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The Effect of Tannic Acid as Antimicrobial Agent on *Pseudomonas aeruginosa* Using *In-vitro* Diffusion Method

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Authors' contributions

This work was carried out in collaboration among all authors. Author Sardjiman designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RR and JS managed the analyses of the study. Author RR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Bacterial infection is a health problem that has long been attention in the world of health. Various antimicrobials have been discovered and developed by researchers to solve pathogenic bacterial infections. *Pseudomonas aeruginosa* is a pathogenic bacteria that is often of concern to researchers because it has the potential to become resistant to a number of antibiotics. Tannic acid is known to have potential antibacterial activity against pathogenic bacteria. The antibacterial activity of tannic acid is interesting to study considering its great potential as an antimicrobial agent for pathogenic bacterial infections.
Aims: To find out the antibacterial potential of tannic acid compounds against *Pseudomonas aeruginosa In-vitro* using the disc diffusion method.
Methodology: This research is quantitative research with experimental methods. This research was carried out at the Pharmacy Laboratory of Kusuma Husada Surakarta University, Indonesia

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between April and May 2023. The sample for this study was a certified bacterial culture of *Pseudomonas aeruginosa* ATCC 27853 originating from a stock culture from the laboratory. We use certified tannic acid from Merck Millipore. The tannic acid in powder form was dissolved in a 1% DMSO solution. The sample solution containing tannic acid in 1% DMSO was dropped on a Kirby-Bauer disk and placed on an agar plate inoculated with bacteria. One petri dish contains five discs with different samples placed at adequate distances. There were 0.5% tannic acid solution, 1% tannic acid solution, and 2% tannic acid solution, negative control 1% DMSO solution and positive control ciprofloxacin 5 µg/ml. Replication was carried out five times.

Results: Tannic acid with a concentration of 0.5% has an average inhibitory zone of 102 mm. A 1% concentration of tannic acid has an average inhibitory zone of 121 mm. A 2% concentration of tannic acid has an average inhibitory zone of 150 mm. The positive control used, namely the antibiotic ciprofloxacin, obtained an average inhibitory zone of 350 mm. The negative control used, namely 1% DMSO solution, had no inhibitory zone at all. Statistical tests using the Kruskal-Wallis test showed that there was a significant difference with a value of $p = 0.000$.

Conclusion: The tannic acid compound is an active compound that has antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 and its application in health can be considered.

Keywords: Antimicrobial agent; tannic acid; Pseudomonas aeruginosa; diffusion method.

1. INTRODUCTION

Currently, many active compound extractions have been found in various medicinal plants. Researchers have applied many of the latest methods and old methods to obtain medicinal plant extracts. These medicinal plant extracts are usually applied directly to patients in the form of extracts or in formulations of various medicinal dosage forms. In recent years, several technological and scientific developments — including improved analytical tools, genome mining and engineering strategies, and microbial culturing advances — are addressing such challenges and opening up new opportunities. Consequently, interest in natural products as drug leads is being revitalized, particularly for tackling antimicrobial resistance [1].

The extensive, inappropriate, irregular, and indiscriminate uses of antibiotics have resulted in the emergence of antimicrobial resistance, making many currently available medications ineffective. This emerging trend is concerning and considered by the WHO to be perhaps the most urgent issue facing medical science. Therefore, there is an increasing demand to develop new antimicrobial agents that are able to decrease the use of antibiotics and to face resistance development. This has directed researchers to isolate and identify new bioactive chemicals from plants to act against microbial resistance. , also considering that approximately 50% of current pharmaceuticals and nutraceuticals are natural products and their derivatives [2].

Pseudomonas is an opportunistic bacteria that can become a pathogen in animals, plants and humans. Human infection especially in immunocompromised patients and hospital-acquired infections. *Pseudomonas aeruginosa* as an opportunistic germ is difficult to eradicate with the presence of antibiotic-resistant bacteria [3].

Tannic acid has antibacterial activity based on a number of previous studies. Cases of *Pseudomonas aeruginosa* bacterial infection require alternative solutions besides treatment guided by many health guidelines. We are interested in knowing the antibacterial activity of tannic acid as an antibacterial agent against *Pseudomonas aeruginosa*. The aim of study is to find out the antibacterial potential of tannic acid compounds against *Pseudomonas aeruginosa In-vitro* using the disc diffusion method.

2. MATERIALS AND METHODS

2.1 Study Design

This research is quantitative research with experimental methods. This research was carried out in May 2023 at the Pharmacy Laboratory of Kusuma Husada University, Surakarta. The sample for this study was a certified bacterial culture of *Pseudomonas aeruginosa* ATCC 27853 originating from a stock culture from laboratory. We use certified tannic acid from Merck Millipore.

Research variables consist of independent variables, dependent variables and confounding variables. Independent variables include the concentration of tannic acid compounds, positive

control ciprofloxacin 5 µg/ml, and negative control 1% DMSO (Dimethyl Sulfoxide) solution used. The dependent variable includes the sensitivity pattern of *Pseudomonas aeruginosa* bacteria to samples and controls in the form of growth barrier diameter. Confounding variables include incubation temperature, culture media, and aseptic methods.

The tools used in this research include LAF, autoclave, incubator, petri dish, measuring needle, Kirby-Bauer disc, sterile cotton swab, micropipette, microscope, slide, vernier calliper, coarse and fine gram balance, measuring cup, beaker glass, Erlenmeyer, test tube, and oven.

Research data was collected after going through the preparation stage, inoculating bacteria onto agar media, administering sample and control fluids to the bacterial culture, and also through an overnight incubation period. *Pseudomonas aeruginosa* were cultured in petri dishes containing nutrient agar. The tannic acid in powder form was dissolved in a 1% DMSO solution. The sample solution containing tannic acid in 1% DMSO was dropped on a Kirby-Bauer disk and placed on an agar plate that had been inoculated with bacteria. The data obtained is data on the diameter of the area inhibiting bacterial growth in the sample and control of the growth of *Pseudomonas aeruginosa* bacteria on agar media.

Petri dishes were given nutrients to solidify and *Pseudomonas aeruginosa* was cultured using aseptic techniques. Each petri dish contains 5 Kirby-Bauer disks, each of which is positive control (ciprofloxacin 5µg/ml), negative control (1% DMSO), tannic acid solution in 1% DMSO with a concentration of 0.5%, 1%, and 2%. Petri dishes are incubated for 24 hours at a stable temperature of 37 degrees Celsius or 98.6 degrees Fahrenheit in an incubator.

2.2 Data Collection

The research data is taken from the growth inhibitory diameter of *Pseudomonas aeruginosa* on agar media on petri dishes. The diameter of the inhibitory zone is a transparent zone that is not covered by bacteria, then the width is calculated using a calliper.

2.3 Ethical Consideration

This research has received Ethical Clearance from the Health Research Ethics Committee, Dr. Moewardi General Hospital, Surakarta, Indonesia with number: 1.619/VIII/HREC/2023. This research was carried out at the Pharmacy Laboratory of Kusuma Husada University, Surakarta, Indonesia.

2.4 Statistical Analysis

Analysis of the data using SPSS software version 20. Data analysis consisted of a normality test (Shapiro-Wilk test) and a homogeneity test (Levene's Test of Homogeneity of Variance). If the data conditions are normal and the variance is homogeneous, then continue with the one-factor Anova test (One Way Anova). If the data is not normal then continue with the Kruskal-Wallis test.

3. RESULTS

Documentation of the results is presented in Fig. 1 below.

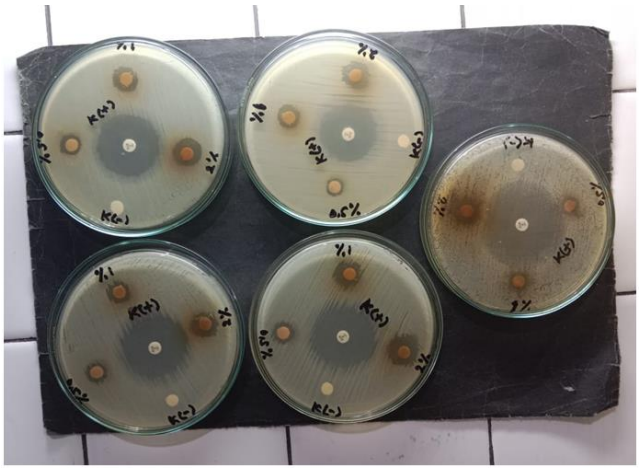

Fig. 1. Documentation of test results for the antibacterial activity of tannic acid against *Pseudomonas aeruginosa in-vitro*

The results of antimicrobial activity of tannic acid against *Pseudomonas aeruginosa In-vitro* using disc diffusion methods showed in the Table 1 below.

Table 1. Results of antibacterial activity of tannic acid against *Pseudomonas aeruginosa*

Replication	Tannic acid 2%	Tannic acid 1%	Tannic acid 0,5%	Positive control Ciprofloxacine	Negative Control DMSO 1%
1	15	12	10	35	5
2	14.5	12	11	35	5
3	16	13	10	35	5
4	15	12	10	35	5
5	14.5	11.5	10	35	5
Average	**15**	**12.1**	**10.2**	**35**	**5**

**Zone of inhibition in millimeters (mm). 5 mm is Kirby-Bauer Disc diameter*

The inhibition zone results showed that 0.5% tannic acid has an average inhibition zone of 10.2 mm. The inhibition zone results showed that 1% tannic acid has an average inhibition zone of 12.1 mm. The inhibition zone results showed that 2% tannic acid has an average inhibition zone of 15 mm. The positive control ciprofloxacin showed an average zone of inhibition of 35 mm. The negative control 1% DMSO solution showed no inhibition zone at all. All samples were replicated five times.

4. DISCUSSION

The emergence of bacterial resistance certainly creates new problems in health. *Pseudomonas aeruginosa* is a bacteria that has the potential to be resistant to commonly used antibiotics. *Pseudomonas aeruginosa* is a bacterium that can potentially cause nosocomial infections and can be multiresistant to various antibiotics [4]. A solution to this multiresistant event will likely be needed.

Plants containing tannic acid are often found in everyday life, including tea, coffee and cola. Tannic acid is a natural tannin from the group of phenolic acids and consists of a central glucose unit and ten gallic acid molecules attached to it. Apart from that, tannic acid is a compound that can be obtained from natural sources of medicinal plants with high yields, making it interesting for researchers to explore [5].

Tannic acid is known to have antiviral and antibacterial effects. The antibacterial activity of tannic acid is known to be active against bacteria such as *Staphylococcus aureus*, *Escherichia coli*, Streptococcus pyogenes, *Enterococcus faecalis*, *Yersinia enterocolitica*, *Listeria innocua*, *Bacillus cereus* and *Pseudomonas aeruginosa* [6].

Based on research data that has been obtained, tannic acid compounds with a concentration of 0.5% to 2% have an inhibitory zone against *Pseudomonas aeruginosa* growth. The inhibitory zone of tannic acid compounds increases linearly from the lowest (0.5%) to the highest concentration (2%). Statistical tests using the Kruskal-Wallis test showed that there was a significant difference with a value of $p = 0.000$. This means that there are differences in antibacterial activity between the treatment groups. This is in accordance with the statement from the literature that tannic acid has antibacterial efficacy against *Pseudomonas aeruginosa* [6].

Tannic acid can inhibit bacterial drug efflux pumps, change fatty acid metabolism, and cause iron depletion [7-9]. Tannic acid is also known to have an antiplaque effect in attempts to clean teeth from dental plaque by gargling with a tannic acid solution [10]. The antimicrobial activity of tannic acid is thought to target the bacterial part, namely peptidoglycan, which is part of the integrity of the bacterial cell wall, while also being able to reduce the formation of bacterial biofilm at sub-MIC concentrations [9].

Tannic acid is known to be a well-known tea stain polyphenol which has a high affinity for various substrates. Tannic acid can also inhibit microbial adhesion to its host. Tannic acid can actively inhibit the development of microbial colonization on the host [10].

The basis of tannic acid's activity comes from hydroxyl groups and its affinity for forming hydrogen bonds with proteins and other biomolecules. Tannic acid is known to have inflammation-reducing activity as an antioxidant. Tannic acid also has antibacterial activity against common pathogenic bacteria. Tannic acid is

known to have the ability to stimulate apoptosis in several types of cancer cells. Apart from that, tannic acid is also known to have antiviral and antifungal properties [11].

Pseudomonas aeruginosa is a gram-negative bacterium that is an opportunistic pathogen in humans because it can cause severe acute and chronic infections in immunocompromised individuals. *Pseudomonas aeruginosa* is known to be difficult to eradicate because its ability to form biofilms makes it resistant to antibiotics. Bacterial biofilms consist of autogenic extracellular polymeric substances which make a sheath to enclose bacteria on surfaces and protect them from environmental stress. Biofilms are also able to inhibit cell phagocytosis. This provides the capacity for *Pseudomonas aeruginosa* to colonize and persist in the long term [12].

The main concern of *Pseudomonas aeruginosa* infections is hospital-acquired infections, especially in critically ill patients and immunocompromised patients. The main problem lies in the ease with which it becomes resistant to antibiotics resulting in high death rates from *Pseudomonas aeruginosa* infections. Various antimicrobial compounds ranging from those that kill germs to antivirulence (disarming) pathogens are still being developed [13].

Tannic acid or tannin is an active compound in tea leaves (*Camellia sinensis*). This compound is also found in other plants. Research by Mahtuti [14] found that tannic acid has good antibacterial activity and is better than chloramphenicol against Salmonella typhi bacteria that cause typhoid. Kurniawan and Zahra [15] in their review stated that tannins have antibacterial activity.

Tannic acid has an anti-biofilm effect on polymicrobials so it has the potential to be an antimicrobial agent. In research, it was found that tannic acid can inhibit biofilm formation in *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Candida albicans with the concentration of tannic acid used was 1% [16]. In other research, it is also known that tannin is able to inhibit the formation of biofilms, where biofilm resilience is a challenging problem for hospitals [17].

As explained previously, tannic acid compounds can reduce the occurrence of biofilms in bacteria, so tannic acid compounds have great potential as potent antimicrobial agents against *Pseudomonas aeruginosa* infections. Management of Pseudomonas infections that focuses on prevention is important whenever possible [18]. Tannic acid compounds can be an alternative infection prevention solution for external infections.

5. CONCLUSION

The results of our research state that tannic acid compounds in concentrations of 0.5%, 1% and 2% have antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853 through *In-vitro* testing using the Kirby-Bauer disks. Tannic acid compounds can be a solution to *Pseudomonas aeruginosa* infections that are difficult to eradicate because they form biofilms and become resistant to antibiotics. The tannic acid compound is an active compound that has antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 and its application in health can be considered.

6. LIMITATION OF STUDY

The study has some limitations about the most effective concentration applicable to humans. This study is limited to the *In-vitro* and in vivo methods and in vivo method is encouraged to do in the future.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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