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"Nipahol": A Locally Formulated Sanitizer/Disinfectant from Nipa Bioethanol for Possible Use Against Covid-19

Roque A. Ulep[1], James Paul T. Madigal[1♥], Thiara Celine E. Suarez[2], Karyl Mae D. Ramos[2], Jayson F. Cariaga[2], and Shirley C. Agrupis[3]

[1]Mariano Marcos State University, City of Batac, 2906 Ilocos Norte, Philippines
[*]Department of Biological Sciences, College of Arts and Sciences, Mariano Marcos State University, City of Batac, 2906 Ilocos Norte Philippines
[2]Current Affiliation: National Bioenergy Research and Innovation Center, Research Directorate
[3]Current Affiliation: Department of Biological Sciences, College of Arts and Sciences
[♥]Corresponding author email: jtmadigal@mmsu.edu.ph

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Abstract. "Nipahol" is high-grade bioethanol produced from nipa sap using the proprietary fermentation and distillation technologies developed by a group of researchers at the Mariano Marcos State University-National Bioenergy Research and Innovation Center (MMSU-NBERIC). To ensure the quality and efficacy of the formulated product, the present study was set to explore the antibacterial potential of the locally formulated nipa disinfectant/antiseptic as an alternative alcohol formulation for possible use during the COVID-19 pandemic using standard microbiological assays. Susceptibility test revealed that at least 70% nipa alcohol formulations showed inhibitory activity against *Staphylococcus aureus* (6.25 mm and 4.25 mm zone of inhibitions). The 95% nipa alcohol concentration showed a bactericidal effect against *Escherichia coli* and *S. aureus*. High percent (%) bacterial cell reduction (90-99.9% log reduction) was observed when alcohol concentration and time increases. A confirmatory antimicrobial susceptibility test conducted by Philippine Department of Science and Technology, Microbiology Division reported that 95% nipa alcohol showed active inhibitory effect to test organisms while partial active observed in 70% nipa alcohol formulation. Glo-Germ Test revealed nipa disinfectant/antiseptic is as effective as commercial alcohol, thus, it can be utilized as an alternative intervention to prevent the spread of infectious microorganisms. The effectiveness of nipa disinfectant/antiseptic formulations is heightened with proper handwashing, strictly following proper hygiene, and health protocols. In conclusion, the formulated nipahol possesses the antibacterial potential to inhibit the multiplication of *E. coli* and *S. aureus*.
Keywords: antimicrobial susceptibility; glo germ test; nipa bioethanol; nipa disinfectant/antiseptic; percent bacterial cell reduction

INTRODUCTION

The emergence of the Coronavirus Disease-2019 (COVID-19) as a global pandemic made it a significant global public health concern. As of September 1, 2020, World Health Organization (WHO) reported a total of 25.89 million reported cases and more than 860 thousand death cases affecting over 200 countries worldwide. It's contagious nature led to an extensive use of hand disinfectants (COVID-19 Coronavirus 2019-nCov Statistics Update Online, 2020; Situation Update Worldwide, 2020).

COVID-19 is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which can persist and remain infectious on surfaces for up to 9 days (Kampf and Kramer, 2004; Chan et al., 2020). The recent study reveals that transmission of SARS-CoV-2 is possible in the form of aerosol and fomite. It can remain viable and infectious in aerosols for hours and on surfaces up to days depending on the inoculum shed (Van Doremalen et al., 2020). Hence, it is crucial to interrupt the transmission chain of the virus through contact isolation and strict infection control tools (Thomas et al., 2014). Health protocols such as wearing face masks and doing appropriate hand hygiene must be strictly observed. Hand hygiene is of utmost importance because hands are more prone in getting contact with the virus from direct contact with patients' respiratory droplets from coughs and sneezes or indirect contact via surfaces which may then facilitate the transmission and spreading of the disease (Van Doremalen et al., 2020). Furthermore, SARS-CoV-2 belongs to the same family of virus as SARS-CoV which caused the 2003 Severe Acute Respiratory Syndrome (SARS)

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outbreak. The studies on SARS-CoV outbreak settings showed that providing efficient handwashing facilities reduced transmission (Seto et al., 2003). Also, the outbreak has triggered the so-called "pandemic pantries", a term that well defines the spikes in stockpiling of emergency supplies all around the world. Upon the recommendation of frequent handwashing and sanitization across the world, supplies of hand sanitizers rapidly vanished from some markets. According to a market research from Nielsen, the sale of hand sanitizers skyrocketed by 300% and 470% in the last week of February and first week of March 2020, respectively, in comparison to the same time in the previous year (Yu et al., 2007). Similarly, in Italy – one of the most affected countries by CoViD-19 - sales of hand sanitizers in supermarkets augmented by 561% during the first three weeks of the pandemic (24th February-15th March 2020) compared to the previous year (Huddleston, 2020). Through years of research, MMSU has been able to develop research-based products that are ready for bulk production. One of the researches was the production of 95% alcohol from nipa sap and molasses. Using the MMSU's proprietary fermentation and distillation protocol, MMSU was able to produce 70% Ethyl Alcohol or the NIPAHOL from the 95% alcohol. These researches were able to help mitigate the shortage of supply of safety agents such as disinfectants and sanitizers amidst COVID-19 pandemic.

By utilizing the existing bioethanol facilities and the available stock of nipa sap, MMSU-NBERIC has already rationed approximately 1,000 liters of 70% Nipahol to various Local Government Units (LGUs) and other agencies in Ilocos Norte and Cagayan Valley. During the Enhanced Community Quarantine, there was a weekly distribution of Nipahol to the different checkpoints in Ilocos Norte and to the barangays in the City of Batac. MMSU continues to fight against COVID-19 by helping to protect the beneficiaries, especially the frontliners and key families/individuals in Regions 1, 2, and CAR, by providing them 70% ethyl alcohol as disinfectant/sanitizer.

The present study conducted research experiments amid pandemic such as formulation of disinfectant/antiseptic from produced 95% nipa bioethanol, ethanol content analysis, and antimicrobial assay to evaluate the efficacy of the locally formulated product. Hence, the said analyses will ensure the quality of the product and its efficacy in preventing pathogenic microorganisms that causes infectious diseases.

The development of an environment-friendly disinfectant/antiseptic as an alternative alcohol formulation that will be utilized to prevent the spread of pathogens and decrease the alarming increase of the rate of infection was taken into action. Hence, this study was set out to explore the antibacterial potential of the locally formulated nipahol disinfectant/antiseptic as an alternative alcohol formulation amid COVID-19 pandemic. Specifically, the present study sought to, (a) determine the susceptibility pattern of the test organisms to nipahol at various concentrations through zone of inhibition (ZOI); (b) determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC); (c) determine the efficacy of the nipa disinfectant/antiseptic in reducing viable counts of bacteria overtime; and (d) assess the germicidal potential of the nipahol concentrations on hands of subjects through the use of Glo Germ.

METHODS

Test isolates

Two bacterial isolates namely *Escherichia coli* and *Staphylococcus aureus*, obtained from the Philippine National Collection of Microorganisms (PNCM), BIOTECH-UPLB, Laguna were used in this study. These isolates were revived in Tryptic Soy Agar plates and re-cultured in Tryptic Soy Broth with agitation at 120 x g using a

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shaker incubator. They were streaked on nutrient agar plates and kept at 4°C until when needed.

Formulation of Disinfectant/Antiseptic as Various Concentrations

MMSU 95% nipa ethanol concentration produced from the distillation is blended with 70-95% by volume along with the distilled water with 25% down to 5% to produce 70-80% nipahol. Moisturizing agent was added (0.1 to 1%) to prevent dry, rough, scaly, itchy skin and minor skin irritations. An alcohol meter was used to check desired concentration of the formulated nipa disinfectant/antiseptic.

Preparation of McFarland Standard and Standardization of Test Organisms

The McFarland 0.5 turbidity standard was prepared according to the method recommended by the National Clinical Committee for Clinical Laboratory Standards (NCCLS). The standard was prepared by adding 0.5ml of 1.175% w/v barium chloride dihydrate ($BaCl_2.2H_2O$) solution to 99.5 ml of 15 w/v sulfuric acid (H_2SO_4). This was mixed well and then aliquoted into test tubes identical to the ones used in preparing inoculum suspensions of the test organisms. The accuracy of the density of the standard was verified using a spectrophotometer. The absorbance of the 0.5 McFarland standard at wavelength 625 nm was 0.08-0.10. The tubes were stored in a well-sealed container in the dark at room temperature until when needed (National Committee for Clinical Laboratory Standards (NCCLS)., 1999).

A sterile loop was used to pick a loopful of inoculum from a 24hr old pure culture of the test organisms. This was then transferred and suspended in a tube of sterile distilled water. The tube was compared with the turbidity standard and the density of the organism was adjusted to that of the standard by adding more bacteria or more sterile distilled water (Cheesbrough, 2005).

Antibacterial Susceptibility Testing

The susceptibility of the test organisms to the hand sanitizers was investigated using the well variant of the agar diffusion method (Vandepitte et al., 2003). The new formulated nipa disinfectant/antiseptic at different concentrations of 60%, 65%, 70%, 75%, 80% were tested using standard Mueller Hinton Agar plates to detect the antibacterial activity of these testing solutions.

A sterile cotton swab was dipped into a tube containing the inoculum and was rotated properly to allow maximum contact. Excess inoculum was removed by pressing and rotating the swab firmly against the inside of the tube above the liquid level. The swab was then streaked over the surface of the medium three times while rotating the plate through an angle of 60° after each application. The swab was also passed round the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed. With the aid of a sterile 6mm cork-borer, four equally spaced holes were bored in the agar plate with a fifth hole in the center of the plate. The agar plugs were discarded using a sterile needle. Fifty microliters (50µL) of each of the sample was then introduced into each of the 4 wells while the central well was filled with an equal volume of commercial alcohol to serve as control.

All plates were incubated for 24 hr at 37°C in an upright position. They were then examined for zones of inhibition which indicate the degree of susceptibility or resistance of the test organism to the antibacterial agent. The test was carried out in duplicates and the average of 2 readings was taken as the zone of inhibition in each case. Inhibition zones were measured with the aid of a digital caliper (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The formulated nipahol which showed activity against test organisms in the agar diffusion test were subjected to further tests to determine their MIC values using the broth dilution method. MIC is the lowest concentration of a specific antimicrobial needed to prevent the growth of a given

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antimicrobial substance *in vitro* (Valgas et al., 2007). The method used for the determination of MIC in this study was adopted from the study Nester et al., 2009 with several modifications.

Various concentrations of the disinfectant/antiseptic were prepared in increasing order (60%, 65%, 70%, 75% and 80%). Two milliliters of each sanitizer was introduced into tubes containing equal volume (2 ml) of standardized test organisms. Each of the concentrations of the sanitizers was used in each case. A tube containing only nutrient broth and bacteria without sanitizer served as negative control while a tube containing just the sanitizer and broth without bacteria served as positive control. The tubes were incubated at 37°C for 18-24 hr and examined for visible growth or turbidity. The concentration of the disinfectant/antiseptic at which no visible growth was observed when compared with the controls was regarded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

MBC is the lowest concentration of a specific antimicrobial substance that kills 99.9% of cells of a given bacterial strain (Oke et al., 2013). MBC was done following the method of CLSI, 2012 with several modifications. MBC was determined by assaying for live organisms in the tubes from the MIC tests which showed no visible growth. A loopful of inoculum from the MIC tubes was streaked on fresh nutrient agar plates without the hand sanitizer incorporated into 0 them. The plates were incubated at 37°C for 24 hr after which they were observed for growth. Absence of growth indicated a bactericidal effect of the sanitizer at that concentration which is the MBC.

Determination of the Percent (%) Microbial Cell Reduction Overtime

Microbial cell reduction assay was conducted following the method standardized by Clinical and Laboratory Standard Institute (CLSI) but with modifications depending on the availability of materials/chemicals.

Freshly standardized test organisms were used in this assay as previously described. The concentration of nipahol used in this assay is the same as the MBC (70%, 75%, 80%, and 95%). One millimeter of the standardized test organisms was mixed to one millimeter of the test sample in 2 ml capacity Eppendorf tubes. This was done to the other test organism and test samples (other concentrations of disinfectant/antiseptic). The tubes were left in contact for 5 minutes and then 15 minutes. Tubes containing 1ml of the standardized organism and 1ml of sterile distilled water served as the control.

After the contact time, 0.1ml of the mixture was spread plated in pre-solidified Nutrient Agar plates. The experiment was done in duplicate, and all plates were incubated for 24 hrs at 37°C. Percent (%) reduction and log reduction were computed using the formulas (1) and (2) (Kar, 2008).

$$Percent\ (\%)\ Reduction = \frac{(A-B)\ x\ 100}{A} \quad \ldots\ldots (1)$$

$$Log\ Reduction = L\log_{10}\left(\frac{A}{B}\right)10 \quad \ldots\ldots (2)$$

Where:

(1) is the number of viable microorganisms before treatment
(2) is the number of viable microorganisms after treatment

Formulated nipa alcohol samples were sent to Philippine Department of Science and Technology, Regional Office 1 (DOST-RO1) for a confirmatory antimicrobial susceptibility test. Ethanol concentration analysis was also done by the DOST RO1 to validate the quality of the alcohol formulation.

Assessment of the Germicidal Elimination Potential of the Formulated Disinfectant/ Antiseptic through Glo Germ Kit

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The Glo Germ cream simulates the behaviour of real germs so an individual could see how they spread. It is an effective tool to emphasize the importance of hand washing, surface cleaning, applying proper hygiene, and employing containment techniques. Using the Glow Germ cream exposed under ultraviolet (UV) light, the test conducted assesses the effectiveness of the alternative alcohol formulation (Nipa disinfectant/antiseptic) developed by the MMSU-NBERIC. It involved four (4) healthy individuals without any comorbidities who underwent a series of tests composed of five (5) interventions—Set 1: With Soap; Set 2: Without Soap; Set 3: With Alcohol; Set 4 Without Alcohol; Set 5: With Soap and Alcohol. Each intervention was applied in different timeframes—5 seconds, 10 seconds, 15 seconds, and 20 seconds—to identify the application procedure that would yield the most effective use of the developed disinfectant/antiseptic.

Due to the pandemic, there is an urgency of the product to be released and the study did not undergo URERB evaluation because their office was not operational due to lockdown. However, the present study obtained informed consent from the subjects and explained the procedures, risks, and benefits of using the formulated disinfectant from nipa bioethanol. They were assured that they will be given appropriate medical care should there be illnesses that will be contracted by using the product. Moreover, the subjects participated voluntarily after giving the consent. Different concentrations of 60%, 65%, 70%, 75%, 80% were tested using standard Mueller Hinton Agar plates to detect the antibacterial activity of these testing solutions.

RESULTS AND DISCUSSION

Susceptibility of Test Organisms to various Nipahol Concentrations using Agar Diffusion Method

Susceptibility test assay was done to determine the sensitivity or resistance of test bacteria to various antimicrobial compounds such as disinfectants and sanitizers. Table 1 shows the susceptibility pattern of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) to the various nipahol formulations in the agar diffusion tests. The 70% and 80% nipahol concentrations were the only formulations that showed inhibition against all the test organisms with the highest activity against *S. aureus* (6.25mm and 4.25mm, respectively). Lowest activity was observed against *E. coli* with 2.25 mm and 2 mm mean inhibition zone. The rest of the formulations showed no activity while the positive control (commercial ethyl alcohol) also gave the lowest activity (2.4mm) against *E. coli* but not in *S. aureus*.

The results suggest that all disinfectant/sanitizer formulations, instead of those formulations which only showed activity against test organisms, will be subjected to further tests to determine their Minimum Inhibitory Concentration (MIC) values using the broth dilution method. MIC was conducted to determine the lowest concentration of the nipa disinfectant/antiseptic needed to prevent the growth of the test organisms.

Minimum Inhibitory Concentrations (MIC) of Nipa Disinfectant/ Antiseptic against Escherichia coli and Staphylococcus aureus

Table 2 shows the MIC of test organisms to various nipa disinfectant/antiseptic formulations after 24 hours of incubation at 37˚C. Results revealed that inhibition activity was only observed at the 95% nipa alcohol formulation against *E. coli* and 75% formulation against *S. aureus*. Data suggests that Minimum Bactericidal Concentrations (MBC) must be done from 70% to 95% formulations to determine the lowest concentrations that kill 99.9% of cells of the test organisms.

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Table 1. Susceptibility Pattern of the Test Organisms to Nipa Disinfectant/Antiseptic

Test Organisms	Mean Inhibition Zone (mm) of the formulated disinfectant/sanitizer against test organisms at various concentrations						
	60%	65%	70%	75%	80%	Control (+)	Control (-)
Escherichia coli	-	-	2.25	-	2.0	2.4	-
Staphylococcus aureus	-	-	6.25	-	4.25	-	-

- *no inhibition*

Table 2. Minimum Inhibitory Concentration (MIC) of Test Organisms to Various Nipa Disinfectant/Antiseptic Formulations after 24 hr of incubation at 37˚C

Disinfectant/Sanitizer Concentrations (%)	Test Organisms		MIC
	Escherichia coli	***Staphylococcus aureus***	
60	+	+	75% and 95%
65	+	+	
70	+	+	
75	+	-	
80	+	+	
95	-	+	

Note: + growth, - no growth

Minimum Bactericidal Concentrations (MBC) of Nipahol against *Escherichia coli* and *Staphylococcus aureus*

MBC was determined by assaying for live organisms in the tubes from MIC tests which showed minimal or no visible growth. Table 3 reveals the MBC of tested organisms to four nipahol formulations (70%, 75%, 80% and 95%). No visible growth or colonies was observed in the plates containing 95% nipahol formulation which indicate the bactericidal activity against *E. coli* and *S. aureus*. Moreover, the rest of the formulations showed decreasing growth over increasing concentrations of nipahol, thus indicating that these formulations showed only a bacteriostatic effect against test organisms.

Table 3. Minimum Bactericidal Concentration (MBC) of four concentrations of Nipahol to Test Organisms to which showed minimal or no visible growth after 24 hr of incubation at 37˚C

Disinfectant/Sanitizer Concentrations (%)	Test Organisms		MBC
	Escherichia coli	***Staphylococcus aureus***	
70	+	+	95%
75	+	+	
80	+	+	
95	-	-	

Note: + growth, - no growth

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Two formulations displayed bactericidal activity against at least one of the test organisms and the rest showed bacteriostatic activity. This is attributable to the presence of alcohols as the main active ingredients in the products.

Alcohols are known to exert disinfectant activity in bacteria by causing protein denaturation, disruption of tissue membranes and dissolution of several lipids (Clinical and Laboratory Standard Institute (CLSI), 2012). Ethanol was the main ingredient of the locally formulated nipa disinfectant/antiseptic, although isopropanol has been reported as being superior to ethanol as an antiseptic, however, efficacy of alcohol-based hand sanitizers or antiseptic is affected by several factors such as the type, concentration and volume of alcohol used, the contact time (Centers for Disease Control and Prevention (CDC), 2002), the test method (*in vitro* and *in vivo*), target organism and matrix (Liu et al., 2010).

Efficacy of Nipa Disinfectant/Antiseptic in Reducing Viable Bacterial Counts

Alcohol Based Hand Sanitizers (ABHS) contains either ethanol, isopropanol, or n-propanol. A concentration of 60%–95% of alcohol by volume is said to exhibit optimum bactericidal activity. The antimicrobial effect of alcohols is attributed to their ability to dissolve the lipid membranes and denature the proteins of microbes. Based from the paper of Huddleston, 2020, alcohols have broad-spectrum antimicrobial activity against most vegetative forms of bacteria (including Mycobacterium tuberculosis), fungi, and enveloped viruses (human immunodeficiency virus [HIV] and herpes simplex virus). However, they are ineffective against bacterial spores that are found most in raw materials.

Table 4. Mean Percentage (%) Cell Forming Unit (CFU) Reduction of viable bacterial count after 5 and 15 minutes contact time to various nipahol concentrations

Disinfectant/Sanitizer Concentrations (%)	Mean CFU Reduction (%) overtime			
	E. coli		*S. aureus*	
	5 mins	15 mins	5 mins	15 mins
70	85.28	99.78	27.60	67.53
75	93.60	84.10	13.50	37.10
80	91.36	99.35	13.79	49.40
95	100%	95.42	67.71	60.70

Confirmatory Antimicrobial Susceptibility of Nipa Alcohol Formulation

To validate the effect of the locally formulated nipa alcohol as disinfectant and antiseptic, samples were sent to Philippine Department of Science and Technology (DOST), Regional Office 1 for the confirmatory antimicrobial susceptibility of 70% nipahol and 95% nipahol against test organisms. DOST Microbiology Laboratory conducted the confirmatory antimicrobial assay. Three test organisms were used in the test namely, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium*. Noticeably, the nipahol 95% showed the highest inhibitory activities against all test organisms while locally formulated 70% nipahol showed partially active effect against the test organisms used. Data suggests that the higher the concentration of alcohol particularly ethyl alcohol, the active the antimicrobial property. Also, the results of the confirmatory antimicrobial assay support the results obtained from the previous tests (MIC and MBC), that a decreasing colony growth observed over increasing concentrations of nipahol. Thus, the confirmatory results suffice the previous data that nipahol formulations (70-80%) give bacteriostatic effect against possible pathogenic bacteria.

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Table 5. Cell Forming Unit Log Reduction of test organisms after 5 and 15 minutes contact time to various nipahol concentrations

Disinfectant/Sanitizer Concentrations (%)	Log Reduction (Log_{10}) overtime			
	E. coli		*S. aureus*	
	5 mins	15 mins	5 mins	15 mins
70	1 log or 90%	2 log or 99.9%	<1 log	<1 log
75	1 log or 90%	1 log or 90%	<1 log	<1 log
80	1 log or 90%	2 log or 99.9%	<1 log	<1 log
95	100%	2 log or 99.9%	<1 log	<1 log

Interpretation data:
- *1 log reduction = 90% reduction*
- *2 log reduction = 99% reduction*
- *3 log reduction = 99.9% reduction*
- *4 log reduction = 99.99% reduction*
- *5 log reduction = 99.999% reduction*
- *6 log reduction = 99.9999% reduction*

Table 6. Confirmatory Antimicrobial Susceptibility of 70% Nipahol and 95% Nipahol conducted by DOST RO1 Microbiology Laboratory

Treatments	Mean Zone of Inhibition, mm			Interpretation
	S. aureus	*E. coli*	*S. typhimurium*	
Nipahol70	12.23[bc]	13.02[b]	12.56[b]	Partially Active
Nipahol95	16.61[a]	16.42[a]	17.40[a]	Active
Level of Significance	**	*	**	
CV, %	7.80	11.14	10.27	

*** - significant at 0.05 p-value; *- significant at 0.01 p-value*
Interpretation guide:

Zone of inhibition (in mm)	*Interpretation*
Less than 10	*- Inactive*
10-13	*- Partially Active*
14-19	*- Active*
Greater than 19	*- Very Active*

Germicidal Potential of Formulated Nipahol as Detected by Glo Germ Test Kit

Keeping hands clean is a fundamental and essential step to avoid getting sick while limiting the transmission of germs to others. The Glo Germ cream simulates the behavior of real germs, so an individual could see how they spread. It is an effective tool to emphasize the importance of hand washing, surface cleaning, applying proper hygiene, and employing containment techniques. Using the Glow Germ cream exposed under ultraviolet (UV) light, the test conducted assesses the effectiveness of the alternative alcohol formulations (NIPAHOL) developed by the MMSU-NBERIC.

Table 7 shows the effectiveness of nipahol formulations with different interventions against resident microbial flora on the hands of different subjects at different time frame. Noticeably, hand washing with

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the use of soap is the 90-100% effective (Very Much Effective) among the interventions applied. However, data reveals that the use of commercial alcohol or nipa disinfectant/antiseptic without proper hand washing with soap (commercial antibacterial soap) does not eliminate the germs. Therefore, the use of alcohol alone could not yield satisfactory results when it comes to germicidal elimination, hence, could not solely substitute the practice of proper hand washing with soap.

Table 7. Effectiveness of nipahol formulations with different interventions against resident microbial flora on the hands of different subjects at different time frame

Interventions	Sample 1	Sample 2	Sample 3	Sample 4
	5 seconds	10 seconds	15 seconds	20 seconds
	A	B	C	D
1. With soap	Somewhat Effective	Effective	Effective	Very Much Effective
2. Without soap	Not Effective	Not Effective	Not Effective	Not Effective
3. With 60% nipahol	Not Effective	Not Effective	Not Effective	Somewhat Effective
4. With 70% nipahol	Not Effective	Not Effective	Not Effective	Somewhat Effective
5. With 80% nipahol	Not Effective	Not Effective	Not Effective	Somewhat Effective
6. With soap and 70% commercial alcohol	Effective	Effective	Effective	Very Much Effective
7. With 70% commercial alcohol	Not Effective	Not Effective	Not Effective	Somewhat Effective
8. With soap and 60% nipahol	Not Effective	Somewhat Effective	Effective	Very Much Effective
9. With soap and 70% nipahol	Not Effective	Somewhat Effective	Effective	Very Much Effective
10. With soap and 80% nipahol	Not Effective	Somewhat Effective	Effective	Very Much Effective

Rating Scale:

Rating (%)	Descriptive Interpretation	Description
100-90	Very much effective	Glo Germ cream is completely eliminated after an intervention was applied
89-80	Effective	Most of the Glo Germ cream is eliminated after an intervention was applied
79-70	Somewhat effective	Approximately half of the Glo Germ cream is only eliminated after an intervention was applied
69-60	Not effective	Only an insignificant amount of Glo Germ cream was eliminated after the intervention was applied

Notably, the use of commercial alcohol and formulated nipahol with or without the use of soap produced similar results in terms of effectiveness (70-79% effective) in eliminating germs following the 20-second application of the said alcohol formulations on an individual's hands. It was also found out that the effectiveness of the interventions and the length of the application time follow a direct relationship, where the effectiveness

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increases as the time of application increases. Both formulations obtained a descriptive rating of Somewhat Effective (70-79%) when they are used by themselves, and a descriptive rating of Very Much Effective (90-100%) when they are applied after proper hand washing with soap. The results reveal that the Nipahol is as effective as the commercial alcohol and can be utilized as an alternative intervention to prevent the spread of germs. Additionally, the effectiveness of the different nipa disinfectant/antiseptic formulations is heightened with proper hand washing using soap.

Centers for Disease Control and Prevention (CDC) recommends handwashing with soap and water whenever possible as it remarkably reduces the amount of all types of microbes and dirt on the skin surface (Centers for Disease Control and Prevention (CDC), 2019; Gerberding et al., 2002). Both the soaps and alcohol-based sanitizers work by dissolving the lipid membranes of microbes, thereby inactivating them. Thus, the sanitizer serves as an alternative when the soap and water are not readily available. The suggested minimum alcohol content of 60% is needed for it to exert the microbicidal effect. As compared to soap, alcohol-based sanitizers do not eliminate all types of germs, including norovirus and Clostridium difficile, the common pathogens that can cause diarrhea (Blaney et al., 2011; Oughton et al., 2009).

The results of this test may vary depending on the following factors: an individual's hand washing technique, amount of alcohol to put on, amount of Glow Germ applied, soap and alcohol brands used. Furthermore, in vitro testing such as microbial kinetic kill assay must be done to evaluate the germicidal potential of the products.

CONCLUSIONS

Proper hand hygiene is one of the essential infection control strategies as it can undeniably lower the likelihood of direct or indirect transmissions of microorganisms. The use of Alcohol-Based Hand Sanitizer (ABHS) is becoming more common because of its rapid action and efficiency in killing microorganisms, mainly when hand washing using soap and water is not practical or convenient. There are, however, some situations in which handwashing is preferred as ABHS are less effective when the hands are visibly dirty or stained and cannot cover certain kinds of pathogens.

The study explored the antibacterial potential of the locally produced nipa alcohol disinfectant/antiseptic. Results revealed that 95% nipa alcohol showed a bactericidal effect against *Escherichia coli* and *Staphylococcus aureus*. Moreover, high percent bacterial cell reduction was observed when alcohol concentration and time increases. However, factors such as target organisms and matrix may vary the efficacy of the product. In addition, log reduction showed that more bacterial cells killed or inhibited (up to 99.9% reduction) overtime (15 minutes contact time).

A confirmatory antimicrobial test supports the findings of the present study that 95% nipa alcohol showed bactericidal activity while nipa alcohol formulations (70-80%) is bacteriostatic. In addition, nipahol is as effective as the commercial alcohol and can thus be utilized as an alternative intervention to prevent the spread of germs as revealed by the Glo Germ kit test. Thus, the formulated Nipahol possesses antibacterial potential to inhibit the multiplication and spread of infectious pathogens such as *E. coli* and S. *aureus*.

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