

Screening and Formulation of Hand Sanitizer with Herbal Extracts of Aloe Vera and Cinnamon

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ABSTRACT

Sanitizers are liquid solutions formulated to reduce microbial growth on the hands, typically featuring alcohol as a primary component. However, various plant-derived bioactive compounds exhibit antimicrobial properties that can benefit the skin without adverse effects, enhancing its softness and overall health while providing a natural glow. This study explores the antimicrobial potential of plants such as tulsi, neem, lemon, and eucalyptus. An herbal sanitizer was developed using Aloe Vera and cinnamon extract, with the Microtiter Plate Technique employed to identify the optimal concentrations for effective use. Results revealed that both Aloe Vera and cinnamon extracts were effective in inhibiting microbial growth at a concentration of 1 mg. Notably, the combination of these extracts showed enhanced effectiveness, suppressing microbial growth at 0.5 mg. The formulated sanitizer using this combination inhibited specific microorganisms at 0.75 mg, while demonstrating a remarkable inhibitory effect on hand microbes at just 0.09 mg. These findings underscore the effectiveness of combining Aloe Vera and cinnamon in sanitizers. Additionally, the natural extracts incorporated into the formulation offer prolonged antimicrobial activity and contribute to maintaining skin health.

Keywords: Sanitizer, herbal extract, Aloe Vera, cinnamon, Microbes.

INTRODUCTION

The rise in infections caused by opportunistic microorganisms has become a major concern, with many pathogens developing multidrug resistance (Sunanda and Kolhapure, 2004). Pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* persist in high-risk environments like healthcare and the food industry (Elizabeth and Sally, 1990), spreading through physical contact, contaminated surfaces, and food (Kimura et al., 2004). Hands harbor resident and transient flora. Resident flora, like *Staphylococcus aureus* and *Staphylococcus epidermis*, affect deeper skin layers and are harder to remove, whereas transient flora affects the skin surface temporarily (Imaël and Juliani, 2012). Pathogenic microbes on hands contribute to respiratory and gastrointestinal diseases (Kimura et al., 2004), as human skin offers favorable growth conditions (Coogan et al., 2008). Hand hygiene through sanitization is essential to prevent disease spread.

Alcohol-based sanitizers, while effective in killing 99.9% of bacteria (Kojo & Qian, 2004), can cause skin irritation and pathogen resistance (Joshi et al., 2008). To address these issues, herbal-based sanitizers have been introduced, leveraging plant extracts with antimicrobial, analgesic, and anti-inflammatory properties (Salma and Zeeshan, 2013; Behrooz et al., 2013; Nguefack et al., 2004). Eucalyptus, neem, and lemon extracts show antimicrobial activity (Dipti and Kamna, 2019; Satoru et al., 2000), while herbs like *Coleus*

Vetiveroides, *Coriandrum sativum*, and *Vetiveria zizanioides* enhance sanitizer formulations (Supinya et al., 2003). Aloe vera and cinnamon extracts also exhibit antimicrobial and pharmacological effects (Philip, 1998; Marjorie, 1999), making them valuable alternatives to chemical sanitizers combined with quaternary ammonium compounds (EI Kamali et al., 2005). Thus, the present study was aimed to screen Aloe Vera and cinnamon extracts for their antimicrobial properties (using minimum inhibitory concentration) against various microbes and their use in the formulation of herbal based hand sanitizer.

MATERIALS AND METHODS

Collection of samples

Samples of Aloe Vera pulp and cinnamon barks were collected. It was taken care that the samples were without any debris and contamination.

Extraction of herbal phytochemicals.

2.5g of Aloe Vera and 5g of Cinnamon were mixed separately with 500ml of absolute

ethanol in 2 glass jars. The jars were incubated at 40°C for 96 hrs until the content in each jar was reduced to 250ml, so as to get the final concentration of 10mg/ml of Aloe Vera and 20mg/ml of cinnamon extracts respectively.

Determination of Minimum Inhibitory Concentration (MIC) of phytochemicals using microtitre plates

Separate cultures of microorganisms *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* were grown in LB broth and were kept ready for MIC screening. 30 ml of LB Broth was prepared by adding tryptone 0.3g, NaCl 0.3g, yeast extract 0.18g, distilled water 30ml. The broth was then autoclaved. 20ml of Resazurin dye was taken; it was used as an indicator for the growth of microorganism which can be visualised by the change in colour. Appearance of a pinkish red colour indicates the growth of microbes. Whereas if the blue colour of the dye is retained, it means there is no growth of microbes in that well.

Fig 1: Wells in the Micro titer plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

From fig 1, wells A1 –A12, B1 – H1, H2 – H12 and B12 – G12 were filled with autoclaved water to prevent the dehydration of the plates. Wells B2 – G2 were maintained as the control. They were filled with 100µl broth and 100µl Resazurin dye only. Wells B3 – G3 were maintained as the negative control. They were filled with 100µl broth, 100µl organisms and 100µl dye. Wells B4 – G4 were incubated with 100µl broth, 100µl organism, 100µl absolute ethanol and 100µl

dye as another control in order to study the comparative inhibition of the respective extracts. Wells B5 – G5 were filled with 100µl broth, 100µl organism and 100µl of the extract and serially diluted to the adjacent wells. From these wells, 100µl of the contents were pipetted into the adjacent well upto the last. 100µl from the last well (column 11) was pipetted out and discarded. The serially diluted concentrations were 1000 µg, 500 µg, 250 µg, 125 µg, 62 µg, 31

µg and 15 µg respectively. Then 100µl dye was added to all the wells. The inoculation of microorganisms was done as follows: Rows B and C – *Escherichia coli*; Rows D and E – *Salmonella typhi* ; Rows F and G – *Staphylococcus aureus*. This was performed for pure (Aloe Vera and Cinnamon) extracts as well as for combinations of the extracts (Aloe Vera + Cinnamon) to estimate the minimum effective concentration of the extract. The plates were observed after incubation and the minimal inhibitory concentration were recorded.

Preparation of sanitizer

After screening MIC, the most effective concentration as found, was selected for the formulation of the sanitizer.

Screening of formulated sanitizer with various microbes.

The prepared formulation was tested and screened for the antimicrobial activity against various microbes. This was done on Micro titre plates. The exact same procedure was followed. Instead of the extract, sanitizer samples were taken and serially diluted. This was screened through the following organisms: *Salmonella typhi*, *Escherichia*

coli, *Pseudomonas aeruginosa*, *Bacillus cersus*, *Streptococcus mutans*, *Staphylococcus aureus* and Hand microbes. The plates were incubated and the results were observed.

RESULTS

MIC results of the individual extracts

Aloe Vera showed inhibition against *E. coli* at 1 mg dilution, *Salmonella typhi* at 1 mg dilution however there was no inhibition against *Staphylococcus* (Table 1). Cinnamon showed inhibition against *E. coli* at 1mg dilution, *Salmonella typhi* at 1mg dilution and *Staphylococcus* at 1mg dilution concentration (Table 2). Aloe Vera & Cinnamon combination showed inhibition against *E. coli* at 1mg dilution, *Salmonella typhi* at 0.5mg dilution and *Staphylococcus* at 0.5mg dilution concentration (Table 3).

Thus, the combination of Aloe Vera and Cinnamon was found to be the most effective in comparison to individual extracts of Aloe Vera and as the minimum inhibition of cinnamon was seen at 0.5mg against the microbes. The Aloe Vera and Cinnamon extracts were mixed to produce a net concentrated extract of 15 mg/ml.

Table 1: MIC of Aloe Vera Extract

Organisms	Duplicates	Minimum Inhibitory Concentration						
		1mg	0.5 mg	0.25mg	0.125mg	0.062 mg	0.031mg	0.015 mg
<i>Salmonella typhi</i>	1	+	-	-	-	-	-	-
	2	+	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
<i>Escherichia Coli</i>	1	+	-	-	-	-	-	-
	2	+	-	-	-	-	-	-

Table 2: MIC of Cinnamon Extract

Organisms	Duplicates	Minimum Inhibitory Concentration						
		1 mg	0.5 mg	0.25mg	0.125 mg	0.062 mg	0.031mg	0.015 mg
<i>Salmonella Typhi</i>	1	+	-	-	-	-	-	-
	2	+	-	-	-	-	-	-
<i>Staphylococcus Aureus</i>	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
<i>Escherichia Coli</i>	1	+	-	-	-	-	-	-
	2	+	-	-	-	-	-	-

Table 3: MIC of Aloe Vera and Cinnamon Extract

Organisms	Duplicates	Minimum Inhibitory Concentration						
		1 mg	0.5 mg	0.25mg	0.125mg	0.062 mg	0.031mg	0.015 mg
<i>Salmonella Typhi</i>	1	+	+	-	-	-	-	-
	2	+	+	-	-	-	-	-
<i>Staphylococcus Aureus</i>	1	+	+	-	-	-	-	-
	2	+	+	-	-	-	-	-
<i>Escherichia Coli</i>	1	+	-	-	-	-	-	-
	2	+	-	-	-	-	-	-

MIC results of sanitizer samples

The MIC results were obtained for the sanitizer samples after preparation of aloe vera and cinnamon combination. The formulated sanitizer was shown to be effective against most of the microbes tested. The observed values are as follows: Inhibition against *Salmonella* was seen at 0.75mg, against *E. coli* at 1.50mg, against

Bacillus at 0.75mg and against *Pseudomonas* at 0.75mg. No Inhibition was seen against *Staphylococcus* and *Streptococcus*. Inhibition against Hand microbes was seen at 0.09mg. Sanitizer with combination of Aloe Vera and Cinnamon showed effective inhibition at concentration 0.75mg on most the tested microorganisms as shown in Table 4.

Table 4: MIC of Aloe Vera and Cinnamon Hand Sanitizer Extract

Organisms	Duplicates	Minimum Inhibitory Concentration				
		1.5 mg	0.75 mg	0.37 Mg	0.18 mg	0.093 mg
<i>Staphylococcus Aureus</i>	1	-	-	-	-	-
	2	-	-	-	-	-
<i>Salmonella Typhi</i>	1	+	+	-	-	-
	2	+	+	-	-	-
<i>Escherichia coli</i>	1	+	-	-	-	-
	2	+	-	-	-	-
<i>Bacillus cereus</i>	1	+	+	-	-	-
	2	+	+	-	-	-
<i>Pseudomonas Aureginosa</i>	1	+	+	-	-	-
	2	+	+	-	-	-
<i>Streptococcus Mutant</i>	1	-	-	-	-	-
	2	-	-	-	-	-
Hand Sample 1	1	+	+	+	+	+
Hand Sample 2	2	+	+	+	+	+

On the contrary to our study the studies by Mohammad Mehdi *et al.*, 2012 on Aloe Vera was found to have an average MIC of 25- 50 µg from Micro titer assay. The study conducted by Liesel Brenda *et al.*, 2008 showed the minimum inhibitory concentration of cinnamon to be 25- 50 µg. Cinnamon is found to be an effective antimicrobial agent based on several tests against 20 microbes and was reported as the best herbal product to be used (Masoumian *et al.*, 2017). Successful results have also been reported at concentrations of neem, tulsi,

mint, lemon etc. ranging from 10-50µg. Addition of lavender and jasmine essential oils were also found to show inhibitory action along with a pleasant fragrance to the product.

In consideration with the samples used, equipment and lab ambience that were available, the results obtained in the current study are sufficient enough to support the fact that the combination of Aloe Vera and Cinnamon in sanitizers is very effective. Further advanced techniques and

methodologies could be employed to get more precise and effective results.

CONCLUSION

Sanitizers provide an effective alternative to hand washing with soap and water, but alcohol-based sanitizers can cause skin dryness and irritation, with their effect lasting only until the alcohol evaporates. Herbal sanitizers, however, offer extended protection as their natural extracts remain on the skin even after alcohol evaporation. Phytochemicals such as alkaloids, phenolics, terpenoids, and tannins in herbal formulations contribute antimicrobial, anti-inflammatory, and immunity-enhancing properties. In this study, a herbal sanitizer formulated with ethanolic extracts of aloe vera and cinnamon showed significant antimicrobial activity. The combination of aloe vera and cinnamon proved more effective against tested microorganisms and hand microbes than either extract alone. Therefore, the combination of these herbal ingredients enhances the overall efficacy of the sanitizer, making it a more effective option than traditional alcohol-based sanitizers.

Declaration by Author

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