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Effect of Green Betle Leaf Extract (*Piper betle* L.) on Indoor Microbiological Quality

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A B S T R A C T

The microbiological quality of indoor air is still a signifikans concern because it causes mortality and morbidity. This study aims to analyze the effect of green betel leaf extract (*Piper betle* L.) on reducing the bactherial in the air. Quasi-research using the One Group Pretest Posttest design, conducted in March-May 2024. Betle leaves were extracted using the maceration method using 96% Ethanol solvent, then diluted with distilled water to obtain a concentration of 15%. Three volume variations (0.5 ml, 1.0 ml, and 1.5 ml) were put into a humidifier and applied to a nine-square-meter bedroom for three hours, with six replication. Sampling was carried out before and after the experiment. All data (N = 48) were analyzed using the One Way ANOVA and Tukey tests at CL 95%. The study results found a decrease in colonies in the treatment with a volume of 1.0 ml and 1.5 ml: 39.3% (from 36.2 to 22.3 CFU / m3) and 17.6% (from 49.2 to 37.0 CFU / m3). The treatment with a volume of 0.5 ml did not show a decrease. The statistical analysis showed a significant effect of betle leaf extract on reducing the bacteria in the air (P = 0.020). The study results have proven that green betle leaf extract can be used as a disinfectant to reduce the bacteria in the air. However, safe use must be applied by paying attention to the air humidity level.

Kualitas mikrobiologi udara dalam ruangan masih menjadi perhatian serius karena menyebabkan angka kematian dan kesakitan. Salah satu cara untuk menurunkannya adalah dengan disinfeksi. Penelitian ini bertujuan menganalisis pengaruh ekstrak daun sirih hijau (*Piper betle* L.) terhadap penurunan angka kuman di udara. Penelitian Quasi menggunakan rancangan One Group Pretest Posttest, dilaksanakan pada bulan Maret-Mei 2024. Daun sirih diekstrak dengan metode maserasi menggunakan pelarut Etanol 96%, selanjutnya diencerkan dengan air destilasi hingga diperoleh konsentrasi 15%. Tiga variasi volume (0,5 ml, 1,0 ml, dan 1,5 ml) dimasukkan ke dalam humidifier dan diaplikasikan pada kamar tidur berukuran sembilan meter persegi selama tiga jam, dengan enam kali pengulangan. Pengambilan sampel dilakukan sebelum dan sesudah percobaan. Keseluruhan data (N=48) dianalisis dengan uji One Way ANOVA dan Tukey, pada CL 95%. Hasil penelitian mendapatkan penurunan angka kuman pada perlakuan dengan volume 1,0 ml dan 1,5 ml, yaitu 39,3% (dari 36,2 menjadi 22,3 CFU/m^3) dan 17,6% (dari 49,2 menjadi 37,0 CFU/m^3). Sedangkan perlakuan volume 0,5 ml, tidak menunjukkan penurunan. Hasil analisis statistik menunjukkan pengaruh signifikan ekstrak daun sirih terhadap penurunan angka bakteri di udara (P= 0,020). Hasil penelitian telah membuktikan bahwa ekstrak daun sirih hijau dapat digunakan sebagai desinfektan untuk menurunkan angka bakteri di udara. Namun, penggunaan yang aman harus diterapkan dengan memperhatikan tingkat kelembaban udara.

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1. Introduction

Indoor air quality is an important concern for health due to the high prevalence of diseases arising from poor air quality (Apte & Salvi, 2016; Wolkoff, 2018, 2024). World Health Organization data (2024) shows that every year, 3.2 million people die prematurely from diseases related to indoor air quality, including pneumonia and tuberculosis.

Good indoor air quality includes air free from microbes, pollutants, and irritants that can affect human health. Poor air quality can cause the air in closed rooms to be filled with airborne microbes (Syaputri et al., 2022).

Three basic strategies recommended to improve indoor air quality are source control to avoid indoor and outdoor emissions, provision of ventilation technology, and adequate air cleaning to achieve further improvements (Apte & Salvi, 2016; Bahri et al., 2021; Landrigan, 2017; K. K. Lee et al., 2020). It is important to maintain indoor air quality through various efforts, including ensuring good ventilation, minimizing indoor pollutants, disinfecting the room and taking steps to reduce exposure to hazardous substances (Apte & Salvi, 2016; Landrigan, 2017; K. K. Lee et al., 2020; World Health Organization, 2024)

Several air disinfection methods can be used, such as spraying, fogging, steam, and ultraviolet light (Romano, 2023). The steam method is applied with a humidifier that releases water vapor through heating so that it can be mixed with disinfectants. Humidifiers are very suitable for use as disinfection tools in the community because of their varied sizes, ease of use, and low cost (Akbar et al., 2023; Nie et al., 2014).

Humidifiers in oxygen therapy prevent respiratory tract irritation by humidifying oxygen (Hany et al., 2021). According to Nugroho et al. (2022), humidifier robots work effectively to freshen the room and prevent bacterial growth if disinfectants are added. Chemical and natural disinfectants can be added to the humidifier. However, natural disinfectants (bio disinfectants) are more recommended because they do not harm long-term use. Residues from chemicals can cause health problems (Broto et al., 2021; E. Lee et al., 2020; Park et al., 2015).

Indonesians have widely used green betel leaves (*Piper betle* L.) in traditional medicine. Betel leaves contain phenol derivative compounds useful as antiseptics, antibacterials, and antioxidants (Andila et al., 2020; Hermanto et al., 2023; Umesh et al., 2021). According to Sadiah et al. (2022), betel leaf extract can affect the quality of bacterial inhibition, has antibacterial effects against several types of bacteria (including Staphylococcus aureus and Escherichia coli), and does not cause toxic effects even at high concentrations.

The study aims to analyze the effect of green betel leaf extract (*Piper betle* L.) on reducing the bacterial in the air. The results of this study are expected to be an alternative solution to improve indoor air quality.

2. Material and Methods

2.1. Research design and Setting.

This study is a Quasi Experiment using One Group Pretest Posttest Design. This design is carried out through pretest, treatment, and posttest (Chan, C., & Holosko, 2020; Marsden & Torgerson, 2012). The study was conducted with six replications from March to May 2024 at the Yogyakarta Health Polytechnic Laboratory and residential housing. The variables studied were the volume of *Piper betle* L. extract (15%) consisting of three levels (0.5ml, 1ml, and 1.5ml). The entire research process has obtained ethical approval from the Health Ethics Commission of the Yogyakarta Health Polytechnic, Number DP.04.03/e-KEPK.1/006/2024, dated January 2, 2024.

2.2. Plant extraction.

Plant samples were collected from traditional markets in Yogyakarta City (7°48′02″S 110°23′29″E, 112 meters altitude). Following Lidya & Yushananta (2024) and Yushananta & Ahyanti (2021), the collected Piper betle L. leaves (local name = sirih) were washed repeatedly with distilled water to remove dirt and dried without sunlight. Four hundred grams of dried sirih leaves were ground and soaked in 500 mL of 96% ethanol for 24 hours in a closed container. Then, it was filtered to separate the filtrate and residue. The entire filtrate was evaporated with a rotary evaporator at a temperature 40-60℃ until a thick extract (100% concentration) was obtained. Dilution of the extract with distilled water to obtain a concentration of 15%.

2.3. Experiment and data analysis.

The experiment was conducted in a bedroom (residential housing) measuring 3x3 equipped with a window for ventilation. The repetition is determined based on the Federer formula, namely (t-1) (n-1) ≥ 15, where t is the treatment and n is the number of repetitions (Eun-Jung Yoon et al., 2023). Based on this formula, the replication of each treatment is six times. The experiment was carried out once every three days for three weeks, following the normal activities of the occupants. Sampling was carried out before and after each experiment, so 48 data were obtained.

A total of 300 ml of distilled water was put into a humidifier (KRIS humidifier 1.5L 15W), and betel leaf extract was added based on the treatment variations (0.5 ml, 1.0 ml, and 1.5 ml), carried out for three hours of exposure. Air microbiology sampling using the settle plate method and Plate Count Agar media. The petri dish was placed open 1 meter from the floor and at least 1 meter from the wall for 1 hour. After one hour, the petri dish was closed and incubated for 24 hours at 37°C to grow airborne bacteria (Andriana et al., 2023; Muhammad & Widayati, 2024; Rosyad et al., 2024). Data analysis was carried out in stages with SPSS (24.0). Univariate analysis to obtain the mean, minimum, and maximum number of bacteria before and after treatment. One-way ANOVA and Tukey tests were applied to determine the effect of betel leaf extract on reducing the number of bacteria in the air. The entire analysis was carried out at a 95% confidence level.

3. Results

The study results (Table 1) showed that in the first treatment (extract volume 0.5 ml), there was an increase in colonies from 31.7 (13.0-67.0) CFU/m^3 to 41.0 (27.0-57.0) CFU/m^3, or an increase of 56.5%. In the second treatment (1.0 ml), there was a decrease in colonies from 36.2 (27.0 - 56.0) CFU/m^3 to 22.3 (10.0 - 43.0) CFU/m^3, or a decrease of 39.3%. Likewise, in the third treatment (oil 1.5 ml), it decreased from 49.2 (21.0-83.0) CFU/m^3 to 37.0 (17.0-79.0) CFU/m^3 or a decrease of 17.6%. Meanwhile, in the control group (0 ml), colonies decreased from 41.2 (27.0-78.0) CFU/m^3 to 40.0 (14.0-66.0) CFU/m^3 or decreased by 2.5%.

Table 1. Pre and post test result

Volume	Pre test CFU/m^3	Post test CFU/m^3	Reduction (%)
0.5 ml			
Mean	31.7	41.0	-56.5
Minimum	13.0	27.0	-215.4
Maximum	67.0	57.0	14.9
1 ml			
Mean	36.2	22.3	39.3
Minimum	27.0	10.0	-7.5
Maximum	56.0	43.0	66.7
1.5 ml			
Mean	49.2	37.0	17.6
Minimum	21.0	17.0	-28.0
Maximum	83.0	79.0	61.3
0 ml			
Mean	41.2	40.0	2.5
Minimum	27.0	14.0	-66.7
Maximum	78.0	66.0	53.3

Normality and homogeneity of variance tests were conducted using the Kolmogorov-Smirnov and Levene tests. Both tests were conducted to meet the basic assumptions of the ANOVA test. The Kolmogorov-Smirnov test results (Table 2) indicate that the three groups of data are normally distributed (P > 0.05). Meanwhile, Levene's test showed that the three groups have the same variance (P= 0.359).

Table 2. Kolmogorov Smirnov and Levene tests results

Kolmogorov Smirnov Test			Levene Test			
Volume	F	P	Levene Statistic	df1	df2	P
0.5 ml	0.216	0.200 (Normal)	1.097	2	15	0.359
1.0 ml	0.275	0.176 (Normal)				
1.5 ml	0.320	0.055 (Normal)				

The one-way ANOVA test results (Table 3) show a difference in the reduction of bacterial counts based on treatment variations (P= 0.020). This result explains the effect of the treatment (volume of betle leaf extract) on the number of bacteria in the air.

Table 3. One-way ANOVA test results

	Sum of Squares	df	Mean Square	F	P
Between Groups	30284.843	2	15142.422	5.175	0.020
Within Groups	43894.422	15	2926.295		
Total	74179.265	17			

The Tukey HSD test results (Table 4) show that the difference in the reduction of bacterial counts is only between the 0.5 ml and 1.0 ml volumes (P= 0.020). Meanwhile, there is no significant difference between the 0.5 ml volume and the 1.5 ml volume (P= 0.076) and between the 1.0 ml volume and the 1.5 ml volume (P= 0.772). Of the three treatment variations, the treatment with a volume of 1.5 ml showed the greatest effect on reducing airborne bacteria (39.2%).

Table 4. Tukey HSD test results

Volume	Mean	0.5 ml (MD)	1.0 ml (MD)	1.5 ml (MD)
0.5 ml	-56.5	-	0.020 (-95.78)	0.076 (-74.16)
1.0 ml	17.6	0.020 (95.78)	-	0.772 (21.61)
1.5 ml	39.2	0.076 (74.16)	0.772 -21.61)	-

4. Discussion

Bedrooms with high humidity (more than 60%) pose a health risk through microbiological exposure (Dewi et al., 2021). Unqualified indoor air humidity can impact respiratory infections, such as tuberculosis and pneumonia (Aurora, 2021; Sari et al., 2022; Trigunarso et al., 2018). The study results showed that a humidifier application of betel leaf extract (*Piper betle* L.) at a concentration of 15% could reduce the bacteria in the air. The average reduction (Table 1), using a volume of 1.0 ml of extract, was around 39.3% (from 36.2 to 22.3 CFU/m^3), and a volume of 1.5 ml was 17.6% (from 49.2 to 37.0 CFU/m^3). The statistical results analysis (Table 3) has proven the effect of betel leaf extract on reducing the bacteria in the air (P = 0.020), which is the largest decrease in the use of a volume of 1.0 ml.

However, in the experiment with a variation of 0.5 ml, there was no decrease in bacteria between before and after treatment. According to Cui et al. (2022), indoor disinfection failure is related to the duration of use, human activities, goods, cleanliness, and composition of household occupants (family members). In addition, disinfectant solutions must be appropriate (volume, exposure time, and concentration) because they can reduce the effectiveness of the disinfectant solution (World Health Organization, 2020). Although it did not show a decrease, the bedroom still met health requirements. According to (Kemenkes RI, 2023), the standard for indoor biological pollutants is <700 CFU/M3.

Betel leaves have antiseptic and antibacterial properties (Kopong and Warditiani, 2022). The antiseptic properties of betel leaves are due to the content of phenol and several of its derivatives, including eugenol and kavikol (Mustam et al., 2022).

Betel leaves contain phenol derivative compounds, which are useful as antiseptics, antibacterials, and antioxidants (Andila et al., 2020; Hermanto et al., 2023; Umesh et al., 2021). Sadiah et al. (2022) explained that betel leaf extract can inhibit bacterial growth (including Staphylococcus aureus and *Escherichia coli*) and does not cause toxic effects even at high concentrations. Bio-disinfectants are also recommended to prevent the risk of health problems due to chemical disinfectant residues (Broto et al., 2021). Park et al. (2015) and E. Lee et al. (2020) have reported lung disorders due to exposure to chemical-based humidifier disinfectants.

However, humidifiers must still be used with attention to air humidity. According to the Kemenkes RI (2023), the environmental health quality standard for indoor air humidity is 40-60% Rh. Less or more than this limit will impact health.

Air humidity has direct and indirect effects on the quality of the indoor environment (E. Lee et al., 2020; Park et al., 2015; Romano, 2023; Wolkoff, 2018). The direct effect is affecting comfort in the home. While the indirect effect is related to the interaction of humidity with indoor air pollutants. High air humidity can increase the concentration of pollutants, create new pollutants due to reactions with water vapor (such as sulfur dioxide to form sulfuric acid), and increase biological pollutants (bacteria, viruses, fungi, mites, mold, and other organisms).

5. Conclusions

This study has proven that green betel leaf extract (Piper betle L.) can reduce bacteria in indoor air by 39.3% (volume 1.0 ml) and 17.6% (volume 1.5 ml). The statistical analysis results showed the significant effect of betle leaf extract on reducing the bacteria in the air (P = 0.020), further reinforce the potential of this natural disinfectant. The largest decrease was observed at a volume of 1.0 ml (concentration of 15%). Furthermore, betle leaf extract can be a safe disinfection alternative. However, the use of a humidifier must still pay attention to the air humidity standard (40-60% Rh) so as not to have other impacts on health.

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