The Pharmaceutical Journal of Sri Lanka 2021 11(1): Page 14 – 26

DOI: http://doi.org/10.4038/pjsl.v11i1.65

Research Article

Formulation and characterization of essential oils based antibacterial hand sanitizer gels

Diviyagahage C. M.[1], Thuvaragan S.[2*], Gnanakarunyan T. J. [3], Srikaran R. [4]

[1]State Pharmaceuticals Manufacturing Corporation of Sri Lanka, Rathmalana, Sri Lanka.
[2]Department of Pharmacy, Faculty of Allied Health Sciences, University of Jaffna, Jaffna, Sri Lanka.
[3]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Jaffna, Sri Lanka.
[4]Department of Chemistry, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka.

*Corresponding author: *sthuvaragan@univ.jfn.ac.lk*

Revised: 29 July 2021; Accepted: 18 December 2021

ABSTRACT

Purpose: The usage of herbal-based antibacterial sanitizers is increasing, and they have considerably shown fewer side-effects than sanitizers with synthetic compounds. In the present study, essential oils obtained from cinnamon and lime were used to formulate hand sanitizer gels, and their efficacy was evaluated.

Method: Three different hand sanitizer gel formulations were prepared using cinnamon oil (Formulation A), lime oil (Formulation B) and a mixture of cinnamon and lime oil (Formulation C). Carbopol 940 was used as the gelling agent. Tests for organoleptic, physicochemical characteristics and stability were conducted on the prepared formulations. The antibacterial activity of the prepared formulations was evaluated using the agar diffusion method. All three formulations were compared with a commercial liquid hand wash product for their *in vitro* antibacterial activity. Three-way ANOVA with Tukey's honestly significant difference test was used to compare the antibacterial activity of the hand sanitizer gel formulations. Stability of the three hand sanitizer formulations were assessed.

Results: Considerable differences in the pH and viscosity were not observed between the prepared hand sanitizer gel formulations. However, considerable changes in the spreadability were observed between the prepared hand sanitizer gel formulations. A statistically significant difference in antibacterial activity was observed among the three hand sanitizer gels and the commercial hand wash ($p<0.05$). Formulation C showed the highest antibacterial activity among the prepared hand sanitizer formulations. Based on the stability studies, Formulation A was found to be the most stable.

Conclusion: Hand sanitizer gel containing a mixture of cinnamon and lime oils showed the highest antibacterial activity among prepared formulations. However, the most stable formulation was the hand sanitizer gel containing cinnamon oil.

Keywords

Essential oil; Cinnamon oil; Lime oil; Hand sanitizer gel

INTRODUCTION

Hygiene can be defined as "maintenance and the practice of cleanliness". Skin hygiene ensures the cleaning of the skin and prevents diseases.(1) Hands are a primary transmission mode of opportunistic pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas spp.* that primarily cause nosocomial infections. Generally, these organisms are known to cause infections in various organs, including the skin.(2)

Hand washing remains the single most effective and cost-efficient method of preventing and reducing the transmission of infections. Many studies have confirmed that hospital-acquired infections can be reduced among healthcare workers by washing their hands between contact with patients.(3) Thus, the hospital-acquired infection rate can be reduced by improving hand hygiene.

Hand sanitizers with synthetic chemicals are available in different forms, such as plain soap (bar or liquid form), alcohol-containing sanitizers, and water-based hand sanitizers. Hand sanitizers contain different chemical antiseptics such as chlorhexidine, chloroxylenol, iodine, triclosan, and quaternary ammonium compounds. These hand sanitizer preparations help reduce the transmission of infectious diseases more effectively.(4)

Adverse-effects due to frequent use of hand sanitizers are mainly skin irritation and the development of resistance among pathogens.(5) The most common skin reactions reported due to the use of alcohol-based hand sanitizers were irritant contact dermatitis and allergic contact dermatitis.(6,7) Further, alcohol is known to cause cracks or peels in the skin.(8) Intrinsic and acquired resistance was developed by different types of bacteria against different antimicrobial agents. Acquired resistance has been observed to certain biocides, notably in Staphylococci.(9)

Development of bacterial resistance and adverse-effects due to frequent usage of biocides, including antiseptics, warrant searching for new molecules with antimicrobial activity. Several studies have been conducted focused on making herbal ingredient based hand sanitizer products with improved quality, lesser expense, and fewer side-effects.(10)

Essential oils (EOs) are aromatic oily liquids obtained from plants. EOs consist of a mixture of compounds such as terpenes and their oxygenated derivatives.(11) Several studies have reported that EOs exhibited significant antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal activities.(12-14) EOs exert their antimicrobial activity by disruption of the bacterial cell wall and cell membrane resulting in cell lysis and leakage of intracellular components.(14-16) EOs are extensively used in the food and cosmetic industries due to their flavouring property.(17)

Cinnamon oil showed good antimicrobial activity in several studies.(14, 18, 19) The antimicrobial activity of some components of cinnamon oil against opportunistic microorganisms, food-borne pathogens, including mycotoxin-producing fungi, has also been reported.(20) Cinnamon oil also has antiallergic, antiulcerogenic, antipyretic, and analgesic activities.(21) The major component present in the cinnamon oil was cinnamic aldehyde.(19) Lime oil also has proven antimicrobial activity.(19,22) Limonene and citral are the chief components of lime oil.(23)

Therefore, the objective of our study was to formulate and characterise hand sanitizers using EOs of cinnamon and lime and

compare their efficacy with a commercial liquid handwash using the two bacterial strains, *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Extraction of essential oils from cinnamon bark and lime: The fresh cinnamon barks were collected from Kurunegala District, Sri Lanka and mature unripe lime fruits were collected from the Jaffna district, Sri Lanka. Soil and earthy matter were removed from the collected plant material.

Peels of lemon were dried in a hot air oven at 45^0C for 12 hours at an air velocity of 1 ms^{-1}. (24, 25) The dried peels were kept in a polythene bag with a desiccant (silica gel) and stored in the dark until use. The dried peels were ground using an electric grinder for the extraction process.

The cinnamon barks were dried in a hot air oven at 35^0C for 8 hours at an air velocity of 1 ms^{-1}.(26)The dried barks were kept in a polythene bag with a desiccant (silica gel) and stored in a dark place until use. The dried cinnamon barks were broken into small pieces using an electrical grinder for the extraction process. The EOs were extracted using the steam distillation method described in previous studies. (27, 28) Prepared EOs were stored in amber-coloured air-tight bottles at 4^0C until use.

Formulations of herbal hand sanitizer gels: Herbal hand sanitizer gel formulations (containing EOs), and blank gel (without EOs) were prepared based on methods given in previous studies (29-30) and standard industrial guidelines.(31) Different formulations of hand sanitizer gels were prepared, as shown in Table 1.

The gel base for each formulation was prepared using Carbopol-940. Carbopol-940 was added to deionised water and stirred using a four-armed impeller mechanical stirrer (Stuart Scientific Stirrer-SS3, United Kingdom) at 1000 rpm for 10 minutes. Then triethanolamine at a concentration of 0.7% w/w was added drop-wise with slow stirring at 500 rpm until pH 7 was reached.(29-31)The mixture was kept aside for 24 hours to complete the swelling of the polymer. The resultant gel base was used to prepare different hand sanitizer formulations.

Table 1: Formulation compositions of different hand sanitizer gels

	Ingredient composition (% w/w)			
Ingredients	**Formulation A**	**Formulation B**	**Formulation C**	**Blank gel**
Carbapol 940	0.50	0.50	0.50	0.50
Deionised water	83.50	83.50	83.50	85.50
Ethanol 95%	10.00	10.00	10.00	10.00
Cinnamon essential oil	2.00	_	1.00	-
Lime essential oil	_	2.00	1.00	-
Glycerin	2.30	2.30	2.30	2.30
Methyl paraben	0.50	0.50	0.50	0.50
Polysorbate 20	0.50	0.50	0.50	0.50
Triethanolamine	0.70	0.70	0.70	0.70

Polysorbate 20 was added to the gel base with continuous stirring at a slow rate to avoid bubbles (500 rpm for 3 minutes). EOs were mixed with ethanol, and then glycerine was added while stirring at 500 rpm.

This mixture was added to the carbapol gel base with high-speed stirring at1000 rpm. Finally, methylparaben was added to the gel. The final mixture was stirred continuously for 15minutes.

Blank gel was also prepared without EOs (using deionised water instead of EO). The prepared formulations were stored in air-tight aluminium cosmetic jars.

Determination of organoleptic characteristics of the prepared hand sanitizer gels: Organoleptic characteristics such as appearance, colour, and odour of the gels were directly determined.(32)

Evaluations of the physicochemical parameters of the prepared hand sanitizer gels: The physicochemical parameters of the prepared hand sanitizer gels were determined in triplicate.

The viscosity of hand sanitizer gels was measured using a Brookfield viscometer (HAAKE Viscometer C, Germany). An aliquot containing 25 ml of herbal hand sanitizer gel was taken into a beaker, and the tip of the viscometer was dipped into the beaker to measure viscosity.(33)

The pH of hand sanitizers was determined using a digital pH meter (HACH, Spain) in triplicate.

The spreadability of the hand sanitizer gel formulations was determined by an apparatus which consisted of a wooden block connected with a pulley at one end. Spreadability was measured based on the slip and drag characteristics of the hand sanitizers. A ground glass slide was fixed on this block. Two grams of the prepared hand sanitizer was placed on this ground slide. The sanitizer was then sandwiched using another glass slide with the same dimension as a fixed ground slide connected with a hook. A weight of 1kg was placed on the top of the two slides for 5 minutes to expel air and provide a uniform film of the hand sanitizer gel between the slides. Excess of the hand sanitizer gel was scraped off from the edges. The top plate was then subjected to a pull of 80 g with the help of a string attached to the hook, and the time (in seconds) required by the top slide to move 7.5 cm was noted. A shorter time was considered as indicating better spreadability.

Spreadability was calculated using the following formula:

$$S = M \times \frac{L}{T}$$

Where S = spreadability, M = mass in kg of the pan (tied to the upper slide), L = length (cm) the glass slide moved, and T = time (seconds) taken to separate the slides from each other.(34)

The homogeneity test was conducted by smearing the hand sanitizer samples on a glass slide. Homogeneity was observed by examining the presence of coarse grains or any phase separation.(35)

Stability studies: Stability studies were conducted to detect the physicochemical parameter changes of the hand sanitizer gels with time. Aliquots of 50 ml hand sanitizer gel samples were stored in air-tight aluminium cosmetic jars at room temperature. Viscosity, pH, spreadability, organoleptic characteristics, homogeneity tests were conducted at the end of the first day (day 0), 5, 10, and 15 days.(36)

Evaluation of *in vitro* antimicrobial activity: *In vitro* antimicrobial activity of the prepared hand sanitizer gels was evaluated using the agar disc diffusion method. All the

procedures for evaluating in vitro antimicrobial activity were done under aseptic conditions (inside a class II biosafety cabinet).

Antimicrobial activity of the prepared hand sanitizer gels was evaluated according to the Clinical and Laboratory Standards Institute guidelines (37), with slight modification of the sample dilution and disc preparation steps described in a previous study (38), against two pathogenic bacterial species which were, Gram-negative *E. coli* (ATCC25922) and Gram-positive *S. aureus* (ATCC25923). The bacterial strains were obtained from the Department of Microbiology, Faculty of Medicine, University of Jaffna, Sri Lanka.
Antimicrobial tests were conducted for the 3 prepared herbal hand sanitizer gel formulations, blank gel and one commercial hand wash product. Sterile water was used as the negative control. Different concentrations of the active ingredient (50 mg/ml, 100 mg/ml, and 200 mg/ml) of formulations A, B, C, blank gel, and the commercial hand wash product were prepared for the tests with sterile water. From each test sample, 10 µL was added onto sterile paper discs. Five discs from each concentration were placed on each culture plate. Three discs of 10 µL sterile water were used as a negative control. The antibacterial activity was evaluated by measuring the diameters of the inhibition zones in millimetres using a sliding calliper. All tests were done in triplicate.

Data analysis: Statistical analysis was carried out using the Statistical Package of Social Science (SPSS) 20. A p-value < 0.05 was considered statistically significant. The results of the physicochemical studies of the hand sanitizer gel formulations were reported as means and standard deviations.

For stability studies, changes of physicochemical parameters with time were analysed. The diameter of the zone of inhibition (in millimetres) was used as the indicator for the antimicrobial activity of the samples. *In vitro* antimicrobial activity of hand sanitizers was compared by the Tukey's honestly significant difference (HSD) test in three-way ANOVA.

RESULTS

Extraction of essential oils from cinnamon and lime: The yield values of EOs obtained from cinnamon barks and mature unripe lime fruits were 2.1% and 1.45 %, respectively.

Organoleptic characteristics of hand sanitizer gel formulations: All three hand sanitizer gel formulations had a translucent and cream-like appearance. Cinnamon oil-containing formulation A was in white colour, and lime oil containing formulation B was pink in colour. Formulation C with a mixture of both EOs appeared in a milky white colour. The odour of formulations A and B was due to cinnamon and lime oils, respectively. Formulation C had a characteristic odour.

Table 2: Physicochemical characterization of prepared hand sanitizers

Tests	Formulation A	Formulation B	Formulation C
Homogeneity	Homogenous	Homogenous	Homogenous
pH	6.87±0.01	6.53±0.01	6.64±0.01
Viscosity (cps)	30.66±0.58	27.33±0.58	29±1.00
Spreadability (g.cm/sec)	85.71±0.00	112.50±11.55	105.88±11.55

Physicochemical parameters of hand sanitizer gel formulations: Results of physicochemical parameter assessment of the hand sanitizer gel formulations, such as homogeneity, pH, viscosity, and spreadability of all three formulations are shown in Table 2.

Evaluation of the antibacterial activity of the prepared formulations: Antibacterial activity of the hand sanitizer gel formulations in terms of inhibition zone in millimetres is shown in Table 3. An inhibition zone was not observed for the sterile water-containing discs (negative control). According to the results, *E. coli* was more resistant to all the hand sanitizers than *S. aureus*. According to three-way ANOVA with Tukey's HSD tests, all three hand sanitizer gels had a statistically significantly different antimicrobial activity than the prepared blank gel (Table 4).

However, the commercial hand wash product demonstrated the highest antimicrobial activity. Formulation C showed the highest antimicrobial activity among the prepared hand sanitizer gel formulations, followed by formulations A and B.

Stability studies: All three formulations were kept in air-tight aluminium jars at room temperature during storage. The stability of the formulations was determined by conducting tests to determine organoleptic and physicochemical characters such as appearance, colour, and odour, homogeneity, viscosity, pH, and spreadability. The appearance, colour, and odour of all hand sanitizer formulations remained stable in the observed duration. Stability test results of physicochemical parameters of the hand sanitizer gels are shown in Table 5. All three formulations appeared homogenous even at the end of 15 days.

Only slight changes were observed in the pH and viscosity of all formulations. However, considerable changes were observed in the spreadability of all formulations.

Based on of changes in physicochemical parameters, formulation A appeared the most stable, followed by formulations C and B.

DISCUSSION

This study aimed to prepare hand sanitizer gels containing EOs, with antibacterial activity and assess their effectiveness against a commercial hand wash.

Cinnamon and lime oils are hydrophobic and are immiscible with aqueous media. Therefore, ethanol (10%) was used in the hand sanitizer gels to facilitate the miscibility of EOs with an aqueous medium. Since the bactericidal effect of ethanol is observed at 60% to 85% (40), 10% ethanol present in the hand sanitizer gel formulations does not contribute any significant bactericidal activity. Polysorbate 20 was used as a dispersing agent, and triethanolamine was used to adjust the pH of the formulations. Glycerine was used as a humectant in these hand sanitizer gel formulations.

Viscosity is a crucial physical characteristic of hand sanitizer gel formulations. Viscosity of these formulations was observed. A considerable viscosity drop was observed when the mixing plant oil and alcohol mixture with the gel base. The viscosities of all the prepared gels were lower than those reported in a previous study by Wani *et. al.* (29)

The pH of the human skin is in the range of pH 5.4 - 5.9.(42) The pH of topical pharmaceutical preparations should be within the range of pH 4 - 7 to avoid skin irritation.(43,44) In our study, the pH of the formulated hand sanitizers ranged from pH 6.53-6.87.

Table 3: Antibacterial activity of different hand sanitizers evaluated by agar diffusion method

The type of hand wash	Concentration of hand wash gel (mg/ml)	Bacterial strain	Mean diameter of the inhibition zone (mm)
Formulation A	50	*Escherichia coli*	0.00±0.00
		Staphylococcus aurous	6.67±0.577
	100	*Escherichia coli*	7.67±0.577
		Staphylococcus aurous	9.33±0.577
	200	*Escherichia coli*	8.67±0.577
		Staphylococcus aurous	11.33±1.155
Formulation B	50	*Escherichia coli*	0.00±0.00
		Staphylococcus aurous	0.00±0.00
	100	*Escherichia coli*	7.33±0.577
		Staphylococcus aurous	7.33±0.577
	200	*Escherichia coli*	9.33±0.577
		Staphylococcus aurous	10.67±0.577
Formulation C	50	*Escherichia coli*	7.33±0.577
		Staphylococcus aurous	6.67±0.577
		Total	7.00±0.632
	100	*Escherichia coli*	9.33±0.577
		Staphylococcus aurous	11.00±1.00
	200	*Escherichia coli*	11.33±0.577
		Staphylococcus aurous	14.00±1.00
Blank hand sanitizer gel	50	*Escherichia coli*	0.00±0.00
		Staphylococcus aurous	0.00±0.00
	100	*Escherichia coli*	0.00±0.00
		Staphylococcus aurous	0.00±0.00
	200	*Escherichia coli*	7.33±0.577
		Staphylococcus aurous	8.33±0.577
Commercial liquid hand wash	50	*Escherichia coli*	12.33±0.577
		Staphylococcus aurous	14.00±1.0011
	100	*Escherichia coli*	16.33±0.577
		Staphylococcus aurous	18.67±0.577
	200	*Escherichia coli*	19.33±0.577
		Staphylococcus aurous	22.33±0.577

Table 4: Multiple comparison of different hand sanitizer formulations by Tukey's HSD

The type of hand wash	The type of hand wash	Mean Difference	Standard Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Formulation A	Formulation B	1.50*	0.199	0.000	0.94	2.06
	Formulation C	-2.67*	0.199	0.000	-3.23	-2.11
	Blank hand sanitizer gel	4.67*	0.199	0.000	4.11	5.23
	Marketed liquid hand wash	-9.89*	0.199	0.000	-10.45	-9.33
Formulation B	Formulation A	-1.50*	0.199	0.000	-2.06	-.94
	Formulation C	-4.17*	0.199	0.000	-4.73	-3.61
	Blank hand sanitizer gel	3.17*	0.199	0.000	2.61	3.73
	Marketed liquid hand wash	-11.39*	0.199	0.000	-11.95	-10.83
Formulation C	Formulation A	2.67*	0.199	0.000	2.11	3.23
	Formulation B	4.17*	0.199	0.000	3.61	4.73
	Blank hand sanitizer gel	7.33*	0.199	0.000	6.77	7.89
	Marketed liquid hand wash	-7.22*	0.199	0.000	-7.78	-6.66
Blank gel	Formulation A	-4.67*	0.199	0.000	-5.23	-4.11
	Formulation B	-3.17*	0.199	0.000	-3.73	-2.61
	Formulation C	-7.33*	0.199	0.000	-7.89	-6.77
	Marketed liquid hand wash	-14.56*	0.199	0.000	-15.11	-14.00
Commercial liquid hand wash	Formulation A	9.89*	0.199	0.000	9.33	10.45
	Formulation B	11.39*	0.199	0.000	10.83	11.95
	Formulation C	7.22*	0.199	0.000	6.66	7.78
	Blank hand sanitizer gel	14.56*	0.199	0.000	14.00	15.11
Formulation A	Formulation B	1.50*	0.199	0.000	1.10	1.90
	Formulation C	-2.67*	0.199	0.000	-3.06	-2.27
	Blank hand sanitizer gel	4.67*	0.199	0.000	4.27	5.06
	Marketed liquid hand wash	-9.89*	0.199	0.000	-10.29	-9.49
Formulation B	Formulation A	-1.50*	0.199	0.000	-1.90	-1.10
	Formulation C	-4.17*	0.199	0.000	-4.56	-3.77
	Blank hand sanitizer gel	3.17*	0.199	0.000	2.77	3.56
	Marketed liquid hand wash	-11.39*	0.199	0.000	-11.79	-10.99
Formulation C	Formulation A	2.67*	0.199	0.000	2.27	3.06
	Formulation B	4.17*	0.199	0.000	3.77	4.56
	Blank hand sanitizer gel	7.33*	0.199	0.000	6.94	7.73
	Marketed liquid hand wash	-7.22*	0.199	0.000	-7.62	-6.82
Blank gel	Formulation A	-4.67*	0.199	0.000	-5.06	-4.27
	Formulation B	-3.17*	0.199	0.000	-3.56	-2.77
	Formulation C	-7.33*	0.199	0.000	-7.73	-6.94
	Marketed liquid hand wash	-14.56*	0.199	0.000	-14.95	-14.16
Commercial liquid hand wash	Formulation A	9.89*	0.199	0.000	9.49	10.29
	Formulation B	11.39*	0.199	0.000	10.99	11.79
	Formulation C	7.22*	0.199	0.000	6.82	7.62
	Blank hand sanitizer gel	14.56*	0.199	0.000	14.16	14.95

Sig.=Significance

Table 5: Physicochemical parameters of different hand sanitizers during storage

Days	Formulations	Homogeneity	pH	Viscosity	Spreadability
0	A	Homogenous	6.87±0.01	30.66±0.58	85.71±0.00
	B	Homogenous	6.53±0.01	27.33±0.58	112.50±11.55
	C	Homogenous	6.64±0.01	29.00±1.00	105.88±11.55
5	A	Homogenous	6.87±0.01	30.67±0.58	81.82±6.19
	B	Homogenous	6.50±0.01	29.00±1.00	94.74±8.25
	C	Homogenous	6.63±0.01	29.67±0.58	100±0.00
10	A	Homogenous	6.86±0.01	31.67±0.58	78.26±6.19
	B	Homogenous	6.44±0.01	31.67±0.58	81.82±6.19
	C	Homogenous	6.62±0.01	30.33±0.58	90.00±8.25
15	A	Homogenous	6.85±0.01	32.33±0.58	72.00±4.81
	B	Homogenous	6.42±0.01	33.33±0.58	78.26±6.19
	C	Homogenous	6.62±0.00	31.00±0.58	81.82±6.19

Therefore, all three hand sanitizer formulations could be safely used. When considering the initial pH of formulations, the mean pH was increased in the order of formulation B < formulation C < formulation A. A considerable pH drop was observed when EOs were mixed with the gel base.

Lime oil is more acidic than cinnamon oil. Therefore, the pH of formulation B was lower than the pH of formulation A. The pH of formulation C was between formulations B and A as it contained a mixture of both cinnamon and lime oils.

The viscosity of the topical formulation is inversely correlated with its spreadability.(45) When considering the initial spreadability of the formulations, the mean spreadability decreased as formulations B > C > A. Formulation A showed a considerable increase in spreadability when EOs and alcohol were mixed with the gel base. The spreadability of all the formulations are higher than those reported previously by Choudhari *et al.*(33)

According to the results of a three-way ANOVA with Tukey's HSD test, the antibacterial activity of all the hand sanitizers is significantly differed from each other ($p<0.05$). Further, all three test gel formulations had statistically significant differences in antimicrobial activities compared to the blank gel (Table 4). According to Turkey's HSD analysis, the antibacterial activity of hand sanitizers was increased in the order of, blank gel < formulation B < formulation A < formulation C < commercial liquid hand wash.

Formulation C showed the highest antibacterial activity among the prepared hand sanitizer gels. This could be due to the synergistic antibacterial effect of cinnamon and lime oils in formulation C. Formulation A had a higher antibacterial activity than Formulation B. This could be due to the higher antibacterial activity of cinnamon oil than lime oil.(19,46)

The purpose of the stability studies was to assess the changes in physicochemical parameters during the storage of the prepared formulations. Even though longer real-time studies are needed to ensure the stability of hand sanitizers. Stability studies were limited to 15 days in this study. Stability studies revealed slight changes in the pH and viscosity among three hand sanitizer gel

formulations. However, considerable changes were observed in the spreadability of the three hand sanitizer gel formulations at the end of 15 days. The pH of the formulations was increased with time. The viscosity of all three formulations was also observed to increase with time. This may be due to the evaporation of water and alcohol increasing the formulations' concentration. Further, increasing pH is known to increase the viscosity of Carbopol gel.(47) The spreadability of all three hand sanitizer gel formulations increased with time. This is due to the drop in the viscosity of the tested formulations during storage.

The changes in the physicochemical parameters during stability studies were assessed. Formulation A was the most stable gel followed by formulations C and B.

However, longer real-time stability studies are needed to evaluate further changes in physicochemical characters and the antibacterial activity of the prepared hand sanitizer gel formulations.

CONCLUSION

The physically most stable hand sanitizer gel among the prepared formulations was Formulation A containing cinnamon oil. Formulation C containing a mixture of cinnamon and lime oils showed the highest antibacterial activity among the prepared hand sanitizer gel formulations. Further studies are needed to improve the antibacterial activity of the prepared hand sanitizer gel formulations by adding more suitable phytoconstituents with antibacterial activity.

Author's Declaration:

The authors declare that all persons listed as authors have read and given approval for the submission of this manuscript.

Acknowledgements:

The authors acknowledge the non-academic staff of the University of Jaffna for their valuable contribution towards completing this research work.

Competing Interests:

The authors declare that they have no competing interests to disclose.

REFERENCES:

1. Mukhopadhyay P. Cleansers and their role in various dermatological disorders. Indian J Dermatol. 2011;56(1):2-6.
2. Black, J.G, 1996. Microbiology: Principles and Applications. 3rd ed. Upper Saddle River, New Jersey: Prentice Hall College.
3. Nicolay CR. Hand hygiene: an evidence-based review for surgeons. International Journal of Surgery.2006; 4(1):53-65.
4. Luby SP, Agboatwalla M, Feikin DR, Painter J, Billhimer W, Altaf A, Hoekstra RM. Effect of hand washing on child health: a randomised controlled trial. The Lancet. 2005; 366(9481):225-233.
5. Pittet D. Clean hands reduce the burden of disease. The Lancet. 2005;366(9481), pp.185-187.
6. Wilhelm KP. Prevention of surfactant-induced irritant contact dermatitis. Curr Probl Dermatol. 1996;25:78-85.
7. Ale IS, Maibach HI. Irritant contact dermatitis. Rev Environ Health. 2014;29(3):195-206.
8. Guin JD, Goodman J. Contact urticaria from benzyl alcohol presenting as intolerance to saline soaks. Contact Dermat. 2001;45:182–183.
9. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999 Jan;12(1):147-79.

10. Londhe J, Jagtap SD, Doshi C, Jagade D. Formulations of herbal hand wash with potential antibacterial activity. In International Journal of Research in Advent Technology. In: Special Issue National Conference on Advances and Challenges in Green Technology, Savitribai Phule Pune University.2015 Feb; (pp. 11-15).
11. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel). 2017;4(3):58.
12. Properzi A, Angelini P, Bertuzzi G, Venanzoni R. Some Biological Activities of Essential Oils. Med Aromat Plants.2013; 2: 136.
13. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, Tundis R, Sharifi-Rad M, Loizzo MR, Ademiluyi AO, Sharifi-Rad R. Biological activities of essential oils: From plant chemoecology to traditional healing systems. Molecules. 2017 Jan;22(1):70.
14. Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. Int. J. Food Microbiol. 2004; 94:223–253.
15. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpenes. Antimicrobial agents and chemotherapy. 2005 Jun;49(6):2474-8.
16. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food and Chemical Toxicology. 2008 Feb;46(2):446-475.
17. Vostinaru O, Heghes SC, Filip L. Safety profile of essential oils. In Essential Oils-Bioactive Compounds, New Perspectives and Applications 2020 Feb 27 (pp. 1-13). Intech Open.
18. Chaudhari LK, Jawale BA, Sharma S, Sharma H, Kumar CD, Kulkarni PA. Antimicrobial activity of commercially available essential oils against Streptococcus mutans. J Contemp Dent Pract. 2012 Jan 1;13(1):71-4.
19. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC Complement Altern Med. 2006 Nov 30;6:39.
20. Bullerman LB, Lieu FY, Seier SA. Inhibition of growth and aflatoxin production by cinnamon and clove oils. Cinnamic aldehyde and eugenol. Journal of Food Science. 1977; 42(4):1107-1109.
21. Kurokawa M, Kumeda CA, Yamamura J, Kamiyama T, Shiraki K. Antipyretic activity of cinnamyl derivatives and related compounds in influenza virus-infected mice. Eur J Pharmacol. 1998 May 1;348(1):45-51.
22. Chanthaphon S, Chanthachum S, Hongpattarakere T. Antimicrobial activities of essential oils and crude extracts from tropical *Citrus spp.* against food-related microorganisms. Songklanakarin J. Sci. Technol.2008 April;30:125–131.
23. Rammanee K, Hongpattarakere T. Effects of tropical citrus essential oils on growth, aflatoxin production, and ultrastructure alterations of *Aspergillus flavus* and *Aspergillus parasiticus*. Food and Bioprocess Technology. 2011;4(6):1050-1059.
24. Kamal GM, Anwar F, Hussain AI, Sarri N, Ashraf MY. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. International Food Research Journal. 2011;18(4):1275-1282 .
25. Bendaha H, Bouchal B, El Mounsi I, Salhi A, Berrabeh M, El Bellaoui M, Mimouni M. Chemical composition, antioxidant, antibacterial and antifungal activities of

peel essential oils of citrus aurantium grown in Eastern Morocco. Der Pharmacia Lettre. 2016;8(4):239-245

26. Chandra, K.A. Study the effectiveness in preprocessing of cinnamon chips. Digital Library, University of Moratuwa.2014 [Online][Accessed January 2021]. Available from: http://dl.lib.uom.lk/bitstream/handle/123/9930/Pre-text.pdf?sequence=1
27. Senhaji O, Faid M, Kalalou I. Inactivation of Escherichia coli O157:H7 by essential oil from *Cinnamomum zeylanicum*. Braz J Infect Dis. 2007 Apr;11(2):234-6.
28. Wong YC, Ahmad-Mudzaqqir MY, Wan-Nurdiyana WA. Extraction of Essential Oil from Cinnamon (Cinnamomum Zeylanicum). Orient J Chem 2014;30(1).
29. Wani NS, BhaleraoAK, Ranaware VP, Zanje R. Formulation and evaluation of herbal sanitiser. Int J PharmTech Res. 2013;5(1):40-43.
30. Shafi S, Singh S, Verma S, Tiwari RK, Tripathi D. Formulation and Development of Mint Containing Herbal Hand Sanitizer. European Journal of Pharmaceutical and Medical Research. 2107; 4(11):454–457.
31. Lubrizol Advanced Materials, Inc. Pharmaceutical Bulletin -Dispersion Techniques for Lubrizol Pharmaceutical Polymers. 4th ed. 2011.
32. Dantas MG, Reis SA, Damasceno CM, Rolim LA, Rolim-Neto PJ, Carvalho FO, Quintans-Junior LJ, Almeida JR. Development and evaluation of stability of a gel formulation containing the monoterpene borneol. The Scientific World Journal. 2016 Jan 1;2016.
33. Choudhari S, Sutar M, Chavan M. Formulation evaluation and antibacterial efficiency of herbal hand wash. Indo American Journal of Pharmaceutical Research. 2016;6(4):5202–5209.
34. Kumar L, Verma R. *In vitro* evaluation of topical gel prepared using natural polymer. International journal of drug delivery. 2010;2(1).
35. Kurniawan DW, Wijayanto BA, Sobri I. Formulation and effectiveness of antiseptic hand gel preparations essential oils galanga (*Alpinia galanga*). Asian Journal of Pharmaceutical & Biological Research. *2012*;*2*(4).
36. Ningsih DR, Zusfahair Z, Kartika D, Fatoni A. Formulation of handsanitizer with antibacterials substance from n-hexane extract of soursop leaves (*Annona Muricata* Linn). Malaysian Journal of Fundamental and Applied Sciences. 2017;13(1):1-5.
37. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard, 12th ed CLSI document M02-A12. Clinical and Laboratory Standards Institute, Wayne, PA. 2015.
38. Abbas SZ, Hussain K, Hussain Z, Ali R, Abbas T.Anti-Bacterial Activity of Different Soaps Available in Local Market of Rawalpindi (Pakistan) against Daily Encountered Bacteria. Pharm Anal Acta.
39. Villa C, Russo E. Hydrogels in Hand Sanitisers. Materials (Basel). 2021 Mar 24;14(7):1577.
40. Sauerbrei A. Bactericidal and virucidal activity of ethanol and povidone-iodine. Microbiologyopen. 2020 Sep;9(9):e1097.
41. Maslii Y, Ruban O, Kasparaviciene G, Kalveniene Z, Materiienko A, Ivanauskas L, Mazurkeviciute A, Kopustinskiene DM, Bernatoniene J. The Influence of pH Values on the Rheological, Textural and Release Properties of CarbomerPolacril® 40P-Based Dental Gel Formulation with Plant-Derived and

Synthetic Active Components. Molecules (Basel). 2020;25(21), 5018.
42. Braun-Falco O, Korting HC. Der normale pH-Wert der menschlichen Haut [Normal pH value of human skin]. Hautarzt. 1986 Mar;37(3):126-9.
43. Ali SM, Yosipovitch G. Skin pH: from basic science to basic skin care. Acta Derm Venereol.2013 May;93(3):261-7.
44. Lambers H, Piessens S, Bloem A, Pronk H, Finkel P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. Int J Cosmet Sci. 2006 Oct;28(5):359-70.
45. Rahmasari D, Hendradi E, Chasanah U. Formulation and evaluation of hand sanitizer gel containing infused of binahong leaf (Anrederacordifolia) as antibacterial preparation. Farmasains: Jurnal Farmasidan Ilmu Kesehatan.2020; 5(2):23-30.
46. Chaudhari LK, Jawale BA, Sharma S, Sharma H, Kumar CD, Kulkarni PA. Antimicrobial activity of commercially available essential oils against Streptococcus mutans. J Contemp Dent Pract. 2012 Jan 1;13(1):71-4.
47. Maslii Y, Ruban O, Kasparaviciene G, et al. The Influence of pH Values on the Rheological, Textural and Release Properties of Carbomer Polacril® 40P-Based Dental Gel Formulation with Plant-Derived and Synthetic Active Components. Molecules. 2020;25(21):5018.