The effectiveness of green betel leaf (*Piper bettle Linn*) extract hand sanitizer gel in reducing bacterial colonies on the palm hand of dental clinical clerkship student

Efektivitas gel *hand sanitizer* ekstrak daun sirih hijau (*Piper bettle* Linn) mengurangi koloni bakteri pada telapak tangan mahasiswa kepaniteraan klinis kedokteran gigi

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ABSTRACT

Objective: To determine the effectiveness of using green betel leaf (*Piper bettle Linn*) extract hand sanitizer gel in reducing the number of bacterial colonies in the palms of hand. **Method**: In this experimental research with posttest only with control group design using dental clinical clerkship students of Faculty of Dentistry Universitas Sumatera Utara. They were evenly divided into 5 groups. They were chlorhexidine gel (group 1), 1% green betel leaf extract (group 2), 3% green betel leaf extract (group 3), 5% green betel leaf extract (group 4), and use of placebo (group 5). Bacterial colonies isolated from the swabs were identified by standard microbiological procedures. All statistical analysis methods were conducted using the one-way Anova and posthoc LSD tests. **Results**: The results of univariate analysis showed that sig <0.05, meaning that there was a difference in the number of bacterial colonies between chlorhexidine, 1%, 3%, and 5% green betel extract gel. The one-way Anova test at 95% confidence level proved that the differences are significant at p-value 0,001. **Conclusion**: There is a difference in the effectiveness of using a hand sanitizer for green betel leaf extract on the number of bacterial colonies in the palms of hand. Hand sanitizer green betel leaf extract gel 5% more effective than 1% and 3% concentrations.

Keywords: hand sanitizer, green betel leaf extract, palm hand, number of bacterial colonies

ABSTRAK

Masalah: Mengetahui efektivitas penggunaan gel handsanitizer ekstrak daun sirih hijau *(Piper bettle Linn)* dalam menurunkan jumlah koloni bakteri di telapak tangan. **Metode**: Pada penelitian eksperimental dengan *posttest only with control group design* ini digunakan mahasiswa kepaniteraan klinik Fakultas Kedokteran Gigi Universitas Sumatera Utara. Sampel dibagi rata menjadi 5 kelompok, yaitu chlorhexidine gel (kelompok 1), ekstrak daun sirih hijau 1% (kelompok 2), ekstrak daun sirih hijau 3% (kelompok 3), ekstrak daun sirih hijau 5% (kelompok 4), dan plasebo (kelompok 5). Koloni bakteri yang diisolasi dari penyeka diidentifikasi dengan prosedur mikrobiologi standar. Semua metode analisis statistik dilakukan dengan uji one-way Anova dan posthoc LSD. **Hasil**: Hasil analisis univariat menunjukkan bahwa sig <0,05 artinya terdapat perbedaan jumlah koloni bakteri antara gel ekstrak sirih hijau 1%, 3%, dan 5%. Uji *one-way Anova* pada tingkat kepercayaan 95% membuktikan bahwa perbedaan tersebut signifikan pada nilai p 0,001. **Simpulan**: Terdapat perbedaan efektivitas penggunaan *hand sanitizer* ekstrak daun sirih hijau terhadap jumlah koloni bakteri di telapak tangan. *Hand sanitizer* gel ekstrak daun sirih hijau 5% lebih efektif dibandingkan konsentrasi 1% dan 3%.

Kata kunci: *hand sanitizer*, ekstrak daun sirih hijau, telapak tangan, jumlah koloni bakteri
Received: 1 May 2021 Accepted:1 June 2021 Published: 1 August 2021

INTRODUCTION

Infection is a process of tissues invasion by micro-organisms, characterized by multiplication of these micro-organisms in the host body to produce disease.[1] Cross infections implies transmissions of infection between fellow patients and health care professionals.[1,2]

Hands are the part of the human body that is most frequently in contact with the outside world which makes contact with microbes easier. Hands turn out to be a nest for bacteria with various types of bacteria that live on the hands, both pathogenic and non-pathogenic.[3] Contamination of hands by bacteria is caused by factors originating from medical personnel, paramedics, and the environment. WHO has reported that hands contain 39,000-460,000 CFU of bacteria/cm2, which have a high potential to cause infectious diseases. Meanwhile, according to the Hand Hygiene of Europe website, humans have about 2-10 million bacteria between the fingertips and elbows.[4,5]

Infection prevention and cross-contamination control are essential to creating a safe environment for patients and health care workers to keep general health, especially in dental practice.[4] All health care workers have the responsibility to carry out aseptic techniques and correct infection control to avoid harm cross-infection.[7] The most important basic technique in preventing and controlling infection of the hands is washing hands with a hand sanitizer. One of the ingredients that has antibacterial properties is piper bettle leaf. Piper betle Linn or betel is a plant known to have antiseptic properties. The main components of green betel leaf oil consist of phenols and their derivative compounds.[6] The

DOI 10.35856/mdj.v10i2.417

antibacterial power of green betel leaf essential oil is caused by the presence of phenol compounds and their derivatives that can denaturate bacterial cell proteins. To damage cell walls, phenolic compounds and their derivatives can denaturate cell proteins and damage cell membranes.[6,7] At high concentrations of phenol can damage the cytoplasmic membrane completely. A part of phenol, several other research results show that terpenoid compounds can also inhibit bacteria growth by disrupting the process of forming a membrane or cell wall, so the membrane or cell wall is not fully formed.

Based on it, this study is aimed to explore the effectiveness of hand sanitizer green betel leaf extract gel in decreasing the number of bacterial colonies of the dental clinical clerkship students.

METHODS

Sampling was carried out in March 2021. One kg of green betel leaf was washed and dried then extracted to become a thick extract and then made as a 1%, 3%, and 5% hand sanitizer. Chlorhexidine was used as the positive control, and placebo as the negative control. Twenty-five clinical clerkship students of Faculty of Dentistry Universitas Sumatera Utara have participated in this research evenly divided in 5 groups.

Palm swabs were taking after washing hands according to the WHO technique within 20-30 seconds, making plate count agar (PCA) media, testing the effectiveness of green betel leaves and culture samples. Several inclusion criteria were the condition of the hands being clean, not wearing hand accessories such as rings and watches, and short fingernails. Swab samples were packaged in a sterile swab transport then taken to the Microbiology Laboratory of the USU Faculty of Medicine. The sample was diluted twice then poured into each PCA medium. The petri dish containing the test sample was left to stand at room temperature and then incubated at 37°C for 48 hours. Observations were performed by counting the number of colonies that were identified using basic microbiological tests.

Data obtained from observations were analyzed statistically using SPSS analysis, one-way Anova test and LSD test.

RESULTS

There were 50 PCA media were used because the samples took replication tests (Table 1). Shapiro-Wilk test shows that the data were normally distributed if the p-value was more than 0.05 (Table 2). The one-way Anova indicated that there was a significant differrence in the number of bacterial colonies on the hand palm of clinical clerkship students of Faculty of Dentistry USU between all treatment groups if the p value was less than 0.05 (Table 3). Furthermore, LSD test shows that there was a significant difference in effectiveness between the two groups if the p-value was less than 0.05.

DISCUSSION

This research used green betel leaf is caused by this plant has antibacterial properties.[8] The part of the betel plant that is widely used is the leaf.

On human hands there is a normal flora that functions protecting the body from pathogenic bacterial infections by competing for nutrients and living space. The lower pathogenic potential compared to transient flora is important to prevent the colonization of bacteria which

Table 1 Average number of bacterial colonies in each group (CFU/ml^3)

Sample	Clorhexidine	Placebo	*Handsanitizer* Green Betel Leaf Extraxt		
			K 1%	K 3%	K 5%
1	3	44	54	21	19
2	2	32	34	18	15
3	2	83	47	21	11
4	1	75	39	18	10
5	3	54	44	20	18
6	1	36	30	16	14
7	4	68	37	24	19
8	2	51	31	20	13
9	3	49	48	22	12
10	2	37	37	19	11
Mean	**2.3**	**52.9**	**40.1**	**19.9**	**14.2**

Table 2 Normality test

		Mean	SD	P-Value
Number of bacteria colonies	Clorhexidine	2.3	0.95	0.287
	Placebo	52.9	17.31	0.434
	K 1%	40.1	7.89	0.673
	K 3%	19.9	2.28	0.984
	K 5%	14.2	3.43	0.177

DOI 10.35856/mdj.v10i2.417

Table 3 One-way Anova test

Treatment Group	Number of Bacteria Colonies (CFU/ml^3)	P-Value
Clorhexidine	2.3±0.95	
Placebo	52.9±17.31	
Hand Sanitizer 1%	40.1±7.89	0.001*
Hand Sanitizer 3%	19.9±2.28	
Hand Sanitizer 5%	14.2±3.43	

Table 4 LSD Test

Treatment Group	Mean Diff	P-Value
Clorhexidine – Placebo	-50,6	0,001*
Clorhexidine – HS 1%	-37,8	0,001*
Clorhexidine – HS 3%	-17,6	0,001*
Clorhexidine – HS 5%	-11,9	0,004*
Placebo – HS 1%	12,8	0,002*
Placebo – HS 3%	33,0	0,001*
Placebo – HS 5%	38,7	0,001*
HS 1% - HS 3%	20,2	0,001*
HS 1% - HS 5%	25,9	0,001*
HS 3% - HS 5%	5,7	0,151

HS: hand sanitizer

have the ability to cause more diseases on the skin. Normal flora can form biofilms in the presence of collaboration between colonies to stimulate antibodies that are cross-reactive antibodies.[9] The types of bacteria found on the hands include H.pylori, E.coli, Sallmonela sp S.aureus, S.haemoliticus, P.aeruginosa, Clostridium welchii, Coliform bacteria, Pseudomonas spp, Staphylococcus epidermis, and Proteus spp.[10]

The use of this antiseptic in this study has a significant positive effect on reducing the number of bacterial colonies. From the results of this study, it can be stated that the use of 1%, 3%, 5% hand sanitizers was proven to significantly reduce the number of bacterial colonies in the hand palms. The ability of 5% green betel leaf extract hand sanitizer may be caused by the active compound content.[10]

The results of phytochemical screening conducted by Pratiwi stated that green betel leaf extract contains 31 active compounds including essential oils, kavikol, cavibetol, estargiol, eugenol, flavonoids, metal eugenol, carvacrol, terpenes, sesquiterpene, phenylpropan and tannin. The essential oil has an antibacterial power 5 times greater than the phenol derivative compounds.[11,12] Phenolic compounds that function as antiseptics works by interacting with bacterial cells through an absorption process involving hydrogen bonds, interfere with work in the cytoplasmic membrane including interfering with active transport and proton strength.[12] The largest group of phenolic compounds are flavonoids and tannins. Flavonoids, which is a natural antioxidant, play a role in preventing cell damage by reactive free radicals.[13] Tannins function as antigens, so tannins for humans can increase immunity, and can avoid bacterial infection.[13]

Based on the results of this study, it can be stated that the greater the concentration of betel leaf extract in a hand sanitizer, the smaller the number of palm bacterial colonies formed. Therefore, 5% green betel leaf extract in hand sanitizer are the most effective materials in reducing the number of bacterial colonies on the hand palms dental clinical clerkship students.

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