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Antibacterial Effects Against Drug-Resistant Microorganisms of *Coscinium fenestratum*

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Abstract

Background: Nowadays, many bacteria resist the drug actions, which are harmful to humans, such as *Staphylococcus aureus*. Many microorganisms have developed themselves to be methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *Enterococcus faecalis* (VRE), which are resistant to methicillin and vancomycin, respectively. New drugs are needed to develop for use with to fight these bacteria. *Coscinium fenestratum* is an herb in Thai pharmacopoeia, which is known for curing illnesses and is also reported for containing the antibacterial substance called berberine. **Materials and Methods:** The methanol *C. fenestratum* (CFM) extract was used in the antibacterial test against resistant bacteria by disk diffusion method. The antibacterial effect was evaluated against MRSA (30 strains), VISA (4 strains), and VRE (4 strains). **Results:** Test results showed that the CFM extract was very effective against 16 strains of MRSA and 4 strains of VISA at sensitive level. It also displayed sensitive level against 17/30 MRSA strains (56.7%) and 4/4 VISA strains. However, it had antibacterial properties against VRE at moderate level. The range of MIC of the *C. fenestratum* extract was 12.5-100 µg/ml. Its antibacterial activity against VISA 21083 was stronger than methicillin and vancomycin. The highest antimicrobial sensitivity of the extract against MRSA was observed against MRSA 20632 and 20636 at the same concentration of 12.5 µg/ml. Therefore, CFM extract was less effective than vancomycin, but it was higher effective than the drug control (methicillin) in most of MRSA strains. **Conclusions:** *C. fenestratum* methanol extract had higher antibacterial activity than methicillin, but it had lower activity than vancomycin. The natural properties and antibacterial efficiency as shown in the experiments suggested for further purifying medicinal substance form of *C. fenestratum* crude extract as an alternative antimicrobial agent.

Keywords: *Coscinium fenestratum*, MRSA, VRSA, VRE, MIC

Introduction

Antimicrobial resistance in pathogenic bacteria, including *Staphylococcus aureus* and *Enterococcus faecalis*, is one of the greatest threats to human health, causing morbidity and mortality worldwide. *S. aureus* is a gram-positive human commensal that persistently or intermittently colonizes the anterior nares of most of the healthy adult population. However, they could cause diverse pathological infection in acute pattern such as bacteremia and skin abscessed as well as chronic pattern such as osteomyelitis, endocarditis, and infected implanted materials. Pathologic *S. aureus* has a collection of virulence factors and the ability to acquire resistance to antibiotics including methicillin and vancomycin.

Therefore, they have developed themselves to be methicillin-resistant *S. aureus* (MRSA) [1], vancomycin-intermediate *S. aureus* (VISA), and Vancomycin-resistant *S. aureus* (VRSA) strains [2]. Community-acquired infection with MRSA mostly affects healthy young people, without health-care related risk factors for MRSA. Patients often display with skin and soft-tissue infections [3]. First reported case of vancomycin (vancomycin-resistant *S. aureus*, VRSA) was in USA [4], which can cause severe illness.

Enterococci which are part of normal flora especially in gastrointestinal tracts are known as opportunistic pathogens and today are accepted as leading cause of nosocomial infections. Various enterococcal species have been identified, but the major two which cause human diseases are *E. faecalis* and *Enterococcus faecium*. Most common and important these infections are bacteremia, endocarditis, urinary tract infections, surgical wound infections, intra-abdominal and intra-pelvic infections. The emerging of vancomycin-resistant Enterococci (VRE) strain could cause its infection to be an untreatable fatal disease [5]. Therefore, the research for discovery of new substances that have antibacterial effect to these MRSA bacteria is now a high priority.

Coscinium fenestratum or yellow vine, called "Hamm" in Thai which is a medicinal plant in the family *Menispermaceae.* It has been used in traditional medicine in many South and Southeast Asian countries including Thailand. It also appears in Thai medicinal texts and has therapeutic properties such as reducing blood sugar levels, blood cholesterol, liver toxicity and antitumor activity [6-8]. The major components in the wood and root of *C. fenestratum* are isoquinoline alkaloids (berberine, palmatine, tetrahydropalmatine crebanine, jatrorhizine, etc.). Among these, berberine has been reported to be the major and active constituent [9]. It is reported that the most important substances are berberine, which has antibacterial activity, such as *Streptococcus mutans*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus* spp. *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* [10], *Neisseria gonorrhoeae* [11]. However, antibacterial activity data is still scantly especially the potential antibacterial activity against new antimicrobial resistance bacteria.

The present *in vitro* experiment study aims to explore the antimicrobial effect of *C. fenestratum* extract to MRSA, VRSA and VRE strains. In order to have the potential to be developed as a useful product. This is the basis for the extraction of biologically active antimicrobial agents in the future.

MATERIALS AND METHODS

Plant material and preparation of crude plant extracts

The stems of *Coscinium fenestratum* Gaertn., Colebr. (Menispermaceae), were collected from the Medicinal Plant Garden, Chanthaburi province, Thailand. Fresh samples were cut into small pieces and air dried at room temperature. One kilogram of the small pieces of dry samples was blended to obtain fine powder. Then, 100 g of fine powder from stems of the *C. fenestratum* samples were macerated in 500 ml of 95 % methanol (Merck, Darmstadt, Germany), and kept at room temperature for 7 days. Filtration through cheesecloth was performed to separate the plant debris (sediments), and the resulting solution was filtrated through filter paper Whatman No.2. The extraction was carried out three times, and the final filtrated solution was concentrated at reduced pressure, using a vacuum rotary evaporator at 60°C, to yield of an methanol *C. fenestratum* extract. This was stored in a refrigerator at 4°C for further sensitivity test.

Bacterial Strains

Thirty strains of MRSA (*S. aureus* MRSA 20625, *S. aureus* MRSA 20626, *S. aureus* MRSA 20627, *S. aureus* MRSA 20628, *S. aureus* MRSA 20629, *S. aureus* MRSA 20630, *S. aureus* MRSA 20631, *S. aureus* MRSA 20632, *S. aureus* MRSA 20633, *S. aureus* MRSA 20634, *S. aureus* MRSA 20635, *S. aureus* MRSA 20636, *S. aureus* MRSA 20637, *S. aureus* MRSA 20638, *S. aureus* MRSA 20639, *S. aureus* MRSA 20640, *S. aureus* MRSA 20641, *S. aureus* MRSA 20642, *S. aureus* MRSA 20643, *S. aureus* MRSA 20644, *S. aureus* MRSA 20645, *S. aureus* MRSA 20646, *S. aureus* MRSA 20647, *S. aureus* MRSA 20648, *S. aureus* MRSA 20649, *S. aureus* MRSA 20650, *S. aureus* MRSA 20651, *S. aureus* MRSA 20652, *S. aureus* MRSA 20653, *S. aureus* MRSA 20654) and 4 strains of VISA (*S.*

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aureus VISA 20622, *S. aureus* VISA 20623, *S. aureus* VISA 20624, *S. aureus* VISA 21083) and Vancomycin-resistant *Enterococcus faecalis* 4737 (VRE) were received from Department of Medical Sciences, Ministry of Public Health, Thailand.

Preparation of Bacterial Cultures

Bacteria were grown in Trypticase soy broth (TSB, Gibco, Grand Island, N.Y.) for 24 hours at 37°C. The cultures were then diluted with TSB broth to the turbidity of 0.5 McFarland units [10^6CFU/ml].

Kirby-Bauer Disc Diffusion Method

The agar disc diffusion method was used for screening antibacterial activity according to the Clinical and Laboratory Standard Institute [12]. Briefly, the *C. fenestratum* methanol extract was prepared at a concentration of 10 mg/ml in dimethyl sulfoxide (DMSO) and diluted to the concentration of 10, 5 and 2.5 mg/ml. Ten □l of the solution was pipetted onto a sterile filter paper discs (diameter of 6 mm) and placed onto Mueller Hilton (MH) agar surface spreaded with 0.1 ml bacteria [10^5-10^6 colony-forming units (CFU)/ml]. The plates were then incubated for 16-18 h at 37°C for bacterial growth. Inhibition zone values were measured including the diameter of the disc. For each disc, the inhibitory zone diameter was measured in at least three dimensions using a standard ruler, whose smallest division was 1 mm. A diameter of less than 8 mm was omitted for clarity of presentation. A disc prepared with only the coresponding volume of DMSO was served as negative control. Indeed, DMSO has never given clear zone over 1 mm (i.e., about 0.5 mm from each side of the paper disc), and usually gave none)]. Antibiotic discs (Methicillin and vancomycin) (Oxoid) were used as positive controls. Antimicrobial activity was expressed as the inhibitory diameters (mm) produced by the tested compounds.

The inhibition zones of the inoculating plates containing 10, 5 and 2.5 □g/disc of the *C. fenestratum* extract were recorded. Organisms inhibited by three concentrations were classified as sensitive (S), those inhibited only by the two higher as moderate (M) and those growing on three plates were considered resistant (R).

MIC Determination

As the extract is naturally dark in nature, it was designed for MIC determination using the disk diffusion method [13]. Briefly, initial *C. fenestratum* methanol extract was prepared in DMSO, and subsequent two-fold dilution was performed with 0.5 ml of DMSO. The extract was diluted to give the final concentrations of 100, 50, 25, 12.5, 6.25 mg/ml. Ten □l of each solutions was pipette onto a sterile filter paper discs (diameter of 6 mm) and placed onto MH agar surface spread with 0.1 ml bacteria bacteria [10^5-10^6 colony-forming units (CFU)/ml]. The plates were then incubated for 16-18 h at 37°C. At the end of incubation period, the diameter of the inhibition zone was measured. The endpoint MIC is the lowest concentration of compounds at which the inhibition zone was less than 8 mm. DMSO was used as negative control. E-test strips of methicillin and vancomycin (AB Biodisk, USA) were used as positive control.

RESULTS

The *in vitro* microbial activity test of the *C. fenestratum* extract by disc diffusion was shown in Table 1. The different 30 strains of MRSA, 4 VISA and VRE in the study showed variable response towards the *C. fenestratum* extract (Table 1). The extract exhibited antibacterial activity against all resistant tested strains. It was displayed S level against 17/30 MRSA strains (56.7%) and against 4/4 VISA strains. However, it had antimicrobial properties against VRE at moderate level. In MIC determination, all MRSA organisms except *S. aureus* MRSA 20654 were resistant to methicillin, but 3/4 strains of VISA and VRE organisms were sensitive to methicillin at the MIC > 128 □g/ml. The range of MIC of the *C. fenestratum* extract was 12.5-100 □g/ml. The number of sensitive strains of the extract, methicillin and vancomycin was 20, 6 and 35 □g/ml, respectively. Therefore, the extract showed weaker antibacterial activity against all resistant microorganism tests compared to vancomycin. However, the extract had antimicrobial activity against VISA 21083 strain that had stronger activity than methicillin and vancomycin. Maximum antimicrobial sensitivity of the extract against MRSA was observed against MRSA 20632 and 20636 at the same concentration of 12.5 □g/ml.

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DISCUSSION

The increasing trend in development of antibiotic resistance could be attributed to frequent, unnecessary and indiscriminate usage of antibiotics and longer duration of hospitalization [14, 15]. The present study, *S. aureus*, which is predominant organism that causes a large percentage of all skin and soft-tissue infections [16, 17]. There has also been a considerable effort to discover plant-derived antibacterial active against MRSA strains. The plant used in the present study, *C. fenestratum*, is a medicinal plant in many Asian countries. The methanol extract has antibacterial activity against *Klebsiella pneumonia*, *Enterococcus* sp., *Escherichia coli* and *S. aureus* and had stronger antibacterial activity than water extract [10]. Our study found that the methanol extract had antibacterial activity against all MRSA, VISA and VRE. As it has berberine as a high content as was previously emphasized by Nair et al [10]. Berberine is an active compound, which is high content in the *C. fenestratum* methanol extract. It has antibacterial activities against most bacteria included *Bacillus subtilis*, *Enterococcus* sp., *E coli*, *K. pneumonia*, *Proteus vulgaris*, *P. aeroginosa*, *P. fulorescens*, *Salmonella typhi*, *Serratia marcescens*, *S. aureus*. Compared with the methicillin, the extract showed lower antimicrobial activity against resistant strains. All of these results demonstrated the efficacy of the *C. fenestratum* extract against MRSA, VRSA and VRE. These results lead to the conclusion that *C. fenestratum* methanol extract may be considered a suitable alternative for the treatment of drug-resistant opportunistic microorganism such as MRSA, VISA and VRE in the future.

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Table 1: *Antibacterial activity of C. fenestratum methanol extract*

Microorganism	**Zone of Inhibition (mm)**			
	C. fenestratum **10 □g/Disc**	***C. fenestratum*** **5 □g/Disc**	***C. fenestratum*** **2.5 □g/Disc**	**Sensitivity[#]**
S. aureus MRSA 20625	3	2	1.5	M
S. aureus MRSA 20626	20	8	8	S
S. aureus MRSA 20627	5.5	2	1	M
S. aureus MRSA 20628	20	15	11	S
S. aureus MRSA 20629	20	13.5	9	S
S. aureus MRSA 20630	6	2	8	S
S. aureus MRSA 20631	5.5	3	1.5	M
S. aureus MRSA 20632	19	11	7	S
S. aureus MRSA 20633	3	2	0	M
S. aureus MRSA 20634	8	4	0	M
S. aureus MRSA 20635	14	9	2	S
S. aureus MRSA 20636	21	12	9	S
S. aureus MRSA 20637	5	3	1	M
S. aureus MRSA 20638	9	3.5	1.5	M
S. aureus MRSA 20639	6	1	0	M
S. aureus MRSA 20640	3	1	0	M
S. aureus MRSA 20641	5	3	2	S
S. aureus MRSA 20642	9	2	1.5	M
S. aureus MRSA 20643	24	17	14	S
S. aureus MRSA 20644	19	14	9	S
S. aureus MRSA 20645	22	14	11	S

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S. aureus MRSA 20646	14	4	0	M
S. aureus MRSA 20647	9	0	0	M
S. aureus MRSA 20648	8	0.5	0	M
S. aureus MRSA 20649	22	16	12	S
S. aureus MRSA 20650	20	13	10	S
S. aureus MRSA 20651	20	12	9	S
S. aureus MRSA 20652	22	14	9	S
S. aureus MRSA 20653	20	11	9	S
S. aureus MRSA 20654	13	6	5	S
S. aureus VISA 20622	19	14	9	S
S. aureus VISA 20623	17	8	4	S
S. aureus VISA 20624	17	10	7	S
S. aureus VISA 21083	18	12	8	S
E. faecalis 4737 (VRE)	9	2.5	0	M

[#]Strain with inhibition zone from 3 concentrations of the extract was classified as sensitive or "S".

Strain with inhibition zone from 1-2 concentrations of the extract was classified as moderate or "M" and resistance

or "R" showed no inhibition zone from all concentrations.

***Table 2**: No of strains classed as sensitive (S) or moderate (M) by disc sensitivity tests*

Substance	MRSA		VISA		VRE	
	S	M	S	M	S	M
C. fenestratum extract	17/30	13/30	4/4	0/4	0/1	1/1

***Table 3:** Antibacterial activity of C. fenestratum extract against microorganisms*

Strain	**No. of sensitive strains**		
	***C. fenestratum* extract**	**Methicillin**	**Vancomycin**
MRSA (30)	16	3	30
VRSA (4)	4	2	4
VRE (1)	0	1	1
Total	20	6	35

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Table 4: *MIC of the extract against resistant strains*

Microorganism	MIC (µg/ml)		
	Methicillin	***C. fenestratum***	**Vancomycin**
S. aureus MRSA 20625	>128	100	4
S. aureus MRSA 20626	>128	50	4
S. aureus MRSA 20627	>128	100	4
S. aureus MRSA 20628	>128	25	4
S. aureus MRSA 20629	>128	50	4
S. aureus MRSA 20630	>128	100	4
S. aureus MRSA 20631	>128	100	4
S. aureus MRSA 20632	>128	12.5	8
S. aureus MRSA 20633	>128	100	4
S. aureus MRSA 20634	>128	50	4
S. aureus MRSA 20635	>128	100	4
S. aureus MRSA 20636	>128	12.5	4
S. aureus MRSA 20637	>128	100	4
S. aureus MRSA 20638	>128	100	8
S. aureus MRSA 20639	>128	100	8
S. aureus MRSA 20640	>128	100	2
S. aureus MRSA 20641	>128	100	4
S. aureus MRSA 20642	>128	100	8
S. aureus MRSA 20643	>128	50	8
S. aureus MRSA 20644	>128	100	4
S. aureus MRSA 20645	>128	100	4
S. aureus MRSA 20646	>128	100	4
S. aureus MRSA 20647	>128	100	4
S. aureus MRSA 20648	>128	100	4
S. aureus MRSA 20649	>128	50	4
S. aureus MRSA 20650	>128	25	4
S. aureus MRSA 20651	>128	50	4
S. aureus MRSA 20652	>128	25	4
S. aureus MRSA 20653	>128	25	4
S. aureus MRSA 20654	4/8	25	2

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S. aureus VRSA 20622	0.5/1	12.5	8
S. aureus VRSA 20623	>128	12.5	8
S. aureus VRSA 20624	0.25/1	12.5	8
S. aureus VRSA 21083	32/64	12.5	32
E. faecalis 4737 (VRE)	32/64	100	2

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