Formulation and evaluation of herbal hand sanitizers based on plants producing essential oil

ABSTRACT

Hand hygiene is important because it can easily be transmitted through direct contact with microbial spores produced during coughing and sneezing. It is important to prevent the spread of the disease, especially in cases such as epidemics, through the use of antibiotics. This can be achieved by establishing and maintaining strict infection control equipment such as good hygiene in hospitals and public places. The aim of this study is to prepare an alcohol-based sanitizer against bacterial infections. After the test, the pH value of the disinfectant is between 6.65 and 6.67. The viscosity of the antiseptic is between 389 cps to 835. The spread ability was good and found in range between 6.55 to 7.18 g-cm/sec. The drug content of Eucalyptus Oil in formulations was found to be 93% in F1 and 97.5% in F2. In-vitro drug release study was found to be 56.2% in F1 and 60.6% in F2, and the stability of the preparation remained stable after 3 months of storage. All these studies showed that the F2 formulation is the best as it has better pH, viscosity, spreading ability and safety.

Keywords: - Herbal ingredients, Hand disinfection, Infection control.

INTRODUCTION

Hands are a primary route for transmitting microbes and infections, making hand hygiene essential for preventing and controlling infections. Bacteria found on hands can be classified as resident or transient. Healthcare workers often carry pathogenic flora, such as Staphylococcus aureus, Enterococcus spp., and Acinetobacter , which can lead to nosocomial infections if proper hand hygiene is not maintained. Simple washing may not effectively eliminate these pathogens, highlighting the necessity for hand sanitizers [1].

Sanitizers are available in various forms—liquid, gel, or foam—and can be categorized as alcohol-based or herb-based. Alcohol-based sanitizers typically contain isopropyl alcohol, ethanol, or n-propanol in concentrations ranging from 60% to 95%. In contrast, non-alcohol-based options often contain benzalkonium chloride, which tends to be less effective. Healthcare professionals, including doctors and surgeons, frequently use hand sanitizers. Misapplication, such as not allowing the sanitizer to dry completely or using an insufficient alcohol concentration, can further reduce effectiveness. The correct usage involves applying the product to the palms and rubbing it over all surfaces until dry [2-4].

While chemical-based sanitizers work by disrupting cell membranes and inhibiting protein synthesis, frequent use can lead to skin issues like dryness and irritation, particularly for sensitive skin. To address these concerns, there is growing interest in developing herbal sanitizers that combine herbal Eucalyptus oil, Clove oil, Glycol, Aloe vera gel and

Preservatives. Many plants contain secondary metabolites with antimicrobial properties, making them valuable for formulating effective herbal hand sanitizers.

Figure 1 Various types of hand sanitizer dosage form

Benefits of Using Herbal Hand Sanitizer:

Ease of availability: Since herbs are easily available in both rural and urban areas, anyone can use them easily.

Cheap: Herbal remedies are less expensive compared to the chemical ingredients used in synthetic hand sanitizers.

Increased efficiency: Herbal hand sanitizers can provide better hand hygiene.

Less side effects: Herbal hand sanitizers have fewer side effects than other hand sanitizers.

MATERIALS AND METHODS

Materials:

Plant information was collected from the herbal garden of the Faculty of Pharmacy, College of Pharmacy. Taxonomic identification and certification was done by the Department of Pharmacy, Jaipur National University. Using Eucalyptus globulus, lemongrass and clove essential oil as raw materials, the soap extracts the essential oil of each plant through water distillation.

Methods:

Volatile oil extraction procedure:

Sample preparation of Eucalyptus leaves:

Gather fresh eucalyptus leaves and rinse them under water to remove dust and dirt. After draining the excess water, weigh 100 grams of leaves and cut them into small pieces with a sharp knife. [5-8].

Hydro distillation procedure:

- First, purified water was placed in a round-bottom flask.
- Next, the chopped leaf samples were added to the flask for distillation.
- Then connect the lower container to the condenser via the connecting pipe and place the mercury thermometer in the container so that it touches the top of the leaf bed.
- The heating mantle was turned on and adjusted to maintain the desired heating rate.
- Observations were made every 30 minutes and the volume of oil extracted, the temperature of the extractor and the readings of the electric meter were recorded. At the end of each distillation experiment the weight of the hydrosol, the eucalyptus leaves used and the water remaining in the extractor were also recorded.
- Finally, store the eucalyptus oil extract in the refrigerator below 4°C.

Extraction of clove oil:

150 grams of whole clove buds and whole ground cloves are hydro distilled for 3 hours with 300 ml of water using a Clevenger apparatus. Heat the apparatus to 100°C using a heater. The essential oil obtained is dried over sodium sulphate and stored in a refrigerator at a temperature below 4°C. [9-11].

Figure 2 Clove **Figure 3 Distillation apparatus**

FORMULATION

Procedure for formulation of herbal Hand Sanitizer:

Alcohol-based hand sanitizers are made by slowly mixing Carbopol 940 into distilled water. Once the combination is taken, the product is left to sit for 24 hours. Denatured alcohol,

polysorbate 20, ethylene glycol and all herbs are added to the aqueous solution. 0.80% potassium hydroxide and 0.20% methyl and propylparaben are added to make the product consistent. The final formulation is stored in an airtight, high-density polyethylene (HDPE) container. The prepared hand antiseptics are first physically tested and then a disinfection test is performed.

Figure 4 Homogenous mixture of Carbopol 940 and distilled water

Table 1: Formulation Table

S. No.	INGREDIENTS	QUANTITIES	
		F1	F2
1.	Deionised water	13.5ml	13ml
2.	Denatured alcohol	23 ml	23.5ml
3.	Eucalyptus oil	0.50ml	0.45ml
4.	Aloe-vera gel	0.50ml	0.45ml
5.	Clove oil	0.30ml	0.20ml
6.	Carbopol 940	0.40g	0.30g
7.	Glycerine	1 ml	0.80ml
8.	Polysorbate 20	0.30 ml	0.25ml
9.	Tri ethanol amine	0.20ml	0.25ml
10.	Fragrance	0.10 ml	0.20ml
11.	Preservatives(Methyl+Propyl paraben)	0.2g	0.6g
	Total weight	40ml	40ml

Figure 5 Herbal hand sanitizer

EVALUATION PARAMETERS

1.Visual Appearance:

The prepared sanitizer was visually examined for clarity, color, transparency, and any visible particles. A smear of the sanitizer was placed on a glass slide and observed under a microscope to check for any particles or grittiness [13].

2. Physical Evaluation:

(a) PH:

The pH of an alcohol-based herbal hand sanitizer formulation was assessed using a digital pH meter. One gram of gel was dispersed in 100 millilitres of distilled water and allowed to sit for two hours. Each formulation's pH was measured in triplicate, and the average values were calculated.

(b) Viscosity:

The viscosity of an alcohol-containing hand sanitizer solution was analyzed using a Brookfield viscometer equipped with a T-bar spindle (S-94) at 37 °C. Fill a 10 mL beaker with 5 g of solution and perform the same procedure. Place the rotor vertically, making sure it does not touch the bottom of the beaker. The spindle was rotated at 50, 60, and 100 rpm to obtain more than 50% torque. Measurements are recorded 60 seconds after the start of the test.

(c) Spreadability:

The spread of alcohol-based disinfectants was tested using a device that operated with two slides, the upper one attached to the scale of the hook and the lower one to the wooden table. [14-16].

The Spreadability of sanitizer was calculated by using formula:

$$S = m \times l/t \text{ (gram cm/sec)}$$

Where,

m = wt. Tied to upper slide

L = length of glass slides

T = time taken to separate the slides

(d) Determination of λ max:

The λ max of various essential oils was determined using the following procedure:

1. Accurately weigh 100 mg of the essential oil and transfer it to a 100 ml volumetric flask.
2. Add sufficient amount of ethanol and shake the flask thoroughly to ensure the oil dissolves completely.
3. Adjust the volume to 100 ml with ethanol.
4. Transfer the prepared dilution into a cuvette.
5. Set the spectrophotometer to a wavelength range of 200 to 400 nm, using either an empty light path or a cuvette filled with distilled water to establish the reference level.
6. Insert the cuvette containing the diluted oil into the sample compartment and record the absorbance.
7. Repeat steps 4 and 5 at the same wavelength and record the absorbance again.
8. Plot the results of absorbance against wavelength.

(e) Drug content estimation:

The drug content of the hand sanitizer was assessed by dissolving an accurately weighed 1000 mg sample in 120 ml of ethanol.

The solution was shaken for 4 hours and allowed to sit for an additional 6 hours to ensure complete dissolution of the formulation. After this, the solution was filtered, and dilutions were prepared for analysis. The drug content was measured using a UV/Visible spectrophotometer at 260 nm for eucalyptus oil and 245 nm for clove oil.

The drug content was then calculated using the linear regression equation derived from the calibration data.

(f) Preparation of dialysis membrane for in-vitro release study:

Drug release studies have been conducted with membrane dialysis treatments. The membranes were washed in water for 4–5 hours to remove glycerol and then treated with 0.3% w/v sodium sulfite solution at 80°C for 1–2 minutes to remove sulfide solution. The membranes were washed in hot water at 60°C for 2 minutes and then acidified with 0.2% v/v sulphuric acid solution. use.

(g) In-vitro release study:

Franz diffusion cell (effective diffusion area 3.14 cm2, cell volume 15.5 ml) was used for drug release studies. Use an equal amount of alcohol-based hand sanitizer (200 mg) on the membrane. The membrane was sandwiched between the transmitter and receiver chambers of the diffusion cell. The receiver was filled with phosphate buffer (7.4) and different samples were removed from the port at 0, 0.5, 1, 2, 4, 6, 8, and 24 h and updated without. Absorbance measurements were performed at 260 nm, 245 nm, 282 nm, and 263 nm.

(h) Stability studies

Store alcohol-based hand antiseptics prepared with essential oils in aluminum tubes in triplicate, protected from light. Samples were stored for 3 months at three different conditions: refrigerator temperature (2-8 °C), room temperature (25 ± 2 °C) and oven temperature (45 °C). After the storage period, samples were evaluated in terms of physical properties, pH, viscosity and drug release. [14-16].

RESULT AND DISCUSSION

Visual appearance:

Colour: Colourless to greenish yellow

Odour: Aromatic & characteristics

pH determination:

Record the pH of each type of disinfectant after dispersion in distilled water and obtain the results.

Table 2: pH determination

S.NO	Formulation	pH Values
1	F1	6.65
2	F2	6.67

The pH of each sanitizer formulation after dispersion in distilled water was noted and the result were taken. Showing in Table 2. The pH of the alcohol based herbal hand sanitizer was found to be between 6.65 to 6.67 which was well within the normal pH range of 5.5-7, Hence the prepared herbal hand sanitizer can be applied to the hand.

Viscosity:

Table 3: Viscosity of Alcoholic herbal hand sanitizer

S.NO	Formulation	Viscosity (cps)		
		50 rpm	60 rpm	100 rpm
1	F1	389	456	782
2	F2	416	482	835

The viscosity of disinfectant alcohol was determined using Brookfield viscometer at 37 ° C. The viscosity values ranged from 389 cps to 835 cps as shown in Table 3.

Spread-ability:

Table 4: Spread ability of Alcoholic herbal hand sanitize

S.NO	Formulation	Spread-ability (g-cm/sec)
1	F1	6.55
2	F2	7.18

The spread of the disinfectant was found to be in the range of 6.55 to 7.18 g-cm/sec, confirming that the disinfectant spread well and evenly (as shown in the table). 4

Drug Calibration Curve:

Calibration Curve for Eucalyptus Essential Oil (Formulation 1)

λ_{max} determination:

λ max is determined using U.V. Spectrophotometer. λ max is found to be 260nm

Construction of calibration curve:

Calibration curves for eucalyptus oil were prepared in ethanol. The resulting eucalyptus oil calibration curve follows the Beer-Lambert law of mass selection.

Table 5: Calibration Curve of Eucalyptus Oil (Formulation 1)

S.NO	Concentration (µg/ml)	Absorbance
1	2	0.184
2	4	0.375
3	6	0.575
4	8	0.781
5	10	0.985

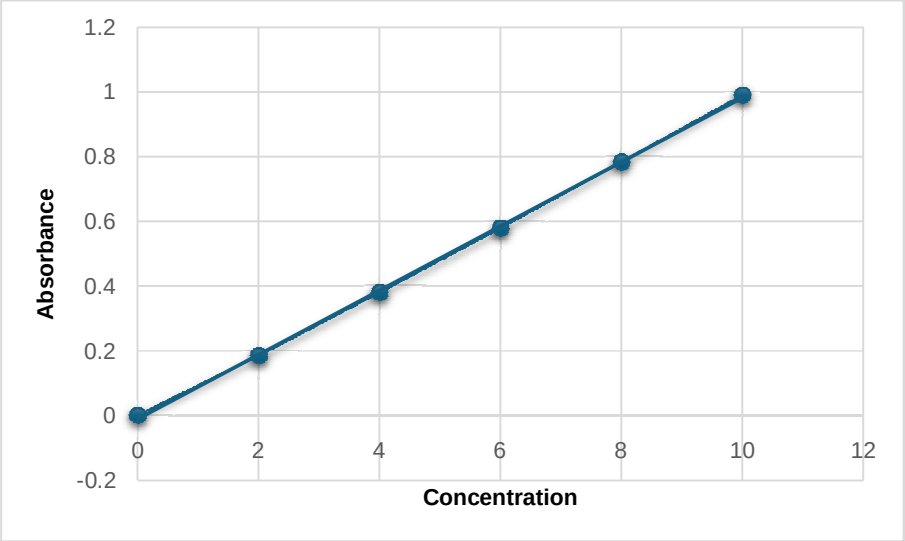

Figure 6 Standard Curve of Eucalyptus oil at λ 260nm (Formulation 1)

Table 6: Calibration Curve of Eucalyptus Oil (Formulation 2)

S.NO	Concentration (µg/ml)	Absorbance
1	2	0.184
2	4	0.380
3	6	0.578

4	8	0.784
5	10	0.990

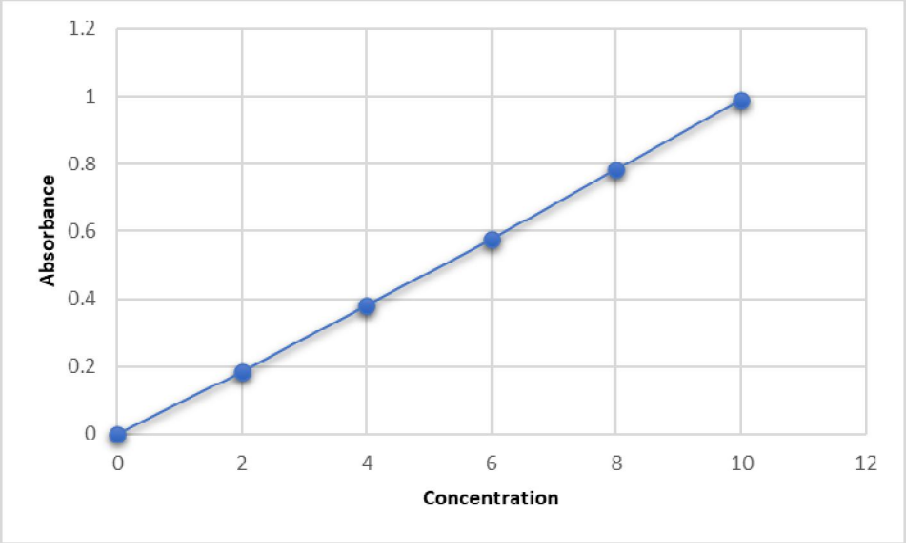

Figure 7 Standard Curve of Eucalyptus oil at λ 260nm (Formulation 2)

Drug Content:

Weigh 1000 mg of detergent accurately, dissolve it in 100 ml of solvent (ethanol) and determine the content of the detergent. The solution was kept for 4 hours and stored for 6 hours to allow the production to be completed. Then filter the solution. Then 2 ml of this solution was diluted again in 100 ml of solvent (ethanol). Then dilutions such as 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml were made.

Table 7: Percent Drug Content of Formulation (Eucalyptus Oil)

S.NO	Formulation	% Drug Content
1	F1	93%
2	F2	97.5%

In-vitro drug release study:

In vitro drug release studies were performed using alcohol-based disinfectants to estimate the amount of drug potential in biofilms. shows a comparison of the in vitro release profile by dialysis.

Table 8: Drug release study data

Time (hrs)	F1	F2
0	0.00	0.00
0.5	73±0.62	9.1±1.23
1	9.4±0.68	12.0±1.63
2	16.2±0.86	14.0±1.11
4	18.1±0.82	20.1±1.0
6	25.5±0.87	30.2±1.2
8	37.1±1.05	48.3±1.08
24	56.2±1.42	60.6±1.46

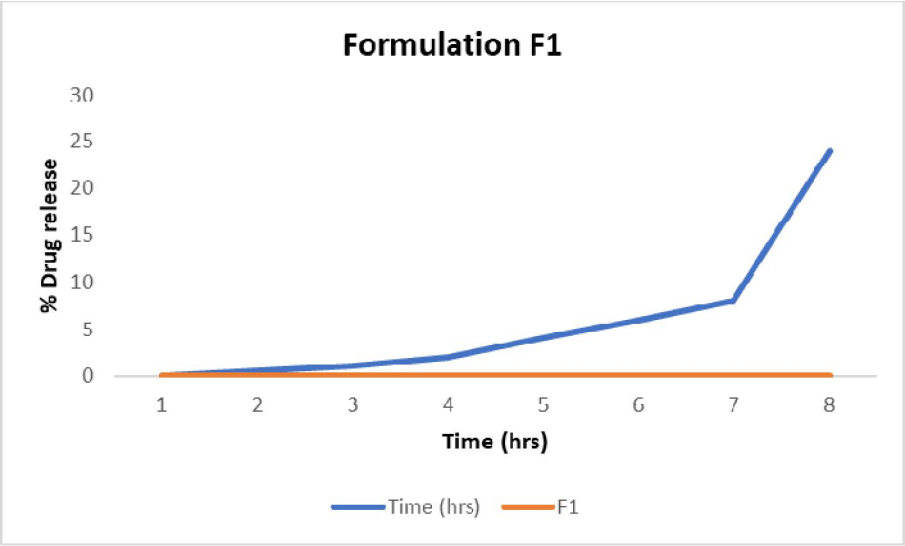

Figure 8 Drug release study of Formulation F1

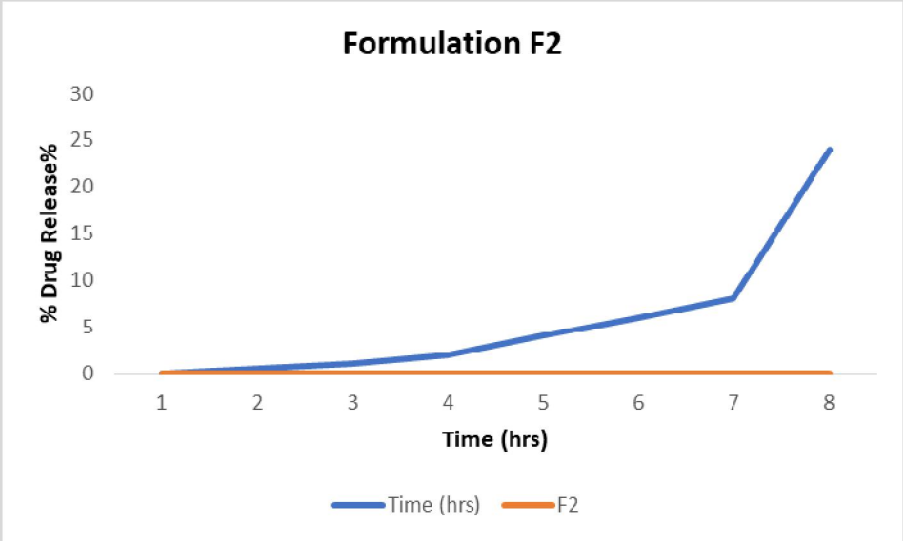

Figure 9 Drug release study of Formulation F2

Stability study:

Table 9: Effect of different storage conditions

Time (month)	**Percent (Entrapment)** **5 ± 3^{0}C**	**Percent (Entrapment)** 25 ± 2^{0}C
0	72.2±0.768	74.02±0.768
1	70.1±0.450	71.11±0.363
2	69.5±0.213	66.8±0.452
3	69.4±0.361	65.52±0.532

Alcohol-based hand sanitizer containing eucalyptus oil, clove oil and aloe vera gel remains stable after 3 months of storage without any change in physical properties, pH value and drug release.

CONCLUSION

In order to provide a natural means of preventing the spread of disease, this study sought to create a unique alcohol-based herbal hand sanitizer that is safe, effective, and devoid of dangerous chemicals. The antibacterial, antifungal, and antiseptic qualities of eucalyptus oil, clove oil, and aloe vera gel, which are all included in the sanitizer, help treat bacterial infections and get rid of pathogens for efficient sanitization. This herbal hand sanitizer was made using an easy-to-follow and economical process. Two formulations were created,

labelled F1 and F2. Among various tests conducted, the F2 formulation proved to be particularly effective against bacterial infections.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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