Jurnal Rekayasa Kimia dan Lingkungan (Journal of Chemical Engineering and Environment)
Volume 14, Number 2, Page 163-170, 2019
ISSN 1412-5064, e-ISSN 2356-1661
https://doi.org/ 10.23955/rkl.v14i2.14275

Effectiveness of Durian Peel Extract as A Natural Anti-Bacterial Agent

Nina Arlofa[1,2*], Ismiyati[2], Muhammad Kosasih[2], Nurul H. Fitriyah[2]

[1]Departement of Chemical Engineering, Faculty of Engineering, Universitas Serang Raya, Jl.Raya Cilegon-Serang Km. 05, Drangong, Taktakan, Kota Serang, Banten, Indonesia.
[2]Master in Chemical Engineering Program, Faculty of Engineering, Universitas Muhammadiyah Jakarta, Jl. Cempaka Putih2 Tengah No.27, Jakarta, Indonesia.

*E-mail: nina73arlofa@gmail.com

Article History
Received: 20 August 2019; Received in Revision: 28 October 2019; Accepted: 4 November 2019

Abstract

Durian (durio zibertinus) is a native tropical fruit from Southeast Asia, especially Indonesia. The peel makes up the highest composition of the fruit (60-75%), and is still considered as a waste that causes environmental problems. This study was conducted to do several analyses on durian peel extract. The first was analyzing the secondary metabolite content. The second was analyzing the anti-bacterial activity against gram-negative and gram-positive bacteria. The third was determining the anti-bacterial effectiveness of durian peel extract as a natural anti-bacterial ingredient in hand sanitizer products by using the paper disc method. Durian peel was extracted with ethanol to obtain an extract solution, which was then separated from the solvent and applied as an ingredient in a hand sanitizer formulation. The analysis results showed that durian peel extract contains triterpenoids, alkaloids, and saponins, which are phytochemical compounds that have anti-bacterial function. Durian peel extract at concentration of 1% wt. inhibited the growth of Escherichia coli, Salmonella thyposa and Sthapylococcus aureus with clear-zone diameters of 7.4 mm, 8.2 mm and 8.6 mm, respectively. In antiseptic tests, hand sanitizer samples containing durian peel extract showed that the interaction between the concentration of durian peel extract and the sampling duration simultaneously gave a significant effect in reducing the number of microorganism colonies.

Keywords: Durian peel, natural anti-bacterial agent, hand sanitizer

1. Introduction

Durian (*Durio zibertinus*) is one of popular fruits in Indonesia. In 2016, Indonesia produced 735,423 tonnes of durians (BPS, 2016). Structurally, a durian fruit consists of three parts, which are 20-35% of meat, 60-75% of peel, and 5-15% of seeds (Djaeni, Prasetyaningrum, 2010). In other words, the peel makes up the largest part of the fruit. However, durian peel is still considered a waste and does not have any economic value. During the durian season, therefore, the peel causes an environmental problem. Durian peel contains lignin (15.45%), holocellulose (73.45%) and α-cellulose (60.45%) (Khedari et al., 2004), which makes it potential to be transformed into bio-briquette with 77.87% carbon content (Nuriana, 2014), second generation bioethanol (Aditya et al., 2016), and low-cost partition particle boards (Khedari et al., 2004). Previous studies demonstrated that the polysaccharide gel of durian peel extract at 1.25%wt. concentration exhibited an inhibitory effect on *S. aureus*, whereas 2.5%wt. extract did similar to *E. coli* (Lipipun et al., 2002). Other studies also showed that durian peel extract inhibited the growth of *Pseudomonas aeruginosa* bacterium at a minimum concentration of 125 µg/ml (Balram Prasad sah et al., 2014), as well as the growth of *Candida albicans* fungus with a minimum concentration of 25% (Amelia, 2010). Anti-bacterial properties of durian peel extract which can inhibit the growth of gram-positive and negative bacteria can be utilized in the health and pharmaceutical industries

Currently, most hand sanitizer or antiseptic gel products utilizes alcohol as their antibacterial active compound. However, an alcohol-based hand sanitizer is often not safe for use. Alcohol can dissolve the oil layer or sebum produced by the sebaceous glands of human skin, that functions as skin protection against microbial infections. Furthermore, frequent alcohol applications

can cause skin dryness and irritation (Brooks, 2005).

Antibacterial activities exhibited by durian peel extract show that it can be used as a safe alternative for natural antibacterial compound, such as that for hand sanitizer. This is important because hand sanitizer uses as health product have been increasing with the increase of public awareness in personal hygiene. The objective of this study is to determine whether the durian peel extract that exhibits antibacterial activities can be used to replace alcohol in hand sanitizer.

Utilization of anti-bacterial properties of durian peel extract has been applied to survagingival plaque. Durian peel extract is a liquid extract with ethanol solvent. Liquid extract of durian peel was made into gel by the addition of carboxy methyl cellulose and aquades. Durian peel extract has significant differences of inhibitory zone from each concentration on supragingival plaque growth. The best concentration range was 6.25%-12.5%. (Permatasari, et al., 2015). In this study durian peel extract was obtained by maceration process. Durian peel extract used is a solid extract after separation with ethanol solvent and water content derived from ethanol or durian peel. With the different extraction processes carried out, it is expected that the effectiveness of durian peel extract as a natural anti-bacterial agent on the hand sanitizer is increasing.

2. Methodology

2.1. Materials and Equipment

The materials used in this research were durian peel (Pandenglang, Indonesia), nutrient agar, nutrient broth, and Mueller Hinton agar (Sigma Aldrich), *Escherichia coli* culture*, Salmonela thyposa* culture, *Staphylococcus aureus* culture from the collection of provincial laboratories in Banten. The instruments used in this research were an incubator, an autoclave for sterilization, a rotary evaporator, and FTIR (Cary 630 FTIR Spectrometer Agilent) for sample analyses.

2.2. Extraction of Durian Peel

Extraction of durian peel by maceration method for 24 hours using ethanol as a solvent. The ethanol solvent was separated using a rotary evaporator set at 175 mbar and 40^{o}C. Samples were further dehydrated in the rotary evaporator set at 72 mbar and 40^{o}C, followed by oven drying at 40^{0}C.

2.2. Phytochemical Test of Durian Peel Extract

Identification of secondary metabolites content in durian peel extract with phytochemical testing and FTIR analysis. Phytochemical screenings consisted of alkaloid, triterpenoid/ steroid, flavonoid, saponin, tannin, and phenol hydroquinone tests.

2.3. Anti-Bacterial Activity Test of Durian Peel Extract

Tests of antibacterial effectiveness of durian peel extract at concentrations of 1%, 2%, 3%, 4%, and 5% of wt. on cultures of *S. areus, S. typhosa,* and *E. coli* using disk diffusion methods (Kirby & Bauer test).

2.4. Manufacture of Hand Sanitizer Gel and its Anti-Bacterial Test

Preparation of antiseptic hand sanitizer gel by homogenizing 0.5% CMC in water with stirring at a speed of 5000 rpm for 60 minutes as the base gel with the addition of durian peel extract at concentrations of 1%, 2%, 3%, 4% and 5% wt.

Tests of anti-bacterial and antiseptic of hand sanitizer containing durian peel extract against a negative control and a commercial hand sanitizer. Anti-bacterial activity tests were performed using disk diffusion methods (Kirby & Bauer test). Antiseptic tests were carried out with a replication method in the following way: hands were washed with tap water then dried. Hand sanitizer gel, at 0.5 ml, was applied to palms of hands and let stand for one minute. Next, thumbprints were place on nutrient-densed solid media at 0-, 30-, and 60-minute intervals. The media were incubated at 37ºC for 24 hours. After incubation, the number of bacterial colonies was counted.

3. Results and Discussion

3.1. Secondary Metabolite Contents of Durian Peel Extract

The extraction of the durian peel was performed using maceration method with ethanol solvent. There was 34.9 grams of

extract obtained from 400 grams of durian peel, which translated into 8.725% yield. The use of ethanol solvent in a previous study to isolate water-soluble polysaccharides, which was further dissolved in acidic ethanol, exhibited antibacterial activities on gram-positive and gram-negative bacteria (Lipipun et al., 2002).

Triterpenoids were detected in Durian peel extract as Liebermann-Burchard test resulted in a deep purple solution. A series of tests using Meyer, Dragendorff, and Wagner reagents resulted in white, red, and brown precipitations, respectively, indicating the presence of alkaloids in the extract. Saponins were also present as indicated by the formation of foam that appeared for ten minutes in hot water and did not disappear with the addition of one drop of HCl (Harborne, 2006). The results are summarized in Table 1.

Table 1. Qualitative analysis results of secondary metabolites in durian peel extract

Tests	Results	Remarks	Figure Number
Triterpenoids	+	Deep purple	1
Flavonoids	-	No color change	2
Phenol Hydroquinones	-	No color change	3
Tannin	-	No color change	4
Saponins	+	A stable foam forming for 10 minutes and resistant to one drop of HCl	5
Alkaloids			
Dragendorff	+	Red precipitation	6
Meyer	+	White precipitation	7
Wagner	+	Brown precipitation	8

Figures 1 - 8 show results of phytochemical tests as described in Table 1. The presence of triterpenoids and alkaloids in durian peel extract is supported by Fourier-transform infrared (FT-IR) spectroscopy analyses as shown in Figure 9. There is a typical O-H stretching band for triterpenoids for Carbon number three (figure 10) at the wavenumber of 3274 cm^{-1}. At wavenumber of 2922 cm^{-1} and 2852 cm^{-1}, there are typical aliphatic C-H stretching bands indicating that the triterpenoids in durian peel extract consist of only aliphatic C-H. The spectra indicate the absence of typical aromatic C-H bands at the wavelengths of 3010-3020 cm^{-1} and C=C bands at the wavelengths of 1600 – 1400 cm^{-1} indicating the absence of aromatic compounds. These results also reveal that the alkaloid contents of durian peel extract are those of the aliphatic group.

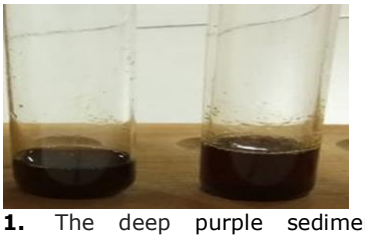

Figure 1. The deep purple sediment was formed in the triterpenoid test

Figure 2. The green, red or yellow colors did not form in the flavonoids test

Figure 3. The green, red, purple or black colors did not appear in the phenol hydroquinones test

Figure 4. The dark blue or black color did not appear in the tannin test

Figure 5. The stable foam was presence even with the addition of one drop HCl in the saponin test

Figure 6. The orange sediment was performed in Dragendorff reagents

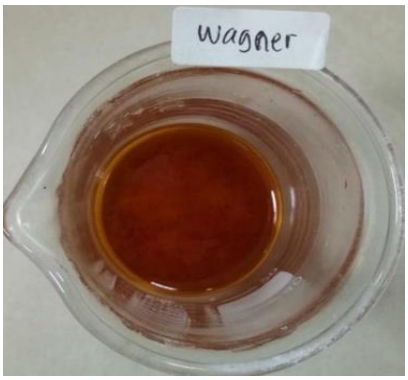

Figure 8. The brown sediment was shown in Wagner's reagent

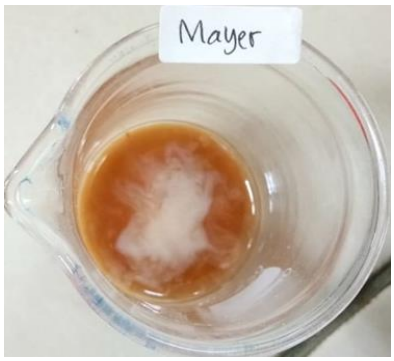

Figure7. The white sediment was appeared in Mayer's reagents

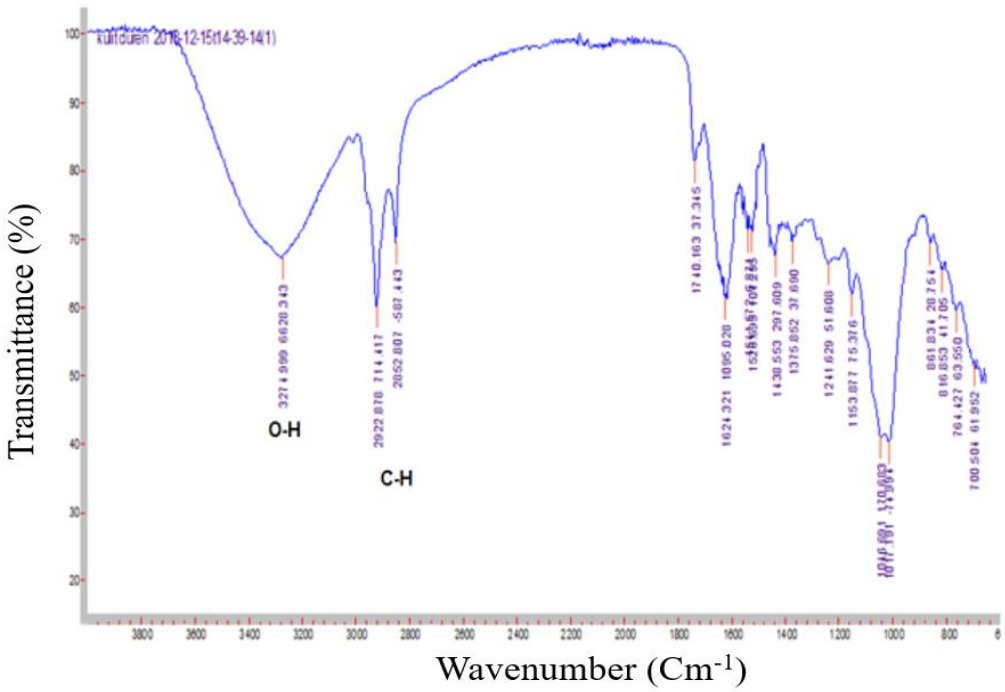

Figure 9. FTIR Spectra of Durian Peel Extract

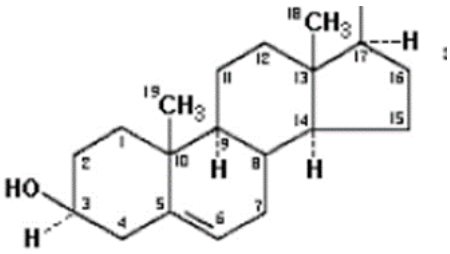

Figure 10. O-H stretching band for triterpenoids for Carbon number 3.

Saponin is a glycosidic triterpenoid, in which the O-H bond on Carbon number 3 is bound to a glucose molecule, and therefore saponin is a derivative of triterpenoid or steroid. Durian peel fiber contains cellulose and hemicellulose (Manshor et al., 2012) that can be hydrolyzed into glucose. With the presence of these precursors, saponin compounds were very likely generated.

3.2. Antibacterial Activity of Durian Peel Extract

The results of antibacterial activity tests in Table 2 show that durian peel extract inhibited the growth of gram-negative and gram-positive bacteria. The gram-negative bacteria used in these tests were *E. coli* and *S. typhosa*, whereas the gram-positive was *S. aureus*. Durian peel extract demonstrated growth inhibition on all bacterial species tested. The paper disc tests results showed that there was a positive correlation between the concentrations of durian peel extract and diameter of inhibition zones; the higher the concentrations of durian peel extract, the larger the area of inhibition zones. The graph of the anti-bacterial activity test results for durian peel extract is shown in Figure 11.

Durian peel extract at 1%wt. concentration produced inhibition zones with an average diameter of 7.4 mm on *E. coli* cultures, whereas at 5% the average diameter was 16.2 mm. On *S. aureus* cultures, 1%wt. of durian peel extract produced inhibition zones with an average diameter of 8.6 mm, while it produced that of 14.5 mm at 5%wt. Addition of durian peel extract on *S. typhosa* cultures produced similar patterns, in which 1%wt. extract generated inhibition zones with an average diameter of of 8.2 mm and 5%wt. extract produced that of 13.6 mm.
Inhibition zone shows the capacity of durian peel extract in inhibiting the growth activity of the bacteria being tested. The larger the area of inhibition zone, the stronger the capacity of durian peel extract in inhibiting bacterial growth. The strength of antibacterial activity based on the size of inhibition zone can be described as very strong at the diameter of 2 cm or greater, strong at 1-2 cm, moderate at 0,5-1 cm, and weak at less than 0,5 cm (Hong et al., 2002). The results of this study show that durian peel extract exhibits strong antibacterial activity at 3% concentrations tested.

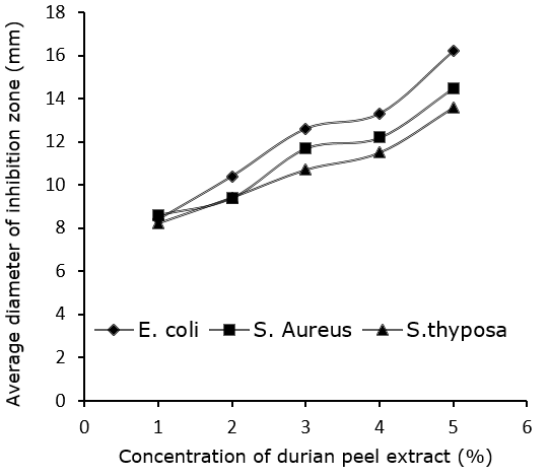

Figure 11. Results of anti-bacterial activity test for durian peel extract.

Inhibitory action of durian peel extract on bacterial growth is a result of its polysaccharide contents (Lipipiun et al., 2002) as well as the presence of alkaloids, saponins, and triterpenoids. Alkaloids are antibacterial because they disrupt the formation of peptydoglycans on bacterial cell walls resulting in incomplete wall formation leading to cell death (Rika et al., 2014). Another antibacterial mechanism of alkaloids is achieved by the presence of nitrogen-containing bases that react with amino acids required in the formation of bacterial cell wall and DNA. These reactions cause changes on the structure and sequence of amino acids that lead to genetic imbalances on the DNA chains, which are manifested in DNA degradation and bacterial cellulolytic processes (Karou et al., 2005).

The antibacterial mechanisms of terpenoids are achieved in the destruction of the bacterial cell membrane (Cowan, 1999). This process takes place when an active antibacterial compound reacts with the active regions of the cell membrane to dissolve the lipid constituents and increase permeability. Bacterial cell membrane consists of phospholipids and protein molecules. The increased permeability allows antibacterial compounds to enter the cells and initiate cellular breakdown or

cytoplasmic coagulation of bacterial cells (Rahman et al., 2017).

Antibacterial property of saponins comes from the ability of these molecules to reduce the surface tension of bacterial cell wall and increase the permeability of the cell membrane. The destruction of the cell membrane leads to bacterial cell death (Harborne, 2006). Saponins diffuse through the cell wall and outer membrane to bind with the cytoplasmic membrane to disturb and reduce the cell membrane stability. Saponins diffusion releases the cytoplasm into the outside environment and leads to bacterial cell death. Antimicrobial agents that disturb the cytoplasmic membranes are considered bactericides (Rika et al., 2014).

3.3. Hand Sanitizer Containing Durian Peel Extract

3.3.1. Formulation of Hand Sanitizing Gel

Preparations of hand sanitizing gel were conducted with several formulas to yield the best product. The compositions of hand sanitizer were varied according to the durian peel extract contents at 1%, 2%, 3%, 4%, and 5%, while carboxymethyl cellulose (CMC) concentration was determined at 0.5% for all samples. These formulations were based on a previous study demonstrating that durian peel extract at 1% concentration was sufficient to inhibit the growth of *S. aureus* bacteria, while that at 1.5% did similar to *E. coli* (Aditya et al., 2016). The concentration of CMC as a gelling agent for hand sanitizer had been determined previously that less than 0.5% resulted in hand sanitizer being too thin, while more than 0.5% would make it too thick (Gandasasmita, 2009).

3.3.2. Anti-bacterial of Hand Sanitizer Containing Durian Peel Extract

The inhibition zones generated by hand sanitizer containing durian peel extract were smaller than those of durian peel extract (Figure 12). This fact might have been caused by absorption capacity of paper disc being less for hand sanitizing gel than durian peel extract solution. There was less durian peel extract absorbed by the paper discs when it was incorporated in hand sanitizer, and therefore there was less amount of active ingredients to produce changes that would disrupt bacterial cell wall structure and increase bacterial membrane permeability. The anti-bacterial activity of the hand sanitizer gel with the addition of 1% durian peel extract could not be detected. This was cause by the small amount of durian peel extract in the gel can be absorbed by the paper disc. The concentration of durian peel extract at 2% inhibited *E. coli* growth as demonstrated by the zone of inhibition at 7.6 mm diameter. The greatest zone of inhibition on *E. coli* with 12.6 mm diameter was achieved at 5% concentration of durian peel extract. On *S. aureus* cultures, 2% durian peel extract produced inhibition zone of 7.2 mm diameter, and the diameter of largest zone was 11.8 mm produced by 5% durian peel extract formulation.

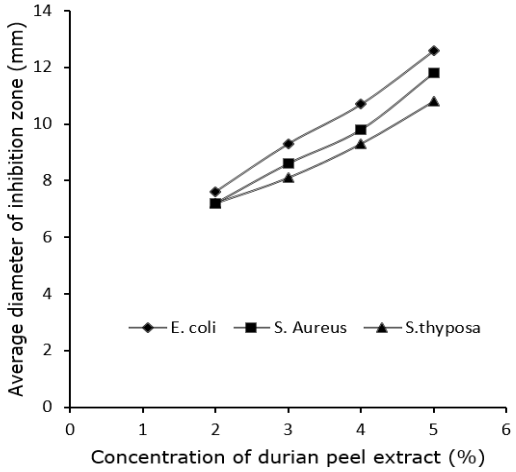

Figure 12. Results of anti-bacterial test on hand sanitizer containing durian peel extract.

Durian peel extract at 2% concentration in hand sanitizer when exposed on *S. typhosa* produced 7.2 mm-diameter zone of inhibition, and the largest zone of inhibition had 10.8 mm diameter at 5% concentration. The Hand sanitizer with the addition of 5% durian peel extract has a strong antibacterial activity. Anova (analysis of variance) results show that durian peel extract in hand sanitizer produces a significant effect (positive with $t < 0.05$) on growth inhibition of *E. coli* with a correlation value of 0.992 (strong correlation). The same results were observed for *S. aureus* and *S. typhosa*, in which additions of durian peel extract in hand sanitizer significantly (positive with $t < 0.05$) inhibited the growths of *S. aureus* and *S. typhosa*, with correlation values of 0.976 and 0.969, respectively. In Anova, t value shows that durian peel extract has a significant influence in inhibiting the growth of *E. coli, S, aureus* and *S. typosa* bacteria.

The higher concentration of durian peel extracts the stronger inhibitory power to the growth of *E. coli, S. aureus* and *S. typosa* bacteria.

3.3.3. Antiseptic of Hand Sanitizing Gel Containing Durian Peel Extract

The results of effectiveness tests, using a replication method, on the antiseptic capacity of hand sanitizing gel containing durian peel extract show that the hand sanitizing gel could reduce the number of skin normal flora (Table 2). The higher the durian peel extract concentration, the lower the number of colonies. There was no colony formed at the concentration of 5%.

Table 2. Results of antiseptic test for hand sanitizer containing durian peel extract

Concentration of Durian Peel Extract (%)	Sampling Duration (minute)	Number of Colonies	
		1st Run	2nd Run
1%	0	14	12
	30	6	5
	60	3	3
2%	0	9	8
	30	5	5
	60	2	1
3%	0	4	5
	30	0	0
	60	0	0
4%	0	2	3
	30	0	0
	60	0	0
5%	0	0	0
	30	0	0
	60	0	0

Anova of multiple regression tests result in a constant of 9.993. The variable coefficient of the durian peel extract concentration (x_1) is -1.733 ($t < 0.05$), which means that there is a significant negative effect of the concentrations of durian peel extract on the variable y that is the number of colonies. The variable coefficient of the sampling duration (x_2) is -4.000 ($t < 0.05$), which also means that there is a significant negative effect of the duration of sampling on the number of colonies. The multiple regression analyses on the antiseptic tests show the interaction between the concentrations of durian peel extract in hand sanitizer and sampling durations which is represented in the following formula:

$y = 9.933 - 1.733\ x_1 - 4\ x_2$

in which:
y = Colony number
x_1= Concentration of durian peel extract (%)
x_2 = Sampling duration (hour)

This equation shows a significant interaction ($t < 0.05$) between durian peel extract concentrations and sampling durations, in which both variables simultaneously lower the number of microbial colonies. The higher the concentrations of durian peel extract and the longer the sampling duration, the lower the number of microbial colonies.

4. Conclusions

This study successfully performed extraction on durian peel in ethanol and prepared hand sanitizing gel containing the extract and CMC. Phytochemistry tests show that durian peel extract contains alkaloids, saponins, and triterpenoids. Durian peel extract inhibits the growth of gram-negative bacteria, *Escherichia coli* and *Salmonella typhosa*, and gram-positive bacteria, *Staphylococcus aureus*, in both direct application of extract and as incorporated in hand sanitizer. The inhibition zones produced by hand sanitizing gel containing durian peel extract are smaller than those produced by direct applications of durian peel extract. This phenomenon is a result of lower absorption capacity of paper discs for hand sanitizing gel compared to the extract solution. Durian peel extract in hand sanitizer has a significant effect (positive, $t < 0.05$) on bacterial growth inhibition of *E. coli*, *S. aureus* and *S. typhosa* with correlation values of 0.992, 0.976 and 0.696, respectively. In the antiseptic tests, there is a significant interaction ($t < 0.05$) between the concentrations of durian peel extract in hand sanitizer and the sampling duration as represented in the following equation: $y = 9.933 - 1.733\ x_1 - 4\ x_2$. This equation shows that concentrations of durian peel extract in hand sanitizer and sampling duration simultaneously lower the number of microbial colonies.

Acknowledgment

We would like to appreciate the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for *Hibah Tesis Magister* research grant in 2019, as well as Master in Chemical Engineering Program at the Faculty of Engineering in Universitas Muhammadiyah Jakarta (UMJ) and LPPM UMJ

for all facilities and supports during the implementation of this thesis research.

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