Study of Natural Antibacterial Body Wash Against Pathogenic Bacteria, Fungi and Covid-19 Virus (SAR-CoV-2) and Its Efficacy to Protect Skin from Pathogenic Bacteria

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Abstract

Background: The onset of COVID-19 worldwide pandemic has increased awareness of personal hygiene. Many studies show that body wash products are effective in killing bacteria and fungi but the effect on viruses is still limited. This paper presents a study on the analysis of an antibacterial body wash containing soap and surfactants also *Mentha piperita* (leaf) extract on killing and protection from bacteria, fungi and Covid-19.

Methods: The antibacterial activity of *Mentha piperita* (leaf) extract was evaluated by Minimum Inhibitory Concentration (MIC). The extract was combined with soap and surfactants to formulate body wash, then evaluated by a quantitative suspension test method to determine bactericidal, fungicidal and virucidal activity. A total of 5 panelists used it on the skin followed by collection of skin swabs after 12 hours. The swabs were grown in selective agar media to determine pathogenic bacteria.

Results: The MIC test showed that the extract is able to inhibit bacteria and yeast. By quantitative suspension test, the body wash was effective in killing >99.999% of *C. acnes* isolate, *C. albicans, A. brasiliensis, P. aeruginosa, S. enterica, E. coli* and *S. aureus*. It was also effective in inactivating Covid-19 virus by reducing 4.09 $\log_{10}$. Skin protection efficacy tested on panelists shows that after 12 hours of application there is no pathogenic bacteria detected.

Conclusion: This study indicates that the combination of soap, surfactant and *Mentha piperita* (leaf) extract in a body wash is effective in protecting skin from pathogenic bacteria, fungi and Covid-19 virus.

Keywords: *Covid-19 virus, Antibacterial, Skin Protection, Body Wash, Mentha piperita*

Introduction

Coronavirus belongs to a group of viruses that can cause illnesses ranging from SARS, MERS and the common cold. In China, COVID-19 was identified as a new virus and the World Health Organization on 11 March 2020 categorized COVID-19 as a pandemic disease [1]. So far, existing drugs have not been able to relieve the disease.

Washing hands using soap regularly in developed countries is part of personal hygiene in everyday life [2]. However, it was in the 19th century that contaminated hands played an important role in the process of spread by infectious agents. In the process of breaking the chain of infection with pathogens, the role of hygiene is very important [2,3,4,5]. Contaminated hands can spread the transmission of many infectious agents, from respiratory and enteric viruses to various non-viral pathogens [2]. Therefore, it is highly recommended to wash hands with soap and water as a form of prevention from the spread of viruses including SARS-CoV-2 [6] via inactivation of the virus [7]. Soaps and detergents are classified as surfactants that can destroy proteins in viruses [8].

Several antiseptic consumer medicinal products are sold on the market, such as hand washers, surface sprays, antibacterial soaps, bath soaps, antibacterial mouthwashes, and cleaners [9]. These products are known to contain active antiseptics such as triclosan, chloroxylenol, and triclocarban which is as an active ingredient registered by the US FDA as an over the counter antiseptic [9]. However, the level of safety and effectiveness is still in doubt. Although antiseptic and antibacterial bath soaps are widely in use it is desirable [10] to replace the harsh antiseptics with natural ingredients which could be effective yet safe alternatives. The extract of mint (*Mentha piperita* L.) has been reported to offer anti-bacterial activities.

Extract of Peppermint leaf (*Mentha piperita* L.) is mainly used in the form of essential oil which is often referred to as mint oil which has a characteristic fragrance. Mint oil has been reported to have a Minimum Inhibition Concentration (MIC) value of 10.5 g/mL and has been reported to have an activity that can inhibit the growth of *S. mutant* bacteria [11]. Mint leaves essential oils (0.5-4%) containing menthone (14-32%) and menthol (30-55%). Mint leaf extract has antibacterial activity against several pathogenic bacteria [12]. This study was designed to analyze Mint extract in antibacterial body wash for removing bacteria, fungi and the Covid-19 virus, as well as protecting the skin from pathogenic bacteria. The body washes formula contains soap and surfactants as well as *Mentha piperita* extract (leaf) as a natural active antibacterial.

Materials and Methods

Materials

Acrylates copolymer (Lubrizol Advanced Material), Allantoin (Akema), Aquades (Paragon Technology and Innovation), *Candida albicans* ATCC10231, Cocamide DEA (BASF), Cocamidopropyl betaine (CAPB), *Cutibacterium acnes* isolate, *Escherichia coli* ATCC8739, Fragrance (Mane), Glyceryl distearate (Kao Indonesia Chemicals), Hydantoin DMDM (YOU Solutions Guangzhou), Lauric acid (Cisadane Raya Chemicals), Mac Conkey Agar (MCA) (Meck), *Mentha piperita* (leaf) extract (Haldin Pacific Semesta), Myristic acid (Soci Mas), NaCl (Unichemcandi Indonesia), Potassium hydroxide (Unid), *Pseudomonas aeruginosa* ATCC9027, Sabouraud Dextrose Agar (SDA) (Merck), S*almonella enterica subsp. Enterica serovar Typhimurium* ATCC14028, Sea salt (Crodarom SAS), SAR-CoV-2, Sodium laureth sulfate (SLES) (Indo Sukses Sentra Usaha), *Staphylococcus aureus* ATCC6538, Stearic acid (Emery Chemicals), Tetrasodium EDTA (Global Chemindo Megatrading), Tryptic Soy Agar (TSA) (Merck), and Tryptic Soy Broth (TSB) (Merck) (All materials come from paragon and Research and Development Microbiology Laboratory, Bio Farma Microbiology Laboratory, Microbac Laboratories).

Methods

Minimum Inhibitory Concentration (MIC)

Second passage of ATCC culture in lyophilized form was subcultured to agar media and then incubated for 24 hours; 48 hours; and 120 hours for bacteria, yeast, and mold respectively. The agar medium used in this study was Sabouraud Dextrose Agar (SDA) for yeast and mold testing and Tryptic Soy Agar (TSA) for bacterial culture. Five single colonies of bacteria, yeast and mold were picked and inoculated to 200 mL Tryptic Soy Broth (TSB) then incubated at the appropriate time and temperature. The microbial suspension was plated to determine the CFU/mL value for bacteria $5x10^5$ CFU/mL and for yeast mold at $2.5x10^3$ CFU/mL.

Mentha piperita (leaf) extract used in this study was in concentrated of 200 mg/mL concentration. The original form was used as a stock solution and diluted with sterile aquadest to create a concentration range from 2 mg/mL to 100 mg/mL. The same amount of extract and microbial inoculum was added to the tubes and then incubated at the appropriate time and temperature referring to the test microorganism. Negative control of each concentration was prepared by adding TSB media to peppermint extract. Positive control was included by mixing microbial inoculum with TSB. Turbidity of the mixtures indicated microbial growth where a turbid suspension indicated micro-

bial growth, while clear one indicated inhibition of growth. The lowest concentration showing clear suspension was determined as MIC.

Percentage Kill Test

The percentage kill test method referred to EN 1276:2019 for bacterial and EN 1650:2008 for yeast and mold. The test was conducted in an external laboratory testing Microbiology Laboratory Bio Farma. The method consists of direct inoculating a product with standard microorganisms concentration then enumerating surviving microorganisms after specified exposure time and temperature. Neat product was evaluated in 60 second contact time to kill *Staphylococcus aureus* ATCC 6538*, Salmonella enterica subsp. Enterica serovar Typhimurium* ATCC 14028*, Escherichia coli* ATCC 8739*, Pseudomonas aeruginosa* ATCC 9027*, Candida albicans* ATCC 10231*, Aspergillus brasiliensis* ATCC 16404, and *Cutibacterium acnes* isolate. These microorganisms represent the various strains of bacteria, fungi and yeast respectively tested. S. *enterica subsp. Enterica serovar Typhimurium* was included because they are known as pathogenic for humans [14]. *C. acnes* was also included due to its role in causing acne in human skin [15]. The product passes the test if the log reduction achieves ≥ 5 for bacteria and ≥ 4 for yeast mold referring to EN 1276:2019 and EN 1650:2008.

Virucidal Efficacy Suspension Test

The virus efficacy suspension test was carried out to test the effectiveness of body wash in killing The test (SAR-CoV-2) was carried out at the external testing laboratory of Microbac Laboratories using a method, namely EN14476:2013+A2:2019. The test virus is added to an interfering substance in a suspension, the test product is then added to the virus suspension for specified exposure time and temperature. After exposure time, samples are retrieved and are neutralised. Serial dilutions are performed to examine viral infectivity. The product is said to meet the criteria if there are at least 4 logs of titer reduction outside the level of cytotoxicity.

Body Wash Formulation

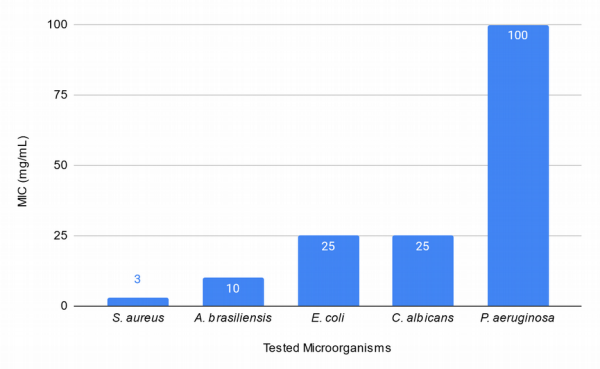
The body washes contained soap, surfactant, humectant, rheology modifier, pearlized agent, extract, anti-irritant, preservative, fragrance, and other additives. The soap consisted of myristic acid, lauric acid, and stearic acid which was neutralized using Multimix with potassium hydroxide at a temperature of 75°C then mixed with the pearlized agent, surfactant, humectant, rheology modifier then cooled down to temperature 40°C to add extract, preservative, anti-irritant, and fragrance. The peppermint extract is produced by the water distillation process. The extract that was used in this formulation is *Mentha piperita* (leaf) extract with a dosage of 0.5% as raw material (as active 0.1%).

12 Hours Protection Test from Pathogenic Bacteria

In this test, a pilot study was conducted on 5 panelists for each 3 test groups. Subjects exposed to dirt and microorganisms such as outdoor workers were recruited for the study. Sterile swab was rubbed on skin to collect samples of microbes. The panelists were instructed not to bathe using body wash before and during testing. The panel was divided in 3 groups consisting of positive control, negative control, and treatment. Before starting the test, panelists were instructed to not to take a bath or shower using body wash for a maximum of 10 hours. In the beginning of the test (0-hr), the treatment group washed their hands using body wash while positive control group did not. Both groups did not wash their hand until the end of sampling time. The negative control panelists washed their hands at every sampling time. Sampling was carried out at 0-hour (before and after hand washing), 5-hr, 8-hr, 10-hr and 12-hr. In the swab method, the hand surface area was marked 10 cm long and 5 cm wide so that the size and the sampling site did not change. Sterile cotton buds were swabbed into the sampling area 3 times back and forth from end to end ensuring that all surfaces were swabbed. Cotton buds were then transferred to a vial containing 20 mL NaCl solution. The NaCl was homogenized first with vortex Thermo Scientific and then inoculated using the pour plate method. 1 mL sample suspension was pipetted with Micropipette 100-1000uL Eppendorf into an empty sterile petri dish, then warm liquid MCA was poured into a petri dish. Mac Conkey Agar (MCA) was used to detect Gram-negative bacteria. The media was incubated at the temperature of 37°C for 48 hours, then observed for the presence of microbial growth. If there is microbial growth on the agar media, it is declared positive (+), whereas if there is no microbial growth on the agar media, it is declared negative (-). Morphology of all growing bacteria was observed by the eye then the bacteria was identified using Vitek 2 in Microbiology Laboratory Bio Farma.

Results

Minimum Inhibitory Testing (MIC)



Graph 1. Minimum Inhibitory Concentration (MIC) of Peppermint Extract

Minimum Inhibitory Testing (MIC) was performed on *S. aureus, P. aeruginosa, E. coli, C.*

albicans and *A. brasiliensis.* with extract concentrations of 2 mg/mL to 100 mg/mL. MIC is determined by the lowest concentration that can inhibit microbial growth. Graph 1 showed that Peppermint extract was effective inhibiting *S. aureus* at 3 mg/mL and required a high concentration of 100 mg/mL to inhibit *P. aeruginosa. E. coli and C. albicans* were inhibited at the extract concentration of 25 mg/mL, while *A. brasiliensis* was inhibited at 10 mg/mL.

Percentage Kill Test

Table 1. Percentage Kill Test of Body Wash

No.	Tested Microorganisms	Microbial Number (CFU/mL)		Log Reduction	Kill Percentage
		Before Contact with Sample	After Contact with Sample		
1	*Staphylococcus aureus* ATCC6538	1.5×10^7	< 140	> 5.03	>99.999%
2	*Escherichia coli* ATCC8739	1.6×10^7	< 140	> 5.06	>99.999%
3	*Salmonella enterica subsp., Enterica serovar Typhimurium* ATCC14028	1.0×10^7	< 140	> 5.04	>99.999%
4	*Pseudomonas aeruginosa* ATCC9027	1.5×10^7	<140	>5.03	>99.999%
5	*Cutibacterium acnes*	1.5×10^7	< 140	> 5.03	>99.999%
6	*Candida albicans* ATCC10231	1.5×10^6	< 140	> 4.02	>99.991%
7	*Aspergillus brasiliensis* ATCC16404	1×10^6	< 140	> 4.02	>99.991%

The body wash in a neat condition showed an ability to kill >99.999% of *S. aureus, Salmonella enterica subsp, Enterica serovar Typhimurium, E. coli, and P. aeruginosa* and *C. acnes;* and >99.991% of *C. albicans and A. brasiliensis* in 60 second.

Virucidal Efficacy Suspension Test

Table 2. Titer Result

Sample	Dilution Tested	Contact Time	Titer ($log_{10}TCID_{50}$/mL)	Volume (mL)	Volume Correction[a]	Viral load ($Log_{10}TCID_{50}$)
Virus Stock of Titer Control	NA		6.18 ± 0.18	-	-	-
Theoretical of load [b]	NA		2.88 ± 0.18	10	200	6.18 ± 0.18
Cell Viability Control	media was sterile (No virus was detected cells were viable)					
Virus Recovery Control (VRC)	N'A	0 minute	3.30 ± 0.19	10	200	6.60 ± 0.19
Neutralizer Effectiveness Control (NEC)		60 seconds	3.30 ± 0.22	10	200	6.60 ± 0.22
Natural Mint Body Wash (Lot No. FH08	Neat (80% intest)	60 seconds	6.30 ± 0.19	NA	NA	6.30 ± 0.19
	Neat (80% intest)		≤ -0.79 *	10	200	≤ 2.51
	1/13		≤ 0.83 *	10	200	≤ 4.13
	1/1334		2.80 ± 0.17	10	200	6.10 ± 0.17

[a] Volume correction accounts for the neutralization and quench of the sample post contact time

[b] Based on the Virus Stock Titer (6.18 Log_{10} $TCID_{50}$/mL) minus Log_{10} (spike ration x dilution = 10 x 200) = 2.88 Log_{10} $TCID_{50}$/mL

[c] A 4.5 mL aliquot of post-quenched sample was spiked with 0.5 mL of stock virus, mixed via vortex, and held in ice-bath for 30 minutes

* No virus was detected; the theoretical titer was determined based on the Poisson Distribution.

NA = Not Applicable

Note 1: The difference in Viral Load between VST (6.18 Log_{10} $TCID_{50}$) and NEC (6.30 Log_{10} $TCID_{50}$) was 0.12 Log_{10} $TCID_{50}$. (VALID)

The log_{10} Reduction Factor (LRF) was calculated in the following manner:

Log_{10} Reduction Factor = Initial viral load (Log_{10}) - Output viral load (Log_{10})

The viral Load was determined in the following manner:

Viral Load (log_{10} $TCID_{50}$) = Titer (log_{10} $TCID_{50}$/mL) + Log_{10}[Volume (mL) x Volume Correction]

Table 3. Cytotoxicity Control

Sample	Dilution*	Cytotoxicity Control
Natural Mint Body Wash (Lot No. FH08)	10^{0}	No cytotoxicity observed
	10^{-1}	No cytotoxicity observed
	10^{-2}	No cytotoxicity observed

*Post-neutral & extinguished sample (PNS) 1:200 is assumed to be not dilute (100).

Table 4. Viral Interference Control

Sample	Virus Titer ($Log_{10}TCID_{50}$/mL)	Log_{10} Titer Difference
Natural Mint Body Wash (Lot No. FH08)	5.70 ± 0.19	0.12 (VALID)
1 X PBS	5.82 ± 0.22	

Conclusion: The test substance that has been neutralized has no interference from the virus.

Table 5. Viral Log_{10} Reduction

Test	Dilution Tested	Contact Time	Input Load ($Log_{10}TCID_{50}$)	Output Load ($Log_{10}TCID_{50}$)	Reduction ($Log_{10}TCID_{50}$)
Natural Mint Body Wash (Lot No. FH08)	Neat (80% intest)	60 seconds	6.60	≤ 2.51	≥ 4.09
	1/13			≤ 4.13	≥ 2.47
	1/1334			6.10	0.50

⩾ Denotes a complete inactivation of the virus challenges
* Large Volume Plating (LVP) was used for the highest dilution only (neat), therefore providing a lower limit detection to enable a log reduction of greater than equal to 4 logs to be demonstrated. LVP was not used in the subsequent dilutions therefore limiting/ reducing the observed endpoint.
Conclusion: The test substance when tested neat met the criteria for EN14476 as an effective virucidal agent against SARS-Cov-2 (COVID-19 virus)

The body wash product used for virucidal testing was coded as Natural Mint Body Wash (Lot No. FH08). According to the EN 14476:2013+A2:2019 guideline, the body washes are effective if there is 4.0 log decrease in virus titer exceeding the cytotoxicity level. Based on the obtained results, the body wash showed ≥ 4.09 log10 reduction at a test concentration of neat (80% in-test), ≥ 2.47 log10 reduction at 1/13, and 0.50 log10 reduction at 1/1334 concentration against SARS-CoV-2 at the test temperature of 21°C using a 0.3 grams/Liter final concentration of bovine serum albumin solution, in compliance with EN14476:2013+A2:2019.

When tested as described, the body wash met the European Standard EN 14476:2013+A2:2019 guideline, when SARS-CoV-2 was exposed to the product at 21°C for 60 seconds, with a Log 10 reduction of ≥ 4.09 for the neat (80% in-test) concentration. Based on the observational data, all controls can be said to meet the criteria for a valid test.

Body Wash Formulation

The appearance of body wash is low viscous gel with vicosity 2000-3000 CP and the color is white opaque. The pH of the body wash is measured around 8.5-9.5.

12 Hours Protection Test from Pathogenic Bacteria

Tabel 6. Hand Swab Test Result after Body Wash Application

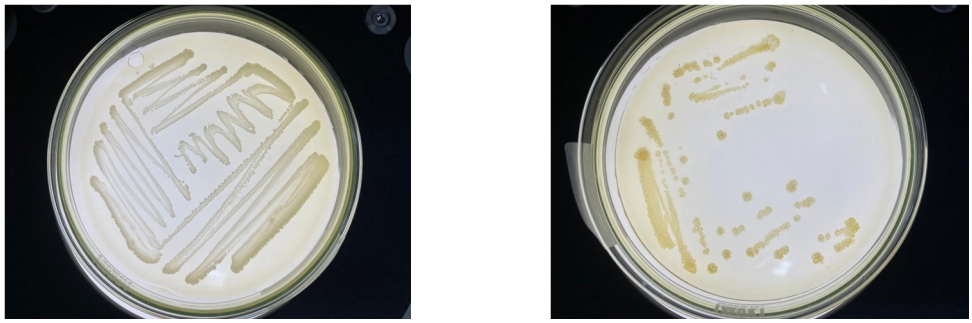
No.	Panelist Code	Group	Growth of Gram Negative Bacteria on Mac Conkey Agar Media					
			0-hr		5-hr	8-hr	10-hr	12-hr
			Before Hand Washing	After Hand Washing				
1	A1R	Control + (No hand washing from the beginning 0-hr)	-	NA	-	-	-	+
2	A2R		-	NA	-	-	-	+
3	A3L		-	NA	-	-	-	-
4	A4R		-	NA	-	-	-	-
5	A5R		-	NA	-	-	-	-
6	B1	Control - (Hand washing at each sampling point)	-	-	-	-	-	-
7	B2		-	-	-	-	-	-
8	B3		-	-	-	-	-	-
9	B4		-	-	-	-	-	-
10	B5		-	-	-	-	-	-
11	A1L	Treatment (Hand washing only at the beginning 0-hr)	-	-	-	-	-	-
12	A2L		-	-	-	-	-	-
13	A3R		-	-	-	-	-	-
14	A4L		-	-	-	-	-	-
15	A5L		-	-	-	-	-	-

+ bacterial detected
- no bacterial detected
NA not applicable

Positive control showed no bacteria growth starting from 0 hours to 10 hours but showed bacterial growth at 12 hours in 40% of panelists. In the treatment group, no microbes were detected from 0 to 12 hours, which was the same as positive controls who washed their hands at every sampling point.

There were 2 types of bacterial colonies obtained from positive control as shown in Picture 1. The first bacterial colony is yellowish-white with a flat surface (a) and the second bacteria is yellowish-white to form wrinkles (b). Vitek 2 identified that bacteria were *Pseudomonas stutzeri*

and *Bacillus pumilus.*



a b

Figure 1. Bacterial isolates from Positive Control on TSA media.

Discussion

Peppermint leaf extract was very active against all tested microbial strains, namely the bacteria *P. aeruginosa*, *S. aureus* and *E. coli*, and the fungus *C. albicans* and *A. brasiliensis*. The extract showed the lowest MIC against *S. aureus* (3 mg/mL), and can be claimed to possess strong antimicrobial activity against Gram-positive bacteria. Whereas, the MIC against Gram-negative bacteria *E. coli* and *P. aeruginosa* were at 25 mg/mL and 100 mg/mL respectively. Peppermint leaf extract also can inhibit *C. albicans* and *A. brasiliensis* at concentrations of 25 mg/mL and 10 mg/mL. Based on the result the extract had the highest antibacterial activity against Gram-positive bacteria. In peppermint leaves, some compounds have antimicrobial activity, namely menthol which is included in one of the terpenoid groups, namely monoterpenes [16]. It has been reported [17] that the leaves of mint have a very strong antibacterial activity against Gram-negative bacteria such as *P. aeruginosa* and *E. coli* as well as Gram-positive bacteria such as *S. aureus*. Terpenoid compounds, especially menthol in mint leaves impart antibacterial activity. Menthol destroys bacterial cells by interferance with the lipid fraction contained in the plasma membrane and thereby changing the membrane permeability. In mint, leaf mushrooms play a role by damaging the cell walls of the fungus because the formation of the cell wall is inhibited by antifungal substances that cause the fungus to lyse [18].

Body wash in a neat condition showed the ability to kill >99.999% of *S. aureus*, *E. coli*, and *Salmonella enterica* subsp. *Enterica serovar Typhimurium*, *P. aeruginosa*, and *C. acnes* isolate and >99.991% *C. albicans* and *A. brasiliensis* in 60 seconds according to EN 1276:2019 for bacteria and EN 1650:2008 for yeast and fungi. It is clear from this study that the body wash met the criteria for general-purpose disinfection. The kill percentage results indicate that the body wash can remove >99.999% of bacteria and >99.991% when applied to the body.

The outer lipid layer of the SARS-CoV and other viruses adsorb on the surface of host. Soaps contains surfactants that can lower the surface tension of the viral cell wall and damage the permeability of the cell membrane thereby allowing soap liquid to penetrate small crevices. Monomers in soap adsorb on viral surfaces due to the amphiphilic nature of soap which results in interactions between the hydrophobic ends of the soap and the hydrophobic lipid membrane. This can prevent charged virus particles to aggregate; the adsorptive properties of the virus are lost they are carried away with water[19]. In this study, the surfactant Sodium Lauryl Ether Sulfate (SLES) was used mainly because of its easy availability and safety profile. The mechanism of SLES in inactivating microbes is by hydrophobic interactions. The hydrophobic group of the SLES surfactant interacts with the microbial surface which is covered by a double layer of the membrane. This is because the interaction between SLES and microorganisms shows the dominant hydrophobic interaction between SLES and viruses. Therefore, the use of SLES surfactant in preparations is sufficient to prevent microbial infection [20].

Based on Virucidal Efficacy Suspension Test the body wash showed 4.09 log10 reduction at a 80% concentration against SARS-CoV -2 at the test temperature of 21°C in compliance with EN14476:2013+A2:2019. The body wash also passed the cytotoxic test so it is safe to use.

In the formulation of body wash, the pH was around 8.5-9.5 because of the content of the soap. The soap consists of potassium laurate, potassium myristate, and potassium stearate from fatty acids neutralized with potassium hydroxide. The pH range of the product can increase the antibacterial activity. The body washes appearance is a low viscous liquid gel with a viscosity of 2000-3000 cP that is built by acrylate copolymer as a thickener so the body wash can dissolve easily in water and contact with the skin. The body washes the color is white opaque due to glyceryl distearate as an opacifier and pearlized agent.

The skin protection ability of body wash was evaluated by a pilot study. It was a preliminary test before going to a larger scale. The test involved 5 panelists for 3 experiment groups. The parameter observed in this test was the absence of bacteria on the skin that was detected on MCA media. The test results showed that there was no microbial growth 12 hours after hand washing while there are microbial growth in positive control. Detected bacteria were *Pseudomonas stutzeri* and *Bacillus pumilus. Pseudomonas stutzeri* is Gram negative bacteria widely distributed in the environment. It was reported as opportunistic pathogen and can cause pneumonia, meningitis, bacteremia, osteomyelitis, ocular infection and joint infection [21]. *Bacillus pumilus* is Gram positive bacteria commonly found in soil. Some reported that the bacteria can cause various infection such as endocarditis, skin infection, sepsis and food poisoning in human [22]. Based on this result, it

can be said that body wash containing peppermint leaf extract is effectively to protect skin from pathogenic bacteria until 12 hours.

Conclusion

The MIC of *Mentha piperita* (leaf) extract against *S. aureus, P. aeruginosa*, *E. coli,* and *A. brasiliensis, C. albicans* was 3 mg/mL, 100 mg/mL, 25 mg/mL, 10 mg/mL, and 25 mg/mL respectively, showing that the extract can inhibit the bacteria and yeast. By quantitative suspension test, body wash was found to be very effective in killing >99.999% *S. typhimurium, C. acnes* isolate*, E. coli, S. aureus* and *P. aeruginosa,* also >99.991% of *C. Albicans* and *A. brasiliensis* within 60 second contact time. It was also effective in inactivating the Covid-19 virus by reducing 4.09 log10 within 60-second contact time. Skin protection efficacy tested on panelists shows that after 12 hours of application there were no pathogenic bacteria detected.

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Conflict of Interest Statement

NONE.

Reference

1. WHO Director-General ‘s remarks at the media briefing on 2019-nCoV on 11 February (2020). (n.d). Retrieved from https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020
2. World Health Organization (2009) World Health Organization WHO guidelines on hand hygiene in health care. 2009. https://apps.who.int/iris/bitstream/handle/10665/44102/9789241597906_eng.pdf;jsessionid=DC6CEAE5C7BC5F46862569D46ABBE53B?sequence=1
3. Alum, Rubino & Ijaz (2010) Alum A, Rubino JR, Ijaz MK. The global war against intestinal parasites—should we use a holistic approach? International *Journal of Infectious Diseases*. 2010;14(9):e732. doi: 10.1016/j.ijid.2009.11.036.
4. Scott et al. (2020) Scott EA, Bruning E, Nims RW, Rubino JR, Ijaz MK. A 21st century view of infection control in everyday settings: moving from the germ theory of disease to the microbial theory of health. *American Journal of Infection Control.* 2020;48(11):1387–1392. doi: 10.1016/j.ajic.2020.05.012.

5. Scott, Bruning & Ijaz (2021) Scott E, Bruning E, Ijaz MK. *Decontamination of environmental surfaces in everyday settings*. In: McDonnell G, Hansen J, editors. Block's Disinfection, Sterilization, and Preservation. Sixth Edition. Philadelphia: Wolters Kluwer; 2021
6. United States Centers for Disease Control & Prevention (2020) United States Centers for Disease Control and Prevention Handwashing: clean hands save lives. 2020. https://www.cdc.gov/handwashing/show-me-the-science-handwashing.html
7. Sturman L.S., Ricard C.S., Holmes K.V. (1990) Conformational change of the coronavirus peplomer glycoprotein at pH 8.0 and 37 degrees C correlates with virus aggregation and virus-induced cell fusion. *J Virol* ;64:3042–3050.
8. Chepurnov A.A., Bakulina L.F., Dadaeva A.A., Ustinova E.N., Chepurnova T.S., Baker J.R. (2003). Inactivation of Ebola virus with a surfactant nanoemulsion. *Acta Trop.* 2003;87:315–320. doi: 10.1016/S0001-706X(03)00120-7.
9. Betterhealth. (2020). Antibacterial cleaning products. https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/antibacterial-cleaning-products [cited 2022 June 16]
10. Food and Drug Administration (FDA). (2013). *Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-thecounter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening the Administrative Record*. Department of Health and Human Services. 21 CFR Parts 310 and 333. Federal Register, 78, p. 242. December 17
11. Golestan Nejad, Z., Gavanji, S., Mohammadi, E., Motamedi, A., Bahrani, M., Rezaei, F., and Bakhtari, A., (2017), Comparison of antibacterial activity of essential oils of Foeniculum vulgare Mill , Mentha arvensis and Mentha piperita against Streptococcus mutans, *Advanced Herbal Medicine*, Vol. 3 No. 1: 3–13.
12. Bupesh, G., Amutha, C., Nandagopal, S., Ganeshkumar, A., Sureshkumar, P., and Murali, K., (2007), Antibacterial activity of Mentha piperita L, (peppermint) from leaf extracts – a medicinal plant, *Acta Agriculturae Slovenica*, Vol. 89: 73–79.
13. Andrews, Jennifer M. (2001). Determination of minimum inhibitory concentration. Journal of Antimicrobial Chemotherapy 48, Suppl. S1 5-16
14. McClelland M, Sanderson KE, Spieth J, *et al*. (2001) Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. *Nature* ;413:852-856.
15. O'Neill AM, Gallo RL. (2018). Host-microbiome interactions and recent progress into understanding the biology of acne vulgaris. *Microbiome* ;6(1):1–16.

16. Oyedeji, O.A.,and Afolayan,A.J.,(2006),*Chemical Composition and Antibacterial Activity Of The Essential Oil Isolated from South African Mentha longifolia (L.) subsp*. Capen.

17. Tabari, et al., (2012). *Comparasion of Antibacterial Effect of Eucalyptus Essence and Combination of Them on Staphylococcus aureus and Escherichia coli Isolates.* Department of Pharmacology, pp. 536-540.

18. Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristiani, M., Daniele, C., *et al*., (2005), Mechanisms of Antibacterial Action of Three Monoterpens, Antimicrobial Agents Chemotherapy, 49 (6), 2474-2478.

19. Roth Y, Opatowski E, Lichtenberg D, Kozlov MM.(2000). Phase Behavior of Dilute Aqueous 468 Solutions of Lipid - Surfactant Mixtures : Effects of Finite Size of :2052–61.

20. Jonges M, Liu WM, van der Vries E, Jacobi R, Pronk I, Boog C, *et al*. (2010). Influenza virus inactivation for studies of antigenicity and phenotypic neuraminidase inhibitor resistance profiling. *J Clin Microbiol*; 48(3):928–40. Epub 2010/01/22. https://doi.org/10.1128/JCM.02045-09 PMID: 20089763; PubMed Central PMCID: PMCPMC2832438.

21. Gilardi, G.L. Infrequently Encountered Pseudomonas Species Causing Infection in Humans. *Ann. Intern. Med.* 1972, *77*, 211

22. Barış Ata Borsa 1, Mehmet Ersoy Aldağ, Birsen Tunalı, Uğur Dinç, Zeynep Güngördü Dalar, Veli Cengiz Özalp. 2016. A sepsis case caused by a rare opportunistic pathogen: Bacillus pumilus. 50(3):466-70. doi: 10.5578/mb.27575.