ANTIBACTERIAL ACTIVITY OF FLAVONOIDS FROM *BAUHINIA SIRINDHORNIAE*

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Abstract

Hexane, chloroform and 95% ethanol extracts from roots and stems of *Bauhinia sirindhorniae* were assayed for antibacterial activity by agar diffusion method. The results revealed that 95% ethanol extracts of roots and stems exhibited activity against *Bacillus subtilis* and *Staphylococcus aureus*. The 95% ethanol extracts from roots and stems were subjected to purification of pure compounds. Fifteen compounds were isolated and characterized. Ten of these compounds were evaluated for antibacterial activities by broth microdilution assay. Results have shown that five compounds were found to be active. (2*S*)-Eriodictyol (**1**), isoliquiritigenin (**4**), isoliquiritigenin 4-methyl ether (**5**) were found to be active against *B. subtilis* with MICs of 50, 100, 200 μg/ml, respectively of and MBCs >200, 100, 200 μg/ml, respectively. They showed activities against *S. aureus* with the same MIC of 200 μg/ml and MBCs of 200, >200, >200 μg/ml, respectively. (2*S*)-Naringenin (**2**) and luteolin (**3**) exhibited antibacterial activity against *B. subtilis* only at MICs of 100, 200 μg/ml, respectively and MBCs of >200, 200 μg/ml, respectively.

Key words: *Bauhinia sirindhorniae*, antibacterial activities, Thai medicinal plant

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ฤทธิ์ต้านแบคทีเรียของฟลาโวนอยด์จากต้นสิรินธรวัลลี

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บทคัดย่อ

การศึกษาฤทธิ์ต้านแบคทีเรียเบื้องต้นด้วยวิธี agar diffusion ของสิ่งสกัดหยาบจากราก และลำต้นของต้นสิรินธรวัลลี ได้แก่ สิ่งสกัดเฮกเซน คลอโรฟอร์ม และ 95% เอทานอล พบว่าสิ่งสกัด 95% เอทานอล จากรากและลำต้นมีฤทธิ์ยับยั้ง *Bacillus subtilis* และ *Staphylococcus aureus* การแยกสารบริสุทธิ์จากสิ่งสกัด 95% เอทานอลจากรากและลำต้นทำให้ได้สารบริสุทธิ์ 15 ชนิด การทดสอบฤทธิ์ต้านแบคทีเรียของสารบริสุทธิ์ 10 ชนิด ด้วยวิธี broth microdilution พบสารบริสุทธิ์ 5 ชนิดที่มีฤทธิ์ต้านแบคทีเรีย ได้แก่ (2*S*)-eriodictyol (**1**) isoliquiritigenin (**4**) และ isoliquiritigenin 4-methyl ether (**5**) สามารถยับยั้งการเจริญของ *B. subtilis* ด้วยค่า MICs ที่ 50, 100, 200 μg/ml ตามลำดับ และ MBCs ที่ >200, 100, 200 μg/ml ตามลำดับ สารทั้งสามชนิดนี้มีฤทธิ์ยับยั้งการเจริญของ *S. aureus* เท่ากัน คือมีค่า MIC ที่ 200 μg/ml และ MBCs ที่ 200, >200, >200 μg/ml ตามลำดับ นอกจากนี้ พบว่า (2*S*)-naringenin (**2**) และ luteolin (**3**) มีฤทธิ์ยับยั้งการเจริญเฉพาะ *B. subtilis* ที่ MICs 100, 200 μg/ml ตามลำดับ และ ที่ MBCs >200, 200 μg/ml ตามลำดับ

คำสำคัญ: ต้นสิรินธรวัลลี, ต้นประดงสามสิบสอง, ฤทธิ์ต้านแบคทีเรีย, สมุนไพรไทย

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Introduction

Bauhinia (Leguminosae-Caesalpinoideae), a genus of erect and climbing shrubs distributed pantropic mostly in the old world. Numerous reports of secondary metabolites from this particular genus reveal those the diverse classes of compounds have been obtained. *Bauhinia sirindhorniae* K. & S.S. Larsen, a tendrilled liana, locally known in Thai as "Sirindhorn-vallee", is a plant indigenous to northeastern Thailand[1,2]. The infusion of its stem is used by Thai local traditional practitioners as an anti-inflammatory in muscular pain.

The present investigation deals with the several biologenetically related compounds from the stems and roots of *B. sirindhorniae.* Antimicrobial activity testing of some isolated compounds was described.

Materials and Methods

Plant material: The stems and roots of *B. sirindhorniae* K. & S.S. Larsen were collected from Nongkhai province, Thailand, in January 2002. Authentication was achieved by comparison with the herbarium specimen (BKF No. 124725) at the Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand.

Extraction and isolation: Dried roots and stems of *B. sirindhorniae* were extracted with hexane, chloroform and 95% ethanol, and then assayed for antibacterial activity by agar diffusion method[3]. Chromatographic separations of the extracts by a silica gel and a gel filtration with the combination of HPLC technique were conducted to give pure compounds. Pure compounds isolated from the 95% ethanol extracts of roots and stem were tested for antibacterial activities by broth microdilution method[3].

Antimicrobial activity testing[3]:

Agar diffusion assay:

Crude extracts were screened by agar diffusion method for its activities against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231 and *Trichophyton mentagrophytes* (clinical isolated).

Test samples: The amounts of crude extracts were 10 mg per disk.

Preparation of the inoculum: Each bacterial strain was cultured overnight on trypticase soy agar (TSA) plate at 37 °C. The isolated colonies were inoculated into a 5 ml trypticase soy broth (TSB) and incubated at 37 °C for 2-3 hours. The turbidity of these inocula was adjusted to match that of a 0.5 McFarland standard No.1 (approximately 10^8 CFU/ml for

bacteria). *Candida albicans* ATCC 10231 was grown on Sabouraud dextrose agar (SDA) slant at 30 °C for 24 hours. The inoculum was prepared by suspending the culture in sterile normal saline solution and turbidity of the inoculum was adjusted to match a 0.5 turbidity standard of McFarland No.1. Spores of *Trichophyton mentagrophytes* grown on SDA slant at 30 °C for 3 days were washed from the slant culture with sterile 0.05% Tween 80. The turbidity of the spore suspension was adjusted to match 0.5 turbidity standard of McFarland No.1 (this produced a fungal suspension containing $1x10^6$ to $5x10^6$ organisms per ml).

Preparation of test plates:

- For testing bacteria

Mueller Hinton agar (MHA) was melted and allowed to cool at 45-50 °C in a water bath. Then 25 ml of the melted agar medium was dispensed into sterile glass petri dishes, with internal diameters of 9 cm, to yield a uniform depth of 4 mm. The agar was allowed to harden on a flat level surface. The plates were dried for 1 hour at 37 °C.

- For testing fungi

Sabouraud dextrose agar (SDA) was used and prepares as described above.

Inoculation of agar plates: A sterile cotton swab was dipped in each inoculum and the excess was removed by rotating the swab several times against the inside wall of the tube above the fluid level. The entire surfaces of the MHA plate and the SDA plate for testing bacteria and fungi, respectively, were inoculated by streaking with the swab for 3 times and each time the plate was rotated 60 degree.

Assay procedure: Within 15 mins after the plates were inoculated, the sample disks were placed individually then gently pressed down onto the agar surface. This was done in duplicate. After maintaining at room temperature for 1 hour, the bacterial and fungal plates were incubated at 37 °C overnight and 30 °C for 48-72 hours, respectively. The sample disks showing inhibition zone were examined further for their minimal inhibitory concentrations (MIC).

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC):

Determination of the MIC and MBC of pure compounds against *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 by broth microdilution test.

Test samples dilution: The samples were dissolved in DMSO and diluted with Mueller Hinton broth (MHB) in a two-fold dilution to give the concentrations ranging from 200 μg/ml to 0.39 μg/ml.

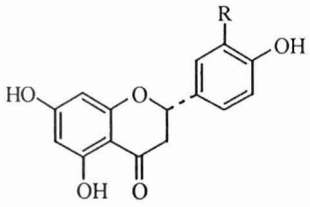
Preparation of the inoculum: The inoculum was prepared as described above. The inoculum was further diluted to 1:100 in MHB.

Assay procedure: A 50 μl volume of each concentration of the sample was dispensed to the corresponding well of the sterile multiwell microdilution plate (96-Flat-shaped wells). Another 50 μl volume of diluted inoculum was added into each well. After incubating the tray at 37 °C for 24 hours, the lowest concentration of the sample that showed growth inhibition was considered as the MIC. This determination was done in duplicate. All inhibitory concentrations were re-checked by addition of each solution showing activity into agar plate, and incubated at 37 °C for 24 hours. The lowest concentration of the test compounds which kill these microorganisms were defined as MBC. Penicillin G was used as a positive control.

Results and Discussion

It was found that some crude extracts obtained from *B. sirindhorniae* possessed antibacterial activities. Chloroform extract of leaves could inhibit *B. subsitis*. The 95% ethanol extracts of leaves, roots and stems showed activities against *B. subtilis* and *S. aureus*. After purification, the extracts of roots and stems yielded 15 pure compounds. The structures of isolated compounds were determined on the basis of spectroscopic data (NMR, IR and MS) and identified as, (2*S*)-eriodictyol (**1**), (2*S*)-naringenin (**2**), luteolin (**3**), isoliquiritigenin (**4**), isoliquiritigenin 4-methyl ether (**5**), (-)-epicatechin, lithospermoside, menisdaurin, (+)-taxifolin, 5,7-dihydroxychromone, 5-hydroxychromone 7-*β*-D-glucoside, (+)-lyoniresinol 3-*O*-*α*-L-rhamnoside, (+)-isolariciresinol 3-*O*-*α*-L-rhamnoside, trimethoxyphenolic-1-*O*-*β*-D-glucoside, protocatechuic acid. Most of them, except lithospermoside, were common plant metabolites. This study provides another data of plant species having lithospermoside that was found in a few plant species. The study on antibacterial activity of the isolated compounds revealed that (2*S*)-eriodictyol (**1**), isoliquiritigenin (**4**), and isoliquiritigenin 4-methyl ether (**5**) showed activity against *B. subtilis* with MICs of 50, 100, 200 μg/ml, respectively and MBCs >200, 100, 200 μg/ml, respectively. Anti-*S. aureus* activity of these three compounds was found to be equal in term of MIC at 200 μg/ml and MBCs of 200, >200, >200 μg/ml, respectively.

(2*S*)-Naringenin (**2**) and luteolin (**3**) exhibited activity against *Bacillus subtilis* with MICs of 100, 200 μg/ml, respectively and MBCs of >200, 200 μg/ml, respectively.



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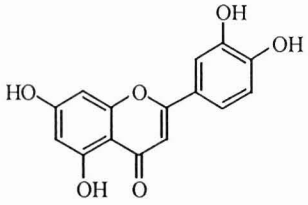
2, R = H



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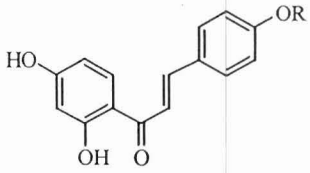

4, R = H
5, R = Me

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