PROFESIONAL HEALTH JOURNAL
Volume 6, No. 1, December 2024 (Hal. 358-364)
Available Online at https:// https://www.ojsstikesbanyuwangi.com
E-ISSN 2715-6249

Test the Effectiveness of Mulberry Leaf Extract (Morus Nigra) Against the Inhibitory Power of Pseudomonas Sp.

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ARTICLE INFORMATION

Article history
Received (21 March 2024)
Revised (1 June 2024)
Accepted (31 July 2024)

Keywords
Morus Nigra, Mulberry, Pseudomonas, Antibacterial

ABSTRACT

Introductions *: Urinary Tract Infection (UTI) is one of the impacts caused by the use of IUDs, namely IUDs. The cause of UTI itself is dominated by the presence of microorganisms that infect the urinary tract. Pseudomonas is one of the bacteria that causes the third largest UTI after E. Coli and Enterococcus. Pseudomonas is capable of infecting the urinary tract when it is inserted, besides that the bacteria can also enter due to improper cleaning techniques. Mulberry leaves are believed to have antibacterial properties such as flavonids, tannins, alkaloids and terpenoids, which can inhibit the growth of bacteria.*

Methods *: The method used in this study uses laboratory experimental methods, by measuring the cause and effect of the independent variable on the dependent variable. Using a post-test only control group design approach.*

Result *: The results showed that there was an inhibition zone formed at the smallest concentration of 60% with the medium category of 8,4 mm and the widest inhibition zone was found at a concentration of 100% with the strong category of 12, 6 mm. The results of the analysis test using One Way Anova proved that there was a significant effect of giving mulberry leaf extract on the inhibition of Pseudomonas with a P value of 0.000. Pos Hoc's test results showed the most influential concentration in each treatment, namely 100%.*

Conclusions *: From the research results, it was found that concentrations of 100% were the most effective concentrations in inhibiting the growth of Pseudomonas bacteria.*

Introduction

The incidence of Urinary Tract Infections according to research conducted (Reu et al., 2018) states that the prevalence of the world's population is 150,000 million people per year experiencing UTIs, and on average it is experienced by women. Meanwhile, according to the Indonesian Ministry of Health in (Vidiasari, 2016). Urinary Tract Infections (UTI) in IUD contraceptive acceptors can generally be found, when there is a traumatic action on the reproductive tract such as childbirth or surgery it can cause increased bacterial colonization (Lin et al., 2015).

Urinary tract infections in women are generally caused by E. Coli and Enterococcus bacteria, but the third highest cause after these two microbes is Pseudomonas sp (Sumolang et al., 2013). Microbes that cause UTIs can enter through medical equipment or can be concluded to be nosocomial infections (Herlina & Mehita, 2019). One of the normal flora microorganisms is

pseudomonas aeruginosa, which is one of the causes of vaginal discharge, although it is a normal flora of the oral cavity, in certain circumstances, the pseudomonas aeruginosa bacteria can turn into a pathogen due to supporting factors, such as poor hygiene.(Geraldin, dkk 2020).

Various pharmacological therapy efforts have been made to inhibit the development of these bacteria, such as cephalosporins (cefixime, ceftriaxon), penicillins (ampicillin), and quinolones (ciprofloxacin). In connection with antibiotic therapy in the community, there is a high level of inappropriate use of antibiotics in society today (Hasan et al., 2019). Considering the emergence of existing resistance, natural treatment therapies are also starting to gain interest among the public. A study conducted by pharmacologists stated the value of natural or herbal medicine as a potential source of bioactive compounds (Hidayatunnikmah et al., 2022). According to WHO, the best source for obtaining various medicines is through herbal plants. In developing countries, almost 80% of individuals use herbal plants as treatment. Morus Nigra or black mulberry is a plant characterized by leaves that fall easily, heart-shaped leaves, dark purplish fruit and a sweet taste. Morus Nigra is known to have many benefits in the health sector, one of which is as an antimicrobial. The flavonoid, alkoloid and polyphenol content in mulberry leaves can act as an antimicrobial.

In line with research conducted by (Hastuti et al., 2012) (Koyuncu et al., 2014) that mulberry leaf extract is able to inhibit bacterial growth. This mulberry leaf extract has been proven effective as an antifungal which has been proven by research conducted by Nina, et al in 2022 on the Candida Albican fungus that the flavonoid glycoside group contained in mulberry leaf extract contains phenol compounds which will then bind to bacterial cell membranes. This will result in changes in the structure of the cell membrane so that cell semipermeability is disrupted and disrupts cell metabolism and results in death (Hidayatunnikmah et al., 2022). The aim in reseacrh does mulberry leaf extract have antibacterial activity against Pseudomonas sp. bacteria?

Method

This research was conducted using a post-test only control group design approach. This approach is a research design where there are two groups, each selected randomly (R). The first group was given treatment (X) and the other group was not given treatment. The group that is given treatment is called the experimental group, and the group that is not given treatment is called the control group. And the effect of treatment is called treatment. If there is a significant difference in the control group, then the treatment given has a significant effect. The test method used in this research used the disc diffusion method. This method is carried out by placing a disk containing an antimicrobial compound on the surface of the agar medium that is inoculated with the test microbes. Then the antimicrobial agent will diffuse into the agar medium which can inhibit the growth of bacteria in the zone surrounding the disc. The effectiveness of antimicrobial compounds can be seen from the inhibition zone formed, according to Tortora, et al in (Rollando, 2019). In this study, the population used was 1 test tube for culturing Pseudomonas bacteria obtained from the Surabaya City Laboratory Center. In this study, the sample used was a culture of Pseudomonas bacteria at 30-300 CFU/ml obtained from the Surabaya Health Laboratory Center. Counting the number of microorganisms using a viable count or standard plate count.

Result

In this study, in vitro testing was carried out using the disc diffusion technique. The sample was divided into 8 dilution series, namely 15%, 30%, 45%, 60%, 75%, 80%, 95% and 100% and repeated 4 times. The positive control group used the antibiotic Ciprofloxacin, while the negative control group used distilled water. The results of the research were then tested using the One-

Way Anova parametric statistical test followed by the Post Hoc LSD test to determine the most effective concentration in inhibiting the growth of Pseudomonas bacteria.

One –Way Anova Test

Table 1. Levene Test

Levene Statistic	df1	df2	sign
6,80	5	18	,071

Table 2. Kolomogorov Smirnof Test

		Hasil
N		24
Normal Parameter	Mean	,0000
	Std. Defiation	1,83590
Most Eterm Differences	Absolute	,275
	Positive	,275
	Negative	-243
Test Statistic		,275
Asymp. Sig. (2t-tailed)		,100

The One Way Anova test was carried out to generalize the data (the sample can be considered representative of the population). In carrying out this test, the data must meet the criteria, namely that it must be normally distributed and homogeneous. To prove that the data is normally distributed, the Levene test (table 1) and Kolmogorof Smirnov (table 2) can be carried out. In this data, it can be said that the data is homogeneous if the P value is> 0.05. The data is homogeneous because the results show 0.07 so it can be continued in the next test to determine whether the data is normally distributed. In this test, data can be said to be normal if P>0.05. Knowing the results of Kolmogorov Smirnof 0.1. Then testing with One Way Anova can be carried out.

Table.3 One Way Anova Test

	Sun of Squares	df	Mean square	f	sign
Treatment	3691,334	5	738,367	171,419	,000

From the test results, a p value of 0.000 was found. This shows that there is a significant effect of mulberry leaf extract on Pseudomonas inhibition because the p value is <0.05.

Table.4 Pos Hoc LSD Test

Kelompok perlakuan	Mean difference	Sig
Positive	43,9000	0,00
Negative	-43,9000	0,00
15%	43,9000	0,00
30%	43,9000	0,00
45%	43,9000	0,00
60%	35,97500	0,00

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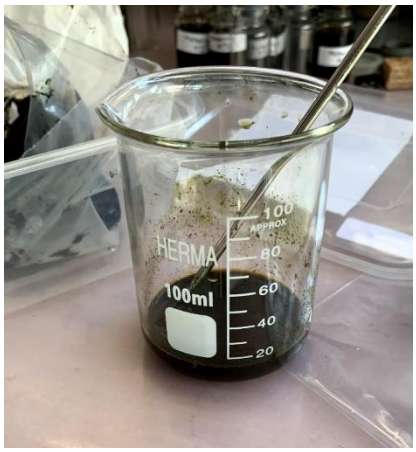
75%	33,97500	0,00
80%	32,65000	0,00
95%	31,62500	0,00
100%	30,55000	0,00

In this test, it was found that a concentration of 100% was the most effective treatment and had the greatest influence in inhibiting the growth of Pseudomonas bacteria with a sig value of 0.00 and an average of 30.55000. In this test, a treatment is said to have an influence if the p-value is 0.00 and it can be said to have the greatest influence if the average value shows the smallest value.

Discussion

effectiveness mulberry leaf extract have antibacterial activity against Pseudomonas sp. bacteria

This study used extracts from mulberry leaves (Morus Nigra). The weight of mulberry leaves used in this study was 1 kg, then dried to obtain a powder form weighing 500 grams and then macerated with 70% methanol solvent and filtered. After that, the determination of the extract concentration was carried out, several concentrations were obtained, namely 15%, 30%, 45%, 60%, 75%, 80%, 95%, 100%. From several concentrations, the results showed that at concentrations of 60%, 75%, 80%, 95%, 100% there was no growth of Pseudomonas. While at concentrations of 15%, 30%, 45%, there was still growth of Pseudomonas. The content contained in mulberry leaves is alkaloids, flavonoids, and polyphenols. Bioactive compounds can be found by extracting the plant. Previous studies have shown that bioactive compounds of alkaloids, flavonoids, and polyphenols can act as antimicrobials. Alkaloids are compounds that have antimicrobial activity, namely inhibiting esterase enzymes along with DNA and RNA polymerase, also inhibiting cell respiration and playing a role in DNA intercalation. The strength of alkaloids as antibacterials works by disrupting the components of peptidoglycan in bacteria and causing the cell wall to not form completely so that bacteria are formed imperfectly because peptidoglycan synthesis is disrupted. Flavonoids work through complex bonds with extracellular proteins that have soluble properties, flavonoids can inhibit the function of bacterial cell membranes and disrupt the integrity of bacterial cell membranes



Pict 1 : Simplicia Dilution Process

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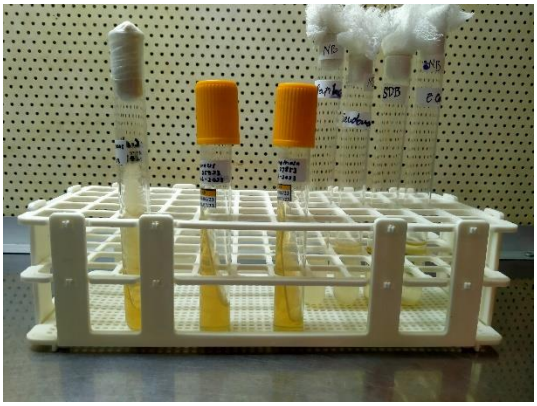
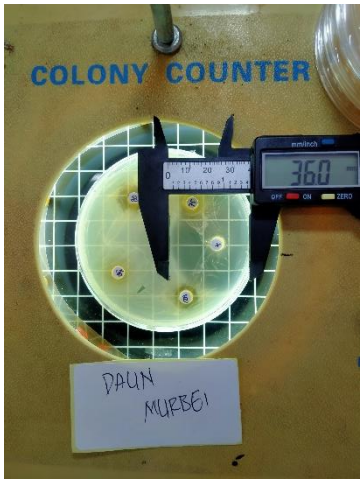


Pict 2 : Pseudomonas Bacterial Isolate

To determine whether or not there is a significant difference between concentrations, a statistical analysis was carried out using the One Way ANOVA Test which showed that there is a significant difference between concentrations in inhibiting the growth of Pseudomonas. In the One Way ANOVA Test process, the Kolmogorov Smirnov test is needed to prove that the data is normally distributed. The data is normally distributed with a value of 0.08, and the Lavene Test to prove that the data is homogeneously distributed and the result is 0.06. The results of the antibacterial activity test of mulberry leaf extract against Pseudomonas showed that mulberry leaf extract with concentrations of 60%, 75%, 80%, 95%, 100% was able to inhibit the growth of Pseudomonas. This is supported by previous research conducted by (Antarini et al., 2021) which stated that an extract concentration of 60% was able to inhibit the growth of Pseudomonas bacteria. The quercetin and anthocyanin compounds found in methanol extract of mulberry leaves are a group of flavonoid glycosides. Where flavonoid glycosides have phenol compounds that act as protein coagulators. Phenolic groups can bind to bacterial cell membranes in their hydrogen bonds, causing changes in protein structure. Changes in the structure of cell membrane proteins can disrupt the semipermeability of cell membranes, disrupting cellular metabolism and resulting in cell death.

The results of the study showed that the inhibitory power was seen to be higher at a percentage of 100% mulberry leaf extract. This shows that the higher the concentration of the extract, the greater the diameter of the inhibition obtained. These results are supported by previous studies which state that the effectiveness of an antibacterial substance in inhibiting growth depends on the nature of the test bacteria, concentration and length of contact time (Swari et al., 2020). This is in line with research conducted by V Miljković, 2018 which stated that morus rubra or similar mulberry is antibacterial. In addition, research conducted by MO Omidiran, 2012 stated that mulberry leaf extracts were screened against Staphylococcus aureus and Pseudomonas aeruginosa. Thus, the results of the study indicate that there is the effectiveness of mulberry against the inhibition of pseudomonas aeruginosa bacteria. With this research, it can be one of the input materials in natural medicine as a therapy for vaginal discharge.



Pict 3 : Results of Measuring the Inhibitory Power of Mulberry Leaf Extract

Conclusion

Mulberry (Morus Nigra) leaf extract has been proven to inhibit the growth of Pseuodomonas bacteria. This can be seen from the results of statistical tests with a p value of 0.00 and the most effective concentration in inhibiting the growth of Pseudomonas bacteria is 100%. Suggestions for further research regarding Testing the Effectiveness of Mulberry Leaf Extract (Morus Nigra) on the Inhibition of Pseudomonas Bacteria are further research using different solvents and methods.

Acknowledgments

Thank you to the thesis supervisor who has guided and directed the process of preparing this research.

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