Anti-inflammatory and Bactericidal Properties of Selected Indigenous Medicinal Plants Used for Dysuria

Bungorn Sripanidkulchai
Unchalee Tattawasart
Pisamai Laupattarakasem
Varima Wongpanich

Follow this and additional works at: https://digital.car.chula.ac.th/tjps
Part of the Pharmacology Commons

Recommended Citation
Available at: https://digital.car.chula.ac.th/tjps/vol26/iss1/4

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Pharmaceutical Sciences by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.
นิพนธ์ต้นฉบับ

ฤทธิ์ต้านการอักเสบและการสลายแบคทีเรียของส่วนสกัดด้วยน้ำชองพืชสมุนไพรที่ใช้รักษาอาการปัสสาวะขัด

บงอร ศรีพานิชกุลชัย 1*, อัญชลี ตัตตะวะศาสตร์ 2 และ วริมา วงภัพาณิชย์ 4

1 ภาควิชาแพทยศาสตร์แผน สุนัข ศูนย์การแพทย์สุนัข มหาวิทยาลัยขอนแก่น 40002
2 ภาควิชาจุลารักษศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น 40002
3 ภาควิชาเภสัชศาสตร์แผน สุนัข ศูนย์การแพทย์สุนัข มหาวิทยาลัยขอนแก่น 40002
* ผู้เขียนที่สามารถติดต่อได้ โทรศัพท์: 04-336-2095 โทรสาร: 04-324-1243 ที่อยู่อีเมล: bungorn@kku.ac.th

บทคัดย่อ

การศึกษาฤทธิ์ต้านการอักเสบ และฤทธิ์สลายแบคทีเรียของส่วนสกัดด้วยน้ำชองพืชสมุนไพรที่ใช้รักษาอาการปัสสาวะขัด พบว่า การฉีดส่วนสกัดจากรากสับปะรด รากมะละกอ และลำต้นของมะเฟืองเข้าทางช่องท้องสามารถยับยั้งการอักเสบที่อุ้งเท้าของหนูขาวซึ่งเกิดจากคาร์ราจีนได้ใกล้เคียงกับฤทธิ์ของยาแอสไพรินที่ให้ทางปาก ส่วนการให้ส่วนสกัดสมุนไพรทางปากพบว่าเฉพาะส่วนสกัดจากรากมะละกอเท่านั้นที่มีฤทธิ์ต้านการอักเสบ ผลจากการศึกษาฤทธิ์สลายแบคทีเรียพบว่า เชื้อ Staphylococcus aureus มีความไวต่อส่วนสกัดจากมะเฟือง และมีค่าความเข้มข้นต่ำสุดในการฆ่าที่เท่ากับหรือต่ำกว่า 15.62 มิลลิกรัม/มิลลิลิตร ส่วนสกัดจากรากแห้วหมู และหญ้าคาสามารถฆ่าเชื้อ Escherichia coli ได้และให้มีความเข้มข้นต่ำสุดในการฆ่าที่เท่ากับหรือต่ำกว่า 62.5 มิลลิกรัม/มิลลิลิตร และพบว่าเฉพาะส่วนสกัดจากมะเฟืองเท่านั้นที่ฆ่าเชื้อ Klebsiella sp. ได้ที่ความเข้มข้น 125 มิลลิกรัม/มิลลิลิตร

กุญแจคำ

ฤทธิ์สลายแบคทีเรีย, ฤทธิ์ต้านการอักเสบ, ปัสสาวะขัด, สมุนไพร
Anti-inflammatory and Bactericidal Properties of Selected Indigenous Medicinal Plants Used for Dysuria

Bungorn Sripanidkulchai '■ , Unchalee Tattawasart 2 , Pisamai Laupattarakasem 3 and Varima Wongpanich 1

1 Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002
2 Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002
3 Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002
* Corresponding author. Telephone: 04-336-2095; Fax: 04-324-1243; Email: bungom@kku.ac.th

Abstract
Aqueous extracts of five plants having ethnomedical application for dysuria were investigated for anti-inflammatory and bactericidal activity. The extracts of Ananas comosus and Carica papaya roots and of Averrhoa carambola stem inhibited rat paw inflammation induced by carrageenan. Intraperitoneal administration of these extracts revealed only the anti-inflammatory activity of C. papaya. For bactericidal studies, Staphylococcus aureus was the most sensitive organism to the extract of A. carambola with a minimal bactericidal concentration (MBC) < 15.62 mg/ml. Escherichia coli, Pseudomonas aeruginosa and S. aureus were similarly affected by the extracts from rhizomes of Cyperus rotundus and Imperata cylindrica with their MBCs of 62.5 mg/ml, whereas Klebsiella sp. was not affected by any of the test plants except A. carambola with MBC of 125 mg/ml.

Key words
Bactericidal, Anti-inflammatory, Dysuria, Herbal medicine, Medicinal plant

Introduction
Traditional medicine has been accountable and well accepted as an alternative treatment in several developing countries including Thailand. Our study on five selected medicinal plants commonly used by traditional practitioners for treatment of dysuria revealed the diuretic effect of the aqueous extracts from the root of Ananas comosus and Carica papaya, commonly known as pineapple and papaya, respectively (1). The treatment of dysuria, which is a common symptom found among the Northeast residents, is based on the use of diuretics, antibacterial and/or anti-inflammatory drugs.

Although there were previous studies related to antibacterial and anti-inflammatory effects of these two plants, not the root part as reported for diuresis (2). The antibacterial activity was shown in A. comosus fruit (3) and in C. papaya fruit and seed (4, 5). Anti-inflammatory activity was also demonstrated in pineapple (6) and papaya latex (7). Besides the two plants, the other three on our list (Cyperus rotundus, Imperata cylindrica and Averrhoa carambola) did not appear to have diuretic activity (1). As their impact on dysuria might be due to effect(s) other than diuretic, we justified a battery of tests for the biological activity related to the basis of treatment. The present study, thus, aimed to...
investigate the anti-inflammatory and bactericidal activity of all five medicinal plants.

Materials and Methods

Plant materials and preparation of the extracts

The aqueous extract of five indigenous plants reported to be used as traditional medicines for the treatment of dysuria in Khon Kaen province were prepared accordingly to the method described by local traditional practitioners (1). The plant parts used in this study were the roots of *A. comosus* (Linn.) Merr. and *C. papaya* Linn., the rhizomes of *C. rotundus* Linn. and *I. cylindrica* (Linn.) Beauv. and the stem of *A. carambola* Linn.

Animals

Adult male Sprague-Dawley rats with a weight range of 140-200 g were purchased from Mahidol University Animal Center at Salaya, Thailand. The animals were then housed in cages of five, at 25°C in the animal unit, Faculty of Medicine, Khon Kaen University, with free access to pellet diet and water for a minimum of 3 days prior to study. The conditions were maintained on a 12 hour light-dark cycle, with an ambient temperature of 25°C. Prior to the beginning of the experiment, all animals were fasted overnight with free access to water.

Chemicals

Acetylsalicylic acid (ASA), from the Thai Government Pharmaceutical Organization, was used as the reference anti-inflammatory drug. All other chemicals were obtained from Sigma Chemical Company.

Carrageenin-induced rat paw edema

The method described by Winter et al. (8) was used to induce inflammation in rats. A 0.1 ml of carrageenin solution (1% w/v in normal saline) was injected into the right hind paw of each rat at the plantar aponeurosis region. The animals were divided into 12 groups. One hour before carrageenin injection, the rats were treated as follows. Group 1 (control) was given distilled water. Group 2 was given a standard drug, acetylsalicylic acid (ASA 300 mg/kg), orally. Group 3-12 (test groups) were given plant extracts at doses equivalent to 5, 10, 20 g of plant dried weight before extraction, per kg body weight of animals. The details on dose and route of plant treatment of these groups were shown in Table 1. An increase in paw volume as measured by plethysmometer (Model 7150, Ugo, Italy) was observed for 3 hours after carrageenin administration and taken as a quantitative measurement of edema.

Antibacterial assay

The following microorganisms were used: *Escherichia coli* ATCC 25922, *Klebsiella* sp. (clinical isolate), *Pseudomonas aeruginosa* ATCC 25922, and *Staphylococcus aureus* ATCC 25923. Test microorganisms were prepared by inoculating 2-3 colonies of overnight bacterial cultures into 2 ml of Mueller Hinton Broth (MHB) (Difco Laboratories, Detroit, MI, USA.) and incubated at 37°C in shaking water bath for 3 h. The cultures were diluted 1:100 with MHB to yield approximately 105-106 CFU/ml of micro-organisms. Lyophilized aqueous extract of plants were redissolved in sterile distilled water to obtain a 250 mg/ml solution. The antibacterial activity was performed by broth microdilution technique (9). MHB was used to make a serial two-fold dilution of the plant extracts in a microtiter plate to yield a final volume of 50 μl in each well. An inoculum (50 μl) of test culture was added into each well by multi-channel micropipette to yield a final concentration of plant extracts ranging from 125 to 15.62 mg/ml. Two sets of control were included. One set was a microorganism control, consisting of the microorganism without plant material. The second set was a sterility control, consisting of the plant material without microorganism. The microtiter plate was incubated at 37°C for 18-24 h. Because of the dark color of plant extracts, the determination of antibacterial activity was conducted by transferring the overnight content from the microtiter plate to blood agar and incubated at 37°C for another 24 h. The lowest concentrations of plant extracts that killed the microorganism were observed and expressed as minimal bactericidal concentrations (MBCs). All tests were performed in duplicate.

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical comparisons within the
same group were performed with Student’s t-test for pair observation. Differences between groups were evaluated at *p*<0.01, using the Student’s unpaired t-test.

**Results**

**Anti-inflammation**

As shown in Table 1, the intraperitoneal pretreatment with the aqueous extracts from five plants revealed anti-inflammatory activity by inhibiting rat paw edema induced by carrageenin. Given orally, all plant extracts except the one from *C. papaya* failed to inhibit the inflammation. After the first hour, the extracts from *C. papaya* (10 g/kg, p.o. and 5 g/kg, i.p.), *A. comosus* (20 g/kg, i.p.) and *A. carambola* (20 g/kg, i.p.), demonstrated comparable anti-inflammatory effects to that of ASA at a dose of 300 mg/kg. However, during the second and third hour these extracts showed stronger activity than that of ASA. The extracts from *C. rotundus* and *I. cylindrica*, given intraperitoneally at a dose of 10 g/kg, exhibited a weaker effect than that of ASA. In contrast, the extract from *A. comosus*, *C. rotundus*, *C. papaya* and *A. carambola* showed rather longer duration of action, since the edematous volume after the third hour could be observed as significantly less than that of ASA.

**Antibacterial activity**

Studies on antibacterial activity showed the growth inhibition of some plant extracts at the concentration used (Table 2). *Staphylococcus aureus* was the most sensitive microorganism to *A. carambola* extract, as indicated by a MBC of 15.62 mg/ml or less. Both *C. rotundus* and *I. cylindrica* extracts similarly inhibited the three tested bacteria, *E. coli*, *P. aeruginosa* and *S. aureus*, with MBCs of 62.5 mg/ml. The extracts from *A. comosus* and *C. papaya* did not show bactericidal activity at the highest concentration used in this study. Except for the extract from *A. carambola*, which moderately killed the test microorganism (MBC value of 125 mg/ml), the rest of four extracts had no bactericidal effect on the test species of *Klebsiella*.

**Table 1.** Effect of plant extracts on carrageenin-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Treatment (route, no. of animals)</th>
<th>Dose a (mg of acetylsalicylic acid or g of dried plant before extraction per kg body weight of animal)</th>
<th>Edematous volume (ml)b (route, no. of animals)</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (p.o., 16)</td>
<td>-</td>
<td>Edematous volume (ml)b (route, no. of animals)</td>
<td>0.21 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>Acetylsalicylic acid (p.o., 16)</td>
<td>300</td>
<td></td>
<td>0.08 ± 0.01*</td>
<td>0.14 ± 0.01*</td>
<td>0.22 ± 0.02*</td>
</tr>
<tr>
<td><em>Ananas comosus</em> (p.o., 8)</td>
<td>20</td>
<td></td>
<td>0.17 ± 0.01*</td>
<td>0.43 ± 0.03*</td>
<td>0.74 ± 0.04*</td>
</tr>
<tr>
<td><em>Cyperus rotundus</em> (p.o., 8)</td>
<td>10</td>
<td></td>
<td>0.06 ± 0.01*</td>
<td>0.06 ± 0.01*</td>
<td>0.02 ± 0.01*</td>
</tr>
<tr>
<td><em>Carica papaya</em> (p.o., 8)</td>
<td>10</td>
<td></td>
<td>0.24 ± 0.02*</td>
<td>0.45 ± 0.02*</td>
<td>0.54 ± 0.01*</td>
</tr>
<tr>
<td><em>Imperata cylindrica</em> (p.o., 8)</td>
<td>5</td>
<td></td>
<td>0.05 ± 0.02*</td>
<td>0.04 ± 0.03*</td>
<td>0.03 ± 0.04*</td>
</tr>
<tr>
<td><em>Avellhosa carambola</em> (p.o., 8)</td>
<td>20</td>
<td></td>
<td>0.07 ± 0.01*</td>
<td>0.02 ± 0.01*</td>
<td>0.01 ± 0.02*</td>
</tr>
</tbody>
</table>

* a mg of acetylsalicylic acid or g of dried plant before extraction per kg body weight of animal

b Values are expressed as mean ± S.E.M., determined at 1, 2, 3 hours after carrageenin injection.

* p < 0.01 statistical significance, relative to control and acetylsalicylic acid treated group, respectively.
Table 2. Minimal bactericidal concentration (mg/ml) of plant extracts.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>E. coli</th>
<th>Klebsiella sp.</th>
<th>Ps. aeruginosa</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ananas comosus</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
</tr>
<tr>
<td>Cyperus rotundus</td>
<td>62.5</td>
<td>&gt;125.0</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
</tr>
<tr>
<td>Imperata cylindrica</td>
<td>62.5</td>
<td>&gt;125.0</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Averrhoa carambola</td>
<td>125.0</td>
<td>125.0</td>
<td>125.0</td>
<td>15.62</td>
</tr>
</tbody>
</table>

Discussion and Conclusions

All of the five plants selected from the list of traditional herbs used for the treatment of dysuria by local practitioners in Khon Kaen province were demonstrated to have significant effects as anti-inflammation, whereas only the extracts from C. rotundus, I. cylindrica and A. carambola had bactericidal activity. Our justification for plant extract administration doses of 5-20 g/kg body weight of test animals in the anti-inflammatory study was based on the calculated amount orally used per day of decoction prepared by traditional practitioners (1, 2). When given intraperitoneally to the rats, the aqueous extracts of A. comosus, C. papaya and A. carambola prevented paw edema induced by carrageenin as ASA did. This finding not only confirmed the previous reports that A. comosus and C. papaya had an anti-inflammatory activity (6, 7, 10) but also demonstrated significant activity of the plant roots. It is interesting to observe that the stem extract of the well-known starfruit plant, A. carambola, showed anti-inflammatory effect in this study. This suggests further pharmacological and chemical studies on parts of this plant other than the edible fruit. Although several compounds such as carotenoids and ionone glucosides had been reported in starfruits (11, 12), other aerial parts of this plant are not thoroughly studied.

The finding that extracts of C. rotundus and I. cylindrica have bactericidal activity on E. coli, Ps. aeruginosa and S. aureus is in agreement with previous reports on antibacterial studies of these two plants, which showed minimal inhibitory concentration (MIC) on Streptococcus mutans MT 5091 at 62.5 and 77.9 mg/ml, respectively (13, 14). In addition, we report for the first time in this study that A. carambola stem extract has a strong bactericidal action on S. aureus, but a moderate effect on a clinical isolate of Klebsiella. Judging from the findings of anti-inflammatory and bactericidal activities, taken together with the previous report on the diuretic effect of these medicinal plants (1), the folkloric claims appear to be supported. Besides, the results reflect a certain degree of harmony on traditional knowledge with modern concept on chemotherapeutic treatment of dysuria. It is also important to point out that the ethnic wisdom on utilization of plants in combination for dysuria, as described by Sripanidkulchai et al. (2) is rational. This wisdom possibly developed from long observations on the differences among the ethnopharmacological effects of these plants as found in our study. Therefore, to make an effective recipe for a certain treatment, active ingredients may have to be collected from several plants. To obtain better scientific support for the effectiveness of traditional application, pharmacological study on the combination of these plants should be further conducted.
Acknowledgements

The authors wish to express appreciation and thanks to Professor Dr. Jit Sitteamorn, and Associate Professor Dr. Nanthawan Bunyaprapatsara for their critical suggestions and encouragement. This work was financially supported by the Canadian International Development Agency (CIDA) and the Research and Development Institute, Khon Kaen University, Thailand.

References


