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NON-SPECIFIC ANTIMICROBIAL STUDY OF BETEL LEAF (PIPER BATLE L) ESSENTIAL OIL ON HAZMAT CLOTHES

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Abstract

Betel leaf or Piper betle L. is a plant that belongs to the Piperaceae family. This plant has more than 100 varieties spread throughout the world. Based on the bacterial test by means of a swab, spray Betel leaf gel 7.5% has the greatest antimicrobial activity. The study aimed to determine the non-specific antimicrobial activity of betel leaf essential oil at a concentration of 7.5% applied to hazmat clothes. The research phase was carried out in the laboratory experimentally by applying betel leaf essential oil with a liquid betel leaf spray gel preparation. The prepared spray gel was applied to the hazmat clothes of health workers in the infection room by spraying it with a concentration of 7.5% betel leaf essential oil. The number of research participants was 30 health workers. The analytical method to see the difference in the number of non-specific germs on hazmat clothes before and after applying 7.5% betel leaf essential oil gel using paired t-test. The results of the study showed significant differences in the number of non-specific germs before and after the application of 7.5% betel leaf essential oil spray gel, indicated by the p-value 0.000 (<0.05)

Keywords: Antimicrobial, betel leaf essential oil, hazmat clothes

INTRODUCTION

Hazmat is one of the Personal Protective Equipment (PPE) that health workers must use in dealing with infectious diseases. Hazmat stands for Hazardous Material, which means this hazmat suit is clothing that can protect against hazardous materials (Masudi & Winarti, 2020) . Hazmat has several arrangements and categories such as protecting the body from exposure to radiant heat by high temperatures and flames, chemical and biological protective clothing and clothing that protects against physical irritation in the workplace. Therefore, hazmat is usually used to protect the entire body of the worker and ensure the safety of its use. However, this clothing has the disadvantage of placing a large burden on the wearer's body due to heat stress (Nishizawa et al., 2018) (Aswad & Loleh, 2021) .

In addition, it is necessary to pay attention to the comfort in wearing this Hamzat clothing. Moreover, this clothing must be used while treating patients for approximately 8 hours each day. Another consideration often used in terms of comfort is the convenience of the user to move, not causing stress, heat and dehydration. The material used must have circulation or looseness so that it is easy to breathe (Fadilla, 2021) . Antiviral hazmat suit is a hazmat suit made by coating the outside with green betel leaf essential oil which is applied to the hazmat suit which has added value in the form of antimicrobial activity. It is hoped that in the future the coating of this antimicrobial material will increase the safety and comfort of hazmat users.

Betel leaf or Piper betle L. is a plant that belongs to the Piperaceae family. This plant has more

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than 100 varieties spread throughout the world. Several studies have shown that betel leaf has several biological activities such as antidepressant, insecticidal, antitumor, antioxidant, antimicrobial, antibacterial, antifungal, hypoallergenic and antiviral. This is because betel leaves contain various chemical compounds such as hydroxychavokol, kavikol, piperbetol, cavibetol, piperol A, methylpiperbetol, and piperol. While the main component of betel leaf is an essential oil known as betel oil (Madhumita et al., 2019) , (Sarma et al., 2018) (Nayaka et al., 2021) .

The main compounds in betel leaf essential oil are estragole, linalool, kavikol, and caryophyllene. These compounds can damage the cytoplasmic membrane and kill cells. Tannins and flavonoids are thought to have a mechanism of action to denature bacterial cell proteins and damage cell membranes and damage lipids in cell membranes through the mechanism of reducing the surface tension of cell membranes. The mechanism of phenol as an anti-bacterial agent acts as a toxin in the protoplasm, damaging and penetrating the walls and precipitating bacterial cell proteins (Liao et al., 2021) .

Antibacterial testing using essential oils against several bacteria such as Staphilococcus aureus, Pseudomonas aeruginosa, and Mycobacterium smegtitis resulted in strong antibacterial activity (Madhumita et al., 2019) . In addition, research conducted by (Sujono et al., 2019) showed that testing of betel leaf essential oil against gram-positive bacteria such as Escherichia coli showed antibacterial activity.

Previous research by Munjiati et al., (2022) showed that the use of spray gel from betel leaf essential oil with various concentrations of 2.5%, 5% and 7.5% on hazmat clothes resulted in fewer microbes at concentrations of essential leaf oil. Betel 7.5%. Phase one trials with small respondents were found to affect the number of bacteria before and after treatment. In this study it was found that there were differences in the administration of betel leaf gel sprays with varying concentrations of 2.5%, 5% and 7.5%. Based on the bacterial test by means of a swab, spray Betel leaf gel 7.5% had the greatest antimicrobial activity with 14 and 18 microbes in the pretest and 2 and 1 posttest colonies (Munjiati et al., 2022) .

Based on this background, it is necessary to conduct further research on antiseptic hazmat clothes made from betel leaf essential oil with a concentration of 7.5% and tested with a larger number of respondents. With this research, the results show how effective it is in reducing the number of microbes in hazmat clothes. The third stage of the research is expected to be a wider trial using the antimicrobial hazmat suit made from betel leaf essential oil with a concentration of 7.5%. The hope is that in the future a hazmat suit will be obtained that can protect health workers from virus and bacterial contamination in a more optimal manner so that it is safer and also comfortable and has a high level of security for health workers.

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MATERIALS AND METHODS

Tools and Materials

The tools used in this study were a stirrer, a set of essential oil distillation tools, vials, mortar and pestle, measuring cups, beakers, spatulas, volume pipettes and pumps, pipettes, hot plates, stirring rods, spatulas and balances.

The materials used were water, 8 kg of betel leaf (Piper betel L), carbopol 940, TEA (triethanolamine), propylene glycol, propyl paraben, methyl paraben, betel leaf essential oil, and distilled water.

Essential oil isolation (Gunawan & Kurniaty, 2021)

The betel leaf is cut into smaller pieces. Betel leaves that have been cut into pieces are put into the distillation apparatus, closed and set. Then it is left for 6-7 hours until the essential oil comes out and is condensed by the condenser and accommodated in the separator. Betel leaf essential oil is separated from water and collected in vials, stored in the refrigerator until used.

Method for making betel leaf spray gel (Rubiyanto, 2020)

The ingredients are weighed according to the weight according to the table listed. Carbopol 940 was put into the mortar and hot distilled water was added. Then stir evenly until dissolved. The solution is added to TEA drop by drop until the carbopol expands. Methyl paraben is put into a beaker and hot distilled water is added. Stir until dissolved and put in the previous carbopol. Betel leaf essential oil is put into a beaker glass and added with propylene glycol to dissolve. Then added with propyl paraben. After that, it was put into the previous carbopol. The solution was added with distilled water up to 100 mL and stirred until the gel formed was not too thick and homogeneous. The spray gel formed is put into a spray bottle and closed. The formulation of the betel leaf essential oil spray gel preparation can be seen in table 1.

Table 1: Formulation of betel leaf essential oil spray gel 7.5%

No	Ingredient	Amount
1	Carbopol	0.35g
2	TEA (Tri ethanolamine)	0.5g
3	Propylene glycol	15 g
4	Propyl paraben	0.05g
5	Methyl paraben	0.18g
6	Betel leaf essential oil	0.75g
7	Aquades	Add 100 mL

Application of betel leaf essential oil gel on hazmat clothes

Prior to the application process, 30 health worker participants were selected who wore hazmat clothes. The participant inclusion criteria were health workers working in the infection room and hazmat clothes only being used for activities in the patient care room for 2 hours.

After determining the participants involved in the research, a pre-test was first carried out to determine the number of microbes in the colony unit before applying the 7.5% essential oil gel.

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The pre-test is carried out using a swab test using a cotton swab that is applied to the entire surface of the hazmat suit. Then the cotton is put into the nutrient medium broth before being inoculated on nutrient agar medium for 24 hours at 37°. The number of microbes was counted using a hemocytometer to determine the number of microbial colonies. After that, betel leaf essential oil spray gel is sprayed all over the surface of the hazmat clothes worn by health workers when treating patients in the infection (isolation) room. Then after the hazmat suit was worn by the health worker for 2 hours, another swab test was carried out to determine the number of microbes.

Data analysis technique

To see the difference in the number of non-specific germs or microbes on the hazmat clothes before and after the application of 7.5% betel leaf essential oil spray gel, paired t tests were used.

RESULTS AND DISCUSSION

The study was conducted with 30 participants who served in isolation rooms or infection treatment rooms. The results of the study explained the number of non-specific germs or microbes on the hazmat clothes of health workers before and after the application of 7.5% betel leaf essential oil gel spray as described in table 2.

Table 2: Results of examination of total germs on linen swabs (hazmat clothes)

No. Sample	Transport Media Volume	Wipe Area (cm^2)	Linen Area (cm^2)	Number of Germs Pre	Number of Germs Post
1	50 ml	300	8000	12	10
2	50 ml	300	8000	11	8
3	50 ml	300	8000	8	4
4	50 ml	300	8000	2	0
5	50 ml	300	8000	8	4
6	50 ml	300	8000	6	4
7	50 ml	300	8000	8	3
8	50 ml	300	8000	6	4
9	50 ml	300	8000	7	5
10	50 ml	300	8000	12	6
11	50 ml	300	8000	14	10
12	50 ml	300	8000	12	8
13	50 ml	300	8000	6	4
14	50 ml	300	8000	11	9
15	50 ml	300	8000	9	4
16	50 ml	300	8000	11	8
17	50 ml	300	8000	10	8
18	50 ml	300	8000	8	2
19	50 ml	300	8000	6	4
20	50 ml	300	8000	10	4
21	50 ml	300	8000	11	10
22	50 ml	300	8000	6	3

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23	50 ml	300	8000	12	4
24	50 ml	300	8000	9	6
25	50 ml	300	8000	11	8
26	50 ml	300	8000	11	8
27	50 ml	300	8000	10	8
28	50 ml	300	8000	8	6
29	50 ml	300	8000	11	8
30	50 ml	300	8000	10	4

Table 2. Below describes the frequency distribution of non-specific germs before and after the application of 7.5% betel leaf essential oil s pray gel on the hazmat clothes of health workers in the Infection Room.

Table 3: Frequency Distribution of Non-Specific Germs Before and After Application of Betel Leaf Essential Oil Gel Spray 7.5% on Hazmat Clothes of Health Workers in the Infection Room of Purbalingga Hospital, Indonesia (n=30)

	Means	S D	SE	Min-Max
Germs on Hazmat Clothes				
Pre	9,20	2.59	0.47	2 - 14
Post	5.80	2.62	0.48	0-10

From table 3 above it can be explained that the average number of non-specific germs on the hazmat suit before application of 7.5% betel leaf essential oil gel spray was 9.20, a standard deviation of 2.59. While the average number of non-specific germs on hazmat clothes after application of 7.5% betel leaf essential oil gel spray was 5.80, a standard deviation of 2.62.

Table 4 below explains the difference in the number of non-specific germs in the hazmat clothes of health workers before and after the application of 7.5% betel leaf essential oil gel spray.

Table 4: Differences in the Number of Germs Before and After Application of Essential Oil Gel Spray 7.5% Betel Leaf on Health Worker Hazmat Clothes in the Infection Room of Purbalingga Hospital, Indonesia (n=30)

Variable	n	Average	S D	SE	p-value
Number of Germs	30				0.000
1. Before		9,20	2.59	0.47	
2. After		5.80	2.62	0.48	

Table 4 above explains that the average number of non-specific germs before the application of 7.5% betel leaf essential oil gel spray was 9.20 and after the application of 7.5% betel leaf essential oil gel spray, namely 5.80. It was concluded that there was a significant difference in the number of non-specific germs before and after the application of 7.5% betel leaf essential oil gel spray marked with a p value 0.000 (<0.05). This study showed that there was an average decrease in the number of non-specific germs before and after the application of 7.5% betel leaf essential oil spray gel on the hazmat clothes of health workers in the Infection Room of Purbalingga Hospital, Indonesia by 3.4 (from 9.20 to 5, 80). The results showed that betel leaf essential oil had a significant effect on inhibiting the growth of non-specific bacteria.

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This is supported by research on betel leaf extract tests to determine the best MIC (Minimum Inhibitory Content) and MIC (Minimum Bactericidal Concentration) using B.subtilis and E.coli bacteria which have been tested for sensitivity to the antibiotics chloramphenicol, vancomycin, and cyprofolksasin. The results of this study showed that the best MIC and KBM were obtained from betel leaf extract respectively, namely 6.25% and 50%. The sensitivity of the test bacteria B.subtilis and E.coli used against the antibiotics chloramphenicol, vancomycin, and ciprofloxacin was susceptibel or susceptible (Fitriana et al., 2019) .

Betel leaf essential oil can also be used for acne treatment. The extracts and essential oils of Piper Betle Leaf contain antibacterial and antifungal activities. The effectiveness of the use of Piper Betle Leaf ethanol extract (Piper betle Linn) in acne treatment can be improved by creating formulations in the form of cream preparations. Formulations in cream preparations will affect the amount and speed of active substances that can be absorbed. The three creams containing Piper Betle Leaf ethanol extracts at percentages of 5%, 10% and 15% for each had inhibitory zones: 9.8 mm, 15.85 mm, 17.35 mm (Meinisasti et al., 2020) . Piper betle (L) is a popular medicinal plant in Asia. Plant leaves have been used as a traditional medicine to treat various health conditions. It is highly abundant and inexpensive, therefore promoting further research and industrialization development, including in the food and pharmaceutical industries. Articles published from 2010 to 2020 were reviewed in detail to show recent updates on the antibacterial and antifungal properties of betel leaves. This current review showed that betel leaves extract, essential oil, preparations, and isolates could inhibit microbial growth and kill various Gram-negative and Gram-positive bacteria as well as fungal species, including those that are multidrug-resistant and cause serious infectious diseases. P. betle leaves displayed high efficiency on Gram-negative bacteria such as Escherichia coli and Pseudomonas aeruginosa, Gram-positive bacteria such as Staphylococcus aureus, and Candida albicans. The ratio of MBC/MIC indicated bactericidal and bacteriostatic effects of P. betle leaves, while MFC/MIC values showed fungicidal and fungistatic effects. This review also provides a list of phytochemical compounds in betel leaf extracts and essential oils, safety profiles, and value-added products of betel leaves. Some studies also showed that the combination of betel leaf extract and essential oil with antibiotics (streptomycin, chloramphenicol and gentamicin) could provide potentiating antibacterial properties (Nayaka et al., 2021) .

CONCLUSION

The average number of non-specific germs on hazmat clothes before application of 7.5% betel leaf essential oil gel spray was 9.20, a standard deviation of 2.59. While the average number of non-specific germs on hazmat clothes after application of 7.5% betel leaf essential oil gel spray was 5.80, a standard deviation of 2.62. There was a significant difference in the number of non-specific germs before and after the application of 7.5% betel leaf essential oil gel spray, indicated by the p value 0.000 (<0.05).

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