ORIGINAL ARTICLE

Formulations and Antibacterial Activity of *Moringa oleifera* Extract in Hand Sanitizer Gel against *Staphylococcus aureus*

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ABSTRACT

Background: Recent studies have demonstrated that the transmission of SARS-CoV-2 is possible in the form of aerosol and fomite. Thus, hand hygiene is of utmost significance. Hand sanitizer is often used as a practical hand washing method.

Purpose: This study aims to study the effectiveness of *Moringa* leaves extract as an antibacterial ingredient in hand sanitizer gel.

Methods: *Moringa* leaves extract was obtained by maceration in 70% alcohol solution. The hand sanitizer formula consists of carbomer, propylene glycol, glycerin, methylparaben, and triethanolamine. The hand sanitizer gel was prepared using three formulas with different extract concentrations. The quality of the hand sanitizer gel was analyzed, including the gel acidity (pH), organoleptic, the inhibitory ability of bacterial growth against *Staphylococcus aureus*, and the gel dispersion.

Results: This study showed that Formula III has the highest inhibitory area against *S. aureus*. The gel acidity (pH) was found at around 4 for all the formulas, safe for use on the skin. The organoleptic test showed no irritation to the skin for all three formulas. However, they demonstrated low dispersity.

Conclusion: *Moringa* leaves extract is effective as an antibacterial in hand sanitizer gel.

Keywords: Alcohol, Gel, Hand hygiene, Hand sanitizer, *Moringa*.

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Conflict of interest: None

INTRODUCTION

Corona Virus Disease 2019 (COVID-19) infection has become a pandemic because the number of confirmed cases worldwide has increased (Atolani *et al.,* 2016). Therefore, it is essential to control and prevent the COVID-19 pandemic spread. Current evidence suggests that COVID-19 infection is transmitted through respiratory droplets or contact. The virus can also be transferred from one surface to another with contaminated hands, facilitating indirect contact transmission. Therefore, hand hygiene is essential to prevent the transmission of COVID-19 infection.[1-3]

Hand sanitizer gel is one of the antiseptic ingredients in the form of a gel often used by the public as a practical handwashing medium.[4] The use of hand sanitizer is more efficient when compared to using soap and water. Therefore, people are interested in using it. The advantages of hand sanitizers are that they can kill germs quickly because they contain alcohol compounds (ethanol, propanol, isopropanol) with concentrations of ± 60% to 80% and phenol groups (chlorhexidine, triclosan). Antiseptics in some brands with an alcohol content of 60–70% without adding other antibacterial substances have more polar properties.[5,6] Continuous use of alcohol is not suitable for hands so researchers are looking for alternatives to reduce alcohol derived from natural ingredients that are relatively sufficient or safer for the skin. One of the ingredients that can be used as an antiseptic in hand sanitizer gel is the *Moringa oleifera* plant.[7]

M. oleifera has an antibacterial effect because it contains saponins, tannins, and flavonoids.[8] Antibacterial substances in the *M. oleifera* plant will inhibit the growth or kill pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeuroginosa,* and *Escherichia coli*. *Moringa* leaves have

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active compounds that can be utilized, including saponins, tannins, flavonoids, alkaloids, and terpenoids obtained from the extraction process. Compounds that play a role in damaging bacterial cell walls include phenols, flavonoids, and alkaloids. These phytochemical compounds have the potential as natural antibacterials for pathogenic bacteria.[9]

METHODS

Extract Preparation

Extraction is done by maceration or immersion method. *M. oleifera* leaf powder was weighed as much as 2 kg, then put into a maceration container and added 15 liters of 70% ethanol, macerated for three days with stirring. The filtered extract was then concentrated using a rotary evaporator in order to obtain a thick extract. Phytochemical screening test for tannin, polyphenol, saponin, flavonoid and alkaloid test was carried out using the procedure in Oshadie *et al.,* (2017) and Shaikh and Patil (2020)**.**

Hand Sanitizer Gel Formulation

The formulation of the hand sanitizer gel preparation with the active ingredient *M. oleifera* extract can be seen in Table 1.

Gel Hand Sanitizer *Moringa oleifera* Extract Preparation

The carbomer base was prepared by dispersing carbomer into hot distilled water until it expands. Methylparaben was dissolved in ethanol until dissolved. Propylene glycol and glycerin were mixed until homogeneous, then added with the *moringa* extract (F1 30, F2 40, F3 50%) stirred until homogeneous, then added to the gel base. Finally, the TEA (triethanolamine) was added into the mixture and stir until homogeneous. The remaining distilled water was added to the volume of 100 mL.

Antibacterial Effectiveness Test Against *S. aureus*

Muller Hilton agar was poured into sterile petri dishes and allowed to solidify. Inoculation of *S. aureus* bacteria by inserting a cotton swab into the test tube medium containing the bacterial culture into the Muller Hilton medium and leveling. Then the disc paper is dipped in the hand sanitizer gel preparation for each concentration and controlled of 1 disc paper for a specific time.

Hand Sanitizer Gel Characteristic Test

Organoleptic Test

Physical observations of hand sanitizer gel preparations of *M. oleifera* extract included odour, colour, and homogeneity of the Preparation.

Homogeneity Test

The homogeneity test was carried out to determine whether the gel preparation was homogeneous or not by dispersing 500 mg of gel on a slide.

Viscosity Test

A viscosity test was carried out using the Rion Viscometer VT 04 series by dipping rotor no two into the gel preparation.

pH test

Test the pH of the gel preparation using a pH meter.

Dispersity Test

The prepared formulation was weighed as much as 500 mg and placed in the middle of the petri dish, which had previously been pasted with millimeter block paper and then covered with another petri dish. Each load weighing 50 g, 100 g, and 150 g was added, allowed to stand for 1-minute, then measured, and the diameter of the gel spread was recorded adhesion test .

This test was carried out by taking 500 mg of gel placed on an object glass, attached to another glass object, and then pressed with a load of 1 kg for 5 minutes. The object glass is mounted on the test equipment, and the load weighing 80 grams is removed.

RESULT AND DISCUSSIONS

The results of phytochemical screening showed a positive reaction to the test for alkaloids, flavonoids, tannins, saponins, and polyphenols. The results of phytochemical screening can be seen in Table 2.

M. oleifera extract is used to preserve and utilize it as an antibacterial based on secondary metabolite compounds present. Studies on the effectiveness of the *M. oleifera* extract hand sanitiser showed an antibacterial effect due to the saponins that function as antiseptics by damaging the protein permeability of the bacteria. In addition, tannins also can cause protein denaturation of the bacteria. The flavonoids in

Table 1: Formulations of Hand Sanitizer Gel with *Moringa* Leaves Extract

Composition	*Function*	*Formula (%) b/v*		
		Formula 1	*Formula 2*	*Formula 3*
Moringa leaves extract	Active ingredient	30	40	50
Carbomer	Gelling agent	0.5	0.5	0.5
Propylene glycol	Stabilizer	5	5	5
Glycerin	Humectant	10	10	10
Methylparaben	Preservative	0.1	0.1	0.1
Triethanolamine	*Alkalizing agent*	2	2	2
Ethanol	Cosolvent	5	5	5
Sterile water	Solvent	Ad 100%	Ad 100%	Ad 100%

Table 2: Phytochemical Test Results

Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Polyphenols	+

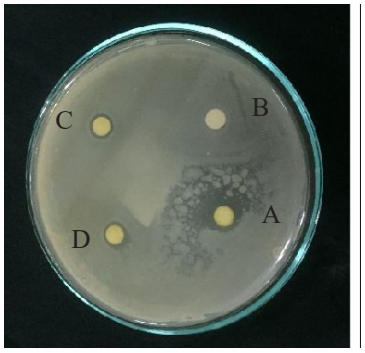

Figure 1: Inhibition zone of Positive Control (A), Formula 1 (B), Formula 2 (C), and Formula 3 (D) Against Staphylococcus aureus

M. oleifera extract acts by inhibiting energy metabolism of bacteria. The alkaloids in also act as antibacterial by interfering with their constituent components, causing cell death of the bacteria.[10-11]

Hand sanitizer gel based on *M. oleifera* extract uses a modified standard gel formulation in general by using a carbomer base.[12] The additional ingredients used are propylene glycol and glycerin that act as humectant in the formula. Ethanol was added as a cosolvent in dissolving methylparaben. Methylparaben functions as a preservative because the gel preparation has high water content. The addition of TEA with a concentration of 2% aims to maintain the pH of the gel preparation to remain stable.[13]

The antibacterial activity test of *M. oleifera* extract aims to determine the ability of *M. oleifera* extract to inhibit bacteria by the inhibition zone. The sensitivity of the tested bacteria to *M. oleifera* extract was seen from the formation of a clear zone around the paper disc (Figure 1). The diameter of the inhibition zone of more than 20 mm had a robust response, 10 to 20 mm had a strong response, the diameter of the inhibition zone of 5 to 10 had a moderate response, and the diameter of the inhibition zone of less than 5 had a weak response. The present study results of the antibacterial test can be seen in Table 3 below.

Table 4: *Moringa oleifera* Extract Gel Hand Sanitizer Characteristic Test Results

Characteristic	*Formula*		
	F1 (n=3)	*F2 (n=3)*	*F3 (n=3)*
Colour	Green	Dark Green	Dark Green
Odor	Typical	Typical	Typical
Homogeneity	Homogenous	Homogenous	Homogenous
pH	4.62 ± 0.06	4.40 ± 0.04	4.25 ± 0.06
Viscosity (dPa.s.)	156.7 ± 20.82	143 ± 6	173.3 ± 5.77
Dispersity (cm2)	12.19 ± 1.57	11.86 ± 0.70	16.40 ± 3.57
Adhesion (seconds)	0.47	0.47	0.83

The hand sanitizer gel formulations showed effective results as antibacterial in Formulas 2 and 3, seen from the inhibition zone produced more than 5 mm, which concluded moderate as an antibacterial. While formula 1 with an inhibition zone of less than 5 mm can be concluded that it is not effective as an antibacterial.

In addition, the results of the observations from this test were visually seen in color, odor, and homogeneity. Gels with a concentration of 30% were green, while those at concentrations of 40% and 50% were dark green (Table 4). This difference is due to the different formulas of each concentration. The form of this gel preparation at a concentration of 30% is not too thick because it contains more water, while at concentrations of 40 and 50% thickens because the amount of water is less. The odor of the three variations of this hand sanitizer gel has a distinctive aroma from *M. oleifera*. A homogeneity test was carried out to determine whether the gel preparation was homogeneous or not. The results were that the three gels with various variations were homogeneous because there were no coarse grains when dispersed on the slide (Table 4). This shows that all the formulated ingredients mixed well without the presence of coarse grain.

The pH test was carried out to determine whether the pH of the *M. oleifera* extract gel preparation was within the skin pH range. Normal skin pH in the range of 4–6 so as not to cause skin irritation. The observations obtained from the three *M. oleifera* extract gel formula variations showed that all three were in the skin pH range. Thus, the *M. oleifera* extract in hand sanitizer gel was acceptable because it did not irritate the skin.

The viscosity test was carried out to determine the gel preparation's viscosity and the gel's resistance to flow at various extract concentrations. A gel is good if it has a viscosity in the

Table 3: Antibacterial activity of *M. oleifera* extract in hand sanitizer gel against *S. aureus*

Repetitions	*Positive control*	*Inhibitory diameter (mm)*		
		Formula 1	*Formula 2*	*Formula 3*
1	9.15	4.79	4.98	5.01
2	9.15	4.53	5.57	5.78
3	6.5	5.35	5.11	6.7
SUM	51.675	14.67	15.66	17.49
MEAN	8.6125	4.89	5.22	5.83

range of 50–1000 dPa.; therefore, it is easy to remove from the tube/container and easier to apply.[14] The viscosity test results of *Moringa oleifera* extract gel preparations at all concentration variations fall within the viscosity range.

The dispersion test aims to determine the dispersity of the gel on the surface of the skin; therefore, the spread of the active substance from the gel preparation of the *Moringa oleifera* extract. In another study using a 2% carbomer base, the resulting dispersion was 12–15 cm.[15] The large dispersity will make the hand sanitizer gel more easily spread on the skin. The active substances contained in the gel base will spread more evenly; thus, the antibacterial effect of *M. oleifera* extract will be more optimal.

CONCLUSION

Moringa leaves extract-based for hand sanitizer gel showed promising results as an antibacterial. The hand sanitizer gel in formulas 2 and 3, with 40% and 50% of *Moringa* leaves extract, has demonstrated a moderate action as an antibacterial hand sanitizer gel.

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CONFLICT OF INTERESTS

There is no conflict of interests found during this study

AUTHORS CONTRIBUTION

Prisca, Desi and Regina contributed equally to the experiment and initial ideas. All authors were involved in the experiments, while Prisca did most of the report's writing. Prisca, Desi, and Regina contributed to the data analysis and the final proofreading of the manuscript.

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