate. The percentage of tyrosinase inhibition was calculated as follows:

$$\% \text{inhibition} = \frac{(A_{\text{control}} - A_{\text{blank}}) - (A_{\text{sample}} - A_{\text{blanks}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100$$

A_{control} = Absorbance at 492 nm of the solution without sample but consisted of enzyme

A_{blank} = Absorbance at 492 nm of the solution without sample and enzyme

A_{sample} = Absorbance at 492 nm of the solution with sample and enzyme

A_{blanks} = Absorbance at 492 nm of the solution without enzyme but consisted of sample

Antibacterial assay

Test organisms

The bacterial strains used were *Streptococcus pyogenes* DMST17020, *Staphylococcus aureus* ATCC25923, *Streptococcus mutans* DMST18777, and *Porphyromonas gingivalis* DMST2136. All the bacteria were obtained from Thailand National Institutes of Health.

All bacteria except *P. gingivalis* DMST2136 were cultured on Tryptic soy agar (TSA) at 37°C for 24 h prior to use. The *P. gingivalis* DMST2136 was cultured on Brucella agar with vitamin K and hemin (BCA) at 37°C in anaerobic atmosphere for 120 h prior to use.

Chemicals

Tryptic soy agar (TSA) and Brucella agar with vitamin K and hemin (BCA) were purchased from Difco™ (USA) and Sigma-Aldrich (USA), respectively. Dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich (USA). The CO₂ pad (Anaerocult® A) was purchased from Merck (USA).

Agar dilution method

The minimal inhibitory concentration (MIC) values were determined by the agar dilution method⁵. In brief, the MPC was dissolved in 10% DMSO (10 ml of DMSO in 90 ml sterile water) then diluted to achieve the concentration of 400 mg/ml, 4 mg/ml, 0.4 mg/ml, 0.04 mg/ml, and 0.004 mg/ml, respectively. Each 0.5 ml of stock solution was mixed with 19.5 ml of molten TSA or BCA agar at 50°C until homogeneous before being poured into the sterile petridish and allowed to solidify in the sterile atmosphere. The final concentrations of tested samples were 10 mg/ml, 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml, respectively.

Microbial suspension in sterile water containing 10⁸ CFU/ml of bacteria was adjusted to McFarland No. 0.5 standard turbidity. A 20- μ l of bacterial suspension was applied and spread on the TSA and incubated at 37°C for 24 h for *S. mutans* DMST18777, *S. pyogenes* DMST17020, and *S. aureus* ATCC25923. *P. gingivalis* DMST2136 was tested for susceptibility on BCA and incubated at 37°C in anaerobic atmosphere for 120 h by using anaerobic jar and Aerocult A®

as CO₂ generator. The lowest concentration that can inhibit the growth of bacteria was reported as MIC. Two independent experiments were performed and each experiment was run in triplicate.

RESULTS AND DISCUSSION: The result shows that the retention time of the standard α -mangostin was 4.16-4.28 min. The mangosteen pericarp extract and MPC showed similar retention time as the standard α -mangostin when using the same HPLC condition as described above. The α -mangostin content in mangosteen pericarp extract and MPC at the same weight were 35.95% and 16.91%, respectively. The loss of α -mangostin in the complex was less than 10%. Also, no new peak was observed in the chromatogram. Thus, the complex between PVP K30 and the mangosteen pericarp extract was in the ratio 1:1 without chemical degradation during the preparing process. The MPC can improve the water solubility problem of the mangosteen pericarp extract. The MPC easily dissolve in the phosphate buffer of the tyrosinase inhibition assay system.

Antityrosinase Activity

The MPC inhibited the tyrosinase enzyme at IC₅₀ = 134 ng/ml. The MPC consisted of 1:1 mangosteen pericarp extract and PVP K30, thus, the mangosteen pericarp extract itself inhibited the tyrosinase enzyme at IC₅₀ = 67 ng/ml (Fig 1).

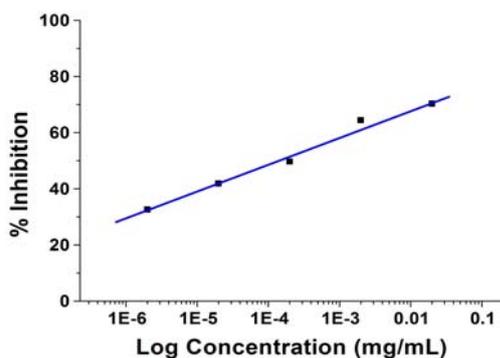


Figure 1 Antityrosinase activity of MPC.

According to the excellent antityrosinase activity, the mangosteen pericarp extract might be used as anti-melasma agent.

Antibacterial activity

The antibacterial activity of the mangosteen pericarp extract against the pathogenic bacteria in the oral cavity was performed by the agar dilution method. The mangosteen pericarp extract displayed antibacterial activity against *S. pyogenes* DMST17020, *P. gingivalis* DMST2136, and *S. mutans* DMST18777 at MIC = 0.01 mg/ml while the antibacterial activity against *S. aureus* ATCC25923 at MIC = 0.1 mg/ml.

The oral cavity pathogenic bacteria including *S. pyogenes*, *S. mutans*, *S. aureus*, and *P. gingivalis* are involved in pharyngitis⁶), tooth decay⁷), wound infection⁸), and periodontal disease⁹), respectively. Therefore, the mangosteen pericarp extract displayed the excellent potential to add in products of mouth spray, oral paste, and toothpaste.

Previous works showed that the antibacterial activity of the mangosteen pericarp extract derived from the presence of the α -mangostin^{1,10,11}). So far no one has reported the tyrosinase inhibition activity of the mangosteen pericarp extract¹. Okunji, *et al.* reported the antityrosinase activity of seeds of *Garcinia kola*, the plant in the same genus as *G. mangostana* in 2007¹²). The antityrosinase activity of the *G. kola* was from biflavanone, 3'', 3''', 4'', 4''', 5, 7, 7'''-octa-hydroxy-3, 8''-biflavanone presented in the plant¹²). However, a large amount of α -mangostin was found in the mangosteen pericarp extract, so, it is very possible that the antityrosinase activity of the mangosteen pericarp extract might be from the α -mangostin.

The limitation of study the tyrosinase inhibitory activity of the mangosteen pericarp extract might be due to the water solubility problem. The complex formation between PVP K30 and the extract can improve the water solubility problem of the mangosteen pericarp extract. Thus, this methodology can be used as the alternative method for studying the biological activities such as antityrosinase activity of the water poor solubility compounds.

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