Sri Lankan J. Biol. 2024, 9 (1): 1-8

DOI: https://doi.org/10.4038/sljb.v9i1.116

Research Article Open Access

Potential of incorporating *Alpinia malaccensis* crude extract into hand sanitizers

Wijewardhana S.P.R.1 and Weerakkody N.S.[1*]

[1]Department of Agricultural and Plantation Engineering, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Sri Lanka.

Abstract

The commercial hand sanitizers are made from alcohol which might not have residual antibacterial activity because alcohol is highly volatile. This study was performed to determine the efficacy of hand sanitizer incorporated with *Alpinia malaccensis* crude extract. Different concentrations (5 mg/ml, 10 mg/ml and 50 mg/ml) of *A. malaccensis* containing hand sanitizers were compared with that of World Health Organization (WHO) formulation and recommended commercially-available hand sanitizers. Disk diffusion assay and synergistic antimicrobial activity test were used as *in vitro* methods to evaluate antimicrobial inhibition. Finger imprint method was conducted as *in vivo* method to evaluate the efficacy of hand sanitizer on resident microflora for 0, 2, 5, 10, and 15 minutes. Disk diffusion assay was tested against *Staphylococcus aureus* 113, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* Typhimurium. The commercial sanitizer (T4) showed a significantly ($p<0.05$) smaller diameter inhibition zone 14.33±0.58 mm for *S. aureus* compared to other treatments, namely 5 mg/ml (T1), 10 mg/ml (T2) of *A. malaccensis* containing sanitizers and WHO formula (T3). There is a possibility to add *A. malaccensis* crude extract to enhance the efficacy of the commercial sanitizer. A significant synergistic antimicrobial inhibition 7.99±0.02 cfu/ml was recorded in 50 mg/ml+55% alcohol hand sanitizer (T2) compared to the control (T4). The finger imprint method did not show any significant differential reduction within the tested time. Alcohol-based hand sanitizers incorporated with herbal *A. malaccensis* could be used to enhance the efficacy of the available commercial sanitizers.

Received: 27 April 2023

Accepted: 31 May 2023

Keywords:

Alcohol-based
Anti-bacterial
Crude extract
Diameter Inhibition Zone
Phytochemical
Synergistic

*Corresponding author:
nweer@ou.ac.lk

https://orcid.org/0000-0001-68679206

1. Introduction

Hand sanitization and hygiene have become vital practices among the general public to overcome cross-contamination of Coronavirus such as SARS-COV2. Different sanitizers including ethanol-based liquid spray, foam, gels, and soap have been heavily used to sanitize hands, to control the infection by SARS-CoV2. The World Health Organization (WHO) recommends alcohol-based hand sanitizers due to their rapid action and broad-spectrum antimicrobial activity against bacteria and viruses (Jing *et al.*, 2020). Furthermore, alcohol-based hand sanitizers are most effective and suitable as infection preventive measures (Fallica *et al.*, 2021). Alcohol-based hand sanitizers contain 60-95% (v/v) alcohol in water and has the ability to denature proteins making up cell walls of microorganisms (Akash *et al.*, 2021). Furthermore, the alcohol-based hand sanitizers in the market have claimed an ability to destroy 99.99% of microorganisms (Suryawanshi *et al.*, 2020; Surwase *et al.*, 2021).

However, alcohols are highly volatile; as a result, alcohols can evaporate from the skin surface; hence, there is no residual antibacterial activity (Shintre *et al*., 2007; Bondurant *et al*., 2019). Various studies have reported that chlorhexidine has persistent antimicrobial activity (Macias *et al.*, 2013; López-Gigosos *et al.*, 2017). Furthermore, chlorhexidine gluconate (CHG), iodophors, p-chloro m-xylenol, triclosan, hexachlorophene, zinc pyrithione, and quaternary ammonium compounds are antimicrobials used in alcohol-based hand-disinfectants (Shintre *et al*., 2007). However, effective concentrations of these antimicrobials can induce skin reactions.

Plants produce naturally derived antimicrobials as secondary metabolites and these are accompanied by anti-infective mechanisms against a broad spectrum of pathogenic microorganisms (Zhang *et al.*, 2019). Furthermore, natural herbs and spices can produce diverse secondary metabolites to protect from the attacks of insects, herbivores, and pathogens (Zhang *et al.*, 2021). Moreover, plants and plant-derived compounds have been used in various industries as food preservatives, pharmaceuticals, cosmetics, and perfumes due to the presence of antifungal, antibacterial, and antioxidant properties. The combination of non-toxic concentrations of different chemical compounds may induce synergistic antimicrobial activity while minimizing side effects (Shintre *et al*., 2007). Therefore, naturally derived antimicrobials can be used instead of chemical compounds.

Alpinia malaccensis (Ran keeriya) is a perennial plant, which is native to Indonesia and Malaysia. This plant is one of the 230 species of the Zingiberaceae family. This plant is a rhizome-producing and grows in the tropical and subtropical regions of Asia (Juwitaningsih *et al*., 2016). Previous studies have identified 1' Acetoxy chavicol acetate (1'ACA) as the bioactive chemical compound in the hexane extract of *A. malaccensis* rhizome (Somarathna *et al.*, 2018, 2020). 1'ACA is the main chemical compound (82.87%) of the crude extract with hexane used as a solvent (Somarathna *et al.*, 2018). Furthermore, 1'ACA for ethanol extract was 65.11% (Somarathna *et al.*, 2018). 1'ACA has strong antibacterial activities against microorganisms specifically against *Staphylococcus aureus* (Weerakkody *et al.*, 2011), and *Listeria monocytogenes* (Somarathna *et al.*, 2018, 2020). 1' ACA has demonstrated efficiency in the elimination of multi-drug resistant bacteria such as *Salmonella enterica*, *Pseudomonas aeruginosa*, *Escherichia coli*, Vancomycin resistant *Enterococcus* (Latha *et al.*, 2009). In addition, 1'ACA is a very effective phytochemical for inhibiting the function of HIV-1 virus activity (Ye and Li, 2006). Therefore, there might be an antiviral activity to act on other viruses. Furthermore, previous studies have been investigated, eye irritation toxicity levels and non-abraded skin irritation test for American white rabbit skin showed that 5 mg/ml-20 mg/ml and 750 mg/ml of crude *Alpinia galanga* extract were non-irritant (Karunarathne *et al*., 2018). The oral toxicity studies in the rat model showed that *A. malaccensis* n-hexane extract 2000 mg/ kg body weight did not produce any adverse effect on behavior, body weight, feed intake, biochemical parameters, and organ histology (Somarathna *et al.*, 2021). Moreover, plant extracts at non-toxic concentrations did not induce DNA damage in A549 cell and therefore nontoxic concentrations of *A. malaccensis* could be used for human consumption without any adverse health effects. Furthermore, the acceptable daily intake (ADI) value for *A. malaccensis n*-hexane extract was calculated NOAEC (No-Observed Adverse Effect Concentration) divided by uncertainty factor 10 and reported ADI as 55.41 mg/day (Somarathna et al., 2021).

There is a requirement to develop a sanitizer having multiple modes of action in controlling a broad spectrum of microorganisms. Therefore, it was speculated that adding the *A. malaccensis* crude extract could exert synergistic antimicrobial activity than solely using Isopropyl Alcohol (IPA). Since the active compound of A. *malaccensis* 1' ACA is not volatile it can contribute to residual microbial effects for a certain time range when incorporated into an alcohol-based sanitizer. To-date, there is no published data on the antibacterial efficacy of hand sanitizer developed by incorporating *A. malaccensis* crude extract. The main objective of this study is to determine the efficacy of the alcohol-based hand sanitizer with *A. malaccensis* crude extract compared to the WHO formulation (75% isopropyl alcohol) and commercial product. Furthermore, as a chemical parameter, pH was investigated during the storage period.

2. Materials and Methods

2.1 Extraction of herbs

Fresh *Alpinia malaccensis* (Ran Keeriya) rhizomes were collected from the medicinal garden of the Nature Secret (Pvt) Ltd, Millewa, Horana, Sri Lanka. Fresh *A. malacccensis* rhizomes were cleaned up using running water and the outer skin was removed. The cleaned rhizomes were sliced and oven-dried at 40 ^{o}C for 10 h (Model NB-7500E, Japan). The slices were ground (Prestige PMG 02, India) for 2 h at 3-minute intervals. The ground powder was stored at -20 ^{o}C until use.

Ethanol was used as the solvent for the extraction. The extract was prepared by adding 20 g of *A. malaccensis* powder to 200 ml of 96% ethanol. The content was agitated (140 rpm) for 24 h at 28^{o}C in a rotary shaker (Bibby scientific limited, Stone, Staffordshire, ST15 OSA, UK). The mixture was filtered using a Buncher funnel with No 1 Whatman filter paper under a vacuum. The filtrate was evaporated to dryness by using a rotary evaporator (Bouchi Labortechnik AG 9230 Flawil, Switzerland) under a vacuum at 40 ^{o}C water bath. Finally, the concentrated extract was redissolved in

ethanol (96%) to make a 0.5 g/ml stock solution, and was stored at 4 ºC until use (Weerakkody *et al.*, 2011).

2.2 Formulation of hand sanitizer

WHO has released two formulations of alcohol-based hand sanitizer consisting of ethanol (formulation 1) or Isopropyl Alcohol (IPA) (formulation 2) with hydrogen peroxide and glycerol. In this experiment test, hand sanitizers were formulated according to formulation 2. The hydrogen peroxide (3%) 4.17 ml was added to a flask containing Isopropyl alcohol (99.8%) 75.15 ml. Next, Glycerol (98%) 1.45 ml was added gradually, and a uniform mixture was prepared. An aliquot of 1 ml and 2 ml from the 0.5 g/ml stock solution was added separately to the above mixtures to make 5 mg/ml and 10 mg/ml formulations respectively. The final volume is made up to 100 ml using deionized water and the mixture was vortexed to get a homogenous solution (WHO, 2010).

2.3 Anti-bacterial efficacy testing

Antibacterial activities of test sanitizers were determined against Gram-positive *S. aureus* 113, *L. monocytogenes*, and gram-negative *E. coli, S. enterica* Typhimurium. Source of culture collection for *S. aureus* 113 and *L. monocytogenes* V7 were obtained from University of Queensland, Brisbrane, Australia. *E. coli* ATCC 1858 and *S. enterica* Typhimurium were obtained from the American Type culture collection (Manassa, USA).

Bacterial strains were confirmed using the Gram staining method and biochemical methods (Catalase test). Baird Parker Agar with Egg Yolk Tellurite Emulsion was used for the identification of *S. aureus* 113. *E. coli* was identified using Violet Red Bile Glucose agar medium. *S. enterica* Typhimurium was identified using Xylose Lysine Deoxycholate agar medium. All bacterial strains were maintained in 80% glycerol at -20 ºC as frozen stock cultures. Working cultures were maintained in Nutrient Agar.

2.3.1 Disc diffusion assay

The antibacterial activity of sanitizers was checked by using the disc diffusion method (Somarathna *et al.*, 2018). A single colony of bacteria was grown in 2 ml Tryptic Soy Broth (TSB) at 37 ºC for 18± 2h. The content was centrifuged at 9000g for 10 min to obtain a bacterial pellet. The supernatant was removed, and the bacterial pellet was re-suspended in 1 ml sterile 0.85% NaCl and serially diluted in 9 ml of sterile 0.85% NaCl solution to obtain $5x10^5$ CFU (Colony-forming unit) per ml. This procedure was carried out for *S. aureus* 113, *L. monocytogenes*, *E. coli*, *S. enterica* Typhimurium separately. From each diluted bacterial suspension 100 µl was spread on Mueller-Hinton agar plates. An aliquot of test sanitizer was pipetted onto sterile paper discs (5.5 mm diameter, Whatman no.1), as shown in the Table 1 which were placed on the agar surface. Incubation was carried out for 18 h at 37 ºC. Each experiment was repeated with triplicate. The antibacterial activity was analyzed by measuring the Diameter of the Inhibition Zone (DIZ) in millimeters.

Table 1: Treatments of disk diffusion assay

Treatments	*A.malaccensis* ethanol crude extract amount	75 % isopropyl Alcohol (99.8%)	H_2O_2 (3%)	Glycerol (98%)	Distilled water
T1	5 mg/ml	75.15 ml	4.17 ml	1.45 ml	Up to 100 ml
T2	10 mg/ml	75.15 ml	4.17 ml	1.45 ml	Up to 100 ml
T3 (WHO standard)	0	75.15 ml	4.17 ml	1.45 ml	Up to 100 ml
T4 (Commercial hand sanitizer)	0				

Table 2: Treatments for in vitro Evaluation of synergistic effect of isopropyl alcohol and *Alpinia malaccensis* crude extract against *Staphylococcus aureus* 113

Treatment No	*A.malaccensis* ethanol crude extract	55% isopropyl Alcohol (99.8%)	H_2O_2 (3%)	Glycerol (98%)	Distilled water	NaCl
T1	10 mg/ml	55.1 ml	1.06 ml	0.092 ml	up to 100 ml	0
T2	50 mg/ml	55.1 ml	1.06 ml	0.092 ml	up to 100 ml	0
T3	0	0	0	0	up to 100 ml	0.85 g
T4	0	55.1 ml	1.06 ml	0.092 ml	up to 100 ml	0

Table 3: Treatments for finger Imprint method

Treatment No	*A.malaccensis* ethanol crude extract	75% isopropyl alcohol (99.8%)	H_2O_2 (3%)	Glycerol (98%)	Distilled water
T1 (*A.malaccensis*)	10 mg/ml	75.15 ml	4.17 ml	1.45 ml	up to 100 ml
T2 (WHO/Without *A.malaccensis*)	0	75.15 ml	4.17 ml	1.45 ml	up to 100 ml

2.3.2 *In vitro* Evaluation of the synergistic effect of isopropyl alcohol and *A. malaccensis* crude extract against *S. aureus* 113

To evaluate the synergistic effect of isopropyl alcohol with *A. malaccensis* crude extract was evaluated according to the previously described method by Shintre *et al*., (2007). Either 10 mg/ml or 50 mg/ml *A. malaccensis* crude extract was added to the base containing 55% Isopropyl alcohol with 6% H_2O_2, 98% glycerol. 55% Isopropyl alcohol-containing corresponding amounts of 6% H_2O_2, 98% glycerol was selected as positive control whereas NaCl was selected as the negative control as in Table 2.

A mixture of 0.5 ml of 10^8 cfu/ml of *S. aureus* culture was placed in a sterile 2 ml Ependorf tube. An aliquote of 100 µl of the test formulation was added to the tube and vortexed for 10 seconds. This was diluted 1:10 with the dilution fluid to neutralize the activity of the test formulation, and then serially diluted with 0.85% NaCl solution, and 100 µl was plated on nutrient agar plate. The plates were incubated at 37 ºC for 24 hours. The number of colonies was counted using the colony counter and cfu/ml was determined.

2.3.3 Finger imprint method

In this method hand sanitizer with *A. malaccensis* crude extract was compared with WHO formulation by screening the microbial load before and after its application to hands. The study was performed on 7 healthy volunteers without any clinical signs of dermal abrasion and infection and nails that were short and clean were included in this study. They were asked to rub both hands thoroughly before the experiment. Sterile nutrient agar was poured into sterile Petri dishes. After solidifying, plates were used for this test.

Under the aseptic condition, four fingers (thumb wasn't used) of both hands were firmly pressed on the surface of the nutrient agar plates and the plates were incubated at 37 ºC for 24 h as the control. Test sanitizers 2 ml of each with 10 mg/ml *A. malaccensis* was applied on the right hand and WHO formulation as in Table 3 (without *A. malaccensis*) was applied on the left hand. The volunteers were asked to rub each palm with fingers in both hands separately for 30 seconds. As earlier, finger imprints were taken on agar plates immediately after 30 seconds (at 0 minutes) and incubated at 37 ºC for 24 hours. A similar test was performed at 2, 5, 10, and 15 minutes (Singla & Saini, 2019). After incubation, colonies were observed and counted using a colony counter. Percentage reduction in the bacterial load (R%) was calculated as follows,

$$\%R = \left[\frac{(BBW-BAW)}{BBW}\right] \times 100 \quad \text{-(Eq.1)}$$

Where BAW is bacterial load after sanitizer use at 0, 2, 5, 10, and 15 minutes respectively and BBW is the bacterial load before sanitizer use (Balkrishna *et al*.,2020).

2.4 Determination of pH

A digital pH meter (AD1000 pH/mV & Temperature Meter) was used to determine pH by eight weeks at room temperature (27ºC). pH measurements were performed in triplicate.

2.5 Statistical analysis

The triplicated data was analyzed by analysis of variance of the general linear model procedure in software Minitab version 17 for disc diffusion assay and finger imprint method. The mean comparison was performed using the Tukey test. While, synergistic antimicrobial activity data was analyzed by comparisons between groups using one-way analysis of variance. A p-value of $P<0.05$ was considered statistically significant.

3. Results

3.1 Disc diffusion assay

Antimicrobial activity of formulated disinfectants is shown in Table 4. There was a significant difference ($p<0.05$) among treatments for *S. aureus* 113. The commercial sanitizer (T4) showed a significantly ($p<0.05$) lower DIZ of 14.33±0.58 mm for *S. aureus* 113 compared to other treatments T1, T2, T3. In addition, there was no significant difference ($p>0.05$) between sanitizers containing *A. malaccensis* (T1 and T2) and sanitizer of WHO formulation (T3). Furthermore, there was no significant difference ($p>0.05$) in DIZ observed for sanitizers against *E.coli*, *L. monocytogens* V7, and *S. enterica* Typhimurium.

3.2 In vitro evaluation of the synergistic effect of isopropyl alcohol and *A. malaccensis* crude extract against *S. aureus* 113

In vitro synergistic antimicrobial activity of *A. malaccensis* in combination with 55% isopropyl alcohol against *Staphylococcus aureus* 113 is shown in Table 5. Our investigation of the synergistic activity between 55% isopropyl alcohol with 10 mg/ml, 50 mg/ml crude extract respectively. There was a significant different ($p<0.05$) among treatments T1 (10 mg/ml *A. malaccensis* crude extract +55% alcohol), T2 (50 mg/ml *A. malaccensis* crude extract +55% alcohol), T3 (control-0.85% NaCl solution) and T4 (55% alcohol) whereas T1 (10 mg/ml *A. malaccensis* crude extract +55% alcohol), T2 (50 mg/ml *A. malaccensis* crude extract +55% alcohol), T4 (55% alcohol) sanitizers showed significant different ($p<0.05$) with T3 (control-0.85% NaCl solution). Any synergistic effect was not observed for *A. malaccensis* containing sanitizer (T1) compared to commercial sanitizer (T4).

Table 5: *In vitro* synergistic rapid antimicrobial activity of *A. malaccensis* in combination with 55% isopropyl alcohol against *Staphylococcus aureus* 113.

Treatment	log cfu/ml*
T1	8.16 ± 0.02^{b}
T2	7.99 ± 0.02^{c}
T3	8.45 ± 0^{a}
T4	8.20 ± 0.1^{b}

T1- 10 mg/ml+55% isopropyl Alcohol hand sanitizer, T2- 50 mg/ml+55% isopropyl Alcohol hand sanitizer, T3- 0.85% NaCl solution, T4- 55% isopropyl Alcohol hand sanitizer

*Within a column, mean values followed by the same lowercase letter are not significantly different ($p>0.05$).

Surprisingly, T2 showed significant different ($p<0.05$) compared to T3 with 0.47 $\log_{10}$ cfu/ml reduction which is < 2 log reduction.

3.3 Finger imprint method

The reduction percentage of microorganisms on fingers by the T1 (WHO formula) and T2 (10 mg/ml *A. malaccensis* extract+ 75% alcohol) sanitizer over time is shown in figure 1. There was no significant difference ($p>0.05$) in reduction % between T1 and T2 hand sanitizer over the time.

3.4 pH

There was no significant difference on pH over the tested period (Figure 2). pH is mild acidic to neutral in the range 5.21-5.99.

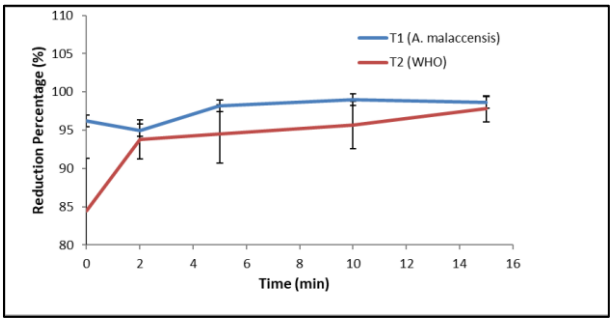

Figure 1: Reduction percentage in the microbial load with the time of Finger imprint method

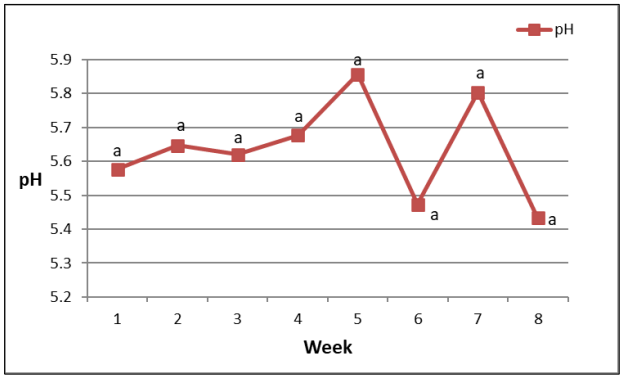

Figure 2: Changes of the pH

Table 4: Antimicrobial activity (Diameter Inhibition Zone) of *A. malaccenssis* crude extract against bacteria

Micro-organism	Diameter of Inhibition Zones(mm)			
	T1	T2	T3	T4
Staphylococcus aureus SA 113	$23.33\pm1.15^{a*}$	23.67 ± 0.58^{a}	23.67 ± 0.58^{a}	14.33 ± 0.58^{b}
Escherichia coli	13 ± 1^{b}	13.67 ± 1.15^{b}	14 ± 2.65^{b}	13 ± 1^{b}
Listeria monocytogenes	12 ± 0^{b}	13.67 ± 0.58^{b}	12.33 ± 0.58^{b}	13.33 ± 0.58^{b}
Salmonella enterica Typhimurium	12.67 ± 0.58^{b}	13.67 ± 0.58^{b}	13.33 ± 0.58^{b}	13 ± 1^{b}

T1-5 mg/ml *A. malaccensis* crude extract+75% Alcohol hand sanitizer T2-10 mg/ml *A. malaccensis* crude extract +75% Alcohol hand sanitizer T3-75% WHO recommended Alcohol hand sanitizer T4-Commercial hand sanitizer.

*[b]Within a row mean values with the same lowercase letter are not significantly different (p).

4. Discussion

Although alcohol-based hand sanitizer can reduce a broad spectrum of microbes effectively and quickly, there are few shortcomings with the effectiveness (Jing *et al.*, 2020). Therefore, antiseptics with persistent and cumulative effects are desirable and persistent effects contribute to reducing contamination and retard the regrowth of resident bacteria on hands or cumulative residual effects could reduce the microorganisms on hand (López-Gigosos *et al.*, 2017). In our study it was evident that *A. malaccensis* crude extract addition could exert synergistic antimicrobial activity than using isopropyl alcohol alone. In addition, it was reported that the killing power of the hand sanitizer with natural ingredients (77.6%) has more enhanced effectivity than market product (67.1%) (Sukamdi *et al.*, 2020).

In our study, we observed that commercial sanitizer (MFD 24.06.2021-EXP 23.06.2024) had poor inhibition on *S. aureus* 113 compared to other treatments. Commercial hand sanitizers may not use standard formula or maybe degrade their antimicrobial properties while storage. Therefore, the addition of *A. malaccensis* crude extract could enhance the efficacy of the available commercial sanitizer. In addition, there was no significant difference between *A. malaccensis* containing sanitizers (T1 and T2) and WHO (T3) standard hand sanitizer. This may be due to the incompatibilities of polar ethanol solvent to perform *A. malaccensis* activity. Zhang *et al.*, (2021) found that the main antibacterial compounds (1'ACA) of *A. galanga* have low polarities because the highest DIZ values are exhibited with non-polar solvents such as n-hexane and chloroform. It can be concluded that to exhibit the antibacterial properties of 1'ACA phytochemical needs a solvent with low polarity because, in a polar solvent, 1'ACA becomes an unstable form. Furthermore, there was no significant difference ($p>0.05$) in DIZ observed for sanitizers against *E. coli*, *L. monocytogens* V7, and *S. enterica* Typhimurium.

A higher percentage of alcohol kills 99-100% of the microorganisms. As a result, WHO formula containing 75% Isoprophyl alcohol couldn't use to determine the synergistic effect due to rapid microbial killing. Therefore, 55% Isoprophyl alcohol was used to study the synergistic activity. Although MBC (minimum Bactericidal concentration) of *A. malaccensis* ethanol extract was >5 mg/ml (Somarathna *et al.*, 2018) for *Staphyloccous aureus* 113, there was no synergistic effect for T1 (10 mg/ml+ 55% isopropyl alcohol sanitizer) compared to T4 (55% isopropyl alcohol sanitizer). Karunarathne *et al*., (2018) showed that the safe effective dosage of *A. galanga* as 750 mg/ml was not irritant on non-abraded skin of New Zealand white rabbits. *A. galanga* and *A. malaccensis* belong to the same genera and with more or less similar chemical composition with major bioactive chemical compound 1'ACA. 1'ACA was the most abundant bioactive (82.87%) chemical compound of the crude extract of *A. malaccensis* (Somarathna *et al.*, 2020). Somarathna *et al*., (2021) showed that administration of 2000 mg/kg body weight dose of *A. malaccensis in vivo* oral acute toxicity did not produce significant toxicity or mortality. Therefore, according to Somarathna *et al*., (2021) and Karunarathne *et al*., (2018) Therefore, increased concentration of *A. malaccensis* crude extract incorporated sanitizer could be formulated in the future.

Somarathna *et al.*, (2018) showed that *A. malaccensis* have significant antimicrobial activity against Gram-positive bacterial strains of *S. aureus* including methicillin-resistant *S. aureus*. Gram-positive bacteria are more sensitive to spice and herb extracts or essential oil than Gram-negative bacteria due to the differences in the cell envelope structure. Cell envelope structure where antibacterial molecules can penetrate through Gram-positive bacterial cell wall and reach the cytoplasmic membrane, which facilitates the leakage of the cytoplasm and coagulation (Bhuvana *et al*., 2020). Therefore, further studies are needed with a higher concentration of extract, to identify synergistic antimicrobial activity on the broad spectrum of microorganisms including fungi and viruses.

Previous studies showed higher DIZ (40±0.5 mm) against *S. aureus* 113 for *A. malaccensis* hexane crude extract dissolved with DMSO (dimethyl sulfoxide) (Somarathna *et al.*, 2018). However, in this study showed DIZ (23.67±0.58 mm) against *S. aureus* 113 ethanol when an extracted crude extract 10 mg/ml with 75% alcohol sanitizer was employed. This may be due to the activity of universal solvent DMSO as it will solubilize the crude extract and exert its maximum antimicrobial activity. However, DMSO or hexane cannot be used as solvents for the preparation of hand sanitizer due to its cytotoxicity. Ethanol was used as a solvent to dissolve *A. malaccensis* crude extract to prepare hand sanitizer. The efficacy of *A. malaccensis* crude extract could change due to various factors such as type of microorganism, age, variety, time of harvesting, time of the day, stage of the development, freshness, or dryness of the plant material, isolation technique, solvent used and climatic condition of the region where plants were grown (Janssen *et al*., 1987).

pH is an important parameter to consider while developing a hand sanitizer to enhance the properties of skin, minimizing irritation, and stabilizing the ecological balance of the skin. A mild pH is one of the ways to minimize damage to the skin.

5. Conclusion

This study generally concludes that, the combination effect of *A. malaccensis* and Isopropyl alcohol could control the growth of *S. aureus* 113 and other microbes residing on the surface of hands. The developed *A. malaccensis* crude extract 10 mg/ml with 75% Isopropyl alcohol sanitizer and standard WHO-recommended Isopropyl alcohol sanitizer had a strong similar bacterial inhibition effect than the available commercial sanitizer. This could be due to deviations of standard WHO formula in commercial hand sanitizers or degeneration of antimicrobial properties while in storage. Synergistic antimicrobial activity was able to be achieved with low concentration of IPA with the *A. malaccensis* crude extract. The developed Alcohol-based herbal hand sanitizer had neutral to acidic pH confirming its potential to be used as a hand sanitizer.

6. Acknowledgments

The authors wish to thank for financial support with Grant No FC/345/14:03 (2021) from the Open University of Sri Lanka.

7. Conflict of Interest

Authors have declared no competing of interest.

8. References

Akash, N., Rani, L., Brundha., Somasundaram, J. (2021) Role of hand sanitizers in infection control practices, *Annals of the Romanian Society for Cell Biology* 25(3): 815–825.

Aodah, A.H., Bakr, A.A., Booq, R.Y., Rahman, M.J., Alzahrani, D.A., Alsulami, K.A., Alshaya, H.A., Alsuabeyl, M.S., Alyamani, E.J., Tawfik, E.A. (2021) Preparation and evaluation of benzalkonium chloride hand sanitizer as a potential alternative for alcohol-based hand gels, *Saudi Pharmaceutical Journal* 29(8): 807–814. https://doi.org/10.1016/j.jsps.2021.06.002.

Balkrishna, A., Singh, K., Singh, H., Haldar, S. and Varshney, A. (2020). GermiX: A skin friendly hand sanitizer with prolonged effectivity against pathogenic bacteria. *AMB Express*, *10*(1), pp.1-8.

Bondurant, S.W., Duley, C.M., Harbell, J.W. (2019) Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application, *American Journal of Infection Control* 47(8): 928–932. https://doi.org/10.1016/j.ajic.2019.01.004.

Bhuvana, K.S., Mandal, P.K., Pal, U.K., Antony, P.X., Kasthuri, S. (2020) Antimicrobial efficacy of Garcinia cambogia fruit extract against food borne bacterial pathogens, In vitro and chicken meat, *Journal of Meat Science*, 15(2):61-66. https://doi.org/10.5958/2581-6616.2020.00016.x.

Fallica, F., Leonardi, C., Toscano, V., Santonocito, D., Leonardi, P., Puglia, C. (2021) Assessment of alcohol-based hand sanitizers for long-term use, formulated with addition of natural ingredients in comparison to who formulation 1, *Pharmaceutics* 13(4):. 1–16. https://doi.org/10.3390/pharmaceutics13040571.

Janssen, A.M., Scheffer, J.J.C., Baerheim Svendsen, A. (1987) Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods, *Planta Medica* 53(5): 395–398. https://doi.org/10.1055/s-2006-962755.

Juwitaningsih, T., Juliawaty, L.D., Syah, Y.M. (2016) Two pyrones with antibacterial activities from alpinia malaccensis, *Natural Product Communications* 11(9): 1297–1298. https://doi.org/10.1177/1934578x1601100928.

Karunarathne, P.U.H.S., Thammitiyagodage, M.G., Weerakkody, N.S., (2018) Safety evaluation of galangal (Alpinia galanga) extract for therapeutic useas an antimicrobial agent, *International Journal of Pharmaceutical Sciences and Research*, 9(11): 4582–4590. https://doi.org/10.13040/IJPSR.0975-8232.9(11).4582-90.

Latha, C., Shriram, V.D., Jahagirdar, S.S., Dhakephalkar, P.K., Rojatkar, S.R. (2009) Antiplasmid activity of 1′-acetoxychavicol acetate from Alpinia galanga against multi-drug resistant bacteria, *Journal of Ethnopharmacology* 123(3): 522–525. https://doi.org/10.1016/j.jep.2009.03.028.

Jing, J.L.J., Yi, T.P., Bose, R.J.C., McCarthy, J.R., Tharmalingam, N., Madheswaran, T. (2020) Hand Sanitizers: a Review on Formulation Aspects, Adverse Effects, and Regulations, *International Journal of Environmental Research and Public Health* 17: 3326.

López-Gigosos, R.M., Mariscal-Lopez, E., Gutierrez-Bedmar, M., Garcia-Rodriguez, A., Mariscal, A. (2017) Evaluation of antimicrobial persistent activity of alcohol-based hand antiseptics against bacterial contamination, *European Journal of Clinical Microbiology and Infectious Diseases* 36(7): 1197–1203.

https://doi.org/10.1007/s10096-017-2908-9.

Macias, J.H., Arreguin, V., Munoz, J.M., Alvarez, J.A., Mosqueda, J.L., Macias, A.E. (2013) Chlorhexidine is a better antiseptic than povidone iodine and sodium hypochlorite because of its substantive effect, *American Journal of Infection Control* 41(7): 634–637. https://doi.org/10.1016/j.ajic.2012.10.002.

Sukamdi, D.P., Kurniawan, M.F., Damarwati, V.L. (2020) Formula optimizaion of antimicrobial hand sanitizer with lemongrass essential oil, *Fmipa*, 33: 353-356.

Shintre, M.S., Gaonkar, T.A., Modak, S.M. (2007) Evaluation of an Alcohol-Based Surgical Hand Disinfectant Containing a Synergistic Combination of Farnesol and Benzethonium Chloride for Immediate and Persistent Activity Against Resident Hand Flora of Volunteers and With a Novel In Vitro Pig Skin Model, *Infection Control & Hospital Epidemiology* 28(2): 191–197. https://doi.org/10.1086/510866.

Singla, D., Saini, K. (2019) Formulation of an Herbal Substitute for Chemical Sanitizer and its Evaluation for Antimicrobial Efficiency, *International Journal of ChemTech Research* 12(03): 114–120. https://doi.org/10.20902/ijctr.2019.120318.

Somarathna, T., Ranaweera, K.K.D.S., Premakumara, G.A.S., Weerakkody, N.S. (2018) Antimicrobial activity and phytochemical screening of Alpinia malaccensis (Ran-kiriya) against food-borne bacteria, *Journal of Applied Microbiology* 125(5): 1276–1285. https://doi.org/10.1111/jam.14039.

Somarathna, T., Fernando, W.M.A.D.B., Ranaweera, K.K.D.S., Premakumara, G.A.S., Weerakkody, N.S. (2020) Antibacterial and antibiofilm activity of Alpinia malaccensis and Terminalia catappa extract combinations on Staphylococcus aureus and Listeria monocytogene strains, *Ijambr*, 8: 73–80.

Somarathna, T., Thammitiyagodage, M.G., Ranaweera, K.K.D.S., Premakumara, G.A.S., Akbarsha, M.A., Kadalmani, B., Weerakkody, N.S. (2021) In Vivo and in Vitro Toxicity Profiles of Hexane Extract of Alpinia malaccensis Rhizome in Rat and Cell Line Models, *Journal of Toxicology*, 2021. https://doi.org/10.1155/2021/9578474.

Surwase, V.B., Savale, M.M., Jadhav, R.S., Akshaykumar., Kadam, B., Shinde, P.P. (2021) Polyherbal Natural Hand Sanitizer Formulation and Evaluation, *Journal of University of Shanghai for Science and Technology*, 23(06): 932–939. https://doi.org/10.51201/jusst/21/05360.

Suryawanshi, V.R., Surani, H.C., Yadav, H.R. (2020) Formulation, Evaluation and Anti-Microbial Efficiency of Alcohol Based Herbal Hand Sanitizer, *Ijesc*, 10(3): 25113–25115.

Weerakkody, N.S., Caffin, N., Lambert, L.K., Turner, M.S., Dykes, G.A. (2011) Synergistic antimicrobial activity of galangal (Alpinia galanga), rosemary (Rosmarinus officinalis) and lemon iron bark (Eucalyptus staigerana) extracts, *Journal of the Science of Food and Agriculture*, 91(3): 461–468. https://doi.org/10.1002/jsfa.4206.

WHO., (2010) Guide to Local Production: WHO-recommended Handrub Formulations Introduction:, *Who*, (April), p. 9.

Ye, Y., Li, B. (2006) 1′S-1′-acetoxychavicol acetate isolated from Alpinia galanga inhibits human immunodeficiency virus type 1 replication by blocking Rev transport, *Journal of General Virology* 87(7): 2047–2053. https://doi.org/10.1099/vir.0.81685-0.

Zhang, D., Gan, R., Farha, A.K., Kim, G., Yang, Q., Shi, C., Luo, Q., Xu, X., Li, H., Corke, H. (2019) Discovery of antibacterial dietary spices that target antibiotic-resistant bacteria, *Microorganisms* 7(6). https://doi.org/10.3390/microorganisms7060157

Zhang, D., Zou, L., Wu, D., Zhuang, Q., Li, H., Mavumengwana, V., Corke, H., Gan, R. (2021) Discovery of 1'-acetoxychavicol acetate (ACA) as a promising antibacterial compound from galangal (Alpinia galanga (Linn.) Willd), *Industrial Crops and Products* 171(July): 113883. https://doi.org/10.1016/j.indcrop.2021.113883.

Balkrishna, A., Singh, K., Singh, H., Haldar, S. and Varshney, A. (2020). GermiX: A skin friendly hand sanitizer with prolonged effectivity against pathogenic bacteria. *AMB Express*, *10*(1), pp.1-8.