Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

In vitro anti-*Staphylococcus aureus* activity of the methanol extract from *Mitrephora thorelii* Pierre's branches and leaves in Lam Dong Province, Viet Nam

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Received on: 16/09/2022, Revised on: 24/10/2022, Accepted on: 06/11/2022, Published on: 05/01/2023.

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Keywords: Antibacterial activity, Lam Dong, *Mitrephora thorelii Pierre*, plants extract, *Staphylococcus aureus*

Vol. 10 (1): 01-06, Jan-Mar, 2023.

DOI: http://doi.org/10.56511/JIPBS.2023.10101

Abstract

We demonstrated that the methanol extract of *Mitrephora thorelii* Pierre's branches and leaves in Lam Dong, Vietnam is anti-*Staphylococcus aureus* strain (Sta1) of Phenikaa University, Hanoi, Viet Nam and *Staphylococcus aureus* ATCC 29213 strain (Sta2) from Thermo Fisher Scientific, USA. Invitro anti-Sta1 and Sta2 activity of the methanol extract of *Mitrephora thorelii* Pierre's branches and leaves were not significantly different. With a rise in extract content, the inhibitory zone's created diameter grew as well. The extract content of 100 mg/mL demonstrated the greatest antibacterial activity with a 25 mm inhibition zone diameter against Sta1 and 24 mm inhibition zone diameter against Sta2. Bacteria were not resistant to the extract at concentrations of 20; 80 and 100 mg/mL after 48, 72, and 96 hours. The lowest inhibitory concentration (MIC) was between 312.5 and 625 µg/mL. The minimum bactericidal concentration was found to be 1.25 mg/mL.

Introduction

Staphylococcus aureus (S. aureus) is a member of the family Staphylococcaceae, the genus *Staphylococcus*, the order Bacillales, and the class Bacilli [1]. There are several different types of Staphylococcus. 30% of people on the planet carried *S. aureus* on a permanent basis [2, 3]. Along with osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections, *S. aureus* is a major contributor to bacteremia, infectious endocarditis, and these conditions. *S. aureus* is a common pathogen that lives everywhere and permanently resides in the upper respiratory, gastrointestinal, and urogenital tracts of 20% to 30% of individuals [4, 5]. *S. aureus* can colonize several animal skin surfaces, including the teat and canal skin. It can cause diseases ranging from mild skin infections to fatal conditions such as sepsis, endocarditis, necrotizing pneumonia, toxic syndrome, plantar fasciitis, necrotizing fasciitis, bone and joint duplication associated with thromboembolic disease, prohibition of fulminant hemorrhage with or without Waterhouse Friderichsen syndrome, orbital cellulitis and endophthalmitis, central nervous system infection predisposing factors different toxins [5-6]. Antibiotic strains of *S. aureus* have been resistant to methicillin, vancomycin, cephalosporins, penicillin, and linezolid. Due to the antimicrobials' selective pressure, *S. aureus* has the capacity to acquire drug resistance more quickly [5]. Finding medications that can effectively prevent and treat diseases from natural herbal sources is a major concern of medical professional biochemists and medics throughout the world due to the risk of drug resistance and adverse effects when using pharmaceutical drugs.

The research results at Tay Nguyen Institute for Scientific Research, VAST, Vietnam have developed the list of medicinal plants of 1003 species collected in Lam Dong [7]. Among those species is *Mitrephora thorelii* Pierre., according to the research results of Hoa and Yen, (2022) showed that they have anti-*Helicobacter pylori* activity [7] , Thuan *et al.* (2014) isolated four flavonoid compounds from *Mitrephora thorelii* leaves are astragalin, juglanin, quercetin, and quercitrin [8]. *Mitrephora thorelii* Pierre is on the list of endangered plant species in Pu Mat National Park [9]. According to the Vietnam Red Book, *Mitrephora thorelii* Pierre is on the list of endangered species. The realize their potential, in this study, we evaluated the in vitro anti- *S. aureus* activity from *Mitrephora thorelii*'s branches and leaves in Lam Dong province, Viet Nam. The research results will contribute to providing scientific evidence on their anti- *S. aureus* activity as well as contribute to the conservation and development of medicinal plants in Vietnam's Lam Dong province.

Material and methods

Plant material

Mitrephora thorelii Pierre leaves and twigs were collected in Duc Trong district of Vietnam's Lam Dong province in October 2013. Plant samples were classified by Dr. Nong Van Duy from Tay Nguyen Institute for Scientific Research, VAST. They are designated No. TN3/044. The sample is stored at the plant resources department of Tay Nguyen Institute for Scientific Research, VAST, Vietnam.

Microorganism sources

Two *S. aureus* strains: *S. aureus* strain (Sta1) was provided by Dr. Nguyen Hong Minh, Phenikaa University, Hanoi. *Staphylococcus aureus* ATCC 29213 (Sta2) imported from Thermo Fisher Scientific, USA. The strains were kept at the Microbiological Technology Department of Tay Nguyen Institute for Scientific Research, VAST, Vietnam.

Chemicals

Methanol, DMSO, Muller Hinton Agar and Muller Hinton Broth (Merck, Germany).

Preparation of crude extracts

Mitrephora thorelii Pierre's branches and leaves (25 g) were air-dried, powdered, and then extracted three times at room temperature using methanol (200 mL/time). To get methanol residue, the methanol solutions were filtered, mixed, and concentrated while under reduced pressure. Until they were employed, the raw extracts were kept at -20°C.

On the day of the assay, an aliquot of the frozen extract was thawed in the refrigerator. Then, the crude extract was weighed and diluted in DMSO (10%) at 100 mg/mL. By serial dilution, the extract was then diluted to reach level of 80 mg/mL and 20 mg/mL.

Antibacterial assay

The agar plate diffusion method was used to test the extract's antibacterial properties [10]. The sterile Petri-plates were filled with sterilized Muller Hinton Agar medium (15 mL) and allowed to harden. The bacteria was grown in Muller Hinton Broth medium and maintained culturing for 24 hours at 37°C. The bacterial population was adjusted to 10^8 CFU/mL. Then, the bacterial broth (100 µL) was swabbed individually on an agar plate using a sterile bud. The wells (6 mm in diameter) were drilled into the agar with a sterile hole. Plant extract was placed into each well aseptically and maintained for 24 hours at 37°C. The positive control was ofloxacin (5 µg) and the negative control was DMSO (10%). The inhibitory zone's size was calculated. The tested bacterium's diameter of the inhibition zone (DIZ) was used to evaluate its antibacterial activity. Millimeters were used to measure the DIZ. Each experiment was performed in triplicate using the methods described in a previous study.

Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) determination

Minimum Inhibition Concentration (MIC) was determined according to the method of Sarker *et. al.* [11]. In this experiment, these extracts were diluted secondary to an initial concentration of 40 mg/mL. The bacterial extracts was cultured overnight and diluted at a density of 10^6 CFU/mL. Add to each well 50 µL of bacterial extract and 50 µL of methanol extract at different dilutions. The control wells contain the bacterial extracts and the medium. Each treatment was repeated 3 times. Incubate at 37°C, overnight. After 24 h, 30 µL of 0.15% resazurin (Across, USA) reagent was added to each well. Incubate at 37°C and observe the color change of the mixture in the well. The MIC value is the lowest concentration in the test range of extracts that can inhibit bactericidal growth (wells do not discolor resazurin).

Determination of minimum bactericidal concentration (MBC)

Spreading plate method was used to calculate the Minimum Bactericidal Concentration (MBC): 100 µL of test solution on wells without resazurin discoloration was spread onto agar plates containing Muller Hinton Agar and grown at 37ºC, checking for bacterial viability after 24 hour. The MBC value is the lowest concentration in the concentration range of extracts that can kill all bacteria (no bacterial colonies appear on the agar plate). The control medium plate

contains only bacterial fluid and medium, without the extract, bacterial colonies appear [11, 12].

Statistical Analysis

The data were analyzed in SPSS 16 software using the ANOVA test. Statistical testing was performed at the level of significance $p \leq 0.05$, using the LSD test.

Results

Anti- *S.aureus* activity of methanol extract from leaves and twigs *Mitrephora thorelii* Pierre

The test findings indicated the methanol extract from *Mitrephora thorelii* Pierre's branches and leaves is anti-*S. aureus* strain (A1) of Phenikaa University, Hanoi, Viet Nam and *S. aureus* strain ATCC 29213 (A2) imported from Thermo Fisher Scientific, USA.

The outcomes demonstrated that for the same concentration of extracts, the inhibitory impact of methanol extract on Sta1 and Sta2 was not substantially different. Using an extract dosage of 100 mg/mL and a cell density of 10^8 CFU/mL for Sta1 and Sta2, the extract's inhibitory area measured 25 mm and 24 mm, respectively.

The diameter of the inhibitory zone varies depending on the concentration of extract used, while the strain, cell density, and amount of extract in each agar hole are all the same. The extraction concentration has a direct correlation with the inhibitory ring diameter. When the extract concentration increased from 20 to 100 (mg/mL), the inhibitory ring diameter increased from 16 to 25 mm for Sta1 and 15 to 24 mm for Sta2.

With the inhibition zone's size of 24 to 25 mm, anti-Sta1 and anti-Sta2 of the extract at 100 (mg/mL) were comparable to the positive control ofloxacin (5 g) (Figure 1, Table 1).

The effect time to antibacterial activity of methanol extract from *Mitrephora thorelii* Pierre

To evaluate the antibacterial ability of the extract over time, we continued to measure the width of the inhibition zone in 3.1 experiment after 48, 72, and 96 hours and compared it with the diameter of the inhibition zone after 24 hour. The findings demonstrated that when we continued to culture the *S. aureus*, the diameter of the inhibitory zone remained unchanged during 48, 72, and 96 hours *S. aureus*. This may indicated that the bacteria are not resistant to the extraction concentrations we used (20 to 100 mg/mL) (Figure 2).

Determinate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract from *Mitrephora thorelii* Pierre

The minimum inhibitory concentration (MIC) is the lowest concentration in the test concentration range of extracts that can inhibit bacterial growth (without discoloring resazurin). Therefore, the lower the minimum inhibitory concentration (MIC), the higher the antibacterial activity. Based on the color change of the resazurin reagent on the 96-well plate, it was possible to determine the minimum inhibitory concentration (MIC) of extract from leaves and twigs of *Mitrephora thorelii* Pierre against *S. aureus*. The results showed that in wells with the extract level of 312.5 µg/mL, the color of the resazurin reagent changed from blue to pink indicating the growth of bacteria in the well. Thus, the methanol extract from the leaves and twigs of *Mitrephora thorelii* Pierre has a value of $312.5\ \mu g < MIC \leq 625\ \mu g/mL$ for S. *aureus*.

The minimum bactericidal concentration (MBC) is the lowest level in the extract's concentration range that can kill all bacterial in the well, with no bacterial colonies appearing on the agar plate. Wells without resazurin discoloration were spread onto Muller Hinton Agar plates, incubated at 37ºC, after 24h of observing the bacterial's survival.

According to the results, *S. aureus* was totally eliminated at an extraction concentration of 1.25 mg/mL because no bacterial colonies could be seen growing on the agar plate. Therefore, the minimum killing concentration of the extract for Sta1and Sta2 was 1.25 mg/mL. Tables 2 and 3 present the findings.

Table 1. The diameter of the inhibition zone of methanol extract from leaves and twigs of *Mitrephora thorelii* Pierre.

Concentration of extract (mg/ml)	Bacterial cell density (CFU/ml)	Diameter of the inhibition zone (mm)	
		Sta1	Sta2
100	10^8 CFU/ml	25	24
80		23	22
20		16	15
ĐC (+)		25	25
ĐC (-)		-	-

Note: Negative (-): DMSO 10%; Positive (+): ofloxacin (5 µg).

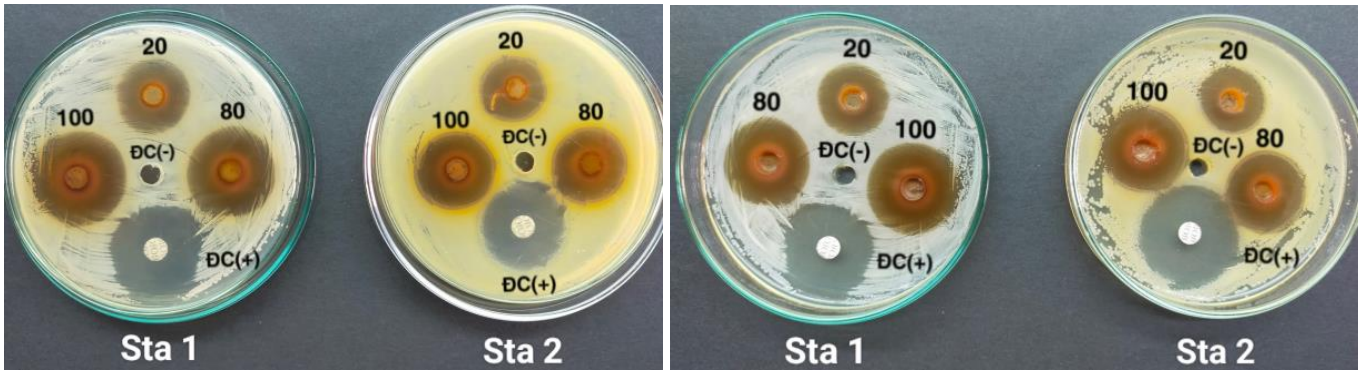

Figure 1. The inhibitory effect on S. aureus of methanol extract from leaves and twigs of *Mitrephora thorelii* Pierre at concentrations of 20; 80; 100 mg/ml; Negative (-): DMSO 10%; Positive (+): ofloxacin (5 µg).

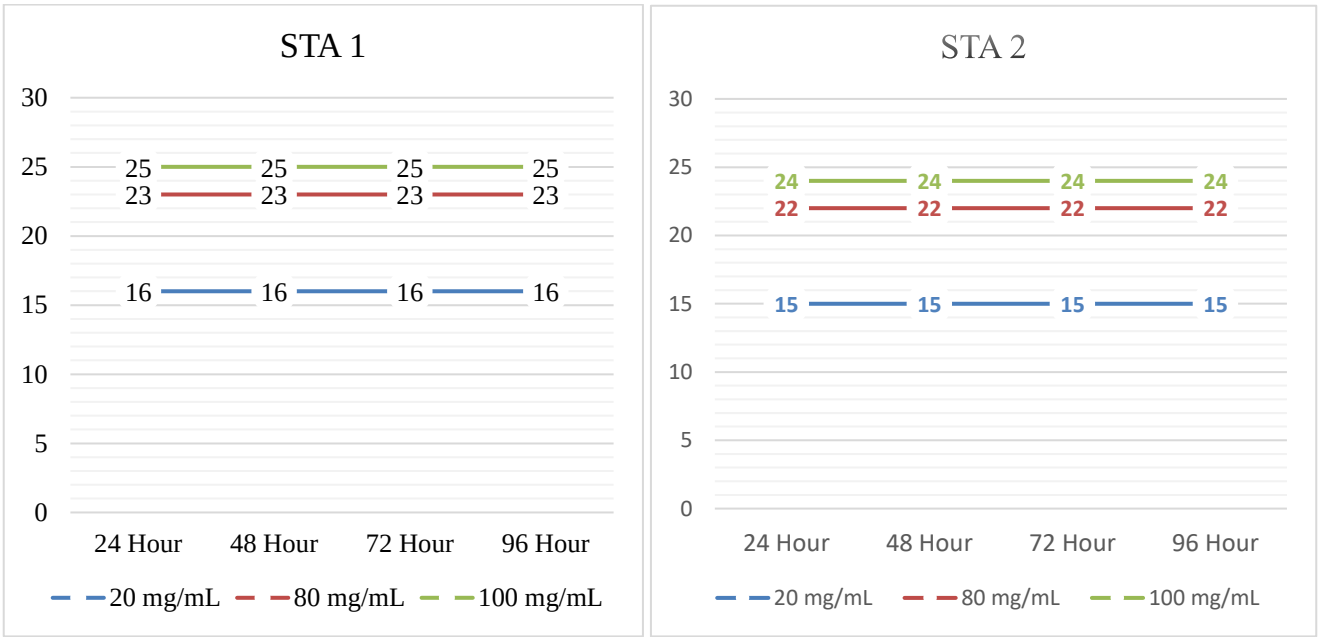

Figure 2. The diameter of the inhibition zone after 48, 72, and 96 hours.

Table 2. Effect of extract concentration on the viability of bacteria.

Concentration of extract (mg/ml)	Bacterial cell density (CFU/ml)	Bacterial viability	
		Sta1	Sta2
80	10^6	-	-
40		-	-
20		-	-
10		-	-
5		-	-
2.5		-	-
1.25		-	-
0.625		+	+
0.3125		+	+
0.15625		++	++

Notes: (-): No bacterial; (+): very few bacterial colonies; (++): colonies grow a lots, thick.

Table 3. Minimum inhibitory and minimum bactericidal concentrations of extract.

Bacterial strain	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
Sta1	0.3125	1.25	4
Sta2	0.3125	1.25	4

Discussion

Numerous scientists investigated various plant extracts for their ability to inhibit the growth of bacteria, yeast, and noticed their antibacterial properties. Numerous active chemical components found in medicinal plant extracts have antibacterial effects. The MICs value has been used for evaluating the extract's antibacterial activity [13]. If the MIC values are less than 500 g/mL, the activity is regarded as significant, and when they range between 500 and 1500 g/mL, it is regarded as moderate. Under these experimental conditions, *Mitrephora thorelii* was found to have significant against Sta1 and Sta2 activity. Furthermore, the smaller the MBC/MIC ratio, the higher the bactericidal effect. MBC/MIC ≤ 4, the extracts have good bactericidal ability [14]. In this instance, the extract from *Mitrephora thorelii* should be noted for its bactericidal properties.

For thousands of years, people have used medicinal herbs to treat various diseases. Isolation and biochemical characterization of pharmacologically active compounds from medicinal plants continue to this day. Numerous investigations have demonstrated that a variety of herbal species are anti-*S. aureus*. The methanol extract from the leaves of *Ficus carica* is resistant to *S. aureus* ATCC 29213 with MIC 2.5 mg/mL [15], anti-*S. aureus* activity of Pouzolzia zeylanica: 40 µg/mL < MIC < 80 µg/mL [16]. Hammer et al. [17] investigated the antimicrobial activity of 52 essential oils and extracts. The results have demonstrated that many essential oils have antifungal and antibacterial activities. In which, essential oil of *Vetiveria zizanioides* was able to inhibit the growth of *S. aureus* NCTC 6571 with MIC is 0.008 % (v/v). *Vetiveria zizanioides* against *S. aureus* was the strongest among the plants tested by the authors. Recently, various reports have confirmed the *S. aureus* activity of essential oils and medicinal extracts [18, 20]. Many experimental studies have shown a correlation between the antibacterial activity of plant extracts and secondary compounds found in plants [22]. Some researchers have suggested that the antibacterial components present in plant extracts such as terpenoids, alkaloids, and polyphenols interact with the enzymes and proteins of the bacterial membrane causing the dispersion of proton flux towards the outside of the cell. leads to cell death or can inhibit bacterial amino acid biosynthetic enzymes [23]. Antibiotic resistance is a worldwide public health threat, affecting the health and lives of people and the overall sustainable development of an entire country. The majority of *S. aureus* strains are resistant to a wide range of medicines. The World Health Organization (WHO) has recommended that research priorities need to be found, finding new methods against these bacteria. The current research results are the basis to encourage further studies on the chemical composition, toxicity, and pharmacology of the *Mitrephora thorelii* Pierre to apply it in the treatment of *S. aureus* effectively.

Conclusion

According to the study's findings, the methanol extract of *Mitrephora thorelii* Pierre, which was found in Lam Dong province, Viet Nam had anti-*S. aureus* species activity. These results are the basis to encourage further studies on the chemical composition, toxicity, and pharmacology of this species in order to apply it in effective treatment against *S. aureus*.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this research article.

Authors Contribution

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

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