FORMULATION AND EVALUATION OF HERBAL BASED HAND SANITIZER

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 between6.55 to 7.18 g-cm/sec. The drug content of Eucalyptus Oil in formulations was found to be 93% in F1 and 97.5% inF2. 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 orusing 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].

Whilechemical-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.

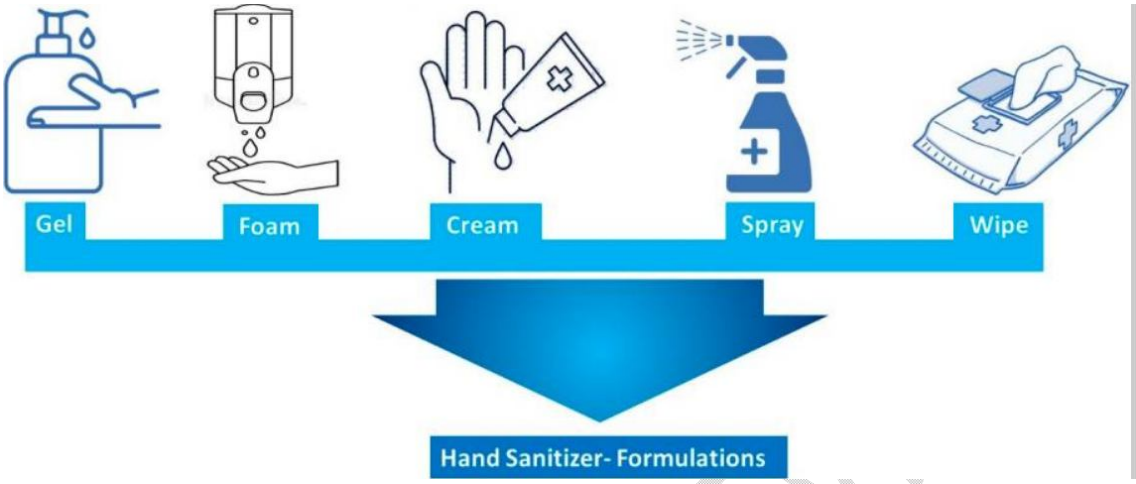

Figure 1 Various types of hand sanitizer dosage form

Benefits of Using Herbal Hand Sanitizer:

Ease of availability: Herbs are easily available in rural as well as urban areas, so they can be easilyused by anyone.

Cheap: Cost of the herbal plants is less as compared to chemical ingredients used in synthetic handsanitizers.

Increased efficiency: Herbal hand sanitizers are more efficient in promoting hand hygiene.

Less side effects: Herbal hand sanitizers have less side effects than other hand sanitizers

MATERIALS AND METHODS

Materials:

The plant materials were collected from the herbal garden, School of Pharmaceutical Science.The taxonomical identification and authentication was done by Department of Pharmaceutical science, Jaipur National University. The hand sanitizer was prepared from the volatile oil of Eucalyptus globulus, Cymbopogon citratus and Syzygiumaromaticum.The volatile oil of each plant was obtained by hydro distillation method.

Methods:

Volatile oil extraction procedure:

Sample preparation of Eucalyptus leaves:

Fresh eucalyptus leaves were collected and rinsed with tap water to remove any dust and dirt. After draining the excess water, 100 grams of the leaves were weighed and chopped into small pieces using 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.
- The round-bottom flask was then connected to the condenser via a connecting pipe, and a mercury thermometer was inserted into the flask to touch 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, recording the cumulative volume of extracted oil, the temperature of the extraction chamber, and the energy meter reading. The weights of the hydrosol, spent eucalyptus leaves, and remaining water in the extractor were also noted at the end of each distillation test.
- Finally, the extracted eucalyptus oil was stored in a refrigerator at temperature below 4℃.

Extraction of clove oil:

A total of 150 grams of whole clove buds and ground clove were separately subjected to hydro distillation using a Clevenger apparatus, with 300 ml of water for 3 hours. The setup was heated to 100˚C using a heating mantle. The essential oils obtained were dried over anhydrous sodium sulphate and subsequently stored in a refrigerator at temperatures below 4˚C [9-11].

Figure 2 Clove **Figure 3 Distillation apparatus**

FORMULATION

Procedure for formulation of herbal Hand Sanitizer:

The alcohol-based herbal hand sanitizer was created by gradually mixing Carbopol 940 into distilled water. After achieving a homogeneous mixture, the product was allowed to sit for 24 hours. Denatured alcohol, polysorbate 20, glycol, and all the herbal extracts were then added to the aqueous solution. To ensure a uniform product, 0.80% Potassium Hydroxide and 0.20% each of the preservatives methyl and propyl paraben were incorporated. The final formulation was stored in airtight high-density polyethylene (HDPE) containers. The prepared hand sanitizer was first subjected to physical evaluation and subsequently tested for antimicrobial efficacy.

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-based herbal hand sanitizer composition was evaluated at 37 ℃ using a Brookfield viscometer equipped with a T-bar spindle (S-94). A 10 mL beaker

containing 5 g of the solution was filled, and the spindle was carefully lowered perpendicularly, ensuring it did not touch the bottom of the beaker. To achieve a torque greater than 50%, the spindle was rotated at speeds of 50, 60, and 100 rpm. Measurements were recorded 60 seconds after initiating the test.

(c)Spreadability:

The spreadability of an alcohol-based herbal hand sanitizer was tested using a laboratory-made devicewith two glass slides, the top one of which was linked to a balance by a hook and the bottom one gluedto a wooden plate [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 λmax 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 study was carried out using treated dialysis membrane. The membranes were washed in water for 4–5 h to remove glycerol and then treated with 0.3% w/v sodium sulphide solution at 80°C for 1–2 min to remove sulfur compounds. The membrane was then washed in hot water at 60°C for 2 min and then acidified with 0.2% v/v sulfuric acid solution. Finally, rinse the membrane with hot water to remove the acid. The treated membrane was stored in the diffusionmedium and refrigerated till use. The membrane was washed with distilled water prior to use for in-vitro study.

(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.

(f)Stability studies

The prepared alcohol-based herbal hand sanitizer, containing essential oils, was stored in collapsible aluminium tubes, away from light, in triplicate. The samples were kept at three different conditions: refrigerated temperature (2-8°C), room temperature (25 ± 2°C), and oven temperature (45°C) for a duration of 3 months. After this storage period, the samples were evaluated for their physical appearance, 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 alcohol based herbal hand sanitizer formulation was determined at 37℃ using a Brook field viscometer. The viscosity values ranged from 389 cps to 835 cps, shown in the 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 Spreadability of the sanitizer was found to be in the range of 6.55 to 7.18 g-cm/sec confirming that the sanitizer may spread smoothly and uniformly shown in table. 4

Drug Calibration Curve:

Calibration Curve for Eucalyptus Essential Oil (Formulation 1)

λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was 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

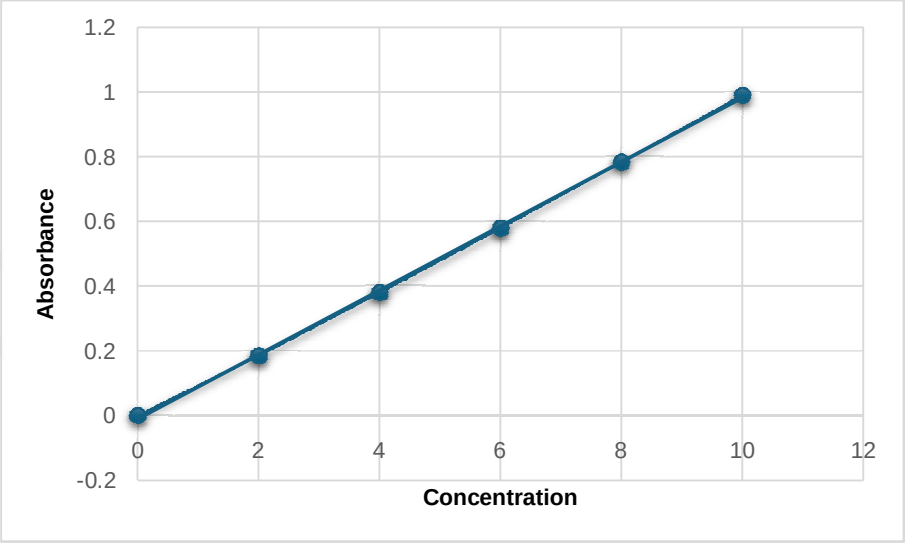

Figure 6Standard Curve of Eucalyptus oil at λ 260nm (Formulation 1)

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

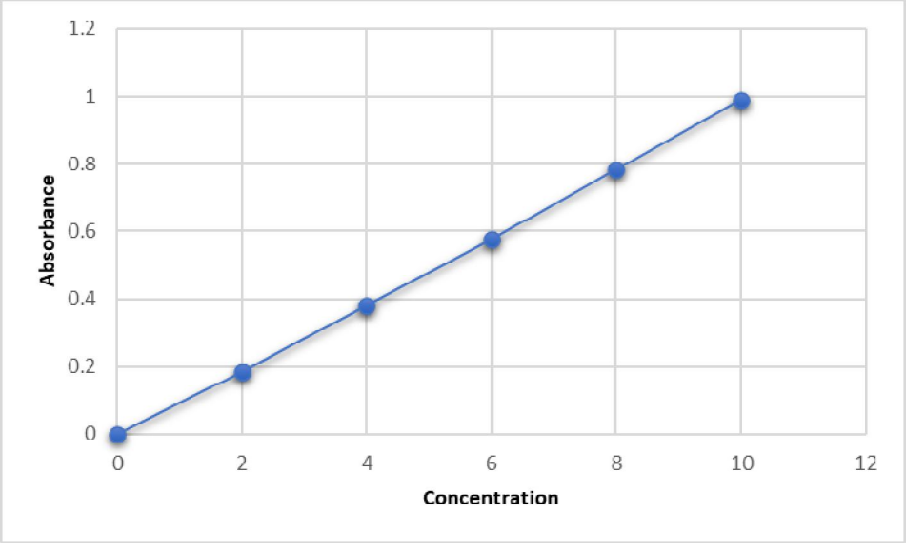

Figure 7Standard Curve of Eucalyptus oil at λ 260nm (Formulation 2)

Drug Content:

The drug content of the hand sanitizer was determined by dissolving an accurately weighed quantity 1000mg in 100ml of solvent (Ethanol). The solutions were kept for shaking for 4 hours and kept for 6 hours for complete dissolution of theformulations. Then the solutions were filtered. Then 2ml of this solution was diluted again into 100mlof solvent (ethanol). Then the serial dilutions were made for example 2µg/ml, 4µg/ml, 6µg/ml, 8µg/mland 10µg/ml.

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: Percent of drug release from hand sanitizer formulations at different time interval

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

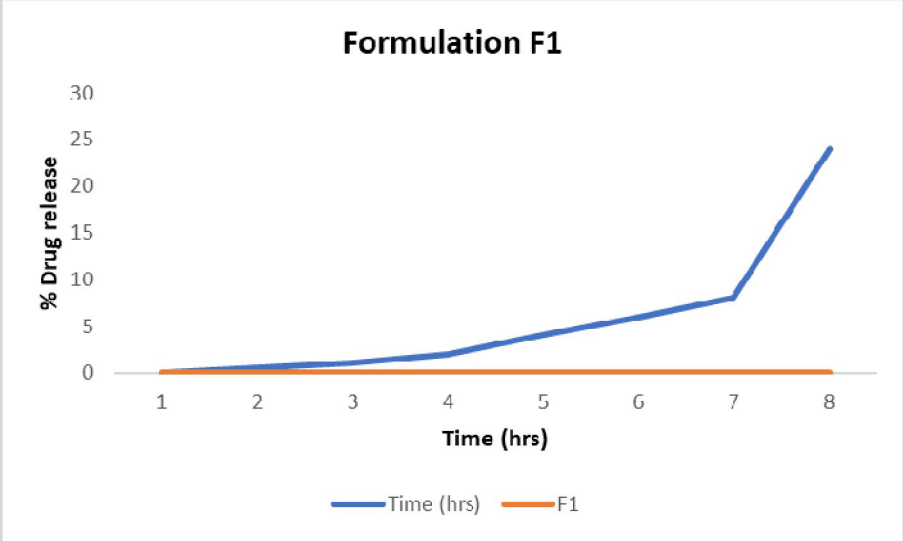

Figure 8 Drug release study of Formulation F1

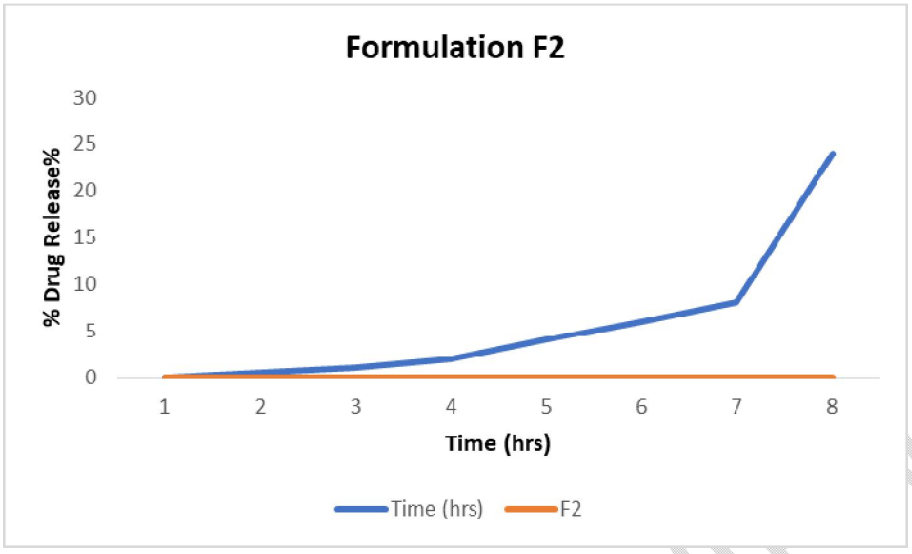

Figure 9 Drug release study of Formulation F2

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

The prepared alcohol based herbal hand sanitizer containing eucalyptus oil, clove oil and aloe vera gel were found to be stable upon storage for 3 months, where no changes was observed in their physical appearance, pH and drug release. Data is present in table 10.

CONCLUSION

This study aimed to develop a novel alcohol-based herbal hand sanitizer that is safe, effective, and free from harmful chemicals, providing a natural way to prevent disease transmission. The sanitizer incorporates eucalyptus oil, clove oil, and aloe vera gel, all of which exhibit antibacterial, antifungal, and antiseptic properties, aiding in the treatment of bacterial infections and the elimination of pathogens for effective sanitization. The methodology used for preparing this herbal hand sanitizer was straightforward and cost-effective. Two

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

REFERENCES:

1. Grace, X. F., Sowmya, K. V., Darsika, C., Jothy, A., & Shanmuganathan, S. (2015). Polyherbalhand sanitizer-formulation and evaluation. Indian journal of pharmacy andpharmacology, 2(2), 143-144.

2. Wani, N. S., Bhalerao, A. K., Ranaware, V. P., & Rahul, Z. (2013). Formulation andevaluation of herbal sanitizer. International Journal of PharmTech Research, 5(1), 40-43.

3. Harsa, M., Misra, B., Chaithra, C., & Ramana, V. (2016). Evaluation of bactericidal activity ofherbal hand sanitizer. International Journal of Ayurveda and Pharma Research, 4(8), 24-28.

4. Enwa Felix, O., Anie Clement, O., &Oghenejobo Micheal& Ilaya Sonia, A. (2015). Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soapsagainst some Bacterial Isolates.

5. Afsar, Z., & Khanam, S. (2016). Formulation and evaluation of polyherbal soap and handsanitizer. International Research Journal of Pharmacy, 7(8), 54-57.

6. Elvira M Gaspar, Renato Duarte and Jose C Santana., 2018. Volatile composition andantioxidant properties of Clove products. Biomedical journal of scientific & technical research,Vol. 9: 7270-7276.

7. Md. Azir Uddin, Md. Shahinuzzaman, Md. Sohel Rana and Zahira Yaakob., 2017. Study ofchemical composition and medicinal properties of volatile oil from Clove buds. Internationaljournal of pharmaceutical science and research, Vol. 8: 895-899.

8. Nzeako BC, Al-Kharousi S.N.Z., Al-Mahrooqui Z., 2014. Antimicrobial activities of clove andthyme extracts. Sultan qaboos university medical journal, Vol. 6: 33-39.

9. Rani L. and Gopinath P., 2017. Anti-bacterial activity of clove oil against staphylococcusaureus. International journal of recent scientific research, Vol. 8: 16134-16136.

10. Singh P.P. and Kaur D., 2016. Antimicrobial activity of clove oil against microbes isolatedwater. International conference and expo on water microbiology and novel technologies: 74.

11. Itrat Malik, Zarnigar, 2013. Aloe vera: a review of its clinical effectiveness. Internationalresearch journal of pharmacy, Vol. 4: 75-79.

12. Sahu P.K., Giri D.D., Singh Ritu, Pandey Priyanka, Gupta Sharmistha, Shrivastava A.K.,Kumar Ajay, Pandey K.D., 2013. Therapeutic and medicinal uses of aloe vera: a review.Scientific Research of pharmacology & pharmacy, Vol. 4: 599-610.

13. Joshi S.P., 1997. Chemical constituents and biological activity of Aloe barbadensis: a review.Journal of medicinal and aromatic plant science, Vol. 20: 768-773.

14. Renisheya Joy Jeba Malar T., Johnson M., Nancy Beaulah S., Laju RS, Anupriya G, RenolaJoy Jeba Ethal T., 2012. Anti-bacterial and antifungal activity of aloe vera gel extract.International journal of biomedical and advance research, Vol. 3: 184-187.

15. Alavijeh Parastoo K., Alavijeh Parisa K. and Sharma D., 2012. A study of antimicrobialactivity of few medicinal herbs. Asian journal of plant science and research, Vol. 2: 496-502.

16. Mounika, A., Vijayanand, P., & Jyothi, V. (2017). Formulation and evaluation of poly herbalhand wash gel containing essential oils Standardization of Inoculums. International Journal ofPharmacy and Analytical Research, 6(4), 645-653