นิพนธ์ต้นฉบับ . Original Article

ฤทธิ์ต้านแบคทีเรียของสารสกัดใบหญ้าขัดมอญด้วยเอทานอลในการต้านเชื้อแบคทีเรียที่แยกได้จากก้อนนิ่ว

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Antibacterial Activity of Ethanolic Extract of *Sida acuta* Burm. F. Leaves Against Bacteria Isolated from Stone Matrices

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Received: 8 December2021 / Edit: 4 March 2022 / Accepted: 28 March2022

บทคัดย่อ

หลักการและวัตถุประสงค์: อุบัติการณ์ของเชื้อแบคทีเรียที่แยกได้จากผู้ป่วยโรคนิ่วไตมีแนวโน้มดื้อต่อยาต้านจุลชีพหลายชนิดเพิ่มขึ้น สารสกัดจากพืชหลายชนิดมีฤทธิ์ในการต้านแบคทีเรีย การศึกษานี้มีวัตถุประสงค์เพื่อประเมินฤทธิ์ต้านแบคทีเรียของสารสกัดใบหญ้าขัดมอญ (*Sida acuta* Burm. F.; SA) ด้วยเอทานอล (EESA) ในการต้านเชื้อแบคทีเรียที่แยกได้จากก้อนนิ่วไต

วิธีการศึกษา: ประเมินฤทธิ์ของ EESA ในการต้านเชื้อแบคทีเรีย 2 กลุ่ม ได้แก่ แบคทีเรียสายพันธุ์มาตรฐาน และสายพันธุ์ที่แยกได้จากก้อนนิ่วด้วยวิธี broth microdilution โดยแต่ละกลุ่มประกอบด้วยแบคทีเรียแกรมบวกและแกรมลบอย่างละ 3 สายพันธุ์ และตรวจหาปริมาณฟลาโวนอยด์ทั้งหมดของ EESA

ผลการศึกษา: ค่าความเข้มข้นต่ำสุดของ EESA ที่สามารถยับยั้งการเจริญของแบคทีเรียแกรมบวกทั้งสายพันธุ์มาตรฐานและสายพันธุ์ที่แยกได้จากก้อนนิ่วไต มีค่าอยู่ในช่วง 1-8 มิลลิกรัม/มิลลิลิตร ส่วนแบคทีเรียแกรมลบทั้งสองกลุ่มมีค่าอยู่ในช่วง 16-32 มิลลิกรัม/มิลลิลิตร โดย EESA มีฤทธิ์ยับยั้ง *Staphylococcus saprophyticus* ได้ดีที่สุด และ EESA มีสารฟลาโวนอยด์เป็นส่วนประกอบ

สรุป: EESA มีฤทธิ์ต้านแบคทีเรียและสารฟลาโวนอยด์ ซึ่ง EESA อาจพัฒนาเป็นยาทางเลือกหนึ่งในการป้องกันและรักษาโรคนิ่วไตที่เกี่ยวกับการติดเชื้อในทางเดินปัสสาวะ โดยเฉพาะ *S. saprophyticus*

คำสำคัญ: ฤทธิ์ต้านแบคทีเรีย, ฟลาโวนอยด์, หญ้าขัดมอญ, แบคทีเรีย, นิ่วไต

Abstract

Background and Objective: The incidence of multidrug-resistant bacteria isolated from stone matrices of kidney stone patients has increased dramatically. Many plant extracts have been studied for antibacterial activity. Therefore, this study aimed to evaluate the antibacterial activity of the ethanolic extract of *Sida acuta* Burm. F. leaves (EESA) against bacteria isolated from kidney stone matrices.

Methods: The antibacterial activity of EESA was evaluated against two groups of bacteria, reference strains and clinical strains isolated from stone matrices by using the broth microdilution method. Each group of bacteria included three Gram-positive and three Gram-negative bacteria. Total flavonoid content of EESA was also determined.

Results: The minimum inhibitory concentrations of EESA against both reference and clinical strains for Gram-positive bacteria were in the range of 1-8 mg/mL whereas those of both groups for Gram-negative bacteria were in the range of 16-32 mg/mL. The best inhibitory activity of the EESA was observed against *Staphylococcus saprophyticus*. The EESA revealed the presence of flavonoids.

Conclusion: The EESA had antibacterial activity and flavonoids. The EESA may be developed as an alternative drug for the prevention and treatment of kidney stone disease with urinary tract infection especially *S. saprophyticus*.

Keywords: antibacteria, flavonoid, *Sida acuta* Burm. F., bacteria, kidney stone

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Introduction

Urolithiasis is well known to be associated with urinary tract infection (UTI)[1-3]. Our previous study showed that in a total of 100 patients with kidney stone disease (KSD), 36 patients had bacteria isolated from urine and/or stone matrices[3] including *Staphylococcus* spp. An increase in antimicrobial drugs resistance among bacterial isolates is leading to the search for some new antibacterial agents. Herbal medicine may help to prevent and treatment of urolithiasis with bacterial infection. These events have forced medical scientists to discover and develop new pharmaceuticals from various natural sources especially Thai medicinal plants. *Sida acuta* Burm. F. (SA) is widely propagated in pantropical areas which are widely used as traditional medicine. This plant is used as a wound-healing agent and diuretic drugs[4-6]. Our previous study, among the reference strains of bacteria, the aqueous extract of *Sida acuta* Burm. F. leaves (AESA) had activity against *S. aureus*[7]. Furthermore, an ethanolic extract had a difference in the solubility of the active component[5]. Therefore, this study aimed to evaluate antibacterial activity of the ethanolic extract of *Sida acuta* Burm. F. leaves (EESA) on both reference strains and clinical strains of bacteria isolated from kidney stone matrices. The total flavonoid content of EESA was also determined.

Materials and methods

Chemicals and reagents

Bacterial culture media were purchased from Oxoid (Hampshire, UK). Gentamicin and quercetin were obtained from Sigma-Aldrich (MO, USA).

Bacterial samples

This study was conducted by following the Declaration of Helsinki and approved by the Institutional Ethical Committee of Khon Kaen University, Khon Kaen, Thailand (HE 521177 and HE 581501). The bacterial samples used in this study were divided into two groups. The first group was the reference strains of bacteria including three Gram-positive (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Staphylococcus saprophyticus* ATCC 15305) and three Gram-negative bacteria (*Citrobacter freundii* ATCC 8090, and *Klebsiella pneumoniae* ATCC 700603, *Salmonella* enterica subsp. *enterica* serovar Vellore. ATCC 15611). The second group was the clinical strains of bacteria isolated from stone matrices including three Gram-positive (*E. faecalis*, *S. aureus*, and *S. saprophyticus*) and three Gram-negative bacteria (*C. freundii*, *K. pneumoniae*, and *Salmonella* spp.). The inclusion and exclusion criteria of the specimen collection were described in our previous study[3].

Determination of the antibacterial activity of EESA

The antibacterial activity of EESA was evaluated by the broth microdilution method[7,8]. Gentamicin was used as control. Minimum inhibitory concentrations (MICs) of EESA against reference and clinical strains of bacteria were recorded. The MIC was the lowest concentration of the EESA or gentamicin that could inhibit the visible growth of bacteria.

Plant extraction

The SA leaves were collected from Khon Kaen province, Thailand, and identified by Prof. Dr. Arunrat Chaveerach. The dried leaves of SA were then ground into a fine powder and extracted with 95% ethanol for 24 h[9]. The SA filtrate was collected after passing through filter paper No.1 and the solvent was removed by using a rotary evaporator (Rotavator R-3, UK). The EESA was used to further analysis.

Determination of total flavonoid content

The total flavonoid content of EESA was determined using the aluminium chloride colorimetric method with quercetin as a standard[10]. The results were expressed as mg quercetin equivalent/g dry weight.

Statistical analysis

Each experiment was performed in triplicate. The total flavonoid contents were presented as the mean ± standard deviation.

Results

The antibacterial activity of EESA

The results revealed that the EESA and gentamicin (control drug) were efficiently suppressing the bacterial growth of reference and clinical strains with variable potency (Table 1 and 2). The MIC values of the EESA against Gram-positive and Gram-negative bacteria were in the range of 1-8 mg/mL and 16-32 mg/mL, respectively. The best inhibitory activity of the EESA was observed against *S. saprophyticus* ATCC 15305 and *S. saprophyticus*.

Total flavonoid content of EESA

The total flavonoid content of EESA was 24.14±0.92 mg quercetin equivalent/g dry weight.

Table 1 Antibacterial activities of EESA and gentamicin against reference strains of bacteria.

Bacterial strains	Minimum inhibitory concentrations	
	EESA (mg/mL)	Gentamicin (µg/mL)
Gram-positive bacteria		
Enterococcus faecalis ATCC 29212	8.00	8.00
Staphylococcus aureus ATCC 29213	8.00	0.50
Staphylococcus saprophyticus ATCC 15305	1.00	≤0.12
Gram-negative bacteria		
Citrobacter freundii ATCC 8090	16.00	1.00
Klebsiella pneumoniae ATCC 700603	32.00	16.00
Salmonella enterica subsp. *enterica* serovar Vellore ATCC 15611	16.00	1.00

EESA = ethanolic extract of *Sida acuta* Burm. F

Table 2 Antibacterial activities of EESA and gentamicin against clinical strains of bacteria isolated from stone matrices.

Bacterial strains	Minimum inhibitory concentrations	
	EESA (mg/mL)	Gentamicin (µg/mL)
Gram-positive bacteria		
Enterococcus faecalis	8.00	0.50
Staphylococcus aureus	4.00	≤0.12
Staphylococcus saprophyticus	1.00	≤0.12
Gram-negative bacteria		
Citrobacter freundii	16.00	2.00
Klebsiella pneumoniae	32.00	1.00
Salmonella spp.	16.00	2.00

EESA = ethanolic extract of *Sida acuta* Burm. F.

Discussion

Both Gram-positive (*E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, and *S. saprophyticus* ATCC 15305) and Gram-negative bacteria (*C. freundii* ATCC 8090, *K. pneumoniae* ATCC 700603, *S. enterica* subsp. *enterica* serovar Vellore. ATCC 15611) were set to analyze because they were the reference strains that matched with clinical strains of bacteria isolated from stone matrices. Our results showed that the EESA inhibited both reference and clinical strains of these Gram-positive and Gram-negative bacteria at different concentrations. The top two sensitive strains to EESA were *S. saprophyticus* and *S. aureus*, respectively, which the bacteria isolated from stone matrices. This result is similar to our previous study[7], the EESA and AESA had antibacterial activity against *Staphylococcus* spp. Several flavonoids in plants have antibacterial activity[11,12]. Our results showed that the EESA contained active gradients such as the flavonoids compound, which was previously reported to have antibacterial activity[13]. In addition, our recent study[9] reported that the SAEE contained catechin, chlorogenic acid, rutin, and ferulic acid. Morever, the antibacterial activity of the EESA was observed that Gram-positive bacteria were higher sensitive than Gram-negative bacteria. The different structures of cell walls between them may be a reason for the antibacterial susceptibility. According to a main target of flavonoids acting on bacteria is the cell membrane via the damage of phospholipid bilayers and som structures[14,15]. This study indicated that the EESA had the potential compounds against the bacteria isolated from stone matrices. For further study, the molecular mechanism of EESA against these pathogenic bacteria is also needed to be researched. The EESA may be used as an alternative agent to prevent and treat KSD with UTI especially *S. saprophyticus*.

Conclusion

The present study showed that the EESA had antibacterial activity and flavonoids. It showed the greatest antibacterial activity against *S. saprophyticus*. Further studies may be undertaken to develop the EESA as an alternative drug for the prevention and treatment of KSD with UTI especially *S. saprophyticus*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research was funded by Khon Kaen University research grant (I62-01-16); and a grant from the Research Fund for Supporting Lecturers to Admit High Potential Students and Research on His Expert Program, Graduate School Khon Kaen University (591H223). We wish to express our deep appreciation to all subjects for providing invaluable clinical specimens.

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