ORIGINAL PAPER

Inhibitory effect of *Ficus racemosa* leaf extract on *Staphylococcus aureus*

Nirawit Pantarak,[a] Techit Thanaputthiphat,[a] Nattamon Niyomdecha[b*]
[a]Princess Chulabhorn Science High School Pathum Thani, Pathum Thani, Thailand, 12140
[b]Thammasat University Rangsit Campus, Faculty of Allied Health Sciences, Department of Medical Technology, Khlong Luang, Pathum Thani, Thailand, 12121

*Corresponding author: nattamon@tu.ac.th

Received: 2 November 2023/ Revised: 11 December 2023/ Accepted: 20 December 2023

Abstract. Current antimicrobial research does not only focus on the identification of new chemical antibiotics but also on the identification of natural compounds present in plant-based traditional medicines. In Thailand, *Ficus racemosa* is an excellent representative of medicinal plants that provide health benefits in several diseases. The objective of this work was to conduct a preliminary evaluation of the antibacterial activity of *F. racemosa* leaf extract against *Staphylococcus aureus*. Fresh leaves of *F. racemosa* were collected and extracted in methanol. The methanolic extract of *F. racemosa* was diluted in sterile distilled water and analyzed for contained phytochemical agents. Antibacterial activity against D-test negative and D-test positive *S. aureus* was evaluated by agar disk diffusion and broth dilution methods to determine inhibition zone, maximum inhibitory titer, and maximum bactericidal titer, respectively. Stability of active agents against *S. aureus* was tested on the day of extract preparation and 15 days later. Chemical analysis suggested the presence of flavonoids and tannins in the methanolic extract of *F. racemosa*. The inhibition of bacteria exhibited a dose-dependent response to the amount of crude extract, and there was no significant difference in inhibition between the two tested strains of *S. aureus* on both day 1 and day 15. Higher concentrated *F. racemosa* extract showed higher antibacterial activity than lower concentrated extract in all measurements. Agar disk diffusion seemed not to be suitable to evaluate antibacterial activity of the *F. racemosa* methanolic extract as it showed only a very narrow inhibition zone. However, the broth dilution assay allowed to determine an inhibitory titer. Our data indicates that methanolic *F. racemosa* leaf extract contains potent antimicrobial compounds. The ability to inhibit the growth of two different drug-susceptible strains of *S. aureus* suggests that these plant-derived compounds are alternative antimicrobial drugs.

Keywords: *Ficus racemosa*, methanolic extract, antibacterial activity, phytochemical agent.

1. Introduction

Many plants, herbal and non-herbal, have been applied in traditional medicine. The effective treatment of many diseases with these plants is due to their phytochemical ingredients. Figs, trees in the genus *Ficus*, family *Moraceae* are well-known medicinal plants with more than 600 species classified worldwide. The various parts of the tree synthesize aromatic substances like steroids, triterpenoids, phenols, tannins, and flavonoids in different proportions (Shahriar et al. 2013; Bagyalakshmi et al. 2019). These phytochemical agents act as a plant defense mechanism against invading organisms. Thus, the fig represents a traditional medicine with pharmaceutical application in the treatment of many diseases (Cheng et al. 2020). However, the efficacy of treatment may differ depending on species, part of plant and extraction method by which the active ingredients are obtained.

In Thailand, *Ficus racemosa* (synonym Ficus glomerata Roxb) is one of the most common species and is known as "Ma Dau Udumbar". Several previous studies demonstrated activity of *F. racemosa* as antibacterial agent (Kingsley et al. 2014; Bagyalakshmi et al. 2019; Pingale et al. 2019). Bagyalakshmi et al. (2019) found that the fruit of *F. racemosa* caused higher

inhibition than the leaf. Pingale et al. (2019) demonstrated that the inhibitory effect of the fruit of *F. racemosa* depended on the type of solvent used for extraction (Pingale et al. 2019). Furthermore, these studies showed that *F. racemosa* can inhibit Gram-positive and Gram-negative bacteria including *Staphylococcus* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* Typhi (Bagyalakshmi et al. 2019; Pingale et al. 2019).

In this study, we aimed to detect phytochemical substances in a methanolic leaf extract of *F. racemosa* and to evaluate whether this extract showed antibacterial activity against D-test positive and D-test negative *S. aureus*. Additionally, stability of the extract was investigated by comparison of the inhibitory results obtained on day 1 and day 15.

2. Materials and Methods

2.1 Plant collection and extraction

Fresh 70 g and 140 g *Ficus racemosa* leaves were collected in Pathum Thani province in the central part of Thailand. The extraction process employed was previously elucidated (Polyium et al. 2014). They were washed and dried in an incubator at 45°C for 2 days, and finely ground to powder by using a mixer grinder. Each of the powders from 70 g and 140 g of dried leaves was then macerated in high-polarity solvent methanol at a ratio of 1:3 (w/v) for 7 days at room temperature to compare the dose-dependent effect. Subsequently, a rotary vacuum evaporator was used to concentrate the crude extract. To prevent false-positive antimicrobial activity from solvent toxicity, the concentrated methanolic crude extract was dissolved in 5 ml of sterile distilled water as opposed to other polarity solvents and passed through a 0.45 µM syringe filter. The extract was kept at −20°C for all experiments.

2.2 Phytochemical screening of F. racemosa leaf extract

The extract was analyzed qualitatively for the presence of tannins and flavonoids (Koodkaew et al. 2018). Tannins were detected by dissolving 0.2 g of the extract in 5 ml of distilled water, boiling for 2 min, filtering, and adding a few drops of 1% $FeCl_3$ to the solution. The appearance of green-black or blue-black color would confirm the presence of tannins. Flavonoids were detected by dissolving 0.2 g of crude extract in 3 ml of 50% methanol, filtration, adding a few pieces of magnesium and boiling of the solution. After the solution had cooled, a few drops of concentrated hydrochloric acid were added. Yellow, red, or orange color apparent under light transmission would indicate the presence of flavonoids.

2.3 Bacteria used in the study

A D-test positive (inducible clindamycin resistance) *Staphylococcus aureus* strain and a D-test negative (non-inducible clindamycin resistance) strain were used in this study. These isolates were obtained from the bacterial collection of the Department of Medical Technology, Thammasat University and had been collected from clinical samples. *S. aureus* was cultured on blood agar before being used in the experiments described below.

2.4 Antimicrobial activity of F. racemosa leaf extract

Agar disk diffusion and broth microdilution methods were used to evaluate the antimicrobial activity of *F. racemosa* leaf extract. Agar disk diffusion method was performed based on the Kirby-Bauer technique. Briefly, a suspension of isolated *S. aureus* colonies in sterile normal saline equivalent to McFarland no. 0.5 turbidity standard was inoculated on Muller-Hinton agar (MHA). Sterile 6-mm diameter paper disks were placed on the inoculated agar, and 25 µl of *F. racemosa* leaf extract or sterile distilled water (negative control) was applied on the disks. Standard antimicrobial disks of clindamycin (DA) and erythromycin (E) were used to confirm antimicrobial phenotype, and a sulfamethoxazole-trimethoprim (SXT) disk was used as positive control. Agar plates were cultured at 35 ± 2°C for 24 h and the

inhibition zone diameter was measured in mm.

In broth microdilution method, *F. racemosa* leaf extract was serially two-fold diluted in 0.5 ml sterile Muller-Hinton broth (MHB). An equal volume of MHB containing 10^6 CFU/ml *S. aureus* was added into each tube. A positive control of *S. aureus* growing in MHB without *F. racemosa* leaf extract and a negative control of MHB with *F. racemosa* leaf extract but without bacteria were done in parallel. The test tubes were incubated at 35 ± 2°C for 24 h, and the turbidity of control and test tubes was determined. The highest dilution of *F. racemosa* leaf extract that could inhibit the growth of *S. aureus* as observed by naked eye was interpreted as maximum inhibitory titer. The maximum bactericidal titer (killing activity ≥ 99.9%) was determined by taking 10 µl culture from each tube that showed inhibition and streaking it on Muller-Hinton agar (MHA). After incubation at 35 ± 2°C for 24 h, the *S. aureus* colonies were counted and reported.

All experiments with *S. aureus* were done at biosafety level 2 (BSL-2) in accordance with the biosafety guidelines. This project was approved by the Thammasat University Institutional Biosafety Committee (003/2566).

2.5 Statistical analysis

Experiments were carried out in duplicate. Data were averaged and described herein as mean±SD.

3. Results

Qualitative analysis of methanolic leaf extract of *F. racemosa* demonstrated the presence of tannins and flavonoids (Figure 1), two phytochemical substances that are important for antibacterial activity.

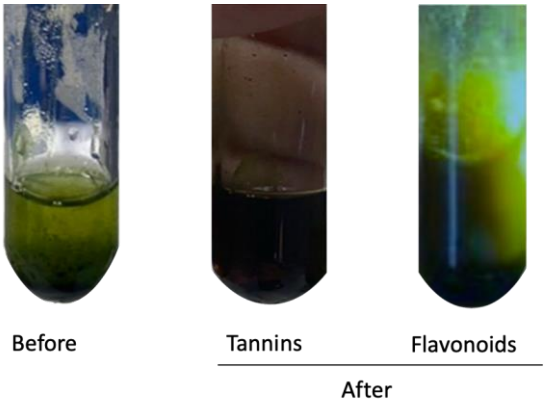

Figure 1. Appearance of tannins and flavonoids in methanolic leaf extract of *F. racemosa*. Methanolic leaf extract of *F. racemosa* before and after chemical detection of tannins and flavonoids. Green-black or blue-black color and yellow color under light transmission indicate the presence of tannins and flavonoids, respectively.

Antibacterial activity of the extract against D-test positive and D-test negative *S. aureus* was evaluated by two assays. Firstly, agar disk diffusion was used to determine the inhibition zone diameter (Figure 2) and, secondly, broth dilution was used to determine the maximum inhibitory titer and maximum bactericidal titer.

The assays showed consistent dose-dependent inhibitory activity against both *S. aureus* strains in comparison of extracts prepared from 70 g and 140 g of *F. racemosa* fresh leaves. The results were the same for the two bacterial strains. Extract prepared from 70 g of leaves could not inhibit *S. aureus* growth (Figure 2); however, a narrow inhibition zone of 12 mm was detected when using 140 g of leaves (Table 1). This inhibition zone was much narrower than the zone observed for the antibiotic sulfamethoxazole-trimethoprim used as a positive control in this study (Figure 2). However, 140 g of *F.*

racemosa fresh leaf extract exhibited an obvious inhibitory effect against *S. aureus* growth in the broth dilution assay. As shown in Table 1, the maximum inhibitory titer of the 140 g leaf extract was 128, and its maximum bactericidal titer was 16. Notably, the antimicrobial activity of leaf extract was still present when tested again 15 days later.

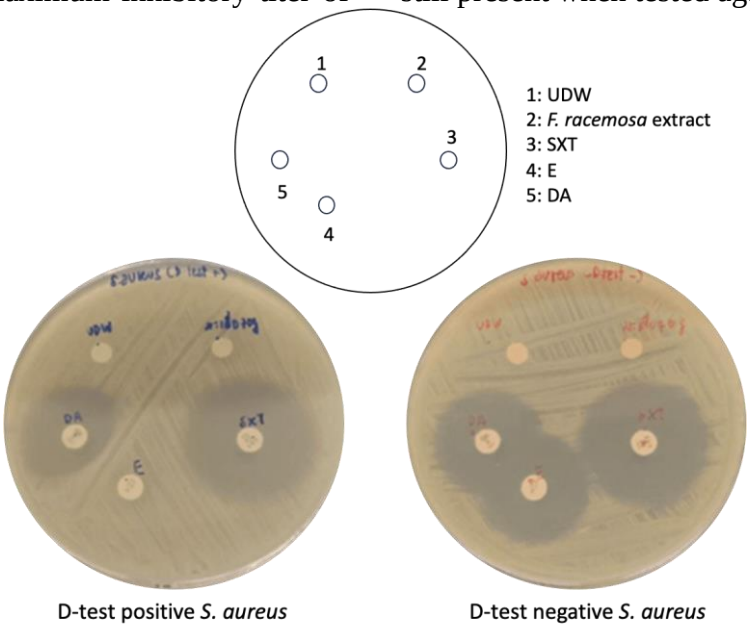

Figure 2. Representative results of the observed inhibition zones and D-test phenotypes from 70 g methanolic leaf extract of *F. racemose* against D-test positive and negative *S. aureus*. Paper disks soaked with 25 µl of ultra-deionized water (UDW) as negative control and 25 µl of methanolic leaf extract of *F. racemosa* were placed together with standard antimicrobial disks on Muller-Hinton agar inoculated with *S. aureus* as outlined in the drawing. The included antibiotics were sulfamethoxazole-trimethoprim (SXT) as positive control and clindamycin (DA) and erythromycin (E) to confirm the phenotype of D-test positive (left lower) and D-test negative (right lower) *S. aureus*.

Table 1. Antimicrobial results of methanolic leaf extract of *F. racemosa* against D-test positive and D-test negative *S. aureus* at day 1 and day 15.

F. racemosa	**Antimicrobial testing[a]**					
	Inhibition zone (mm)[b]		**Maximum inhibitory titer**		**Maximum bactericidal titer**	
	SA (D+)	**SA (D-)**	**SA (D+)**	**SA (D-)**	**SA (D+)**	**SA (D-)**
Day 1						
70 g	6 ± 0	6 ± 0	16 ± 0	16 ± 0	ND	ND
140 g	12.5 ± 0.71	11.5 ± 0.71	128 ± 0	96 ± 45.25	16 ± 0	16 ± 0
Day 15						
70 g	6 ± 0	6 ± 0	16 ± 0	16 ± 0	ND	ND
140 g	12 ± 0	12 ± 0	128 ± 0	128 ± 0	16 ± 0	12 ± 5.66

[a]Data are presented as mean ± SD.
[b]Inhibition zone of sulfamethoxazole-trimethoprim positive control was 20.5 ± 0.71 mm.
Abbreviation: SA (D+), D-test positive *S. aureus*; SA (D-), D-test negative *S. aureus*; ND, Not detectable.

4. Discussion

To the best of our knowledge, this work is the first shown comparison of the antibacterial properties of the *F. racemosa* plant against both antibiotic-sensitive and antibiotic-resistant pathogens. Additionally, it investigated the stability of the antimicrobial action of stored extracts. Here, tannins and flavonoids were qualitatively detected in a methanolic fresh leaf extract of *F. racemosa*. These substances have been documented to

play a potent role in antimicrobial activity (Pękala-Safińska et al. 2021). Tannins induce microbial protein precipitation, thus leaving bacteria devoid of their function (Akiyama et al. 2001) Flavonoids can inhibit bacterial DNA and RNA synthesis, inactivate bacterial adhesins and cell envelope transport proteins, and disrupt the bacterial cell membrane (Cowan 1999; Cushnie and Lamb 2005). Together with antibiotics, tannins and flavonoids showed promising synergistic effects, a wide spectrum of antimicrobial activity, and the ability to inhibit various microbial virulence factors including adhesion proteins, biofilm, and toxins (Daglia 2012).

The inhibition zone diameter observed in our study was in the same range of antimicrobial activity against *S. aureus* as obtained with other tannin- and flavonoid-rich medicinal plants (Neumann et al. 2022). Studies consistently indicated a low antimicrobial activity of *Ficus* extracts if tested by the agar disk diffusion method (Vittaya and Chalad 2011; Shahriar et al. 2013). Conversely, the broth dilution approach showed an inhibitory effect against both D-test positive and D-test negative *S. aureus* that varied depending on the dose of *F. racemose* dried leaf extract (70g vs. 140g). These outcomes were noted once when testing on day 15, demonstrating the antibacterial effect's stability. Therefore, we suggest the use of the broth dilution method as a suitable approach for determining the antibacterial activity of *Ficus* extracts to mitigate the risk of obtaining inaccurate negative outcomes from agar disk diffusion assay.

The observed inhibitory effect of *F. racemose* against *S. aureus* was in line with findings from another research (Bagyalakshmi et al. 2019; Pingale et al. 2019). However, Bagyalakshmi et al. (2019) indicated that the extract derived from the fruit had more activity compared to the leaf, as illustrated by the comparison of minimum inhibitory concentration (MIC) values. The MIC from the fruit extract was 0.07 mg/ml while deriving from the leaf extract was 0.625 mg/ml (Bagyalakshmi et al. 2019). Unlike previous investigations, our study did not measure the dry weight of the crude extract before dissolving it in sterile distilled water and testing. As a result, instead of presenting the extract MIC, inhibitory and bactericidal titers were reported. Due to this constraint, it was not possible to conduct a direct quantitative comparison of the MIC and the minimum bactericidal concentration (MBC).

To summarize, this investigation highlights the consistent antibacterial effectiveness of *F. racemosa* leaf extract against both antibiotic-sensitive and antibiotic-resistant strains of *S. aureus*. Furthermore, forthcoming research should assess other variables that could potentially influence inhibitory activity, such as different *Ficus* species and the specific plant components utilized for extraction, the extraction process employed, and the kind of solvent utilized (Cheng et al. 2020). However, these aspects were not able to be examined in this study due to limitations.

Acknowledgments

The authors would like to thank Ms. Pornpan Chomwong and Ms. Rattanaporn Benmas for their valuable suggestions and Dr. Samorn Pato, Director of Chulabhorn Science College, Pathum Thani, for his support of this project.

References

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2001) Antibacterial action of several tannins against *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy 48(4): 487-491.

Bagyalakshmi B, Nivedhitha P, Balamurugan A (2019) Studies on phytochemical analysis, antioxidant and antibacterial activity of *Ficus racemosa* L. leaf and fruit extracts against wound pathogens. Vegetos 32: 58–63. doi: 10.1007/s42535-019-00007-6.

Cheng JX, Zhang BD, Zhu WF, Zhang CF, Qin YM, Abe M, et al (2020) Traditional uses, phytochemistry, and pharmacology of *Ficus hispida Lf*: A review. Journal of Ethnopharmacology 248: 112204. doi: 10.1016/j.jep.2019.112204.

Cowan MM (1999) Plant products as antimicrobial agents. Clinical Microbiology Reviews 12(4): 564-582. doi: https://doi.org/10.1128/cmr.12.4.564.

Cushnie TT, Lamb AJ (2005) Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26(5): 343-356. doi: 10.1016/j.ijantimicag.2005.09.002.

Daglia M (2012) Polyphenols as antimicrobial agents. Current Opinion in Biotechnology 23(2): 174-181. doi: 10.1016/j.copbio.2011.08.007.

Kingsley JD, Tirkey PM, Ritika C, Jayanthi A (2014) Evaluation of antimicrobial activity of *Ficus racemosa* Linn leaves extract. International Journal of Pharmacy and Technology 6(1): 6310-6317.

Koodkaew I, Boonraksa K, Sengseang N (2018) Preliminary phytochemical determination and phytotoxic effects of *Mimosa pudica L*. leaf crude extract. Khon Kaen Agriculture Journal 46 (1) : 129-136 (in Thai).

Neumann N, Honke M, Povydysh M, Guenther S, Schulze C (2022) Evaluating tannins and flavonoids from traditionally used medicinal plants with biofilm inhibitory effects against MRGN *E. coli.* Molecules 27(7): 2284. doi: 10.3390/molecules27072284.

Pękala-Safińska A, Tkachenko H, Kurhaluk N, Buyun L, Osadowski Z, Honcharenko V, et al (2021) Studies on the inhibitory properties of leaf ethanolic extracts obtained from Ficus (Moraceae) species against *Aeromonas spp*. strains. Journal of Veterinary Research 65(1): 59-66. doi: 10.2478/jvetres-2021-0007.

Pingale T, Duse P, Ogale S (2019) Antibacterial and antifungal approaches of *Ficus racemosa*. Pharmacognosy Journal 11(2): 355-357. doi: 10.5530/pj.2019.11.53.

Polyium U, Kuttiyawong A, Panya N (2014) The potential development of Ficus hispida Linn. use to traditional herbs and cancer prevention for generate knowledge and heritage of local wisdom: Final research report. N.p.: Rajamangala University of Technology Phra Nakhon, Bangkok (Thailand). Faculty of Science and Technology (in Thai).

Shahriar M, Islam S, Parvin S, Hoque S (2013) Thrombolytic activity and antimicrobial properties of *Ficus hispida.* Journal of Scientific Research 5(2): 393-397. doi: 3329/jsr.v5i2.11988.

Vittaya L, Chalad C (2011) Antimicrobial activity of *Ficus hispida Linn*. extract. RMUTSV Research Journal 3(2): 35-39 (in Thai).