Antibacterial Effect of *Pulsatilla chinensis* towards *Staphylococcus aureus*, *Shigella dysenteriae*, and *Salmonella typhi*

Sim Chee Fong,[1] Yanti Mulyana,[2] Dolvy Girawan[3]
[1]Faculty of Medicine Universitas Padjadjaran, [2]Department of Microbiology Faculty of Medicine, Universitas Padjadjaran, [3]Department of Internal Medicine Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung,

Abstract

Background: *Pulsatilla (P.) chinensis* is a kind of traditional Chinese medicine (TCM) that has antibacterial effect. It is used to treat diarrhea, dysentery, and other diseases. The *P. chinensis* is composed of some potent antibacterial substances including protoanemonin, saponin, oleanolic acid. The study aimed to determine the antibacterial effect of *P. chinensis* towards *staphlococcus aureus, Shigella dysenteriae,* and *Salmonella typhi.*
Methods: This was an experimental descriptive study that was conducted in July 2014 using two methods, diffusion and dilution method. In diffusion method, 5 holes were made on the agar that bacteria were growing and different concentrations of *P. chinensis* infusion were placed in different hole. The inhibitory effect was measured by the inhibition zone. In dilution method, 8 test tubes with decreasing concentration of *P. chinensis* infusion were mixed with the bacteria suspension and Mueller Hinton Solution. The minimal inhibitory concentration (MIC) was measured by the last clear test tube. The test tube with solution that showed absence of bacteria on culture indicated the minimal bactericidal concentration (MBC).
Results: In diffusion method, *P. chinensis* infusion showed inhibitory effect towards *S. aureus* and bacteriostatic effect towards *S. dysenteriae* and *S. typhi.* In dilution method, there was no antibacterial activity detected.
Conclusions: *P. chinensis* infusion has inhibitory effect on *S. aureus* and bacteriostatic effect on *S. dysenteriae* and *S. typhi.* **[AMJ.2016;3(2):292–7]**

Keywords: Antibacterial, infusion, *Pulsatilla chinensis*, traditional Chinese medicine

Introduction

The traditional Chinese medicine (TCM) remains the most common traditional medicine and some TCMs have been proved to have antibacterial effect since long time ago. A study conducted in Taiwan[1] showed that TCM have potential to become natural antibiotic and even have synergistic effect with synthetic antibiotics. The intestinal bacteria can alter human health and many diseases development are associated with imbalance in intestinal microorganism.[2] The common pathologic intestinal bacteria that are normally encountered can be *Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi.* These bacteria can cause the disease such as diarrhea and dysentery.

The *Pulsatilla chinensis* is one of the TCMs that have antibacterial effect on the intestinal bacteria. In TCM, it has been used to treat diarrhea, dysentery, and other diseases. The *Pulsatilla chinensis* is composed of some potent antibacterial chemical substances including the protoanemonin, saponin, oleanolic acid, and anemonin. These chemical substances have antibacterial properties and have been proved in the previous studies.[3-8] If *Pulsatilla chinensis* proved to have antibacterial effect on pathogenic intestinal bacteria, it is not just provide a new alternative treatment for diarrhea and dysentery, but also for other intestinal infectious diseases. This study aimed to determine the antibacterial effect of *P. chinensis* towards *staphlococcus aureus, Shigella dysenteriae,* and *Salmonella typhi.*

Methods

The research method was laboratory

Correspondence: Sim Chee Fong, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km.21, Jatinangor, Sumedang, Indonesia, Phone: +6287726143636 Email: cheefong0320@gmail.com

Table 1 The Inhibition Zone Produced by the Pulsatilla chinensis Infusion on Mueller Hinton Agar where Staphylococcus aureus was growing

Test	Diameter of Inhibition Zone (mm)				
	Concentration of Pulsatilla chinensis Infusion				
	0%	10%	15%	20%	25%
1st Test	-	9.17	9.33	9.83	11.83
2nd Test	-	9.17	9.33	10.67	12.00
3rd Test	-	9.50	9.83	10.83	12.00
Mean	-	9.28	9.50	10.44	11.94

Note: - = No inhibition zone

microbiology experiment and the type of study was experimental descriptive study. The study was conducted using two methods, diffusion method and dilution method. First, *Pulsatilla chinensis* would be cut into small pieces and water-bathed with purified water. The amount of 25mg *Pulsatilla chinensis* was boiled with 100ml of purified water to get infusion with concentration of 25%. The infusion was then mixed with purified water to get different concentrations of infusion which were 20%, 15%, and 10%. The bacteria used in the experiment included *Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi.* All the bacteria used were directly taken from the Microbiology laboratory of Universitas Padjadjaran.

In dilution method, selected bacteria (*Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi*) would be grown in Mueller Hinton agar. Five holes were made on the agar and different concentrations (25%, 20%, 15%, 10%, and 0%) of *Pulsatilla chinensis* infusion were placed in the different hole. The inhibitory effect would be measured by the zone around the holes where bacteria was not growing. In dilution method, 8 test tubes with decreasing concentration of Pulsatilla chinensis infusion (6.25%, 3.125%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1%, 0.05%) were mixed with the bacteria suspension and Mueller Hinton Solution. There was one test tube that acted as the negative control which was only filled with Mueller Hinton solution and *Pulsatilla chinensis* infusion. There was also another test tube that acted as the positive control which was only filled with bacteria suspension and Mueller Hinton solution.

The minimal inhibitory concentration (MIC) was measured by the last clear test tube because the clear test tube indicated that there was no bacterial growth. The solution from all test tubes was cultured on Mueller Hinton agar. The test tube solution with least concentration of infusion and showed no cultured bacterial growth would indicate the minimal bactericidal concentration (MBC).

By using the diffusion method, the antibacterial effect was measured by the inhibition zone presented on the agar in which bacteria was growing. Besides that, MIC and MBC were measured to determine the minimal concentration of infusion that can inhibit and kill the bacteria.

Results

In the diffusion method, the inhibition zone was presented in the Mueller Hinton agar

Table 2 The Bacteriostatic Zone Produced by the Pulsatilla chinensis Infusion on Mueller Hinton Agar where Shigella dysenteriae was growing

Test	Diameter of Bacteriostatic Zone (mm)				
	Concentration of Pulsatilla chinensis Infusion				
	0%	10%	15%	20%	25%
1st Test	-	13.83	16.33	19.16	22.67
2nd Test	-	12.17	16.67	17.83	20.33
3rd Test	-	12.83	16.00	18.33	19.67
Mean	-	12.94	16.33	18.44	20.89

Note: - = No inhibition zone

Table 3 The Bacteriostatic Zone Produced by the *Pulsatilla chinensis* Infusion on Mueller Hinton Agar where Salmonella typhi was growing

Test	Diameter of Bacteriostatic Zone (mm)				
	Concentration of *Pulsatilla chinensis* Infusion				
	0%	10%	15%	20%	25%
1st Test	-	12.17	13.33	16.17	17.17
2nd Test	-	12.00	12.67	13.83	16.67
3rd Test	-	11.17	13.00	14.33	17.17
Mean	-	11.78	13.00	14.78	17.00

Note: - = No inhibition zone

where *Staphylococcus aureus* was growing. For *Shigella dysenteriae* and *Salmonella typhi*, the inhibition zone was not detected but there was a presence of bacteriostatic zone. For MIC, all the 3 bacteria tested was growing in all test tubes except 9th test tube which acted as a negative control. For MBC, the solutions of all 10 test tubes for 3 bacteria cultured. The result was there was a presence of bacterial growth in all test tube solutions except the 9th test tube of all 3 bacteria.

In the diffusion test on *Staphylococcus aureus, Pulsatilla chinensis* infusion showed inhibitory effect on bacteria growth. There was visible inhibition zone on the agar and it had concentration dependence. In the diffusion test of *Shigella dysenteriae*, there was no inhibition zone but there was bacteriostatic zone. The zone showed there was a secondary bacterial growth in which it seemed like initial bacteria that had been inhibited but was growing afterward. The bacteriostatic zone had concentration dependence.

In the diffusion test of *Salmonella typhi,* the result was the same as the one in *Shigella dysenteriae* which there was a bacteriostatic

Table 4 The Result of MIC Test on *Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi*

Test	Test Tube Turbidity									
	Test Tube for *Staphylococcus aureus*									
	1st	2nd	3rd	4th	5th	6th	7th	8th	Negative control	Positive control
1st Test	+	+	+	+	+	+	+	+	-	+
2nd Test	+	+	+	+	+	+	+	+	-	+
3rd Test	+	+	+	+	+	+	+	+	-	+
	Test Tube for *Shigella dysenteriae*									
	1st	2nd	3rd	4th	5th	6th	7th	8th	Negative Control	Positive Control
1st Test	+	+	+	+	+	+	+	+	-	+
2nd Test	+	+	+	+	+	+	+	+	-	+
3rd Test	+	+	+	+	+	+	+	+	-	+
	Test Tube for *Salmonella typhi*									
	1st	2nd	3rd	4th	5th	6th	7th	8th	Negative Control	Positive Control
1st Test	+	+	+	+	+	+	+	+	-	+
2nd Test	+	+	+	+	+	+	+	+	-	+
3rd Test	+	+	+	+	+	+	+	+	-	+

Note: + = Cloud Solution, - = Clear Solution

Table 5 The Result of MBC Test on *Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi*

Test	Result of Culture from Test Tube									
	Test Tube for *Staphylococcus aureus*									
	1st	2nd	3rd	4th	5th	6th	7th	8th	Negative control	Positive control
1st Test	+	+	+	+	+	+	+	+	-	+
2nd Test	+	+	+	+	+	+	+	+	-	+
3rd Test	+	+	+	+	+	+	+	+	-	+
	Test Tube for *Shigella dysenteriae*									
	1st	2nd	3rd	4th	5th	6th	7th	8th	Negative Control	Positive Control
1st Test	+	+	+	+	+	+	+	+	-	+
2nd Test	+	+	+	+	+	+	+	+	-	+
3rd Test	+	+	+	+	+	+	+	+	-	+
	Test Tube for *Salmonella typhi*									
	1st	2nd	3rd	4th	5th	6th	7th	8th	Negative Control	Positive Control
1st Test	+	+	+	+	+	+	+	+	-	+
2nd Test	+	+	+	+	+	+	+	+	-	+
3rd Test	+	+	+	+	+	+	+	+	-	+

Note: + = Bacterial presence, - = Bacterial absence

zone. The zone showed a secondary bacterial growth and had concentration dependence.

In MIC test of *Staphylococcus aureus*, all the test tubes showed cloud solution which indicated that there was a bacterial growth except the test tube which acted as negative control. For the MIC test of *Shigella dysenteriae* and *Salmonella typhi,* the result was same as the one for *Staphylococcus aureus*.

For the MBC test, the solution from all test tubes of *Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi* had been cultured on Mueller Hinton agar. The solution from all test tubes contained bacterial growth except the test tube which acted as negative control for the three bacteria.

Discussion

The study was conducted to test whether there is any antibacterial activity from *Pulsatilla chinensis* infusion. The infusion was chosen because for most people, it is common to serve the *Pulsatilla chinensis* by boiling it. Extraction method in this study is more similar to the way society extracts the medicine.

In the study, there was inhibition zone in *Staphylococcus aureus* growing agar and also there was bacteriostatic zone on the agar which *Shigella dysenteriae* and *Salmonella typhi* were growing. The absence of inhibition in gram negative bacteria (*Shigella dysenteriae and Salmonella typhi)* may be caused by the more resistance of gram negative bacteria compared to gram positive bacteria.

The gram-negative bacteria are more resistant because they are surrounded by outer membrane that contains lipopolysaccharide. This outer membrane can act as protective barrier and may exclude certain drugs and antibiotic from penetrating the cell.[9] In fact, a study conducted by Saeed et al.[10] in 2009 showed that out of 54 gram negative bacteria obtained from various pathological laboratories and hospitals, 50 of them are resistant to one or more antibiotics. This result can show that there is high resistance in gram negative bacteria and it may be the cause that the *Pulsatilla chinensis* infusion only inhibits gram positive bacteria but not gram negative bacteria.

Besides that, while testing MIC and MBC, the result showed that there was no inhibitory and bactericidal activity by *Pulsatilla chinensis* infusion towards all 3 bacteria in the study.

The absence of inhibition in test tubes may be due to the low concentration of *Pulsatilla chinensis* infusion that was used. The maximum concentration obtained during the study was 25%. In the MIC test, the highest concentration of infusion was 6.25% which was very low and this could decrease the effectiveness of the infusion. The low concentration of infusion may have less chemical components that are required to kill the bacteria. Besides that, in MIC method, the infusion was in direct contact with the bacteria suspension and the effectiveness of inhibition may be different with inhibition of infusion in agar that bacteria were growing.

Besides that, the process of extracting the chemical substances from *Pulsatilla chinensis* also led to the absence of inhibition in bacterial growth. This is because some chemical substances do not or are difficult to be extracted by water from dried *Pulsatilla chinensis* using infusion method. Certain chemical substances such as saponin need to be extracted by organic solvent such as ethanol, chloroform, or others. If using the correct extract method, the chemical substances may be extracted completely from the *Pulsatilla chinensis* and the result can improve.

Other causes that led to absence of bacterial growth inhibition are the quality and quantity of chemical substances that are presented in the *Pulsatilla chinensis*. The *Pulsatilla chinensis* was mentioned as having chemical substances that can affect the bacteria growth such as protoanemonin, oleanolic acid, and saponin.[3] The exact amount and quality of these chemical substances had not been tested by any study before this study was conducted. Therefore, it is possible that the low concentrations or affected quality of these substances are presented in *Pulsatilla chinensis* and cause no bacteria growth inhibition.

Another possible cause that brings about the presence of bacterial growth is the process of drying of *Pulsatilla chinensis.* In the process of producing *Pulsatilla chinensis*, the herb may be already contaminated by spore forming *Bacillus spp.* or *Clostridium spp*. The spore of these species will not die even boiled in the water. A study conducted by Rice et al.[11] in 2004 showed that the spores of some *Bacillus spp.* were not inactivated even rolling boiled for 1 to 3 minutes in opened container. The spore of these bacteria may reactivate itself when environment is favorable. The activated spore may contaminate the infusion and cause the present of bacterial growth in the test tube and culture.

The limitation of the study was the use of dried *Pulsatilla chinensis* herb. More water is needed to be added while the dried herb was being boiled since it will absorb the water and causes the low concentration of infusion obtained. Another limitation was that the herb used was dried and processed when brought. The use of processed dried herb causes the quality of dried herb cannot be ensured and there is a possibility of mixing of other herbs. One more limitation was that the herb cannot be found in Indonesia and it has to be imported from another country. This also causes the quality cannot be ensured as well as the possibility of mixing herb presence.

In conclusion, the *Pulsatilla chinensis* infusion has inhibitory effect on *Staphylococcus aureus* and bacteriostatic effect on *Shigella dysenteriae* and *Salmonella typhi*. In the study, there is no MIC and MBC for *Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi* since there is no inhibitory or bactericidal effect that has been observed in MIC and MBC test.

The recommendation for researcher referring on this study is to use the extraction method to make sure the substances in the Pulsatilla chinensis are fully extracted by organic solvent. Another recommendation is to conduct the study to investigate exact quantity and quality of substance presence in the Pulsatilla chinensis.

References

1. Liu CS, Cham TM, Yang CH, Chang HW, Chen CH, Chuang LY. Antibacterial properties of Chinese herbal medicines against nosocomial antibiotic resistant strains of Pseudomonas aeruginosa in Taiwan. Am J Chin Med. 2007;35(6):1047–60.
2. Chen T, Xiong S, Jiang S, Wang M, Wu Q, Wei H. Effects of traditional Chinese medicines on intestinal bacteria: a review. Indian J Tradit Know. 2012;11(3):401–7.
3. Hou J, Jin Y. The healing power of Chinese herbs and medicinal recipes. New York: Haworth Integrative Healing Press; 2004. p. 530–1
4. Bobadilla Fazzini RA, Skindersoe ME, Bielecki P, Puchalka J, Givskov M, Martins sos Santos VA. Protoanemonin: a natural quorum sensing inhibitor that selectively activates iron starvation response. Environ Microbiol. 2013;15(1):111–20.
5. Arabski M, Węgierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of saponins against clinical E. coli strains and

eukaryotic cell line. J Biomed Biotechnol. 2012;2012: 286216.
6. Soetan k, Oyekunle M, Aiyelaagbe O, Fafunso M. Evaluation of the antimicrobial activity of saponins extract of Sorghum Bicolor L. Moench. Afr J Biotechnol. 2006;5(23):2405–7.
7. Fontanay S, Grare M, Mayer J, Finance C, Duval RE. Ursolic, oleanolic and betulinic acids: antibacterial spectra and selectivity indexes. J Ethnopharmacol. 2008;120(2):272–6.
8. Wolska K, Grudniak A, Fiecek B, Kraczkiewicz-Dowjat A, Kurek A. Antibacterial activity of oleanolic and ursolic acids and their derivatives. Cent Eur J Biol. 2010;5(5):543–53.
9. Silhavy T, Kahne D, Walker S. The bacterial cell envelope. New Jersey: Cold Spring Harbor Perspectives in Biology; 2010. p. 17.
10. Saeed A, Khatoon H, Ansari FA. Multidrug resistant gram-negative bacteria in clinical isolates from Karachi. Pak J Pharm Sci. 2009;22(1):44–8.
11. Rice E, Rose L, Johnson C, Boczek L, Ardunio M, Reasoner D. Boiling and bacillus spores. Emerg Infect Dis. 2004;10(10):1887–8.