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THE EFFECT OF GIVING GREEN BETEL LEAF EXTRACT ON THE GROWTH OF Bacteroides IN VAGINAL SECRET CULTURE

PENGARUH PEMBERIAN EKSTRAK DAUN SIRIH HIJAU TERHADAP PERTUMBUHAN Bacteroides PADA KULTUR SECRET VAGINA

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Abstract

Green betel leaves contain compounds that can treat vaginal discharge, especially those caused by bacteria. To determine the effect of green betel leaf extract on the growth of Bacteroides in vaginal secretion cultures, this study used a quantitative experimental method, where vaginal secretion samples were taken from the subjects and then tested quantitatively. There were two data collection techniques used in this study: using a questionnaire and testing vaginal secretion samples with green betel leaf extract. The test for using was diffusion (paper disc) for the antibacterial test. The media used was MHA. The positive control used was a blank disc, the extract concentration series used were 20%, 25% and 30%. The diameter of the inhibition zone was observed and then analyzed. The results of the t-test analysis showed that the significance value was 0.00 <0.05, so H0 was rejected Ha was accepted or it could be concluded that there was a difference in the effect of giving green betel leaf extract on the growth of Bacteroides in vaginal secretion culture. Antibacterial activity test of green betel leaves (Piper betle L) against Bacteroides bacteria that cause Bacterial Vaginosis, namely resistant at a concentration of 20%, intermediate at a concentration of 25% and sensitive at a concentration of 30%. Green betel leaf extract (Piper betle L) has inhibitory power against Bacteroides bacteria that cause Bacterial Vaginosis. Can increase the concentration series of methanol extract of green betel leaves, find the minimum bactericidal concentration (MBC) and the maximum inhibitory concentration (MIC) of green betel leaf extract against Bacteroides bacteria that cause Bacterial Vaginosis

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Keywords : Green Bettle Leaf, Bacteroides, Paper disc

Abstrak

Daun sirih hijau memiliki kandungan senyawa yang mampu untuk mengatasi keputihan, terutama yang disebabkan oleh bakteri. Untuk mengetahui pengaruh pemberian ekstrak daun sirih hijau terhadap pertumbuhan Bacteroides pada kultur secret vagina.Penelitian ini menggunakan metode kuantitatif eksperimental, dimana dilakukan pengambilan sampel secret vagina pada subjek untuk kemudian diuji secara kuantitatif. Ada dua teknik pengumpulan data yang dilakukan dalam penelitian ini yaitu menggunakan kuisioner dan pengujian sampel secret vagina dengan ekstrak daun sirih hijau. Pengujian digunakan dengan metode difusi (paper disc) untuk uji antibakteri. Media yang digunakan MHA. Kontrol positif yang digunakan disk kosong, seri konsentrasi ekstrak yang digunakan 20%, 25% dan 30%. Diameter zona hambat diamati kemudian dianalisis. Teknik analisis yang dipakai meliputi uji normalitas, uji homogenitas dan uji hipotesis dengan menggunakan uji-t yang diolah menggunakan SPSS versi 25. Hasil analisis uji-t menunjukkan bahwa nilai signifikansi 0.00 < 0.05, maka H0 ditolak Ha diterima atau dapat disimpulkan bahwa terdapat perbedaan pengaruhpemberian ekstrak daun sirih hijau terhadap pertumbuhan Bacteroides pada kultur secret vagina. Uji aktivitas antibakteri daun sirih hijau (Piper betle L) terhadap bakteri Bacteroides penyebab Bacterial Vaginosis yaitu resisten pada konsentrasi 20%, intermediet pada konsentrasi 25% dan sensitif pada konsentrasi 30%.Ekstrak daun sirih hijau (Piper betle L) mempunyai daya hambat terhadap bakteri Bacteroides penyebab Bacterial Vaginosis.Dapat menaikan seri konsentrasi dari ekstrak metanol daun sririh hijau, mencari konsentrasi bunuh minimum (KBM) dan konsentrasi hambat binimum (KHM) pada ekstrak daun sirih hijau terhadap bakteri Bacteroides penyebab Bacterial Vaginosis.

Kata Kunci : Daun sirih hijau, Bacteroides, Paper disc

1. INTRODUCTION

Reproductive health is a state of complete physical, mental, and social well-being, not merely the absence of disease or disability, but also related to the reproductive system, its functions, and processes (Susilowati, 2017). Reproductive health begins with maintaining personal hygiene, including vaginal hygiene, which aims to keep the vagina clean, healthy, normal, and free from disease. One reproductive health issue among adolescents is vaginal discharge or flour albus (Astuti et al., 2018).

Vaginal discharge can be caused by two factors: fungal infection and bacterial infection. Bacterial vaginosis is an abnormal condition characterized by changes in the vaginal flora, characterized by a shift in the balance of the vaginal flora, dominated by Lactobacillus, replaced by anaerobic bacteria, including Gardnerella vaginalis, Mobiluncus, Prevotella, Bacteroides, and Mycoplasma spp. (Abebaw Bitew & Mengist, 2021). The main risk factors for bacterial vaginosis are women of all ages, regardless of their educational, economic, and sociocultural background. In this case, it is generally more common among women with lower educational, economic, and sociocultural backgrounds, including issues with personal hygiene of the genitals, the use of hormonal contraceptives, and IUDs (Martasaphira et al., 2019).

The prevalence of bacterial vaginosis varies from country to country, but is estimated to range from 8% to 75% in women of reproductive age (Adane et al., 2017). According to the

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National Center for Biotechnology Information (NCBI), approximately 75% of women worldwide will experience vaginal discharge, and 45% will experience it twice or more. For women in Europe, the rate of vaginal discharge is 25%, with 40-50% experiencing recurrence (Kurniasari, 2019). Exhaustion and physical and psychological stress, such as excessive academic demands, poor exam results, and mounting assignments, can affect the functioning of hormones in a woman's body, including triggering an increase in estrogen, a hormone that triggers vaginal discharge in women (Baety, 2019).

The Indonesian Ministry of Health stated in 2013 that adolescents' knowledge of reproductive health was inadequate. This is evident in the percentage of adolescent girls who knew about Adolescent Reproductive Health (ARH), which was only around 35.3% (Nur Baety, 2019). Lack of information and knowledge about reproductive system changes during adolescence can lead to anxiety and embarrassment due to being different from their peers. This can lead to various reproductive health problems, one of which is vaginal discharge in adolescent girls (Dhuangga et al., 2012).

Bacterial vaginosis is an abnormal condition characterized by a shift in the balance of vaginal flora, dominated by Lactobacillus. This normal flora is replaced by anaerobic bacteria, including Gardnerella vaginalis, Mobiluncus, Prevotella, Bacteroides, and Mycoplasma spp. (Abebaw Bitew & Mengist, 2021).

Bacteroides itself is a genus of Gram-negative, rod-shaped bacteria. Bacteroides species are non-spore-forming, anaerobic, and motile or non-motile, depending on the species. Their DNA composition is 40-48% GC. Unusually for bacterial organisms, Bacteroides membranes contain sphingolipids. They also contain meso-deaminopimelic acid in their peptidoglycan layer. Bacteroides suppresses the growth of normal vaginal flora, leading to pH imbalance and abnormal vaginal discharge.

According to Andareto, (2015) the content of green betel leaves (Piper betel L.) is very good for treatment. There are several contents in green betel leaves including essential oils, hydroxycavicol, cavicol, cavibetol, allyprocatechol, eugenol, caryophyllene, cyneole, candinene, diastse, starch, terpennena, sesquiterpene, phenyl propane, tannin, sugar, and anti-fungal. Because of the very rich content, betel leaves are often used in traditional medicine to treat various diseases such as: swollen gums, vaginal discharge, canker sores, dengue fever, smooth menstruation, asthma, sore throat, eliminate underarm odor, and nosebleeds.

Research conducted by Nur Baety (2019) showed a significant effect between pre- and post-test interventions given by wiping with boiled green betel leaves to treat vaginal discharge. Another study conducted by Anas et al. (2018) found that green betel leaf extract (Piper betel L.) had a better inhibitory effect on the growth of Streptococcus mutans bacteria compared to red betel leaf extract (Piper crocatum).

Based on the results of a survey through a questionnaire on female students of SMKS Islam Pajarakan, out of 101 female students in grades XI and XII, 62 students experienced symptoms of bacterial vaginosis for more than 1 week and all students had never undergone treatment therapy for the symptoms of vaginosis they experienced. It was also known from the results of the questionnaire conducted on 62 students, only 10 students knew about the benefits of green betel leaves in treating vaginal discharge caused by bacteria (bacterial vaginosis). From this phenomenon, researchers were interested in conducting research on the effect of administering betel leaf extract on the growth of Bacteroides. Researchers hope to provide theoretical benefits in the form of knowledge about the benefits of green betel leaves for vaginal discharge symptoms caused by bacteria (bacterial vaginosis) and practical benefits in the form

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of alternative herbal treatments that can be applied in reducing symptoms of vaginosis caused by Bacteroides.

2. RESEARCH METHOD

This research uses an experimental design with a quantitative approach to examine the effects of green betel leaf extract on the growth of Bacteroides colonies. The research was conducted at the Community Health and Environmental Laboratory of UPT Puskesmas Pajarakan, Probolinggo Regency, from March to April 2025. The population of this research consists of female students from SMK Islam Pajarakan who show symptoms of bacterial vaginosis, with 62 students identified with the condition. A sample was selected using purposive sampling based on inclusion and exclusion criteria, resulting in 54 students divided into three treatment groups, each consisting of 18 subjects.

Data were collected through questionnaires to gather information on the respondents' symptoms and conditions, as well as laboratory tests to measure the inhibitory effect of green betel leaf extract on Bacteroides growth. Normality testing was conducted using the Kolmogorov-Smirnov Test, which showed that the data were not normally distributed ($p < 0.05$), leading to the use of the Kruskal-Wallis Test to examine differences between the treatment groups. Data analysis was performed using SPSS, with a significance level of $p < 0.05$.

This research adheres to ethical research principles, providing informed consent to all participants, maintaining data confidentiality, and ensuring that the research process complies with applicable ethical guidelines.

3. RESULTS AND DISCUSSION

Research Results

Based on the antibacterial activity test results of green betel leaf, it was found that at a 20% concentration of betel leaf extract, the largest average inhibition zone diameter was 1.6 mm and the smallest average inhibition zone diameter was 0 mm, both classified as resistant. At a 25% concentration of green betel leaf extract, the largest average inhibition zone diameter was 12 mm, which falls into the intermediate category, and the smallest was 2 mm, which falls into the intermediate category. At a 30% concentration, the highest concentration was 30 mm, which falls into the intermediate category, and the lowest concentration was 9 mm, which falls into the intermediate category.

The examination data in this study are presented in tabular form and analyzed statistically using the Statistical Product and Service Solutions (SPSS) program. The initial SPSS analysis was to conduct a normality test to determine whether the data were normally distributed or not. The normality test was carried out using the Kolmogorov-Smirnov test where the significance level of the effect of green betel leaf extract on the growth of Bacteroides colonies was $p = 0.001$ ($p < 0.05$), which means the data were not normally distributed.

This was followed by the Kruskal-Wallis test. The Kruskal-Wallis test results showed a p-value of 0.001 (<0.05), indicating a significant difference between the treatment groups in the inhibition zones produced by Bacteroides bacteria.

Based on the data above, the resulting p-value is 0.001, which indicates that the p-value $< \alpha$ (0.05). This confirms that Ha (alternative hypothesis) is accepted and H0 (null hypothesis)

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is rejected, indicating a difference in the growth of Bacteroides colonies before and after being given green betel leaf extract discs.

Discussion of Results

An antibacterial sensitivity test using the disc diffusion method showed that methanolic betel leaf extract inhibited the growth of Bacteroides colonies at certain concentrations. At a concentration of 20%, the average growth was 0.33 mm from three replicates, categorizing it as resistant. At a concentration of 25%, the average growth was categorized as intermediate, and at a concentration of 30%, the average growth was categorized as sensitive. This indicates that the concentrations of methanolic betel leaf extract that can inhibit the growth of Bacteroides colonies are 25% and 30%.

According to Yosepha (2024) in the antibacterial activity test of betel leaf extract with a comparison of solvent concentration variants, namely 40% ethanol, 70% ethanol, and 96% ethanol. It was carried out using the disc paper diffusion method with 3 types of extracts tested using Staphylococcus aureus bacteria. The antibacterial activity test of betel leaf extract with ethanol concentration variants used the disc diffusion method which aims to determine how large the inhibition zone produced by betel leaf extract in inhibiting the growth of Staphylococcus aureus bacteria around the disc paper.

Other studies show that: 1. The inhibitory effect of red betel leaf extract at concentrations of 2.5%, 5%, 7.5%, and 10%, namely 0.6cm, 1.1cm, 1.2cm, and 1.2cm; 2. Green betel leaf extract has no inhibitory effect at concentrations of 2.5%, 5%, 7.5%, and 10%. The conclusion of this experiment is that red betel leaf extract has a better inhibitory effect than green betel leaf extract. (Tristika Aulia et al, 2015)

The research findings indicate the effect of green betel leaf extract on the growth of Bacteroides colonies. Overall, Bacteroides growth began to be inhibited at a concentration of 25%, categorized as intermediate. However, Bacteroides colonies were only completely inhibited at a concentration of 30%, categorized as sensitive. This indicates that the concentration of green betel leaf extract also influences the growth of Bacteroides colonies.

According to researchers, this is likely because betel leaves contain active ingredients that can inhibit bacterial growth, and methanol can dissolve these active ingredients. Therefore, the main study used betel leaf-methanol extract. In addition to Bacteroides, green betel leaf extract is also effective in inhibiting the growth of other bacteria that cause bacterial vaginosis.

4. CONCLUSION

Based on the results of the research, the effect of green betel leaf extract on the growth of *Bacteroides* in vaginal secretion cultures can be concluded as follows: In the control group, the negative control showed a 0 mm inhibition zone diameter, categorized as resistant, while the positive control had a 50 mm inhibition zone, categorized as sensitive. In the treatment groups, the average inhibition zone diameter in the 20% treatment group was 0.32 mm, with the highest result being 1.6 mm and the lowest being 0 mm. In the 25% treatment group, the average diameter was 6.26 mm, with the highest result at 12 mm and the lowest at 2 mm. In

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the 30% treatment group, the average inhibition zone was 20.9 mm, with the highest result being 30 mm and the lowest being 9 mm. Furthermore, there was a significant effect of administering green betel leaf extract on the growth of *Bacteroides* colonies, with a p-value of 0.001..

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