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Comparison of the Number of Bacterial Colonies Before and After Using Hand Sanitizer from *Acacia nilotica* Leaf Extract

(*Perbandingan Jumlah Koloni Bakteri Sebelum dan Sesudah Menggunakan Hand Sanitizer dari Ekstrak Daun Acacia nilotica* L.)

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ABSTRACT

Background: The use of hand sanitizers was an act of necessity during the Covid-19 pandemic. With this action, it is expected to control the number of bacterial colonies on the palms of the hands. One of the herbal ingredients that can inhibit the growth of germs is *Acacia nilotica* L leaf extract so that it can be used as an active ingredient in hand sanitizer. **Objective**: To determine the number of bacterial colonies before and after using a hand sanitizer with the active ingredient of acacia leaf extract. **Materials and Methods**: The study used random sampling analysis before and after treatment. *Acacia nilotica* leaf ethanol extract formula was 10%, 20% and 30%. Each concentration was used by 11 men and 11 women as a hand sanitizer with movements according to WHO standards and left for 30 seconds. Check the count of germs on hands before and after using hand sanitizer. **Results:** The highest decrease in the number of bacterial colonies was found at a concentration of 30% reaching 64.84% in men and 89.61% in women. There were differences in the ability to decrease the number of bacterial colonies from each concentration (P 0.003 < 0.005). **Conclusion**: *Acacia nilotica* L leaf extract can be a candidate for active hand sanitizer ingredients.

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ABSTRAK

Latar belakang: Penggunaan *hand sanitizer* merupakan tindakan keharusan selama masa pandemi Covid-19. Tindakan ini dapat mengendalikan jumlah koloni bakteri pada telapak tangan. Salah satu bahan herbal yang dapat menghambat dan atau membunuh pertumbuhan kuman adalah ekstrak etanol daun *Acacia nilotica* L sehingga dapat digunakan sebagai bahan aktif *hand sanitizer*. **Tujuan**: Mengetahui jumlah koloni bakteri sebelum dan sesudah menggunakan *hand sanitizer* dengan bahan aktif ekstrak etanol daun akasia. **Bahan dan Metode**: Penelitian menggunakan metode analisis random sampling sebelum dan sesudah perlakuan. Formula Eksttrak etanol daun *Acacia nilotica* 10%, 20% dan 30%, masing-masing konsentrasi digunakan oleh 11 orang pria dan 11 orang wanita sebagai hand sanitizer dengan gerakan sesuai standart WHO dan didiamkan selama 30 detik. Dilakukan pemeriksaan hitung jumlah bakteri pada tangan sebelum dan setelah menggunakan hand sanitizer. **Hasil**: Penurunan jumlah koloni bakteria tertinggi terdapat pada konsentrasi 30% mencapai 64,84% pada pria dan 89,61% pada wanita. Terdapat perbedaan kemampuan penurunan jumlah koloni bakteri dari masing-masing konsentrasi (P 0,003 < 0,005). **Kesimpulan**: ekstrak etanol daun *Acacia nilotica* L dapat menjadi kandidat bahan aktif hand sanitizer.

Kata kunci: *Acacia nilotica L*; *Hand sanitizer*; Koloni bakteri; Covid 19

INTRODUCTION

The transmission of the Covid-19 virus is very massive, since it was first discovered in Wuhan (RRC) in December 2019 and has been proven to be able to move between humans by the route of transmission through droplets that enter the respiratory tract or droplets that stick to the hands. One way of prevention is by hand hygiene (WHO, 2020). Using hand sanitizers with active ingredients such as alcohol or triclosan will kill bacteria on the hands (Desiyanto and Djannah, 2013). Besides, using plants as active ingredients that can inhibit or kill bacteria has been widely proven. One of the potential types of plants that can be used is *Acacia nilotica* L, which is commonly found in Baluran National Park, East Java. Ethanol extract of A. nilotica leaves gave 99.6% effective results in reducing the number of bacterial colonies (Kirui et al., 2015), it was also found that leaf extract at a concentration of 50% could reduce the number of aerobic bacteria in water up to 99.7% after 24 hours contact and reduce the number of MPN Coliforms to 99.5% (Jannah, Prabowo, and Kriswandana, 2019). Ethanol extract from *Acacia nilotica* leaves at a concentration of 25% can kill bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* (Jannah, Latifah, and Zuraida, 2020).

The active ingredients contained in the ethanolic extract of the leaves of *Acacia nilotica* L saponins, terpenoids, flavonoids, phenols, carbohydrates, tannins and steroids (Ukwuani-kwaja et al., 2016). This study aims to determine whether there is a difference in the number of bacteria on the hands before and after using a hand sanitizer with an active ingredient of ethanolic extract of *Acacia nilotica* leaves at concentrations of 10%, 20% and 30%.

MATERIAL AND METHODS

Materials

The research was carried out from April to June 2021, at the Microbiology Laboratory, University of MH Thamrin Jakarta. The leaves of *Acacia nilotica* L were used from trees that were at least one year old obtained from the Baluran National Park, East Java, 96% ethanol solvent. Peptone Buffer (Merck), Carbomer 940, TEA, Methylparaben, Glycerin, Nutrient Agar Media (MERCK CM0405 Lot No. 1460487) and sterile Aquadest.

Methods

Preparation of ethanol extract of the leaves of *Acacia nilotica* L.

The leaves are washed and dried by airing, then put in an oven at 40^{o}C, blended until smooth. The powder was added with 96% ethanol solvent and stirred using a stirrer for ± 3 hours. The suspension was allowed to stand for 24 hours and then filtered. Then the simplicia dregs were added again with 96 ethanol until it was submerged. The stirrer was carried out for 1 hour, then filtered again with filter paper. The collected filtrate is evaporated to form a thick extract.

Hand sanitizer with *Acacia nilotica* L leaf extract

Hand Sanitizer is made with the following formula and concentration that can be seen in table 1.

Table 1. Formula hand sanitizer with leaf extract of *Acacia nilotica* L.

Ingredients	Concentration 10%	Concentration 20%	Concentration 30%
Acacia leaf extract (g)	10	20	30
Aquadest steril (ml)	90	80	70
Carbomer 940 (g)	2	2	2
TEA (ml)	2,5	2,5	2,5
Methylparaben (g)	0,2	0,2	0,2
Glycerine (ml)	10,25	10,25	10,25

Using hand sanitizer

The research was experimental before and after treatment. Respondents were third-semester students of the Department of Health Analyst Univ. MH. Thamrin randomly selected, as many as 66 people. Hand sanitizers with 10% active ingredients were used for 11 male students and 11 female students. the same is done for the concentration of 20% and 30%

The technique for using hand sanitizer follows the manual from WHO as shown figure 1

Figure 1. Techniques for using hand sanitizer (Worl Health Organization 2009)

Counting the number of bacterial colonies

The respondent's hand swab was carried out using a sterile cotton stick using peptone buffer medium. Then it was implanted using the pour plate method, incubated in an incubator at 37^0C for 24-48 hours. Colonies growing on agar medium from each concentration were counted using a colony counter. Paired T-tests were carried out from the data obtained using the SPSS version 17.0 application.

RESULTS AND DISCUSSION

The results of calculating the number of bacteria on the respondent's hands before and after using a hand sanitizer with an active ingredient of ethanol extract of *Acacia nilotica* L leaves, there was a decrease in the number of bacteria from each concentration (Table 2 and Table 3)

Table 2. Results of examination of bacterial count before and after using hand sanitizers with active ingredient ethanol extract of *Acacia nilotica* L. leaves in 33 male respondents

The concentration of *A. nilotica* leaf ethanol extract	Number of Bacterial Colonies						Decreased Persentation {mean ($X_0 - X_1$) : mean X_0} x 100%
	Before using *hand sanitizer* (X_0)			After using *hand sanitizer* (X_1)			
	Min	Max	Mean	Min	Max	Mean	
10%	260	334.000	3.521,17	98	298.000	2.943,55	16,42%
20%	163	97.000	33.839,75	102	57.600	15.340,25	54,55%
30%	2600	460.000	89.892,73	216	120.000	31.606	64,84%

Table 3. Results of examination of bacterial count before and after using hand sanitizer with active ingredient ethanol extract of *Acacia nilotica* L. leaves in 33 female respondents

The concentration of *A. nilotica* leaf ethanol extract	Number of Bacterial Colonies						Decreased Persentation {mean ($X_0 - X_1$) : mean X_0} x 100%
	Before using *hand sanitizer* (X_0)			After using *hand sanitizer* (X_1)			
	Min	Max	Mean	Min	Max	Mean	
10%	217	140.000	12.853,25	116	80.000	75.074,17	41,59%
20%	260	146.000	31.010,42	110	87.000	18.029,58	51,58%
30%	167	720.000	73.478,27	98	43.000	7.633	89,61%

Hand sanitizer with ethanol extract of *A. nilotica* L leaves could reduce the number of germs on the respondent's hands at all concentrations. From the results of calculating the paired T-test, significant results were obtained, where the p-value was $0.003 \leq 0.005$, which means that there is a difference in the number of germs before and after washing hands using hand sanitizer (Table 4).

Table 4 Paired T test results Total plate count before and after using hand sanitizer

Paired Samples Test

		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	before - after	45241,814	123973,786	14817,702	15681,309	74802,320	3,053	69	,003

This proves that the active ingredients in the extract can inhibit or kill these bacteria. As found by Kalaivani et. Al (2011) the active compound Niloticane from stem and leaf ethyl acetate extracts could inhibit the growth of gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* at doses of 4 and 8 g/mL and doses of 16 and 33 g/mL could attenuate the activity of gram-negative *Klebsiella pneumoniae* and *Escherichia coli* (Kalaivani et al., 2011). Other researchers obtained alkaloids from leaf extracts inhibiting the growth of *Aspergillus niger, Bacillus subtilis, Candida albicans, Escherichia*

coli, Micrococcus luteus, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Staphylococcus aureus, and *Staphylococcus epidermidis* (Rather et al., 2015).

In our previous study, phytochemical tests were carried out on the ethanolic extract of *Acacia nilotica* leaves, the active ingredients were obtained as shown in the table 5.

Table 5. Identification of the active ingredients in leaves extract of *Acacia nilotica*

Identification	Result
Total Phenol	42,15 mg in 1 g extract
Total Flavonoid	9,06 mg in 1 g extract
Phytochemical screening	
Alkaloid	-/Negative
Flavonoid	+/Positive
Tannin	+/Positive
Saponin	+/Positive
Terpenoid	+/Positive
Anthraquinone	-/Negative
Glycoside	+/Positive

Source : (Jannah S.M, Prabowo, and Kriswandana 2019)

Phenol works as a bactericide by moving the liquid phase (extract) towards the fat phase contained in the cell wall so that the surface tension of the cell wall decreases and causes the permeability of the bacterial cell wall to be disturbed. Furthermore, the phenolic compounds in the cell wall enter the cell and bind to the H atom in the bacterial protein complex resulting in disrupted bacterial cell protein synthesis (Shaheed et al., 2009). Disruption of the permeability of the bacterial cell cytoplasmic membrane also possess in loss of cellular pH gradient, decreased ATP levels, and loss of proton motive force, which lead to cell death of both gram-positive and gram-negative bacteria.

The total flavonoid measured as quercetin was 0.906%. Lipophilic nature will easily penetrate bacterial membranes, especially gram-negative bacteria. Flavonoids will form complex compounds with extracellular proteins by denaturing protein bonds in cell membranes, causing increased permeability of cell membranes and damaged bacterial lysosomes. After that, the bacteria undergo lysis and the secondary metabolite compounds contained in the extract penetrate the cell nucleus which causes the bacteria to not grow (Riedel et al., 2019).

From the results of qualitative phytochemical screening, it was found alkaloids. These compounds that are alkaline with nitrogen atoms will cause coagulation of proteins in the cell wall resulting in disrupted peptidoglycan components in the cell wall, damaged cell wall layers, and inhibited bacterial growth until

it ends in cell death (Kathleen Park Talaro, 2001). Tannins are secondary metabolites that are also found in the leaf extract of *A. nilotica*. Tannins are bacteriostatic that only able to inhibit the growth of bacteria through an adhesin factor in bacteria and form complex bonds with polysaccharides on the bacterial cell wall so that they can inhibit bacterial growth (Riedel et al., 2019). Tannins also will inactivate the reverse transcriptase enzyme and DNA topoisomerase, which is followed by the inactivation of the function of the genetic material as a result, bacterial cells cannot reproduce. It also interferes with the permeability of bacterial cell membranes causing cells to shrink and stunted growth (Moa Megersa, 2014). From the results of the above study, the active ingredients found in *A nilotica* leaves can reduce the number of microbes on the hands and have the potential as an active ingredient for hand sanitizers.

Hand sanitizers cannot replace washing hands with running water and soap, as stated by Alex (Finch, 2020). Hand sanitizers minimize levels of microorganisms by chemically destroying them just as disinfectants kill germs on surfaces around the body (Finch 2020). The continuous use of alcohol has the potential to cause skin irritation. Then, using ethanol extract of *A. nilotica* leaves with the ability to reduce the number of high bacteria will be safer to use as a substitute for alcohol. The alcohol content in the extract has been removed by evaporation. To remove the ethanol in the leaf extract, heating is carried out at a temperature of 80-90^0 C, as it is known that the boiling point of ethanol is 78,4^{0}C. Although no examination was carried out to prove that the extract was free of ethanol, the number of germs that decreases was due to the active ingredients of the extract.

Normal flora on human skin includes *Staphylococcus epidermidis.* It is one of the commonest species, that making up some 90% of the aerobes and occurring in densities of 10^3 –10^4/cm^2. *S. aureus* may be present in the moister regions (Goering et al., 2019). The greatest percentage of decreasing was found at a concentration of 30%, in male respondents was up to 64.84% and 89.61% in the hands of female respondents. If it is seen that the number of germs on men's hands is relatively higher than women's hands. It may possible because of men that have more activities, so they touch more objects that will cause soiling of the hands. Besides that, it may be men do not pay much attention to the cleanliness of their skin (Hijriyati et al., 2016).

CONCLUSION

There was a significant difference in the number of bacteria before and after using hand sanitizer with the active ingredient of *Acacia nilotica* L. leaf ethanol extract. *Acacia nilotica* leaf ethanol extract at a concentration of 30% was able to reduce the number of germs on hands up to 89% that is very high, compared to other dilutions. Therefore, it can be a candidate as an active hand sanitizer.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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