Antibacterial Effect of *Jatropha multifida L.* Leaf Infusion towards *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Background: *Jatropha multifida* is one of the medicinal plants commonly found in Indonesia. This plant is used in the community to heal open wounds, however, scientific evidence is lacking. The two most common bacteria which often cause infection in open wounds are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study aimed to determine the antibacterial effect of *J. multifida* leaf infusion towards *S. aureus* and *P. Aeruginosa in vitro*.
Methods: This was an experimental laboratory study conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran in 2014. The modified Kirby-Bauer antimicrobial diffusion procedure on Mueller-Hinton agar was applied to determine the inhibitory zone. In determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), the modified technique of tube dilution was used.
Results: The results of this research showed that the infusion of *J. multifida* leaves had inhibitory effects on the growth of *S. aureus* dan *P. aeruginosa* at the concentration of 100% and 75%. The minimum inhibition concentration and minimum bactericidal concentration could not be determined.
Conclusions: There is evidence confirming the bacteriostatic antibacterial effect of *J. multifida* leaves which inhibits the growth of *S. aureus* and *P. Aeruginosa*. Further study is needed to explore *J. multifida* leaves.

Keywords: Antibacterial effect, infusion, *Jatropha multifida* leaf, *P. aeruginosa, S. aureus*

Introduction

Indonesia is a country rich in natural resources and biological diversity, one of which is found in medicinal plants. Since historical times, Indonesians have been using various types of traditional medicinal plants to cure various ailments, one of which is coral plants or *Jatropha multifida Linn* (*J. multifida*).[1] Benefits of *J. multifida* have not been well known to common Indonesian. The parts of this plant that can be used are its leaves, sap, and seeds oil, which have been utilized to treat helminthiasis, infections in open wounds, and various inflammatory conditions of the skin.[2]

Generally, an extract of the parts of a particular plant is used to test the plant's potential effects and therapeutic benefits. One of the more readily made extracts is a liquid extract, which is produced by using solvents as extractors. Solvents that are commonly used are water and ethanol 95%. The most frequently used methods to create liquid extracts are by dedoctum and infusum, which utilize water as the solvent.[3] In this study, the infusum method was used because it is cheaper, faster, and simpler, which makes it more available to common people.

Open wounds happen when the skin or the mucosal surface experiences destruction. This destruction causes increased exposure to infectious agents, one of which is bacteria. One of the bacteria that commonly infect open wounds is *Staphylococcus aureus*.[4] Beside *S. aureus, Pseudomonas aeruginosa* is another bacteria that commonly cause open wound infection and also nosocomial infection.[5]

Based on these facts, the author is interested to study the antibacterial effect of *J. multifida* leaves, in the form of an infusion, against *S. aureus*, which represents Gram-

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positive bacteria, and *P. aeruginosa*, which represents Gram negative bacteria, as bacteria that commonly cause open wound infection.

Methods

This was an experimental laboratory study, approved by the Research Ethics Committee Universitas Padjadjaran. The study was carried out in the Microbiological Laboratory Faculty of Medicine Universitas Padjadjaran in Jatinangor in October 2014.

The study comprised of four stages: (i) making of *J .multifida* leaf infusion, (ii) making of bacterial suspension, (iii) determination of antibacterial effect with antimicrobial infusion method of Kirby Bauer that had been modified on Mueller Hinton agar, and (iv) determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) with modified tube dilution method.

On the first stage, *J. multifida* leaf infusion was made. *J. multifida* leaves were washed, and then dried and finely cut. 100% concentration was achieved by putting 20 g of leaves into 20 g of water. It was heated at 90°C for 15 minutes and stirred every 5 minutes. After cooling it down, the mixture was filtered using sterile gauze until no more water was left. The 100% infusion was diluted to make 75%, 50%, and 25% infusions.

On the second stage, bacterial suspension was made. The bacterial colony was grown on Mueller Hinton agar in a petri dish and incubated for 24 hours at 37°C. After incubation, bacteria were taken using an inoculating loop for 4–5 times and then inserted into a reaction tube filled with distilled water to produce a suspension until the turbidity achieved McFarland standard 0.5.[6] This suspension was equivalent to 1.5 x 108 CFU/mL.

In the third stage, the antibacterial effect was determined. A milliliter of bacterial suspension was added into a petri dish, then the dish was filled with 24 mL Mueller Hinton agar at 40–50°C. It was then homogenized and let to cool down until it solidified with ± 4 mm thickness. Five holes with 10 mm diameter and 4 mm depth were made on the solidified agar. Four holes were filled with 0.3 mL of *J. multifida* leaf infusion with different concentrations: 100%, 75%, 50%, and 25%. The fifth hole was filled with distilled water as a positive control. The dish was then incubated for 24 hours at 37°C in the incubator. After 24 hours, the diameter of bacterial growth inhibition zones at each hole was measured. These zones appeared as clear areas surrounding each hole.

On the fourth stage, MIC and MBC were determined. To determine MIC, ten reaction tubes and J. multifida infusions with different concentrations: 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, and 0.78%. The first to the eight tubes were filled 1 mL of infusion, each one with a different concentration.

A milliliter liquid Mueller Hinton agar that had been added with bacterial suspension (0.5 McFarland turbidity) was added to the eight tubes that contained infusion, and then the tubes were shaken for homogenization. The end concentrations would be the following: 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, and 0.39%. The ninth reaction tube was filled with the same liquid Mueller Hinton agar (with bacterial suspension) as positive control and the tenth tube was filled

Table 1 Diameter of Inhibitory Zones of *S. aureus* and *P. aeruginosa* with Different Concentrations of *J. multifida* Leaf Infusion

Bacteria	Concentration (%)	Diameter of inhibitory zone (mm)			Average (mm)
		I	II	III	
S. aureus	100	25	22	17	21.3
	75	20	19	13	17.3
	50	-	-	-	-
	25	-	-	-	-
P. aeruginosa	100	20	18	18	18.7
	75	14	15	14	14.3
	50	-	-	-	-
	25	-	-	-	-

with liquid Mueller Hinton agar and 100% *J.* multifida infusion as a negative control.

Next, all ten reaction tubes were put into the incubator for 24 hours at 37°C. After incubation, the turbidity of each tube was observed to examine the MIC. Tubes that were clearer than the negative control indicated the presence of an antibacterial effect.

To determine MBC, as much as 1 inoculating loop of the mixture from each tube was taken and smeared on Mueller Hinton agar. It was next incubated for 24 hours at 37°C for observation. The above procedure was repeated for 3 times with new infusion but identical treatment.

Results

Determination of the antibacterial effect of *J. multifida* leaf infusion against *S. aureus* and *P. aeruginosa* was done by measuring the diameter of the inhibitory zones.

Table 1 shows that at 100% and 75% concentrations, *J. multifida* leaf infusion has an antibacterial effect against *S. aureus* and *P. aeruginosa*. The antibacterial effect was bacteriostatic because there was still bacterial growth in the inhibitory zones. The growths were observed through Gram staining.

After MIC test observation for 24 hours with the tube dilution method, it was concluded that MIC of J. multifida leaf infusion against *S. aureus* and *P. aeruginosa* could not be determined because there were still visual observations of turbidity in every tube, indicating bacterial growth (Table 2).

From MBC test observation for 24 hours, using the culture from the previous MIC test, it was concluded that the MBC of *J. multifida* leaf infusion against *S. aureus* and *P. aeruginosa* could not be determined because there was still bacterial colony growth in MH agar regardless of the concentration of the infusion added (Table 3).

Discussions

This study has shown that inhibitory zones

Table 2 Bacterial Growth after MIC Test Against *S. aureus* and *P. aeruginosa* by *J. multifida* Leaf Infusion at Different Concentrations

Bacteria	Concentration (%)								Control	
	50	25	12.5	6.25	3.13	1.56	0.78	0.39	C(-)	C(+)
	+	+	+	+	+	+	+	+	-	+
S. aureus	+	+	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	-	+
P. aeruginosa	+	+	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	-	+

Note: + : Bacterial colonies were present, - : Bacterial colonies were absent, (+): MH broth with tested bacteria suspension, (-) : 100% J. multifida leaf infusion with MH broth

Table 3 Bacterial Growth in MBC Test against *S. aureus* and *P. aeruginosa* by *J. multifida* Leaf Infusion at Different Concentrations.

Bacteria	Concentration (%)								Control	
	50	25	12.5	6.25	3.13	1.56	0.78	0.39	C(-)	C(+)
	+	+	+	+	+	+	+	+	-	+
S. aureus	+	+	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	-	+
P. aeruginosa	+	+	+	+	+	+	+	+	-	+
	+	+	+	+	+	+	+	+	-	+

Note: + : Bacterial colonies were present, - : Bacterial colonies were absent, (+): MH broth with tested bacteria suspension, (-) : 100% J. multifida leaf infusion with MH broth

have been produced surrounding the infusion holes with 100% and 75% infusion concentrations, however, bacterial growth still exists in the hole after examination with Gram staining. It is possible that the antibacterial contents in the infusion are not enough to kill the bacteria, as such only the bacteriostatic effect is observed.

The bacteriostatic effect is not observed in the MIC and MBC test. It is shown by the presence of bacterial growth in all of the tubes except in the negative control tube. This could be due to the concentration of the infusion. Even though the infusion with the highest concentration (100%) has been used, the concentration becomes only 50% after the mixing with Mueller Hinton broth. On the other hand, 50% infusion is failed to produce any inhibitory zones surrounding the holes in the antibacterial effect test.

Several studies have been done to examine the antibacterial effect of the different parts of *J. multifida* such as the leaves, barks, and sap. *J. multifida* leaf extract can inhibit the growth of M. tuberculosis at the concentration of 128 µg/mL.[1] Interestingly, the bark extract of *J. multifida* has an inhibitory effect against several types of fungi and bacteria and has the potential to be an antimalarial drug.[7] The sap of *J. multifida* can inhibit the growth of various bacteria.[8] In other studies, the cream made of *J. multifida* sap can help the healing process of S. aureus infection on the external wounds on rats.[4]

There is a difference between this study and the previous studies in the form of the sample used. This study has used an infusion with water as its solvent, while other studies have made extracts with organic solvents such as ethanol. The advantages of using the infusion method are cheaper, faster, and simpler in terms of procedures and tools needed; meanwhile, extraction process requires prior knowledge about the contents of the plant and their respective suitable solvent. Suitable solvents are used to separate the active substances from the plant and dissolve them. After that, the separation of the active substances from the solvent is relatively easier to do to achieve a pure extract. This shows that extract production is more complicated and expensive than the infusion method and hence more difficult to be adopted by the common people. However, the infusion method also has some limitations, one of them being that the amount of the active substance extracted from the plants is less if compared to extract. Several studies have shown that the active substances that have Ann antibacterial effect in *J. multifida* leaves are flavonoid, saponin, and tannin.[1,9,10] Flavonoid and tannin could dissolve in water and hence they are extractable by infusion,[11] however, the extracted amount is still much smaller than if ethanol 96% is used. The use of ethanol 96% as the solvent can extract flavonoids and tannin twenty-five times more than when water is used.[11]

This study has some limitations. There are difficulties in producing infusion concentration higher than 100% because not all the leaves can be submerged in the water. The study could not use dried leaves because the storage was prone to contamination. Further studies to compare the antibacterial effect between *J. multifida* leaf infusion and its extract are interested to be explored.

To conclude, *J. multifida* leaf infusion has an antibacterial effect, which is bacteriostatic against *S. aureus* and *P. aeruginosa.*

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