SCREENINGS TEST OF TRADITIONAL MEDICINAL HERBS FOR BACTERICIDAL ACTIVITY AND THEIR EFFECTS DETERMINATION

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ABSTRACT: Multidrug resistant pathogenic bacteria are an increasing problem that leads to be the difficulty and high cost of treatment. Therefore a novel effective antibiotic is needed. Thai traditional medicinal herbs may be a source of novel drug which have potential antibacterial effect. This study investigated the antibacterial activity of twenty seven Thai medicinal herbal extract using agar diffusion method for screening test and serial dilution method to determined minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). We found that three of them, *Garcinia mangostana* extract, *Mesua ferrea* extract and *Kaempferia galangal* extract showed high antibacterial activity. Among them, *Garcinia mangostana* extract showed the most potency in inhibition on selected indicator strains. It has strong antibacterial activity against MRSA with low MIC which was at 3.91-7.81 µg/ml and MBC was at 3.91-15.63 µg/ml. *Mesua ferrea* extract and *Kaempferia galangal* extract showed MIC and MBC at 15.63-62.50 µg/ml and 125-250 µg/ml respectively. *Garcinia mangostana* Linn. would be a promising novel antimicrobial agent for MRSA infection. Further study is needed to investigate the bacterial response at molecular level.

Keywords: medicinal herb, *Garcinia mangostana*, MRSA, MIC, MIB

INTRODUCTION

Misunderstanding of antibiotic usage such as over prescription or under dose leads to the problem of drug resistance because a certain strain of bacteria has ability to adapt itself by changing in chromosome level especially in plasmid and transposon. These changes conduct the bacteria to resist against the antibiotic and able to transfer this ability to the other microorganism. This problem grows up to be important, increasing of incidence and causes of mortality. Treatment in case of resistant is so difficult and expensive, has more side effect and has mortality than non-resistance case.

Herbs, plants that have curative powers, were used both prevention and treatment such as culinary, dietary supplements, for treatment illness, viral infection, cancer and inflammatory diseases. Include usage in treatment of bacterial infection. Until the advent of antibiotics in mid-20th century then the usage of medicinal herb for bacterial infection was declined. But in recent time herbal usage tend to be more popular since several researcher interested in some advantages of herbs such as it was the natural product.

In this study we point to investigate the antibacterial activity of traditional medicinal herbs which may be a novel drug for curing patients infected with drug resistance pathogenic bacteria in the future.

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MATERIALS AND METHODS

Screening of antibacterial activity

Agar well diffusion method

This method will be used to detect the antibacterial activity of Thai medicinal herb extracts. The agar plate media which are Tryptic Soy Agar (TSA) for Gram-negative bacteria, Mueller-Hinton agar for MRSA and Nutrient Agar (NA) for others Gram-positive bacteria will be prepared. The indicator strains (Table 1) grown on plate overnight at 37ºC will be adjusted in normal saline solution (NSS) to 0.5 Mcfarland and then swab on agar plate. After drying, agar will be punched using Cork Borer No.3 (7 mm. in diameter). Two wells for each sample then 25 µl of the crude extract of traditional medicinal herbs will be added into each well. 50% ethanol will be used as a control. Incubate the plate at 37°C for 24 hours. Measure the diameter of the clear zone around the well in order to identify the antibacterial effect of each agent [1].

Table 1 Indicator strains used in screening test

Gram-negative strain	Gram-positive strain
Salmonella typhimunium	*Bacillus cereus*
Salmonella Typhi	Vancomycin resistance *Enterococcus sp.* (VRE) (DMST 4737)
Shigella boydii	Methicillin Resistance *Staphylococcus aureus* (MRSA DMST 5199)
Shigella sonnei	MRSA ATCC 4738
Shigella dysenteriae	MRSA ATCC 20651
Shigella flexneri	MRSA ATCC 20654
Escherichea coli O157 SW4	*Staphylococcus aureus*
Ampicillin Resistance *Escherichea coli* (ARE)	*Enterrococcus* spp.

Table 2 List of Thai medicinal herbs/formulations in screening test

No.	Scientific Name	Thai Name	Family
1	*Garcinia mangostana* Linn.	เปลือกมังคุด	Guttiferae
2	*Smilax corbularia* Kunth	หัวข้าวเย็นเหนือ	Smilacaceae
3	*Dioscorea membranacea* Pierre	หัวข้าวเย็นใต้	Dioscoreaceae
4	-	หัวข้าวเย็นเหนือ + ใต้	-
5	*Kaempferia galangal* Linn.	เปราะหอม	Zingiberaceae
6	*Mesua ferrea* Linn.	บุนนาค	Guttiferae
7	*Atractylodes lancea* (thung.) DC.	โกฐเขมา	Compositae
8	*Lepidium sativum* Linn.	เทียนแดง	Cruciferae
9	*Dracaena loureiri* Gagnep.	จันทน์แดง	Dracaenaceae
10	*Angelica dahurica* Benth.	โกฐสอ	Umbelliferae
11	*Ligusticum sinense* Oliv. cv. Chuanxiong	โกฐหัวบัว	Umbelliferae
12	*Artemisia annua* Linn.	โกฐจุฬาลำพา	Compositae
13	*Angelica sinensis* (Oliv.) Diels	โกฐเชียง	Umbelliferae
14	*Anethum graveolens* Linn.	เทียนตาตั๊กแตน	Umbelliferae
15	*Cuminum cyminum* Linn.	เทียนขาว	Umbelliferae
16	*Myristica fragrans* Houtt.	จันทน์เทศ	Myristicaceae
17	*Foeniculum vulgare* Mill. var. dulce Alef.	เทียนข้าวเปลือก	Umbelliferae
18	*Abroma augusta* Willd.	เทียนดำ	Sterculiaceae
19	*Syzygium aromaticum* (L.) Merr. & L.M. Perry	กานพลู	Myrtaceae
20	*Mimusops elengi* Linn.	พิกุล	Sapotadeae
21	*Amomum testaceum* Ridl.	กระวาน	Zingiberaceae
22	Pra-sa-proa-yai	ประสะเปราะใหญ่	-
23	*Piper sarmentosum* Roxb. ex Hunter.	ช้าพลู	Piperaceae
24	*Plumbago indica* Linn.	เจตมูลเพลิงแดง	Plumbaginaceae
25	*Piper pendulispicum* DC.	สะค้าน	Piperaceae
26	*Zingiber officinale* Rosc	ขิง	Zingiberaceae

Traditional medicinal herb extracts

Twenty-six traditional medicinal herbal extracts used in this investigation were selected according to their traditional use (Table 2). All traditional medicinal herbs use in this study were bounty of Dr. Arunporn Itharat, Faculty of Medicine, Thammasat University, Pathumthani, Thailand, who grant these materials as agents for the investigation.

Determination of minimal inhibitory concentration (MIC)

Broth microdilution method will be used to determine the MIC and the MBC of agents according to protocol with few modifications [2].

- Preparation of agents: prepare serial dilution (two-fold dilution) of agents in medium from 1:2 to 1:1024 (initial concentration is 1 mg/ml), positive and negative control which are media with indicator strain and blank, respectively.

- Growing the bacterial strains in proper broth and then inoculate them into 96-well microplates, 100 µl of each microorganism was added to each well. Plates were aerobically incubated at 37ºC. After incubation for 18–24 hrs, bacterial growth was determined by measuring the absorbance at OD 625 nm.

- MIC was defined as the lowest concentration of agent that inhibits bacterial growth to the level that < 0.05 at 625 nm (no visible growth).

- The antibacterial effect was indicated by the absence or presence of turbidity and a pellet on the well bottom.

To confirm MIC and to establish MBC, 100 µl of each culture medium with no visible growth was

Table 3 Minimum inhibitory concentration (MIC) of the herbal plants on MRSA strains

Herb extract	MIC (µg/ml)			
	MRSA DMST 5199	MRSA ATCC 4738	MRSA ATCC 20651	MRSA ATCC 20654
G. mangostana	3.91	3.91	7.81	7.81
M. ferrea	15.63	31.25	31.25	31.25
K. galangal	62.50	62.50	31.25	31.25

Table 4 Minimum bactericidal concentration (MBC) of the herbal plants on MRSA strains

Herb extract	MBC (µg/ml)			
	MRSA DMST 5199	MRSA ATCC 4738	MRSA ATCC 20651	MRSA ATCC 20654
G. mangostana	3.91	7.81	7.81	15.63
M. ferrea	125.00	125.00	250.00	250.00
K. galangal	125.00	125.00	250.00	250.00

inoculated to medium plate, incubate 18-24 hrs. Then determine the viability of the strain. MBC was defined as the lowest concentration which inhibits 99.9% of the bacteria.

RESULTS

Preliminary Screening test

Twenty-six traditional medicinal herb extracts were tested against sixteen indicator strains by agar diffusion technique. Eleven traditional medicinal herb extracts showed antibacterial activity against selected gram positive and/or gram negative indicator strains. Fifteen herb extracts showed no effect to bacterial strains (data not shown).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration determination

Five herb extracts with wide range of antibacterial activity which are *Garcinia mangstana* Linn., Pra-sa-proa-yai formula, *Mesua ferrea* Linn., *Lepidium sativum* Linn. and *Kaempferia galangal* Linn. were selected for determination of their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using five indicator strains which are *Bacillus cereus*, *Staphylococcus aureus*, methicillin resistance *Staphylococcus aureus* (MRSA), *Enterrococcus* sp. and vancomycin resistance *Enterococcus* sp. (VRE). *Garcinia mangstana* Linn. extract showed minimum inhibitory concentration against *Bacillus cereus* and *Staphylococcus aureus* at dilution of 1:8 which will be 125 µg/ml. It showed lower minimum inhibitory concentration against MRSA and *Enterrococcus* sp. at dilution 1:16 which will be 62.50 µg/ml. The extract from *Garcinia mangstana* Linn. showed lowest minimum inhibitory concentration against VRE which is around 31.25 µg/ml.

Mesua ferrea Linn. extract showed minimum inhibitory concentration against *Bacillus cereus* at dilution of 1:16 or 62.50 µg/ml. It showed lower potency against MRSA and *Staphylococcus aureus* with minimum inhibitory concentration at dilution 1: 2 which is around 500 µg/ml but it did not have any effect against *Enterrococcus* spp. and VRE.

Kaempferia galangal Linn. extract showed MIC against *Bacillus cereus* at 250 µg/ml and against both *S. aureus* and MRSA strains at dilution 1:8 or around 125 µg/ml. The result was as same as *Mesua ferrea* Linn. Extract. *K. galangal* Linn. extract did not show inhibitory activity on *Enterrococcus* sp. and VRE. Contrast to the others, Pra-sa-proa-yai formula and *Lepidium sativum* Linn. extract showed only the inhibitory effect against *Bacillus cereus* (MIC was 250 µg/ml) but had no effect to the others bacterial strains.

Minimum bactericidal concentration determination

Three most effective extracts, *Garcinia mangstana* Linn., *Mesua ferrea* Linn. and *Kaempferia galangal* Linn. were selected for determination of their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on four isolated strains of MRSA (MRSA strain DMST 5199, ATCC 4738, ATCC 20651 and ATCC 20654) by using serial dilution followed by measuring the absorbance of OD_{625} in each well.

The values of absorbance were measured as soon as the bacterial strain was added into each well which was defined as H_0. The absorbance was measured after incubation at 37ºC every hour until 5 hour of incubation.

MIC is defined as the lowest concentration of agent that inhibits the bacterial growth to the level that the absorbance lower than 0.05 at 625 nm (no visible growth). The results showed that *Garcinia mangostana* Linn. has higher antibacterial activity than the others (Table 3). *G. mangostana* has ability to inhibit MRSA at concentration 3.91 – 7.81 µg/ml

with no visible bacterial growth while *M. ferrea and K. galangal* have lower capability to inhibit growth. There was no any concentration that the absorbance value is lower than 0.05 and the visible bacterial growth was found at higher concentration (15.63-62.50 µg/ml), more than 4 to 16 times compared to *G. mangostana*.

The minimum bactericidal concentration (MBC) were determined by spreading the bacterial strain onto agar plate and incubate for 18 hours to verify the MBC. It was found that *Garcinia mangostana* Linn. extract showed lowest MBC among these agents (Table 4).

DISCUSSION

Garcinia mangostana Linn., a plant in family Guttiferae which is well-known as "Queen of fruits" that is abundant in tropical zone such as Southeast Asian countries like Thailand, Indonesia, Malaysia and The Philippines. There are extensive studies about effective compounds in many parts of *G. mangostana* Linn. like pericarp, whole fruit, trunk, branches, bark and leaves. Particularly, pericarp which was used as traditional medicine for a long time, has been extensively studied. The extract of *G. mangostana* Linn. composed of many active components. The major active components isolated from this plant are xanthones which have remarkable biological activities [3]. α-, β- and γ-mangostins, garcinone E, 8-deoxygartanin and gartanin are the most well-known xanthones [4, 5] which have many activities such as the antioxidant properties [6], the anti-cancer properties. They also showed cytotoxicity activity against human colon cancer DLD-1 cells at low concentration (5–20 µM) [7]. The antipro-liferative effects were involved with cell-cycle arrest by affecting the expression of cyclin sp cdc2, p27 and apoptosis induction through the activation of intrinsic pathway following the down-regulation of signaling cascades involving MAP kinases and the serine/threonine kinase Akt. The extract of *G. mangostana* Linn. also showed the antibacterial activity against bacteria that induces acne [8]. The minimum inhibitory concentration values at 3.91 µg/ml, while the minimum bactericidal concentration values against *P. acnes* and *S. epidermidis* were 3.91 and 15.63 µg/ml, respectively [8].

In this study, we investigated the antibacterial activity of traditional herbs on various bacterial strains. From the results from the agar diffusion, *G. mangostana* Linn. extract showed obviously larger clear zone on all five selected bacterial strains than those of *M. ferrea* extract and *K. galangal* extract. MIC of *G. mangostana* Linn. extract (3.91-7.81 µg/ml) against MRSA was lower than that of *M. ferrea and K. galangal* (15.63-62.50 µg/ml) which is around 4-16 times. MBC of *G. mangostana* Linn. (3.91-15.63 µg/ml) was lower than that of *M. ferrea and K. galangal* (125- 250 µg/ml) which is around 16-32 times. The MIC and MBC in these experiments were not determined as the lowest concentrations with absorbance lower than 0.05. These due to the color of the medium and extract without bacteria showed the absorbance value.

Our results correspond to the investigation by Priya et al. [9]. They investigated the antimicrobial activity of *Garcinia mangostana* extract powder using macrodilution broth technique against *Staphylococcus aureus* and found that the MIC value was 200 µg/ml. Our result showed the MIC of *Garcinia mangostana* against MRSA was 3.91-7.81 µg/ml. This might be due to the difference in the efficiency of extraction method.

The activity of mangosteen's pericarp extract might be due to the potency of α-mangostin which is the major component and has broad-spectrum antibacterial activity against several Gram-positive and Gram-negative bacteria. Pedraza-Chaverri and colleagues have reviewed the medicinal properties of *Garcinia mangostana* including pericarp, fruit, bark, leaves and synthetic derivatives of α-mangostin [5]. *G. mangostana* pericarp contained at least 50 different active substances. Further study should be done in order to reveal the exact components of *Garcinia mangostana* pericarp which has antibacterial effect.

CONCLUSION

Traditional medicinal herb is an alternative treatment for curing diseases. This study investigated the antibacterial activity of twenty six medicinal herbal extracts. It was found that three herbal extracts, *Garcinia mangostana* extract, *Mesua ferrea* extract and *Kaempferia galangal* extract, showed promising activity. Among these herbal extracts, *Garcinia mangostana* extract showed the most potency in inhibition on selected indicator strains.

There are several studies mentioned in *Garcinia mangostana*'s pericarp extract as traditional medicinal plant. The active components isolated from this plant are xanthones, α-, β- and γ-mangostins, garcinone E, 8-deoxygartanin and gartanin. The most well-known is xanthone which has widely spectrum effects on several diseases. One of them is antibacterial activity. The crude pericarp extract of *Garcinia mangostana* Linn. has strong antibacterial activity against MRSA with MIC which was at concentration 3.91-7.81 µg/ml

and MBC was at concentration 3.91-15.63 µg/ml. It was able to inhibit all MRSA strains used in this study. *Mesua ferrea* extract and *Kaempferia galangal* extract showed MIC and MBC at 15.63-62.50 µg/ml and 125-250 µg/ml respectively. *Garcinia mangostana* Linn. extract would be a promising novel antimicrobial agent against MRSA infection.

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