PARAGONCORP

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

ID -180

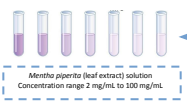
Rosmayanti, Wiwin[1] ; Indiarto, Nur Huda Arif[1] ; Muizzuddin, Neelam[2].*
[1]Paragon Technology and Innovation, Tangerang, Indonesia; [2]Skin Clinical Research Consultants, LLC, New York, United States

Introduction:

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 of bacteria, fungi and Covid-19.

Materials & Methods:

Minimum Inhibitory of *Mentha piperita* (leaf) extract



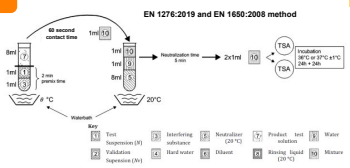
Second Passage of
- *Pseudomonas aeruginosa* ATCC9027
- *Staphylococcus aureus* ATCC6538
- *Escherichia coli* ATCC8739
- *Candida albicans* ATCC10231
- *Aspergillus brasiliensis* ATCC16404

Bacterial inoculum: $5x10^5$ CFU/ml
Yeast mold inoculum: $2.5x10^5$ CFU/ml

The lowest concentration showing clear Suspension was determined as MIC

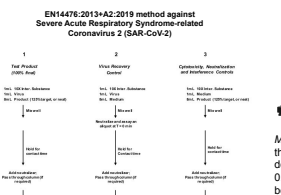
Percentage Kill Test

Tested Microorganisms:
- *Staphylococcus aureus* ATCC6538
- *Pseudomonas aeruginosa* ATCC9027
- *Escherichia coli* ATCC8739
- *Salmonella enterica* subsp. Enterica serovar Typhimurium ATCC14028
- isolate *Cutibacterium acnes*
- *Candida albicans* ATCC10231
- *Aspergillus brasiliensis* ATCC16404



Virucidal Efficacy Suspension Test

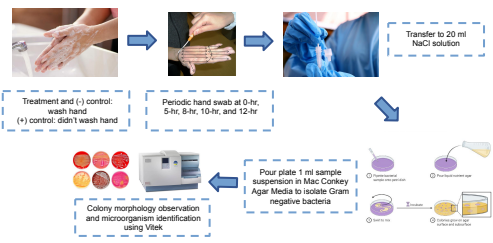
EN14476:2013+A2:2019 method against Severe Acute Respiratory Syndrome-related Coronavirus 2 (SAR-CoV-2)



Body Wash Formulation

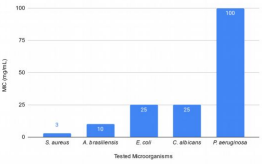
Mentha piperita (leaf) extract is produced by the water distillation process. The extract dose that was used in this formulation is 0.5% as raw material (as active 0.1%). The body wash contained soap and surfactant are processed with Multimix. The appearance of body wash is low viscous gel with viscosity 2000-3000 CP having white color. The pH of the body wash is measured around 8.5-9.5.

12 Hours Protection Test from Pathogenic Bacteria



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.

Results & Discussion:



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. In peppermint leaves, some compounds have antimicrobial activity namely menthol which is included in one of the terpenoid groups, namely monoterpenes [1]. It has been reported [2] that the leaves of mint have a very strong antibacterial activity against Gram-negative as well as Gram-positive bacteria.

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

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

The body wash showed ≥ 4.09 log10 reduction at a test concentration of neat (80% in-test), ≥ 2.47 log_{10} reduction at 1/13, and 0.50 log_{10} reduction at 1/1334 concentration against SARS-CoV-2. The body wash met the European Standard EN 14476:2013+A2:2019 guideline and all controls met the criteria for a valid test. 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 [3].

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. 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*. *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 [4]. *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 [5].

Conclusions:

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, yeast and mold. 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 log_{10} within 60 second contact time. Skin protection efficacy tested on panelists shows that after 12 hours of application there were no pathogenic bacteria detected.

Acknowledgements:

Thank you to the Research and Development Laboratory of PT Paragon Technology and Innovation which has assisted in providing funds, and raw materials for formulations body wash the place for carrying out this research.

References:

1. 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
2. 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.
3. 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.
4. Gilardi, G.L. Infrequently Encountered Pseudomonas Species Causing Infection in Humans. Ann. Intern. Med. 1972, 77, 211
5. 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.