

Antibacterial Activity of *Prismatomeris tetrandra* K. Schum Root Extract against Antibiotic Resistance Bacteria[†]

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Abstract

Antibiotic resistance bacteria has become an increasing problem now today due to many factors. This study investigates the efficacy of *Prismatomeris tetrandra* K. Schum root extract as a new source of antibacterial activity for antibiotic resistant bacteria using agar well diffusion method. The results showed that *S. aureus* TISTR517 exhibited more sensitivity to *P. tetrandra* K. Schum root extract than other Gram-positive bacteria indicator strains. On the other hand, Gram-negative bacteria exhibited resistance to *P. tetrandra* K. Schum root extract. The study further showed the activity between *P. tetrandra* K. Schum root extract and gentamycin (10 µg), it revealed that MRSA142 was resistant to gentamycin (10 µg) but sensitive to *P. tetrandra* K. Schum root extract. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was evaluated by using *S. aureus* TISTR517 and MRSA142 as indicator strains. The MIC value was 0.59 mg/mL and 1.17 mg/mL for *S. aureus* TISTR517 and MRSA142, respectively. MBC assay demonstrated that the MBC value was 9.75 mg/mL and 150 mg/mL for *S. aureus* TISTR517 and MRSA142 respectively. The mode of action was investigated with the presence of *P. tetrandra* K. Schum root extract in the culture broth. The action of *P. tetrandra* K. Schum root extract was revealed of bacteriostatic activity due to the Optical density (OD) at 600 nm and Colony-Forming Units (CFU) of indicator strains were continuously decreased.

Keywords: Antibacterial activity, MRSA, mode of action, *P. tetrandra* K. Schum, *S. aureus*

Introduction

The rising of antibiotic resistance of bacteria is a major problem that affects worldwide, especially developing countries. The origin of antibiotic resistance was recorded to show from loss ability of drug for against bacterial growth [1]. Developing of antibiotic resistance may be from many factors such as inappropriate doses of antibiotic during the treatment procedure. Moreover, the antibiotic resistance in human may be continued for a long time.

Plants have been used as an alternative drug because it is providing many kinds of secondary metabolite such as alkaloid, glycoside, flavonoid, quinones and steroids [2]. So, many medicinal plants are used in medicine instead antibiotic drugs against infectious diseases [3]. The author stated that plant extract showed antibacterial activity against Gram-negative bacteria [4]. The leave extract of 9 plant species showed good activity against both Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*) [5]. Then, the extract of *Punica granatum*, *Syzygium aromaticum*, *Zingiber officinales*, and *Thymus vulgaris* showing against *B. cereus*, *E. coli*, *P. aeruginosa* and *S. typhimurium*

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while only the extract of *Cuminum cyminum* show efficacy against *S. aureus* [6]. Moreover, leave extract of *Arum discoridis* exhibited to against 6 antibiotic resistant clinical pathogens [7]. Philippine medicinal plants, namely are *Psidium guajava* L. and *Phyllanthus niruri* showing narrow spectrum against Gram-positive bacteria while *Piper betle* L. and *Ehretia microphylla* Lam. exhibited inhibition both Gram-positive and Gram-negative bacteria [8]. Moreover, the extract of *Kaempferia pandurata* and *Senna alata* showed the strongest activity against MRSA [9]. Hexane extract of bark of *Cinnamomum cassia* (L.) J. Presl. (Lauraceae) inhibited the growth of MRSA [10]. From the research of [11] they found that 9 plants extract showing against multidrug resistant bacteria. The dried leaves and vernal stem extract of *S. dulcis* exhibited the highest inhibition zone against *S. aureus*. [12]. The ethanol extract of *C. sappan* stem exhibited strong antioxidant and antibacterial activities against the 6 pathogenic bacteria [13]

Three southern border provinces of Thailand have a diversity of local and medicinal plants using for treatment of bacterial infections. This study therefore aims to determine the activity of *P. tetrandra* K. Schum root extract against antibiotic resistance bacteria.

Materials and methods

Root extract

P. tetrandra K. Schum was collected from Pattani Province, Thailand. *P. tetrandra* K. Schum subsp. Malayana (Rigl) J. T. Johanss (BKF no. 194771) is one of medicinal plants used as folk medicine for treating skin diseases in combination with other local plants. Of this, root sample was cleaned with tap water and dried in an oven at 50 °C for 3 days. Then, the dried materials were reduced to a coarse powder with a blender. Root powder was mixed with 95 % ethanol (ratio 1:4) and incubated at room temperature for 3 days. The mixed was filtered by using cheesecloth and Whatman filter paper No.4. The filtrate was concentrated by rotary evaporator. Subsequently, the total dried weight of root extract was transferred and dissolved with acetone. The solution was finally stored at room temperature for 4 days on the purpose of safe acetone remover. The yield was calculated by a percentage based on root dry weight. Before used, the dry extract was diluted with DMSO.

Bacterial strains and media

Eight indicator bacterial strains were used such as *Staphylococcus aureus* (TISTR 517), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (TISTR 884), *Salmonella typhimurium* (TISTR292), *Escherichia coli* (TISTR 887), *Escherichia coli* (ESBL182), *Pseudomonas aeruginosa* (TISTR 1467) and MRSA 142. All indicator strains were inoculated on Luria-Bertani agar (LB agar) and incubated at 37 °C for 18 h. Single pure colony of each indicator strain was picked and transferred into LB broth and incubated at 37 °C for 18 h.

Antibacterial activity

The antibacterial activity was determined by agar well diffusion method. The indicator strains used for the antibacterial activity test were displayed in **Table 1**.

Agar well diffusion method

Each indicator strain (10^6 CFU/mL) was inoculated onto the LB agar plate. Wells with a diameter of 0.6 cm were cut in the agar using sterile tips. The *P. tetrandra* K. Schum root extract of 100 µL (crude extract with 30 mg/mL) was then added to each well and allowed to diffuse into the agar during a 5 h pre-incubation period at room temperature, followed by aerobic incubation at 37 °C for 24 h.

Determination of minimum inhibitory concentrations (MIC's) of the *P. tetrandra* K. Schum root extract

The MIC was determined following the methods described by [6] with slightly modified. MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h of incubation. The *P. tetrandra* K. Schum root extract which showing a strong antibacterial activity at 30 mg/mL was determined their MIC by using the agar well diffusion method and evaluate their efficiency

in controlling bacterial strains causing antibiotic resistance. The *P. tetrandra* K. Schum root extract was diluted with DMSO solution to different final concentrations of 1.17, 2.34, 4.69, 9.75, 18.75, 37.5, 75, 150 and 300 mg/mL. Each indicator strain (10^6 CFU/mL) was inoculated onto the LB agar plate. Wells with a diameter of 0.6 cm were cut in the agar using sterile tips. The 100 μ L of different concentration of *P. tetrandra* K. Schum root extract was added to each well and allowed to diffuse into the agar during a 5 h pre-incubation period at room temperature, followed by aerobic incubation at 37 °C for 24 h. The zones of inhibition were measured by Vernier caliper.

Determination of minimum bactericidal concentrations (MBC's) of the effective *P. tetrandra* K. Schum root extract

The MBC was determined following the methods described by [6] with slightly modification. The 3 lowest concentrations of the MIC plates of *P. tetrandra* K. Schum root extract not showing the invisible growth of bacteria and then the inhibition zone of theirs were streaked onto sterile LB plates. The plates were aerobic incubated at 37 °C for 24 h, then determined for bacterial growth in corresponding to *P. tetrandra* K. Schum root extract concentration. The MBC was defined as the lowest concentration of *P. tetrandra* K. Schum root extract that did not exhibiting of bacterial growth.

Mode of action

The mode of action was according to the method suggested by a previous study [14]. The root extract of *P. tetrandra* K. Schum (final concentration of 30 mg/mL) was added in mid-log phase growing cells of *S. aureus* TISTR 517 and MRSA 142 in LB broth. Growing cells of *S. aureus* TISTR517 and MRSA142 in LB broth without crude protein was used as a control. The optical density of culture broth was recorded at 600 nm and the number of viable cells was done by plating on LB agar.

Results and discussion

Antibacterial activity

The result from the agar well diffusion method showed the inhibitory effect of *P. tetrandra* K. Schum root extract. According to the inhibition zone of each indicator strain shown in **Table 1** and **Figure 1**, *S. aureus* TISTR517 was the most susceptible to *P. tetrandra* K. Schum root extract. This result indicates that the *P. tetrandra* K. Schum root extract had the strongest activity against Gram-positive bacteria. It therefore concludes that the *P. tetrandra* K. Schum root extract showed a narrow inhibitory spectrum against indicator strains. Our finding supported by a previous study showed that *Cuminum cyminum* extract exhibited a narrow inhibitory spectrum against Gram-positive bacteria [6]. Also, Philippine medicinal plants namely; *Psidium guajava* L. and *Phyllanthus niruri* demonstrated a narrow spectrum against Gram-positive bacteria [8]. Our study showed that Gram-negative bacteria resistance to the extract because of its outer membrane consisting of lipopolysaccharide which can protect permeate of substance into the cell [15]. Interestingly, literature reviews reported that composition (e.g., terpenoid, alkaloid and phenolic compound) of the plant extract can interact with protein and enzyme of bacteria cell membrane resulting of damage and leakage of protons into the outer cell and may inhibit amino acid biosynthesis process causing cell death [16]. Moreover, hydrophobicity of the plant extract can interact with protein of microbial cell membrane and mitochondria causing changes of its structure and may also interfere with permeability [17]. In addition, some Gram-negative and Gram-positive bacterial pathogen can produce biofilms formation to protect itself from antibiotic therapy. In Gram-positive bacteria, virulence factors such as toxin, enzyme, adhesion and other protein result in its survival under antibiotic treatment condition [18]. Based on the above findings, it shows antibiotic resistant to bacteria.

Table 1 Antibacterial activity of *P. tetrandra* K. Schum root extract.

S. no.	Indicator strain	Zone of inhibition (mean of 3 trials) (mm)
1	<i>B. cereus</i> ATCC 11778	21.66
2	<i>M. luteus</i> TISTR884	18.33
3	<i>S. aureus</i> TISTR517	24.00
4	MRSA142	22.00
5	<i>S. typhimurium</i> TISTR292	-
6	<i>E. coli</i> TISTR887	-
7	<i>E. coli</i> ESBL182	-
8	<i>P. aeruginosa</i> TISTR1467	-

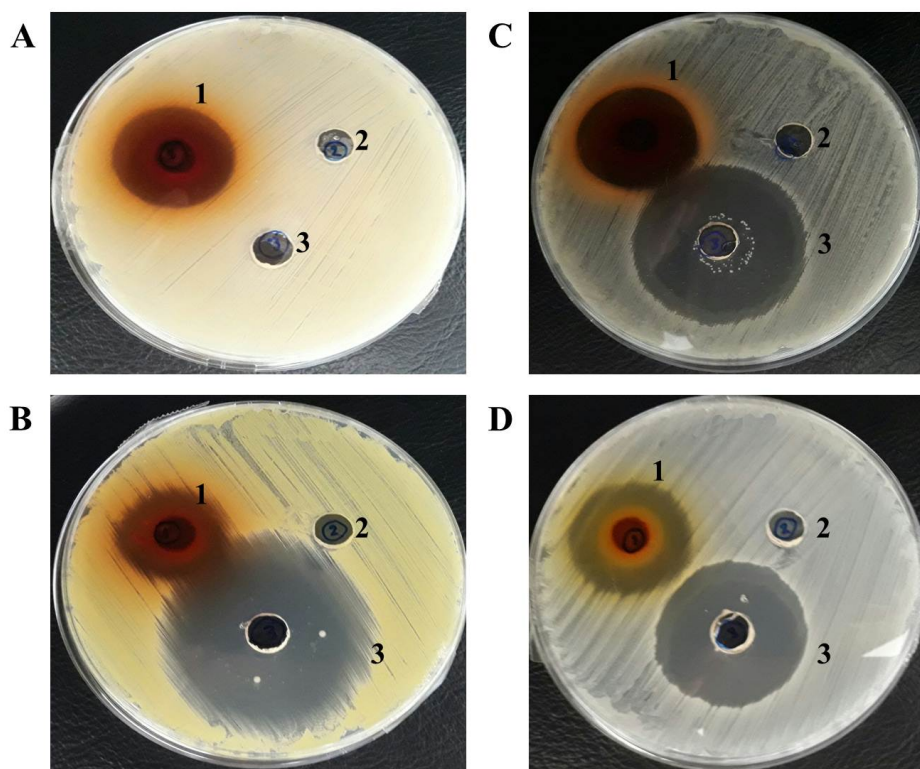


Figure 1 Antibacterial activity of *P. tetrandra* K. Schum root extract (well 1) against A) MRSA142 B) *M. luteus* TISTR 884 C) *B. cereus* ATCC11778 D) *S. aureus* TISTR517 compared with gentamycin (10 µg, well 3) and control (DMSO, well 2).

MIC and MBC of *P. tetrandra* K. Schum root extract

The MIC and MBC assay of *P. tetrandra* K. Schum root extract was determined by agar well diffusion method to evaluate bacteriostatic and bactericidal property. The strongest concentration of *P. tetrandra* K. Schum root extract was reported in **Table 2** and **Figure 2**. MIC value was 0.59 and 1.17 mg/mL for *S. aureus* TISTR517 and MRSA142, respectively. This result shown similarly with the MIC of green tea extract exhibited against *S. aureus* ATCC25923 and MRSA were 400 and 400 µg/mL, respectively [19]. Leaf extracts of *S. alata* and *K. pandurata* showed the lowest MIC against MRSA were 512 and 256 µg/mL, respectively [9]. MBC assay demonstrated that MBC value was 9.75 and 150 mg/mL for *S. aureus* TISTR517 and MRSA142, respectively.

Table 2 MIC's and MBC's of *P. tetrandra* K. Schum root extract against *S. aureus* 517 and MRSA142.

Root extract	Conc. (mg/mL)	Zone of inhibition (mean of 3 trials) (mm)	
		<i>S. aureus</i> 517	MRSA142
<i>P. tetrandra</i> K. Schum	0.15	0	0
	0.29	0	0
	0.59	14	0
	1.17	18	11
	2.34	20	13
	4.69	22	15
	9.75	25	15
	18.75	25	17
	37.50	25	17
	75.00	25	21
	150.00	26	23
	300.00	30	26

*Diameter of well = 6 mm; *All experiments were done in triplicate.

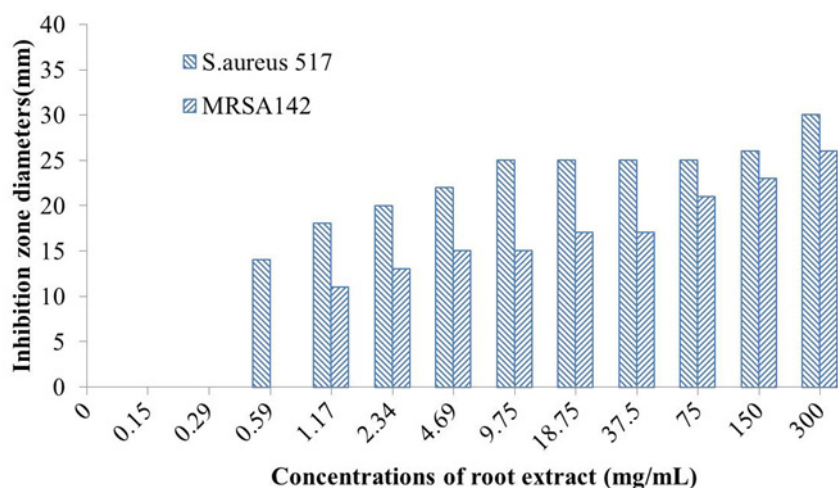


Figure 2 MIC's of *P. tetrandra* K. Schum root extract against *S. aureus* 517 and MRSA142.

Mode of action

This method was initially introduced by *P. tetrandra* K. Schum root extract at 300 mg/mL concentrations added to mid-log phase of culture medium. Our study found that cell viability of *S. aureus* TISTR517 and MRSA142 were continuously decreased with optical density at 600 nm and when counting the number of cell viability of indicator strains by plating on LB agar, it caused the reduction in Colony Forming Unit (CFU) of growing cultures with the passage time (Figure 3). From this study, it shows that the mode of action of *P. tetrandra* K. Schum root extract as bacteriostatic activity because number of cell viability of indicator strains were continuously decreased. This result was similarly shown with the action of bacteriocin from *Brevibacillus laterosporus* SA14 against *S. aureus* TISTR517 and MRSA142 [20].

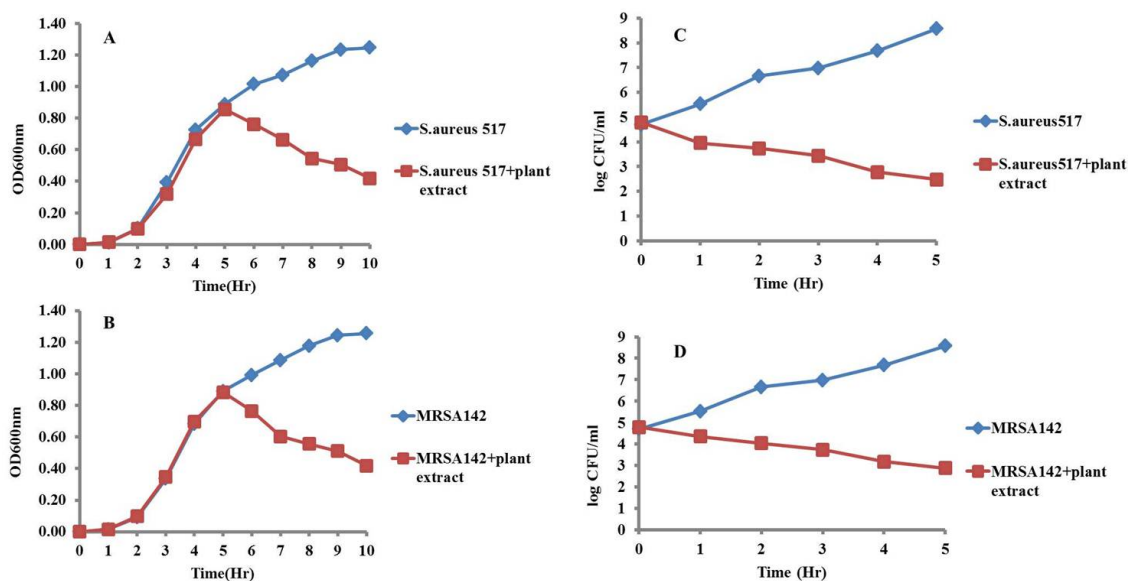


Figure 3 Mode of action of *P. tetrandra* K. Schum root extract to the growing cells of the indicator strains (A, C) *S. aureus* TISTR517 and (B, D) MRSA142. For control, the culture of indicator strain was grown without *P. tetrandra* K. Schum root extract.

Conclusions

Based on the results obtained from this study, it was found that Gram-positive bacteria strains exhibited sensitivity to *P. tetrandra* K. Schum root extract. Of this, *S. aureus* TISTR517 was shown a higher sensitivity than other indicator strains. MRSA1424 showed resistance to gentamycin but sensitive to *P. tetrandra* K. Schum root extract while other indicator strains sensitivity to gentamycin. The highest concentration of *P. tetrandra* K. Schum root extract as a bacteriostatic property with MIC value was 0.59 and 1.17 mg/mL for *S. aureus* TISTR517 and MRSA1424, respectively. This study further showed MBC value was 9.75 and 150 mg/mL for *S. aureus* TISTR517 and MRSA1424, respectively. The mode of action of *P. tetrandra* K. Schum root extract showed bacteriostatic activity against indicator strains. Therefore, future studies are recommended to investigate the activity of *P. tetrandra* K. Schum root extract on the cytotoxicity, the composition of phytochemical, total phenolic and flavonoid contents.

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