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Antibacterial activity of fennel (*Foeniculum vulgare*) leaf from the slopes of Mount Bromo against Methicillin-resistant *Staphylococcus aureus*

Anindya Fatihatur Rahmah [1], Eko Budi Koendhori [2,*] and Joni Susanto [3]

[1] *Medical Education Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[3] *Department of Anatomy, Histology, and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*

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Abstract

Staphylococcus aureus is an infection-causing bacterium that is associated with many deaths in the world and is complicated by its adaptable condition that causes cases of antibiotic resistance. Fennel (*Foeniculum vulgare*) leaves are known to contain antibacterial compounds, so it is necessary to find out whether fennel leaves have an effect on Methicillin-resistant *Staphylococcus aureus* (MRSA). This study aims to prove the antibacterial activity of ethanol extract of fennel leaves against MRSA bacteria. This study is a true experimental study to test the antibacterial activity of ethanol extract of fennel leaves against MRSA. The fennel leaves used were obtained from the slopes of Mount Bromo. MRSA bacteria were obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Airlangga. The antibacterial test used the agar well diffusion method. SPSS version 27 was used for data analysis to perform non-parametric tests with the Kruskal–Wallis test and the Mann–Whitney post hoc test. The test results showed that the several series of ethanol extract concentrations of fennel leaves used in the test showed that a concentration of 80% formed an inhibition zone against MRSA with an average diameter of 7.12 mm. The conclusion of this study is that fennel leaf extract obtained from the slopes of Mount Bromo has the ability to inhibit the growth of MRSA.

Keywords: Antibacterial agents; *Foeniculum vulgare*; Ethanol Extract; MRSA

1. Introduction

Infection is a disease caused by microorganisms known as pathogens such as fungi, bacteria, viruses, or parasites [1]. Among the various types of infection-causing pathogens, those caused by bacteria are of significant concern. In 2019, bacterial infections accounted for 13.6% of deaths worldwide. Globally, deaths related to infections were estimated to reach 13.7 million, with 7.7 million of those deaths linked to bacterial infections. These bacterial infections are caused by various types of bacteria, but only one bacterium was associated with over 1 million death cases that year, namely *Staphylococcus aureus* [2].

S. aureus is a gram-positive coccus bacterium that has become the most clinically relevant species compared to 52 species and 28 subspecies of the genus *Staphylococcus* [3]. This bacterium is a commensal bacterium as well as a major cause of endocarditis, bacteremia, osteomyelitis, as well as skin and soft tissue infections [4]. The issue of *S. aureus* infection incidence continues to evolve with the emergence of antibiotic-resistant bacteria, making it challenging to treat infections, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) [5].

MRSA is a bacterium of *S. aureus* that has developed resistance to all beta-lactam antibiotics, including monobactam and cephalosporins, which are groups of antibiotics commonly used to treat *Staphylococcus* infections [6]. Although the prevalence of MRSA infections varies greatly among countries, MRSA has been detected in most countries around the

* Corresponding author: Eko Budi Koendhori

world [7]. Currently, the antibiotic used and is the first-line treatment for MRSA infections is vancomycin. However, considering that MRSA has a good adaptation capability, it is not unlikely that in the future there will be resistance to vancomycin [8].

Fennel (*Foeniculum vulgare*) is a traditional and popular herb with a long history of usage as medicine [9]. Various parts of this plant are used in the treatment of various diseases, such as in the treatment of diabetes, bronchitis, chronic cough, kidney stones, and digestive system problems. Fennel is also used to treat various bacterial, viral, fungal, and mycobacterial infections [10]. Fennel leaves contain chemical compounds with antibacterial properties, including flavonoids, phenols, tannins, and saponins [11].

Previous study has indicated that fennel leaves originating from Tunisia have the ability to inhibit the growth of *S. aureus* [12]. However, there has been no study discussing the antibacterial activity of fennel leaf extracts from the slopes of Mount Bromo against MRSA. This study was conducted to determine the effects of ethanolic extracts of fennel leaves from the slopes of Mount Bromo on MRSA.

2. Material and methods

This research is a true experimental study with a posttest-only control group design using in vitro testing with the agar well diffusion method. In this study, 7 repetitions were performed with 5 repetitions based on Federer's formula and an additional 2 reserve repetitions. Samples were obtained through simple random sampling.

The sample in this study is fennel leaves obtained from the slopes of Mount Bromo, Probolinggo Regency. The bacteria in this study were obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Airlangga. The research was carried out from February to November 2024 in the Pharmacy Laboratory and the Microbiology Laboratory of the Faculty of Medicine, Universitas Airlangga.

The research materials include 96% ethanol, the antibiotic vancomycin, Mueller–Hinton agar, distilled water, DMSO, and McFarland standards. The research instruments used include petri dishes, graduated cylinders, filter paper, test tubes, Bunsen burner, sterile blank disks, micropipettes and tips, round inoculator, sterile cotton swabs, calipers, autoclave, incubator, vortex mixer, rotary evaporator, and water bath.

The fennel leaves that have been picked are then dried and ground into a fine powder. The powdered leaves then undergo a maceration process by being soaked in 96% ethanol before the filtration process. The evaporation process is carried out using a rotary evaporator to separate the fennel leaf extract from the solvent. A water bath is used to evaporate the extract that has been reduced to a thick extract.

The agar well diffusion method was performed by measuring the inhibition formed by MRSA. The test for the diameter of the inhibition zone of antibacterial activity of ethanolic extract of fennel leaves against MRSA using the agar well diffusion method was carried out using extracts with a series of concentrations. On the agar medium that had been inoculated with bacteria, wells were made and then filled with extracts at concentrations of 20%, 40%, and 80%, along with two control treatments: a positive control (vancomycin) and a negative control (DMSO). Incubation was performed for 24 hours at 37°C. Subsequently, a clear zone will form around the wells in the growth medium of the test bacteria that is not colonized by bacteria, which can be measured using a digital caliper.

The data was analyzed using SPSS (Statistical Product and Service Solutions) version 27 to conduct a normality test using the Shapiro–Wilk test and Levene's test to test for homogeneity. Furthermore, a non-parametric statistical test was performed using the Kruskal–Wallis test and the Mann–Whitney post hoc test.

3. Results and discussion

The determination of the diameter of the inhibition zone is performed using the disk diffusion method by adding the test solution into wells that were created on Mueller–Hinton agar inoculated with bacteria, then incubated at 37°C for 24 hours in an incubator. The determination of the diameter of the inhibition zone is carried out by measuring the clear area surrounding the well created in the agar medium using a digital caliper.

In this study, the determination of the inhibition zone diameter of fennel leaf extract against MRSA bacteria was carried out by testing three series of concentrations, which were 20%, 40%, 80%, a positive control of vancomycin, and a negative control of solvent or DMSO. Vancomycin was used as a positive control because it is still the drug of choice in

the treatment of infections caused by MRSA [13]. DMSO was used as a negative control because DMSO serves as the solvent for diluting the test concentration series.

Table 1 Diameter of the inhibition zone of ethanol extract from fennel leaves

Treatment	n	Mean ± SD (mm)
Positive Control (+)	7	15.00 ± 1.73
Negative Control (–)	7	0 ± 0
Ethanol Extract of Fennel Leaves 20%	7	0 ± 0
Ethanol Extract of Fennel Leaves 40%	7	1.20 ± 3.18
Ethanol Extract of Fennel Leaves 80%	7	7.12 ± 4.93

Table 1 presents the average inhibitory zone diameter area at several different concentration series against MRSA. Based on the results in Table 1, inhibitory zones were formed at concentrations of 40% and 80%, with average diameters of 1.20 mm and 7.12 mm, respectively. In the positive control treated with the antibiotic vancomycin, the average diameter of the inhibitory zone was 15.00 mm. Based on these results, it can be concluded that the antibiotic vancomycin is still more effective in inhibiting the activity of MRSA bacteria than the ethanol extract of fennel leaves at the tested concentration series.

The data obtained from the study was subsequently analyzed using the Shapiro–Wilk normality test and the Levene's test for homogeneity. The results of the normality and homogeneity tests yielded a p-value < 0.05, indicating that the data was not normally distributed and not homogeneous. Therefore, a non-parametric statistical test, the Kruskal–Wallis test, was subsequently performed.

Table 2 Results of Mann–Whitney post hoc test data analysis

Comparison between treatments		P-value
Positive Control	Negative Control	<0,001
	Concentration of 20%	<0.001
	Concentration of 40%	<0.001
	Concentration of 80%	0.002
Negative Control	Concentration of 20%	1000*
	Concentration of 40%	0.710*
	Concentration of 80%	0.026
Concentration of 20%	Concentration of 40%	0.317*
	Concentration of 80%	0.009
Concentration of 40%	Concentration of 80%	0.038

* there is no significant difference (p ≥ 0.05)

The results of the non-parametric Kruskal–Wallis statistical test indicate that the data has a p-value < 0.001, suggesting a significant difference among all treatment groups (p-value < 0.05). Furthermore, to determine which data has significant differences, a non-parametric Mann–Whitney post hoc statistical test was conducted. The non-parametric Mann–Whitney post hoc test indicates that Control (+) compared to Control (-) and all series of concentrations of ethanol extract of fennel leaves had a value of p < 0.05, meaning there is a significant difference among those treatment groups. A significant difference (p < 0.05) was also shown in the comparison of Control (-) with 80% Concentration, 40% Concentration with 80% Concentration, and 40% Concentration with 80% Concentration. Meanwhile, in the comparison of Control (-) with Concentration 20%, Concentration (-) with Concentration 40%, and Concentration 20% with Concentration 40% had a p value ≥ 0.05, which means there is no significant difference between those treatment groups. These results indicate that the antibacterial activity of the ethanol extract of fennel leaves against MRSA was

present at a concentration of 80%, while the series of test concentrations of 20% and 40% did not show any antibacterial activity.

Other studies have shown the antibacterial activity of fennel extract at different concentrations. A study by Sahu et al. [14] reported that fennel extract exhibits activity against MRSA with an MIC of 500 μg/mL and an MBC of 1000 μg/mL. This study also mentioned that fennel extract has the ability to eradicate MRSA biofilm, demonstrating the potential of active compounds in *F. vulgare* extract in addressing MRSA comprehensively.

The antibacterial activity of fennel leaves is influenced by the content of compounds present in them**.** Flavonoids act as bacteriostatic and bactericidal agents by damaging the cytoplasmic membrane and inhibiting energy metabolism and nucleic acid synthesis in microorganisms [15]. Polyphenols exert their antibacterial activity, among other things, through the process of inhibiting biofilm formation [16]. Tannins will chelate iron necessary for bacterial growth and deactivate the enzymes used in cell wall synthesis, making bacteria more susceptible to osmotic lysis [17]. Saponins play a role in the mechanism of disrupting bacterial cell wall permeability, which affects the lack of nutrients for the bacteria [18].

4. Conclusion

Through testing using the diffusion method, the ethanol extract of fennel leaves (*F. vulgare*) obtained from the slopes of Mount Bromo indicated the ability to inhibit the growth of MRSA. These results suggest that fennel leaves could be considered as one of the alternative natural antibacterials. Further studies related to active compounds, determination of the mechanism of action, and toxicity tests can be conducted to ensure its safety and broader utilization.

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

This research received ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (no. 180/EC/KEPK/FKUA/2024).

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