JURNAL BIOLOGI

El-Hayah

Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index
e-ISSN: 2460-7207, p-ISSN: 2086-0064

Antibacterial Activity of Combination *Cold Plasma* and Parijoto (*Medinilla speciosa*) Against *Staphylococcus aureus* and *Pseudomonas aeruginosa* on Diabetic Ulcer

Fajar Farra Anastasia [1)], Itsna Risqii Aziz [1)], Vanny Oktaviola [1)], Arya Iswara [2)]

[1] *DIV Medical Laboratory Technology, Faculty of Nursing and Health Sciences, Muhammadiyah University of Semarang*
[2] *Medical Laboratory Technology Study Program, Faculty of Nursing and Health Sciences, Muhammadiyah University of Semarang*

[*] *Corresponding author*
Email: fajarfarra98@gmail.com and aryaiswara@unimus.ac.id
DOI: 10.18860/elha.v7i4.10313

Article Info

Article history:
Received 05 May 2019
Received in revised form
15 August 2019
Accepted 12 Dec 2019

Keywords:
Cold plasma,
Medinilla speciosa,
diabetic ulcer,
Staphylococcus aureus,
Pseudomonas aeruginosa

Abstract

This study aims to determine the potential of cold plasma (cold plasma) as an antibacterial if combined with Parijoto (Medinilla speciosa) extract against the dominant bacteria in diabetic ulcer infection, namely Gram-positive Staphylococcus aureus and Gram-negative bacteria Pseudomonas aeruginosa with a concentration of 25%, 50 %, 75%, and 100%. This study was an experimental test in vitro using the well diffusion method. The well diffusion method uses an MHA given a 5mm diameter and inserted a 100uL sample then incubates 35 ± 2 ° C for 16-18 hours. The results showed antibacterial activity from Cold Plasma with a 10 mm shot distance for 3 minutes with inhibitory zones against S. Aureus 3 mm and against P. aeruginosa 3.35 mm, a combination of Cold Plasma antibacterial activity with parijoto extract with a zone of inhibition of S. aureus 11.7 mm (25%), 14.1 (50%), 16.5 (75%), 17.1 (100%) and against P. aeruginosa 7.7 mm (25%), 9.3 mm (50%), 10.1 mm (75%), 11.7 (100%). the results of the Cold Plasma inhibition zone and Cold Plasma combination with parijoto extract 100% concentration of S. aureus were greater than the inhibition zone for P. aeruginosa. Cold Plasma combination inhibition zones with starch extracts against S. aureus compared to Vancomicin (VA) antibiotics with a 19.9 mm inhibition zone included in the intermediate category, Cold Plasma combination inhibition zone with parijoto juice against P. aeruginosa bacteria compared with Meropenem antibiotics (MRP) with a 9.7mm inhibition zone included in the sensitive category.

1. INTRODUCTION

The 2010 World Health Organization (WHO) reported that 60% of the causes of death of all ages in the world are due to Non-Communicable Diseases. Diabetes mellitus ranks 6th as a non-communicable disease that causes death. Indonesia is the 7th country with the highest prevalence of diabetes (Ministry of Health, 2013). Diabetes mellitus is a chronic metabolic disease characterized by an increase in blood glucose levels that leads to time and to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. Uncontrolled diabetes mellitus can cause interference with the immune system with a cellular response that causes infection by bacteria and causes impaired macrophage function resulting in delayed healing and increased sensitivity to infection (Ammons, 2013).

Ulcers are a common problem in diabetics. Ulcers can cause inflammation or make the tissue damaged (Gardner dan Frantz RA, 2012). In this condition, Gram-positive substances such as *Staphylococcus aureus* and Gram-negative bacteria such as *Pseudomonas aeruginosa* can produce biofilms (Jenkins et al., 2014). Treatment of ulcers with bacterial infections is recommended by giving antibiotics. Antibiotics have the ability to inhibit bacterial growth. However, giving antibiotics for a long time and the wrong use can lead to resistance (Lipsky et al., 2012). Therefore, alternative solutions are needed to overcome problems in the treatment of diabetic ulcers infected by bacteria.

Since about ten years ago, in developed countries, efforts to implement plasma in the health sector were attracting attention. This effort has given birth to a new study called Plasma medicine. Plasma is the fourth phase of the substance after solid, liquid and gas. Plasma is commonly known as an ionized gas. Plasma produced in the air consists of reactive mixed atoms, excited molecules, charged particles, Reactive Oxygen, and Nitrogen Species (RONS). Cold plasma is used in sterilization applications, food decontamination, dermatology, and dysentery. Cold plasma has become the spotlight as an alternative to non-systemic infection antibiotics, because plasma treatment shows renewable effectiveness of various microorganisms, including antibiotic and spore-forming biofilm strains (Wahyuningtyas et al., 2017). The mechanism of medical plasma work in influencing the biological function of cells is based on two main principles, namely: (1) That plasma biological effects are significantly affected by the cell fluid environment, (2) That RONS generated or transferred into the liquid phase plays a major role in biological response influenced by plasma (Weltmann, 2017).

Indonesia is rich in natural products which have an important role for health, for example, Ginger (*Zingiber offcinale Rosc.*), Noni (*Morinda citrifolia*), and Parijoto (*Medinilla speciosa*) which have the potential as natural antibiotics (Winarti et al., 2005). Parijoto (*Medinilla speciosa*) is a typical shrub plant, curved, single, and cross-faced leaves, the fruit is purplish pink and tastes sour and sate (Wibowo et al., 2012). Parijoto is a typical plant of the Colo region, Kudus Regency, Central Java (Wachidah, 2013).

This study combines cold plasma with Parijoto extract (*Medinilla speciosa*) which is used as an antibacterial alternative to diabetic ulcer infection.

2. MATERIALS and METHODS

Bacterial Culture

Pure cultures of *P.aeruginosa* and *S. aureus* bacteria were obtained from the Microbiology Laboratory of the Faculty of Nursing and Health Sciences at the University Muhammadiyah of Semarang. Rejuvenated in *Brain Heart Infusion* (BHI) media for 6 hours, then made a bacterial stock on the *Heart Infusion Agar* (HIA) media.

Making Bacterial Culture

Pure cultures of *P. aeruginosa and S. aureus* were standardized with standard *Mc Farland* 0.5 turbidity.

Antibacterial Activity Test

Suspension of bacteria *P.aeruginosa* and *S.aureus*, inoculated into MHA media. After 10 minutes, *Argon Cold Plasma was* fired indirectly with a voltage of 14.65 kV and a frequency of 12.72 kHz (Nasruddin, 2016). Spawning for 3 minutes at a distance of 10 mm. In other agar media 4 wells were made on MHA media with a depth of 0.5 cm, then each well was filled with 100 µl parijoto extract with concentrations of 100%, 75%, 50%, and 25%. The media was given 2 treatments, namely by firing Cold plasma and without shooting Cold plasma. MHA media were incubated 35 ± 2 °C for 16-18 hours. For positive controls, Vancomicin and Meropenem antibiotics were used, while negative controls used antibiotics Oxacilin and Metronidazole. The inhibitory zone activity is seen by measuring the diameter of the inhibitory zone of bacteria formed.

Data Analysis

processed using SPSS with the *Kruskal Wallis Test.*.

3. RESULTS

Making Parijoto extract

Parijoto is cleaned then smoothed using a blender. Then filtered and squeezed use gauze to separate the extract from the pulp. After obtaining Parijoto juice with a concentration of 100%, Parijoto juice was made concentrations of 75%, 50% and 25% with the addition of aquabidest.

Antibacterial Activity Test

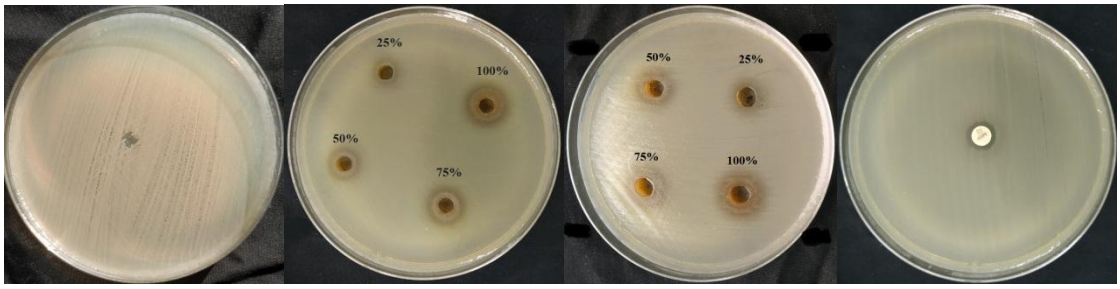
The results of the study of antibacterial activity of Cold plasma, Parijoto Juice and a combination of both showed the presence of inhibitory zones tested in vitro by measuring inhibitory zones in the agar juice so that the wells were filled with parijoto extract with concentrations of 25%, 50%, 75%, and 100% measurements using millimeters are presented in Table 1 and Figure 1.

Table 1. Antibacterial Activity Test Results

Bacteria	Inhibited Zone Bacteria (mm)								
	Cold Plasma	Parijoto juice				Combination Cold plasma with Parijoto extract			
		25%	50%	75%	100%	25%	50%	75%	100%
P. aeruginosa	3,35	7,6	9	11,3	11,9	7,7	9,3	10,1	11,7
S. aureus	3	10,4	13,3	14,9	15,9	11,7	14,1	16,5	17,1

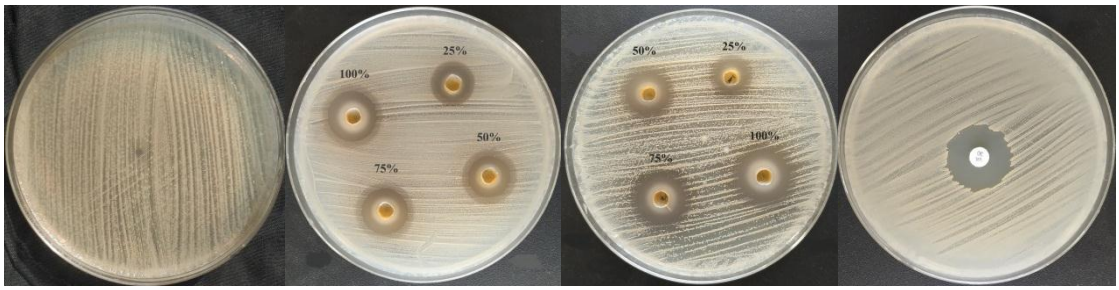
Tabel 2. Antibacterial Activity Test Results P.aeruginosa dan S.aureus with antibiotics

Bacteria	Positif Control
P.aeruginosa	Meropenem (9,7 mm)
S.aureus	Vancomicin (19,9mm)



(a) (b) (c) (d)

Figure 1. Inhibition zone with cold plasma (a), parijoto extract with a concentration of 25%, 50%, 75%, 100% (b), combination of cold plasma with parijoto extract with a concentration of 25%, 50%, 75%, 100% (c), and positive control (d) on *P.aeruginosa*



(a) (b) (c) (d)

Figure 1. Inhibition zone with cold plasma (a), parijoto extract with a concentration of 25%, 50%, 75%, 100% (b), combination of cold plasma with parijoto extract with a concentration of 25%, 50%, 75%, 100% (c), and positive control (d) on S.aureus

Based on Table 1 and Figure 1 above, it can be seen that the antibacterial activity against *P.aeruginosa* treated with Parijoto extract showed the best antibacterial activity with the largest inhibition zone produced by a concentration of 100% at 11.9 mm. While the *S.aureus* treatment of the combination of *cold plasma* with Parijoto extract has the largest bacterial inhibition compared to the antibacterial activity test with *cold plasma* and Parijoto juice with an inhibitory zone of 17.1 mm at a concentration of 100%. This shows that the higher the concentration and the duration of shooting affect the formation of inhibitory zones. The difference in inhibitory zones of *P. aeruginosa* and *S.aureus* bacteria can occur due to differences in cell wall structure between Gram negative and Gram positive bacteria which causes different responses to various treatments and content of a material (Astrini et al., 2014). The zone of Vancomicin (VA) positive control inhibition is 19.9 mm and Meropenem (MRP) is 9.7 mm. The inhibitory zone results of *P.aeruginosa* and *S.aureus* were compared with standard inhibitory zone diameters according to the *Clinical and Laboratory Standards Institute* (CLSI).

4. DISCUSSION

This study aimed to determine the effect of the combination *of cold plasma with* parijoto extract on *P.aeruginosa* and *S.aureus* wherein each bacterium was tested for antibacterial activity with Cold plasma, Parijoto extract and a combination of both. This research is based on the measurement of clear zones around the media so that the resulting antibacterial compounds diffuse into the agar layer and inhibit bacterial growth and are called inhibitory zones (Perdana, 2016). The ability of plasma to produce biological molecules, namely Reactive Oxygen and Nitrogen Species (RONS) plays a major role in the biological

response influenced by plasma. The mechanism of medical plasma work in influencing the biological function of cells is based on two main principles, namely that plasma biological effects are significantly affected by the cell fluid environment and RONS that is generated or transferred into the liquid phase plays a major role in biological response (Wahyuningtyas et al., 2017). The presence of a water content in the ray juice acts as a liquid phase which results in an increases in the biological response of increased bacteria. Coupled with the activity of flavonoid compounds on bacterial growth carried out by damaging the bacterial cell wall that reacts with alcohol groups in flavonoid compounds so that flavonoids enter the cell nucleus, react with DNA so that the lysis bacteria and die (Rustama and lingga, 2005).

5. CONCLUSION

The results of testing the combination of cold plasma and Parijoto extract can be concluded that from concentrations of 25%, 50%, 75%, and 100% Parijoto extract has antibacterial activity against *S.aureus* and *P. aeroginosa*. The concentration of 100% has the best antibacterial activity with inhibitory zones of 11.7 mm and 17.1 mm.

6. ACKNOWLEDGEMENTS

Thank you to the Ministry of Research, Technology, and Higher Education for providing funding and the Muhammadiyah Semarang University which facilitated this research so that it could run well and smoothly.

7. REFERENCES

Ammons MC, 2013. Lactoferrin: a Bioinspired, AntiBiofiltherapeutic. Biofouling;29(4):443-55.

Astrini, D., Wibowo, M.S., Nugrahani, I. 2014. Aktivitas Antibakteri Madu Pahit Terhadap Bakteri Gram Negatif dan Gram Positif Serta Potensinya Dibandingkan Terhadap Antibiotik Kloramfenikol, Oksitetrasiklin dan Gentamisin. Bandung. *Acta Pharmaceutica Indonesia*, Vol 39(3&4):75-83.

Jenkins TC, Knepper BC, Jason Moore S. 2014. Comparison of The Microbiology and Antibiotic Treatment Among Diabetic and Non-Diabetic Patients Hospitalized for Cellulitis or Cutaneous Abscess;9(12):788-94.

Kementrian Kesehatan Republik Indonesia. 2014. Situasi Dan Analisis Diabetes.

Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, et al., 2012. Infection Diseases Society of America Clinical Practice Guideline for the Diagnosis And Treatment of Diabetic Foot Infections.Clinical Infectious Diseases; 54(12):132-173.

Nasruddin. 2016. Aplikasi Plasma Dingin untuk Perawatan Luka dalam Jurnal keperawatan muhammadiyah 1(1). Magelang. Universitas Muhammadiyah Magelang.

Perdana R., Setyawati, T. 2016. Uji In-Vitro Sensitivitas Antibiotik Terhadap Bakteri Salmonella typhi di Kota Palu. *Jurnal Ilmiah Kedokteran*, Vol 3(1):11-22.

Rustama MM, Lingga MA. 2005. Uji Aktivitas Antibakteri dari Ekstrak Air dan Etanol Bawang Putih (*Allium sativum L.*) terhadap Bakteri Gram Negatif dan Gram Positif yang Diisolasi dari Udang Dogol (*Metapenaeus monoceros*), Udang Lobster (*Panulirus sp.*), dan Udang Rebon (*Mysis Acetes*). *Jurnal Biotika*,Vol 5(2):35-40.

Wachidah, L N. 2013. Uji Aktivitas Antioksidan Serta Penentuan Kandungan Fenolat dan Flavonoid Total dari Buah Parijoto (*Medinilla speciosa Blume*). Skripsi. Jakarta : UIN Syarif Hidayatullah.

Wahyuningtyas ES, Putri IK, Nasruddin, 2017 Efektivitas Perlakuan Kombinatif Plasma Medis, Madu dan Pembalut Luka Berlubang Banyak Untuk Penyembuhan Luka.

University Research Colloquium 2017 Universitas Muhammadiyah Magelang

Weltmann K-D, Thv. Woedtke. 2017. Plasma Medicine Current State of Research and Medical Application, Plasma Phys. Control. Fusion; 59, 014031 (11pp).

Wibowo HA, Wasino, Setyowati DL. 2012. Kearifan Lokal Dalam Menjaga Lingkungan Hidup (Studi Kasus Masyarakat di Desa Colo Kecamatan Dawe Kabupaten Kudus). Journal of Educational Social Studies 1(1);25-30