ANTIBACTERIAL ACTIVITY OF SOIL WATER AND SOAP WATER DURING SERTU PROCESS

Syaza Azhari[i], Aina Abdul Rahman[ii] & Mohd Mahyeddin Mohd Salleh[iii]

[i] (Corresponding author) Senior lecturer, Faculty of Science and Technology, Universiti Sains Islam Malaysia. syaza@usim.edu.my
[ii] Research assistant, Faculty of Science and Technology, Universiti Sains Islam Malaysia. ainaabdulrahman95@gmail.com
[iii] Senior lecturer, Faculty of Syariah and Law, Universiti Sains Islam Malaysia. mahyeddin@usim.edu.my

Abstract

In this study, five samples of natural soils, two commercial soils and one commercial clay soil were evaluated pertaining to their antibacterial activity during sertu process. The antibacterial activity of all samples was obtained using total plate count method. Clay soil (S1) showed to be the best among those natural and commercial soils. S1 contain high in copper (Cu) ion compared to the other soil samples which helps in antibacterial activity as Cu ion has the ability to damage directly the bacterial proteins. Cu ion is well known due to its antibacterial strength since it can change the bacterial membranes permeability, forming highly damaging hydroxyls radicals and damage the bacterial cells through interference with DNA. Commercial clay soap (S8) showed the best antibacterial activity among the samples with no bacterial growth on Plate Count Agar (PCA) from beginning of washing. It is best to start sertu process with soil water followed by another 6 times of washing with air mutlak. Hence, the sertu process can be done either using soil or clay soap as long as the process follows the rules set by Islamic law.

Keywords: *natural soils, commercial soils, commercial clay soil, antibacterial activity, sertu process*

INTRODUCTION

The study of antibacterial properties towards soil minerals has been increase recently in modern and traditional techniques. Previous study proved that the bactericidal properties of clay are due to the exchangeable cations absorbed to the particles such as Al, Zn, Cu, Mg and Ni (Haydel et al., 2008). However, not all natural clay minerals have the antibacterial properties. Therefore, some modifications to clay minerals have been investigated such as mixing the clay with water, which contain a unique balance of salts to permeate with the antibacterial properties (Gaskell and Hamilton, 2014).This technique clearly resembling the cleansing or sertu process in Islam.

Sertu is purification of any parts of the body that came in contact with *mughallazah* najis (dog or swine) by seven steps of cleansing, whereby the first step is mixing the water with the soil followed by the subsequent six times cleansing using *mutlak* water (Al-Khin, Al-Bugha & Al-Sharbaji, 2007*).* The Islamic ruling of *sertu* is based on several arguments mentioned in the hadith recorded by Imam Muslim in his *saḥiḥ* as follows :

عَنْ أَبِي هُرَيْرَةَ، عَنِ النَّبِيِّ — صلى الله عليه وسلم – : طَهُورُ إِنَاءِ أَحَدِكُمْ إِذَا وَلَغَ فِيهِ الْكَلْبُ، أَنْ يَغْسِلَهُ سَبْعَ مَرَّاتٍ، أُولاَهُنَّ بِالتُّرَابِ

> "Abū Hurairah R.A. narrates that the Prophet S.A.W. said: "The purification of the utensil belonging to any one of you, after it is licked by a dog, lies in washing it seven times, using sand for the first time" (Muslim, t.th., Kitāb al- Ṭahārah, Bāb Ḥukm Wulūgh al-Kalb).

Moreover, in another *hadith* narrated by Imam Muslim, the Prophet S.A.W. commands that the water in a container that has been licked by a dog should be discarded, and after that the container must be washed seven times (Salleh, Halim & Abd Aziz, 2020). According to Ibn Daqīq al-ʿAid (n.d), the two *hadith* clearly show that dogs are categorized as *najāsah*. Although the texts of the *hadith* only mention the licking of dogs as *najis*, but according to the majority of scholars, the other parts of the dogs, such as sweat, skin, hair, and bone are also classified as *najis* based on the method *of qiyās.* This is because if the dog's mouth, which is considered as the cleanest part of the dog, is deemed as *najis* (because of the mouth licking habit of dogs), then the other parts of the dog would definitely be considered as *najis* as well (Al-Zuhaili, 2007). In sect *Shāfi'ī mazhab*, the *najāsah* of swine is equated with the dogs according to the method of *qiyās,* because of the condition of swine that is much worse than a dog (Al-Shirazi, n.d).

Generally, soil is a natural organic particle which remain the most significant target for researchers to develop and discover the antibacterial and antibiotics properties. Several techniques such as disc total plate count method and disc diffusion method can be used to identify the antibacterial properties in soil. Lihan et al., (2015) found that besides the antibacterial properties, soil also exhibit the capabilities to hinder the growth of fungus and bacteria such as Staphylococcus aureus, Escherichia coli, Salmonella typhi and Enterobacter aerogenes.

Apart from that, research by Eriatna (2017) has been done on the antibacterial study of clay soap and commercial soap without the addition of clay against the bacteria in dog's saliva through total plate count method. The result showed that soap containing clay give the best antibacterial activity compared to the soap without the additional of clay. This is due to the fact that clay contain high cation exchange

capacity (CEC) where the greater CEC resulting stronger ionic bond between the clay surface and the bacterial cell wall that give better antibacterial properties.

The antibacterial activity towards the clay liquid soap also has been done showed that the clay liquid soap able to inhibit Staphylococcus aureus through the inhibition zone by disc diffusion method (Faikoh, 2017). The research stated that, the main component in clay mineral such as iron (Fe), zinc (Zn), copper (Cu), and nickel (Ni) are the significant influence that responsible in the mechanism of antibacterial properties in the clay liquid soap. The ion exchange in clay can disrupt the permeability of the cell membrane that give a significant role in bacterial damage as well as kill the bacteria.

Hence, research has been conducted to extract antibiotics from natural products such as soil since it contains hundred million of microorganism including bacteria, fungi, protozoa, that are essentials to human. It has been found that the presence of antibiotics named malacidins was able to attack and kill many types of super bugs (Hover et al., 2018). The malacidins is an active antibiotic used to treat multi-drugs resistance pathogens such as Staphylococcus aureus. Staphylococcus aureus is a gram-negative bacteria, mainly found in dog saliva that can caused wide range of illness. Therefore, a lot of researches has been conducted to determine the antibiotics that might contain inside the soil to be further used in therapeutics and antibiotics fields.

METHODOLOGY

Sampling of Soil And Clay Soap

Seven different types of soil which are clay (S1), sand (S2), loam (S3), top (S4), mix (S5) and two commercial soils (S6 and S7) were used in this study. The soils were collected around Nilai, Negeri Sembilan and the commercial soils were obtained from laboratory (Global Dibagh, Selangor, Malaysia). Commercial clay soap (S8) was purchased from a local store in Negeri Sembilan, Malaysia.

Preparation of Soil Sample and Clay Soap

50.0 g of soil was collected into a plastic bag, sealed and labeled. The large fractions of the soil samples were removed and crushed using a mortar. The soils were mixed with a clean tap water with ratio of 1:5 (10 g soil: 50 ml distilled water) in the Erlenmeyer flask to undergo *sertu* process (Azhari et al., 2019).

Preparation of Dog's Saliva Bacteria

Dog's saliva swab samples inoculated in 10 ml of Brain Heart Infusion (BHI) broth were obtained from Merci Veterinary Clinic and Surgery in Desa Melati, Nilai. Then, the swab samples were vortexed for 30 sec and 1 ml of saliva samples were transferred

into 9 ml of BHI broth to grow the bacteria and were incubated at 37°C for 24 hr. Next, 1 ml of 24 hr bacterial culture in the BHI broth undergo serial dilution in 9 ml of peptone water and was homogenized using vortex mixer and the step was repeated until five times dilution ($x10^{-5}$). Then, 0.1 ml of bacteria suspension ($x10^{-5}$) was spiked onto Plate Count Agar (PCA) using 100 µL micropipette and was swabbed using sterile cotton swab. The PCA was further incubated for 24 hr at 37°C and the bacterial count (cfu/ml) was calculated as below:

$$\text{Bacterial count (CFU/ml)} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume of culture plate}}$$

Sertu Process

Sertu process was carried out by first washing of the sample bottle with soil water and six times with tap water. 1 ml of diluted bacteria suspension ($x10^{-5}$) was added to a sterilize universal bottle and was homogenized using vortex mixer. Then, 6 ml of soil water was added to the sample bottle to undergo *sertu* process and was homogenized and swabbed onto the PCA using cotton swab. After that, the step was repeated for 2nd until 7th of washing using 6 ml tap water and each step was swabbed onto the PCA. All PCA plates were incubated at 37°C for 24 hr. The bacterial count of each PCA plate were calculated using bacterial count (CFU/ml) formula.

RESULTS AND DISCUSSION

The antibacterial activity of five natural soil (S1, S2, S3, S4 and S5), commercial soil (S6 and S7), and commercial clay soap (S8) samples were determined by total plate count method. The best antibacterial activity was observed and obtained by the 7th washing of *sertu* process.

Total Bacteria of Dog's Saliva

The amount of pure and diluted (10^{-5}) dog's saliva bacteria were observed on PCA plates incubated at 37°C for 24 hr as shown in Figure 1 (a) and (b). The purpose of dilution is to reduce the amount of the bacteria count in the saliva. Figure 1 (a) showed too numerous to count (TNTC) bacterial colonies of pure dog's saliva due to many bacteria that might exist in dog's saliva such as *Dipylidium caninum, Echinococcus spp, Staphylococcus aureus, Escherichia coli,* and *Salmonella typhi* (Yusof et al., 2017). *Capnocytophaga canimorsus* might contain in dog's saliva that can lead to organ failure and ultimately cause death. It can be transferred easily to human body either through dog licking or biting. Therefore, the bacteria need to be diluted for the safety purpose in order to avoid any infection to the researchers during the analysis process. The bacterial count in diluted saliva as shown in Figure 1(b) was 1.02×10^6 CFU/ml.

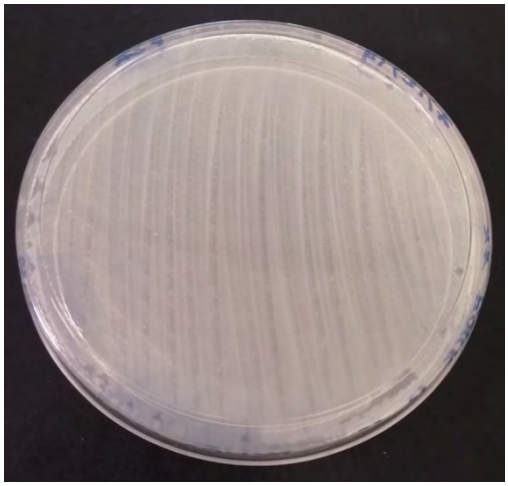
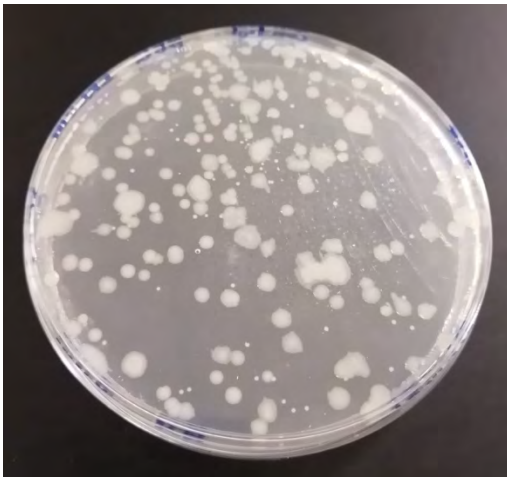
a)  b) 

Figure 1 (a) Pure dog's saliva bacteria and (b) diluted (10^{-5}) dog's saliva growth on PCA after incubated at 37°C for 24 hr

In this research, the focused was on the determination of soil water and commercial clay soap against the dog saliva's bacteria. The cleansing steps carried out as stipulated in hadith where it required seven times of washing. The first wash is a mixture of water with soil and subsequently followed by six times of washing using *air mutlak*. The definition of *air mutlak* is a clean water and capable of cleansing. The types of *air mutlak* that have been used in this research was tap water.

Antibacterial Activity of Natural Soil Water

The results of antibacterial activity of natural soil water samples during *sertu* process by swabbing the bacteria onto the PCA were showed in Table 1. The *sertu* process was carried out with seven times of washing where the first washed was using soil water followed by six times of washing with tap water. The number of total bacteria (CFU/ml) of each sample were calculated for every sequence of 1st, 2nd, 3rd, 4th, 5th, 6th and 7th times of washing step and the control of each samples were also determined in this study.

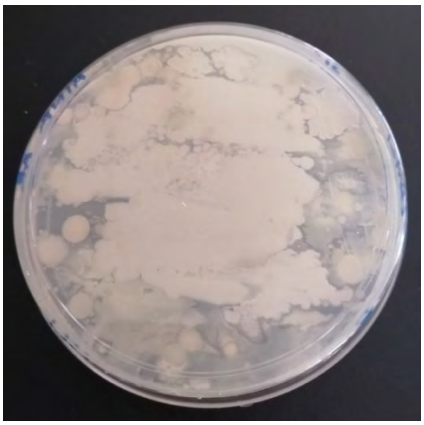
The bacterial count in all control soil water samples resulted as TNTC and no growth of bacteria for control commercial clay soap (Figure 2 (a), (b), (c), (d), (e), (f), (g) and (h). However, it is unknown that it is either good or bad bacteria that can help to remove the bacteria. Soil is a complex ecosystem that hold tremendous numbers of various living organisms such as microorganisms. Besides, the microbes existed on the PCA might contain some good and bad bacteria that may help for antibacterial activity. Lihan et al. (2015) stated that many microbes that occupy in soil have the ability to produce useful antibiotics as protective substances against pathogenic microbes.

Table 1: The bacterial count for clay (S1), sand (S2), loam (S3), top (S4) and mix soil (S5) during *sertu* process

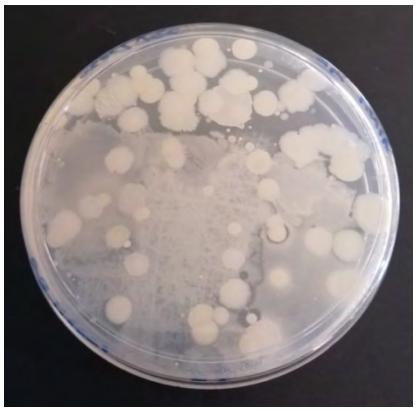
Soil	Bacterial Count (CFU/ml)							
	1st wash	2nd wash	3rd wash	4th wash	5th wash	6th wash	7th wash	Control (Soil)
Clay (S1)	TNTC	4.0 x 10^6	1.0 x 10^6	0	0	0	0	TNTC
Sand (S2)	TNTC	8.0 x 10^6	1.0 x 10^6	0	0	0	0	TNTC
Loam (S3)	TNTC	TNTC	8.0 x 10^6	3.0 x 10^6	2.0 x 10^6	1.0 x 10^6	1.0 x 10^6	TNTC
Top Soil (S4)	TNTC	7.0 x 10^6	5.0 x 10^6	0	0	0	0	TNTC
Mix Soil (S5)	TNTC	8.0 x 10^6	3.0 x 10^6	3.0 x 10^6	3.0 x 10^6	2.0 x 10^6	0	TNTC

For natural soil water samples (S1, S2, S3, S4, and S5), the antibacterial activity during *sertu* process for the samples were showed in Table 1. After seven times of washing step of *sertu* process, the microbial count in five natural soil water samples were decreased. S1, S2 and S4 showed the best antibacterial activity with no growth (0 CFU/ml) of bacterial count during 4th time of washing as shown in Table 1. S5 showed lower bacterial count compared to S3 during 7th time of washing, 0 CFU/ml and 1.0 x 10^6 CFU/ml respectively.

a)



b)



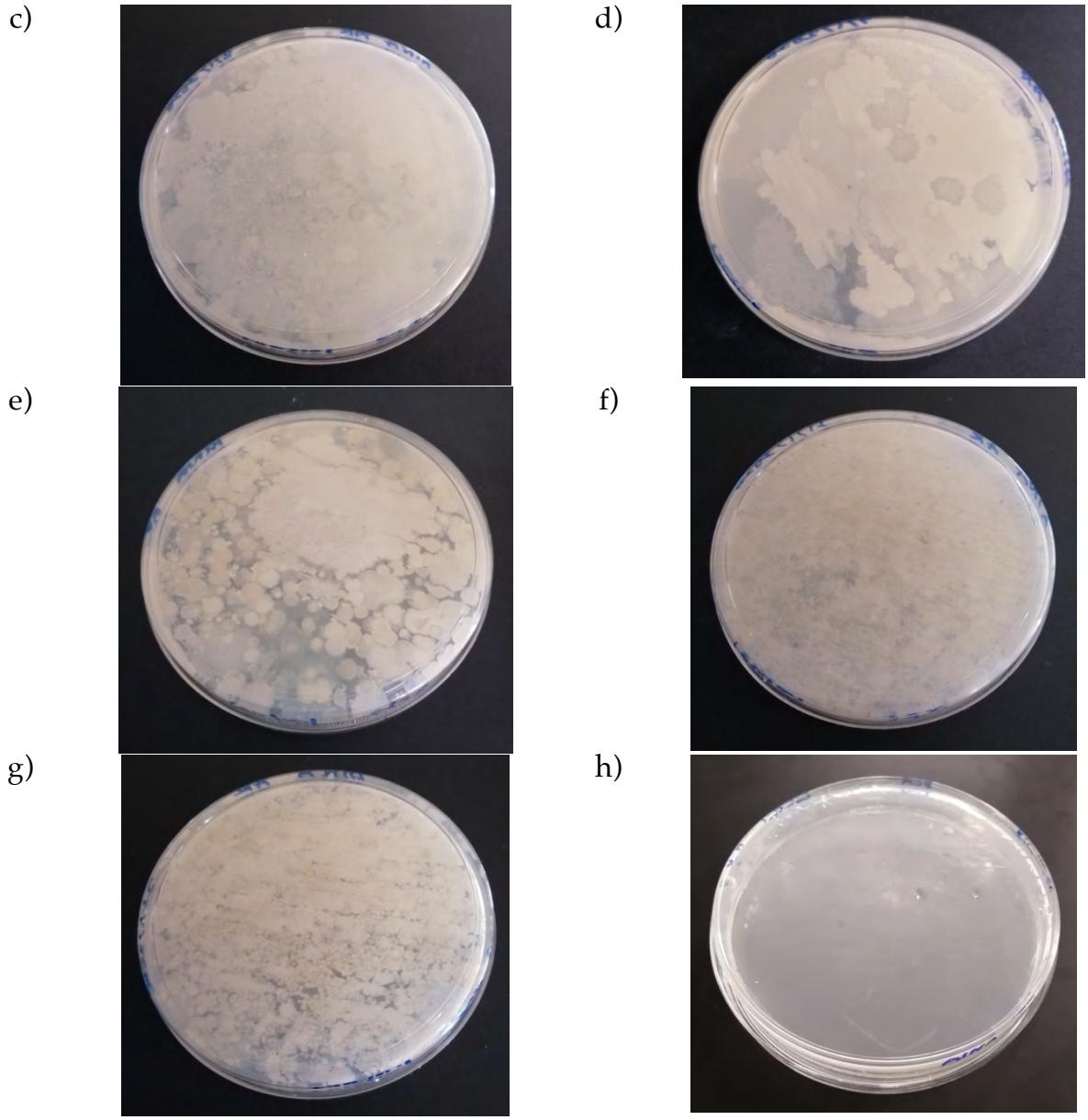


Figure 2: The growth of bacteria in (a) clay soil (S1), (b) sand soil (S2), (c) loam soil (S3), (d) top soil (S4), (e) mix soil (S5), (f) commercial soil A (S6), (g) commercial soil B (S7) on, (h) commercial clay soap (S8) on PCA after incubated at 37°C for 24 hr.

Azhari et al. (2019) stated that clay soil contain high in Cu ion compared to the other soil samples which helps in antibacterial activity as Cu ion has the ability to damage directly the bacterial proteins. Cu ion is well known due to its antibacterial strength since it can change the bacterial membranes permeability, forming highly damaging hydroxyls radicals and damage the bacterial cells through interference with DNA (Villapun et al., 2016). In addition, S1 was also high of NO_3 ion which also helps for antibacterial activity as NO_3 ion usually bind with silver ion forming silver nitrate that prevents division of cell and damages the cell wall of bacteria (Mcdonnell and Russell, 1999).

There is an electrostatic reaction between the cations and negatively charged cell wall of bacteria and undergo anti-adhesion reaction. The bacterial cell wall is said to be negative because of several factors. In gram positive bacteria the reason of negative charge is due to the presence of teichoic acid that are linked to the underlying plasma membrane. The teichoic acid is negatively charge due to presence of phosphate group. Meanwhile, in gram negative bacteria, there is presence of lipopolysaccharides that help in conferring the net negative charge of the cell wall of GN-B. Apart from that, the slightly acidic value of S1 also enhance the antibacterial activity by disrupting the cell wall of bacteria.

Some researchers have claimed that the bactericidal properties of clay are referring to the exchangeable cations absorbed to the particles such as Al, Zn, Cu, Mg and Ni (Haydel et al., 2008). Clay mixed with water contain a unique balance of salts that clearly resemble the way of Islamic cleansing or *sertu* process (Gaskell and Hamilton, 2014). Gram-positive bacteria have thicker cell wall peptidoglycans compared to Gram-negative bacteria.

Antibacterial Activity of Commercial Soil and Commercial Clay Soap

The antibacterial activity of commercial soils (S6 and S7) and commercial soap (S8) were presented in Table 2. During 6th time of washing for S6 and S7 resulted 0 CFU/ml. Azhari et al. (2019) stated that commercial clay soap contain high concentration of Ca ion that help the soil exhibit good antibacterial properties as Ca ion was an active membrane against *Staphylococcus aureus* by disrupting the bacterial membranes or cell walls and inhibit the bacteria growth (Xie and Yang, 2016).

Table 2: The bacterial count for commercial soil A (S6), commercial soil B (S7) and commercial clay soap (S8) during *sertu* process

Soil	Bacterial Count (CFU/ml)							
	1st wash	2nd wash	3rd wash	4th wash	5th wash	6th wash	7th wash	Control Plate
Commercial Soil A (S6)	TNTC	TNTC	2.8 x 10^7	1.1 x 10^7	4.0 x 10^6	0	0	TNTC
Commercial Soil B (S7)	TNTC	TNTC	9.0 x 10^6	7.0 x 10^6	2.0 x 10^6	0	0	TNTC
Clay Soap (S8)	0	0	0	0	0	0	0	0

As development of clay soap for Islamic cleansing has been diversified for industrial and environmental uses, the antibacterial activity of clay soap (S8) was determined in this study (Table 2). The result showed that there was no bacterial growth on PCA for commercial clay soap (S8). This indicate that the commercial clay soap present better antibacterial properties than the natural soils (S1, S2, S3, S4 and S5) and commercial soils (S6 and S7).

As a result, S8 has the most antibacterial properties among the samples. This is due to the fact that S8 may contain some beneficial substances in the soap formula that inhibit the growth of bacteria as the soap formulated with high composition of clay content to give the best antibacterial result against dog's saliva. According to National Council for Islamic Affairs of Malaysia (JAKIM), the percentage of clay content in permitted soap for *sertu* process must be more than the other ingredients (JAKIM, 2015).

Azhari et al. (2019) stated that S8 presented the highest concentrations in all anions and cations. S8 also contains high concentration of organic matter and metal content such as Ca, Cu, K, F and Br ions that help for antibacterial activity. Therefore, high clay content in a soap sample possesses high ion exchange and adsorption capacity and high specific surface area as well as beneficial for antibacterial treatment (Angkatavanich et al., 2009). Thus, it is safe and permitted to use soap for *sertu* process as long as the clay content of the soap is high and approved by JAKIM.

Eriatna (2017) reported that soap containing clay give the best antibacterial activity compared to the soap without additional clay. This is due to the fact that clay contain high cation exchange capacity (CEC) which the greater CEC resulting stronger ionic bond between clay surface and the bacteria cell wall that give better antibacterial properties. A research study on the antibacterial activity towards the clay liquid soap showed that the clay liquid soap can inhibit *Staphylococcus aureus* through the inhibition zone by disc diffusion, the main component in clay mineral such as iron (Fe), zinc (Zn), copper (Cu) and nickel (Ni) are the main influence that responsible in the mechanism of antibacterial properties in the clay liquid soap. The ion exchange in clay can disrupt the permeability of the cell membrane that give a significant role in bacterial damage as well as kill the bacteria (Faikoh, 2017).

The Significance of Using Tap Water and Soil Water During 7th of Washing

Table 3 indicate that there is no growth on PCA plate from washing using tap water only, while the bacterial growth on PCA plate from *sertu* process using soil water during 7th of washing resulted as TNTC. Referring to this result, it is best to start *sertu* process with soil water followed by another 6 times of washing with *air mutlak*. This process is clearly approved that the cleansing or *sertu* in Islam is able to remove dirt (*najs mughallazah*) and avoid any disease from that dirt. Thus, this is the reason why

does Islam obligated undergo *sertu* using soil water first followed by six times of washing to remove the remaining contaminant that left on the surface of the skin.

Table 3: The bacterial count of washing using water only and washing with soil during 7th of washing

Sertu Process	Bacterial Count (CFU/ml)						
	1st wash	2nd wash	3rd wash	4th wash	5th wash	6th wash	7th wash
Wash with water only	2.0×10^7	8.0×10^6	3.0×10^6	1.0×10^6	1.0×10^6	1.0×10^6	0
Wash with soil during 7th wash	1.6×10^7	4.0×10^6	3.0×10^6	2.0×10^6	1.0×10^6	1.0×10^6	TNTC

CONCLUSION

Concisely, it is proven that clay soil (S1) is deemed to be the most effective in terms of removing bacteria from *najs mughallazah* compared to the other natural soil which are sandy soil (S2), loamy soil (S3), top soil (S4) and mix soil (S5) due to its high electrical conductivity. Apart from that, the mild acidic value of S1 also enhance the antibacterial activity by disrupting the cell wall of bacteria. Therefore clay soil type is recommended during *sertu* process. Commercial soils (S6), (S7) and commercial clay soap (S8) is made to increase its efficiency of cleansing. The commercial soils and clay soap show greater antibacterial properties as there are additional chemicals that added to increase the ability of removing and inhibit the bacteria. As a conclusion, the antibacterial of commercial clay soap (S8) showed the strongest antibacterial properties against dog's saliva bacteria and permitted to be used for *sertu* process. Besides, S1 was found to be the best natural soil water against bacteria as the sample has the strength in terms of cation and anion concentrations towards antibacterial properties. Therefore, the *sertu* process can be done either using soil or clay soap as long as the process follows the rules set by Islamic law.

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