Anti-oxidant and anti-bacterial properties of leaf extract of *Pithecellobium dulce*

Watsika Vichaidit*, Panumart Thongyoo*

*Department of Chemistry, Faculty of Science and Technology, Rangsit Campus Thammasat University, Pathumthani, Thailand 12120

*tpanumas@tu.ac.th phone +66-59410465 fax +66-25644483

ABSTRACT

*Pithecellobium dulce* is one of Thai medicinal plants belonging to the *Fabaceae* family and applied extensively as a folk medicine for the treatment of wounds, dental caries, and diarrhea. This research highlighted the anti-oxidative and anti-bacterial activities from the methanolic extract of *Pithecellobium dulce*. This methanolic extract was assessed an anti-bacterial activity against *Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA) by using disk diffusion and minimum inhibitory concentration (MIC) methods. Interestingly, the methanolic extract demonstrated a promising anti-bacterial activity against *Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC values ranging from a concentration of 6.25 mg/ml to 50 mg/ml. Additionally, the methanolic extract also exhibited a very potent anti-oxidative property via DPPH assay. According to their anti-bacterial and anti-oxidative activities, the leaf extract from *Pithecellobium dulce* might possibly be applied as natural preservative ingredients in food and pharmaceutical industries.

**Keywords:** *Pithecellobium dulce*, Antibacterial, Antioxidant, Anti-inflammatory
1. INTRODUCTION

For the last century, a variety of medicinal plants have been applied for the treatment of human diseases, especially infectious diseases from microorganism. The increasing number of bacterial resistance to antibiotics has become a great concern worldwide. For this reason, the exploration of novel alternative medicines for the treatment of infectious diseases is urgently required. In Thailand, biodiversity is relatively high according to their plant and animal types distributed in different regions. To date, a vast number of medicinal plants have been studied to explore novel natural antimicrobial compounds. Much interest has been focused so far on the exploration of the new active compounds from natural product especially from plants for the treatment of human diseases. *Pithecellobium dulce* [1,2] is one of Thai medicinal plants belonging to the Fabaceae family. *P. dulce* is an evergreen tree widely distributed throughout India and also found in Southeast Asia, South Africa and Australia. This plant has been used for hedges, street trees and used extensively as a folk medicine. Leaves of *P. dulce* are used for the treatment of wounds, dental caries, and diarrhea. The bark of the plant is used as for the treatment of dysentery, febrifuge and eye inflammation. In addition, the fruits of *P. dulce* have been consumed as a dietary supplement. The fruits are used as for the treatment of gastric ailments. To this report, we focused on the evaluation the inhibitory effect of the extract of *P. dulce* against a panel of bacterial strains ranging from *S. aureus*, *B. cereus*, *E. faecalis*, *E. coli*, including the evaluation of anti-oxidative activity of the methanolic extract by DPPH.

2. MATERIALS AND METHODS

**General experimental procedure**

The NMR spectra were recorded in CDCl3 using BRUKER-NMR 400MHz spectrometer at 400 MHz for 1H NMR and at 100 MHz for 13C NMR using TMS as internal standard, and chemical shifts are expressed in δ values. Analytical and preparative TLC was carried out on pre-coated silica gel 60F254 and RP-18F254 plates (Merck, 0.25 or 0.50 mm thickness).

**Plant material**

The leaves of *P. dulce* used in this experiment were collected at Prachuabkirikhun Province, Thailand, in April 2010. A voucher specimen (LS 150650) was deposited at Department of Chemistry, Thammasat University, Thailand.

**Plant preparation**

The leaves of *P. dulce* (2 kg) were grinded with a mortar, and subsequently extracted with methanol (1500 ml) for 2 weeks. The decanted crude methanolic extract was filtered by Whatmann filter paper 1 and subsequently concentrated in vacuo to afford the crude methanolic extract (200 mg).

**Bacterial species**

*Staphylococcus aureus* (TISTR 1466), *Bacillus cereus* (TISTR 687), *Enterococcus faecalis* (TISTR 379), *Escherichia coli* (TISTR 780), which were obtained from Thailand Institute of Scientific and Technological Research, and Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300) from the American Type Culture Collection (ATCC).

**Determination of anti-bacterial activity**

Four concentrations of the extract (0.05 g/mL, 0.10 g/mL, 0.20 g/mL, and 0.40 g/mL) were prepared and assessed an anti-bacterial activity by using a disc diffusion method as a screening test [3]. Stock culture of test bacteria were grown in nutrient broth medium at 37°C for 22 h. The culture suspensions were prepared and adjusted to approximately 1.5 x 10^8 cfu of bacteria/mL (0.5 McFarland turbidimetry). One hundred microliters of the inoculum were spread over plates containing sterile nutrient agar. Circular discs of 6 mm diameter impregnated with 50 microliters of each concentrations of extract were placed on the surface of the media. The plates were incubated at 37°C for 24 h. Each concentration of extracts were carried out in triplicate. After incubation, the diameter of the zone of bacterial growth inhibition around each disc were measured and recorded in millimeter. Cephalexin (50 mg) was used as a positive control and distilled water was used as a blank.

**Determination of minimum inhibitory concentration**

0.85% normal saline was added in sterile plate. A stock concentration of 100 mg/mL for crude extracts in methanol was pipetted into the first row of the plate and prepared a serial dilution. To each well 30 µL of 3.3x strength isosensitised broth and 10µL 5x10^7 cfu/mL of bacteria were added. Cephalexin (1.5 mg) was used as positive control and methanol was used as solvent control. The plates were incubated at 37°C for 24 h. After that, 10 µL of resazurin indicator was added in each well. The plates were incubated at 37°C for 3 h. and recorded for the result. From result, blue color was recorded as positive. The lowest concentration at which color did not change
from blue to pink was recorded as the MIC value. The average of three from four values was calculated to MIC value [4].

**Determination of minimum bactericidal concentration**

A nutrient broth from well recorded in the MIC assay was cultured on nutrient agar. The plates were incubated at 37°C for 24 h. After incubation, the highest dilution (least concentration) that inhibited the colony formation on nutrient agar was recorded as MBC value.

**Determination of anti-oxidant activity by thin layer chromatography (TLC) analysis**

The methanol extract of *P. dulce* was loaded on TLC plates. The plates were developed in methanol: ethyl acetate (0.5:9.5, v/v) to separate compound from crude extract. The plates were developed, subsequently dried by air, and sprayed with 0.05% of DPPH solution in methanol to evaluate an anti-oxidative activity. The appearance of yellow color was recorded as anti-oxidant activity [5].

3. RESULTS

The evaluation of an anti-bacterial activity against a panel of bacterial strains was assessed by disc diffusion method as displayed in Table 1. It was found that the methanolic extract of *P. dulce* demonstrated a promising anti-bacterial inhibition with the highest activity at 0.4 g/mL against *E. coli*, *S. aureus*, MRSA, *B. cereus* and *E. faecalis*. At the concentration of 0.2 g/mL, this extract exhibited an anti-bacterial activity only against *E. coli* and MRSA. Additionally, at the lowest concentration studied in this experiment (0.1 g/mL) the methanolic extract showed an inhibition only against *E. coli* as shown in Table 1.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Gram stain</th>
<th>Diameter of inhibition zone (mm) against various concentration of extract (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>Methicillin-resistant <em>S. aureus</em> (MRSA)</td>
<td>+</td>
<td>N</td>
</tr>
</tbody>
</table>

N = no inhibition zone, diameter disc (6 mm)

The determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *P. dulce* methanolic extract was assessed and the result was shown in Table 2. Interestingly, all five bacterial strains used in this experiment were sensitive to the methanolic extract from *P. dulce* with different MIC values, ranging from 12.5 mg/mL to 100 mg/mL. It is very important to note that the methanolic extract from *P. dulce* demonstrated a promising inhibition against medicinally important bacterial strain named as MRSA with that MIC value of 25 mg/mL, and against *S. aureus* with the MIC value of 50 mg/mL. The MBC of each bacterial strains was displayed in Table 2. Additionally, the methanolic extract of *P. dulce* also exhibited an anti-oxidative activity as confirmed by DPPH.

<table>
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<th>Bacterial strains</th>
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<th>MIC and MBC values are in mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>100 100</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>+</td>
<td>50 100</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>+</td>
<td>100 100</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>12.5 100</td>
</tr>
<tr>
<td>Methicillin-resistant <em>S. aureus</em> (MRSA)</td>
<td>+</td>
<td>25 100</td>
</tr>
</tbody>
</table>
4. CONCLUSIONS

*P. dulce* is one of Thai medicinal plants and applied extensively as a folk medicine for the treatment of wounds, dental caries, and diarrhea. It was observed that methanolic extract of *P. dulce* demonstrated anti-bacterial and anti-oxidative properties against a panel of bacterial strains, ranging from *E. coli*, *S. aureus*, MRSA, *B. cereus* to *E. faecalis* with impressive MIC and MBC values. Additionally, the methanolic extract of *P. dulce* also showed an anti-oxidative activity as confirmed by DPPH. This finding has paved the way of the exploration of pure active compounds from *P. dulce* with therapeutic applications.

ACKNOWLEDGEMENTS

This work was funded by The National University Project of Thailand’s Office of Higher Education Commission. We also thank to Dr. Ratthada Janglun for invaluable advice and Center of Scientific Equipment, Faculty of Science and Technology, Thammasat University for mass spectrometer and NMR spectrometer.

REFERENCES