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Formulation and Evaluation of Antibacterial Herbal Paper Soap Containing Essential Oil of Ocimum Basilicum L.

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ABSTRACT

With the objective to offer a practical, environmentally friendly, and efficient personal hygiene solution, the herbal paper soap developed. A blend of Ocimumbasilicum L. essential oil was used to create a new herbal paper soap. Significant antibacterial activity was shown by the soap against a range of harmful bacteria, suggesting that it could be a safe and efficient substitute for traditional soap. This innovative herbal paper soap combines skin care advantages, efficacy, and sustainability in a special way. The current project's goal is to create and assess antibacterial herbal paper soap that works against a variety of harmful bacteria, including strains that are Gram-positive and Gram-negative. The formulation of herbal paper soap was the focus of this project. Among the components used to make paper soap includeOcimumbasilicum L. essential oil, glycerine, tragacanth,colorant, distilled water, and sodium lauryl sulphate. Using clevenger equipment, the essential oil was extracted using hydro distillation. After making the liquid soap, it was applied to the water-soluble paper's surface and allowed to dry. The prepared soap had good physical qualities, such as texture, foam, and a pH higher than 8, and it also showed strong antibacterial action against pathogenic bacterias like E. coli, S. aureus, P. aeruginosa. Among the two formulations F2 showed comparatively better antibacterial activity and other physical characteristics.

KEYWORDS: Ocimumbasilicum L, Hygiene, Herbal paper soap, Clevenger apparatus, Essential oil, Antibacterial, Water soluble paper, Primary emulsion

I. INTRODUCTION

Hand hygiene is the critical step to take with a purpose toprevent the risky germs and illnessesdue to them.Hands are the number oneway of transmission for microorganism and infections. Generally, hand hygiene refers to the practice of washing with water, soap or another liquid. It is well known that a good hand hygiene can reduce the spread of viruses and other germs. The soap helps to reduce health care associated transmission of contagious diseases more effectively. Soap is a substance used with water for washing and cleaning made of natural oils or fats with strong alkali and having a perfume and coloring agents. Soap can be defined as sodium or potassium salt of long chain of carboxylic acid which has cleansing property in water. [1,2]

Paper soap is a soap product innovation. It is a sheet of soap that is dry, light weight and can dissolve with small amount of water within seconds. It is convenient and pocket-sized cleaning product that can be used on hands. Herbal paper soap is a versatile hygiene product that combines the benefits of herbal ingredients. These formulations incorporate a blend of herbal extracts which are known for their cleansing, moisturizing, antimicrobial and nourishing property. According to WHO (World health organization), centre for disease control and prevention, also other health care authorities carried out large scale measures to combat proper hygiene, researchers estimated that just a 40% increase in proper hand washing and sanitizing measures could lead to significant reduction in the spread of disease.

Paper soaps are popular product among users. There is fewer wastage with liquid soap than it with hard soap. It has also better germ control activity.Common hand soap was made in a variety of colours and scents. Compared to freshly made

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

liquid soap, this one had good moisturizing qualities. These soaps are convenient for hand cleaning because of their superior scents and antimicrobial properties. Regarding sanitary practices, soap is a well-known cleanser that aids in the removal of bacteria and germs from the skin and the preservation of bodily health and sanity. In order to overcome the disadvantages of soap bars, herbal paper soap strips are formulated. Herbal soap strips are simple, convenient to use, economical and can be used by people of all ages and sex. The paper soap is easy to carry and therefore is used mostly by travellers. They are undoubtedly outstanding amongst any other travel cleansers. The paper soap has herbal oil which is added together with cleansers and moulded into thin paper sheets. [3,4,5,6]

1.1. Objectives

1) To create a formulation that offers new potential benefits to users.
2) Explore the use of innovative ingredients that can enhance the effectiveness of formulation
3) Ensure that the formulation effectively kills or inhibits the growth of bacteria when the paper soap is used, providing a reliable level of hygiene.
4) To utilize materials that are environmentally friendly and biodegradable to minimize ecological impact.
5) To create a product that is easy to use, ensuring that consumers can have convenient access.

II. PLANT PROFILE

Figure.1: Ocimumbasilicum L.

Common names: Sweet Basil, Purple Basil
Binomial name: OcimumbasilicumL

2.1. Scientific classification:

Kingdom: Plantae
Phylum: Streptophyta
Class: Equisetopsida
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Species:basilicum

2.2. Morphological Description [7]

Basil is a herb that is often annual, or occasionally perennial. Depending on the type, plants can grow to heights ranging from 30 to 150 centimetres (1 to 5 feet).

Leaves: They are shiny and oval, elliptic with smooth or slightly serrated edges, arranged oppositely along the angular stems. Leaves can be green or purple and vary in size from 3 to 11 cm in length and 1 to 6 cm in width.

Stem:The stem is upright and square-shaped, featuring hair between the segments. Stems may be vertical or positioned outward.

Flowers: They are small and white, purple, and arise from a central inflorescence, or spike, that develops from the main stem at the top of the plant. Uncommon for Lamiaceae, the four stamens and the pistil aren't tucked beneath the upper lip of the corolla, but instead rest atop the lower lip.

Seeds: They are either brown or black in color and have an elliptical shape. Upon contact with water, they generate mucilage.

Root: The plant possesses a taproot.

2.3.3Phytochemical constituents present in essential oil of O.basilicum L.[22]

Eugenol
L-Fenchone
1-isopropyl-4-methylenecyclohex-1-ene
α-Pinene
Estragole
p-mentha-1(7), 8-diene
Methyl-2-phenyl-prop-2-enolate
Eucalyptol
(E)-methyl cinnamate
Isocaryophillene
trans-α-Bergamotene

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

Eudesma-3,7(11)-diene
α-Cubebene
beta-bisabolene
α-Caryophyllene

Table.1: Phytochemicals present in O.basilicum L. essential oil

III. METHODOLOGY

1.Collection and Authentication of leaves of Ocimumbasilicum L.

Herb was collected from local areas and authentified by the botanist of Union christian college, Aluva.

Figure.2: Collected Ocimumbasilicum L.

2.Extraction of essential oil from O.basilicum leaves by hydro distillation using Clevenger apparatus.[8,9]

- Place the plant material and water in the round-bottom flask.
- Boil the plant material and water in the flask.
- The steam rises and condenses in the condenser.
- The condensed liquid falls into the burette, where the oil floats on top of the water.
- The water is gradually returned to the flask through a diagonal conduit.
- After a few hours, measure the volume of oil collected in the burette

Figure.3: Essential Oil of Ocimumbasilicum L

3.1. Method for preparation of Herbal paper soap:

1.Preparation of primary emulsion by Forbes bottle shaking method.[10,11,12,13]

The bottle shaking method, also known as the Forbes method, is a technique for preparing primary emulsions of volatile oils and other low-viscosity liquids.In this method, the oil or water is first carefully and vigorously shaken with the calculated amount of gum. Once fully emulsified, the second liquid (oil or water) is immediately added and the bottle is shaken vigorously again to form the primary emulsion.More of water is introduced in small quantities with consistent agitation after every addition to provide the required volume. The ratio of oil, water, and gum is usually 2:2:1 for volatile oil.

- ✓ Put one part powdered tragacanth or other gum in a dry bottle.
- ✓ Add two parts oil to the bottle.
- ✓ Cap the bottle and shake carefully.
- ✓ Add the required amount of water all at once.
- ✓ Shake the mixture until a primary emulsion is formed.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

Figure.4: Forbes bottle method

Figure.5: Primary emulsion

2.Addition of other excipients.**[14]**

- ✓ Sodium Lauryl Sulphate (after dissolving in hot water at 40^0C) was added to the primary emulsion.
- ✓ Required quantity of glycerine was added.
- ✓ Addition of colorant to the mixture.
- ✓ pH adjustment (optional), use a pH meter to check the pH level. Adjust with triethanolamine or borax if necessary (pH range from 8 to 10).
- ✓ Make up the volume using distilled water.

3. Filter and bottle: Filter the liquid soap into clean, sterilized bottles.

4.Paper soap strips were prepared by using seven drops of liquid soap on each side of the e water soluble paper.

5.Hang the paper overnight for air drying, cut into small rectangular pieces and store it in containers.

3.2. Water Soluble Paper

Figure.6: Smart solve water soluble paper

Water-soluble paper is an innovative material designed to dissolve in water, offering a sustainable alternative to tissue paper, filter paper etc. This type of paper, also known as "hydro soluble paper". It is made from made from Sodium Carboxy Methyl Cellulose and wood pulp. Water-soluble paper is not toxic, ecofriendly and 100% biodegradable.

Formulation of optimized antibacterial herbal paper soap

Sl. No	Ingredients	Quantity
1	Tulsi oil	2.25 ml
2	Tragacanth	1.125 g
3	SLS	5 g
4	Glycerine	15 ml
5	Colour	1.5 ml
6	Distilled water	q.s to 50 ml

Table.2: Formulation of optimized liquid soap

Formulations prepared

Sl No	Ingredients	F1	F2
1	Tulsi oil	2.25 ml	2.25 ml
2	Tragacanth	1.125 g	1.125 g
3	SLS	10 g	5 g
4	Glycerine	30 ml	15 ml
5	Colour	3 ml	1.5 ml
6	Distilled water	q.s to 100 ml	q.s to 50 ml

Table.2: Formulations of liquid soap

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

Figure.7: Formulated F1 liquid soap

Figure.8: Formulated F2 liquid soap

3.3. Evaluation parameters of antibacterial herbal paper soap [1,4]

1)Physicochemical parameters

Various physicochemical parameters which are mentioned below were performed to establish quality of the tulsi oil, water soluble paper, herbal paper soap.

Sl. No	Parameter	Test
1	Colour	By vision
2	Odour	By smell
3	Texture	By touch

Table.5: Organoleptic parameters

2)Size and shape

Measure the size and shape of the soap strips.

3) pH

Determined by using Digital pH Meter.Switch on the pH meter and allow it to stabilize for a few minutes if necessary and calibrate the pH Meter. Rinse the electrode with distilled water to remove contaminants. Dip the electrode into a calibration buffer solution (e.g., pH 4.0, 7.0) and wait for the reading to stabilize. Adjust the meter according to the buffer solution's pH.The Formulations were dissolved in 100 ml of distilled water and store for two hours. Pour the test solution into a clean beaker. Ensure the solution covers the pH electrode's sensing bulb. To measure the pH, rinse the electrode with distilled water and gently blot it dry using lint-free tissue. Immerse the electrode in the sample solution and wait for the reading to stabilize. Note the pH value displayed.

4)Foam Height

Dissolve sample of paper soap in a specific volume of distilled water (e.g. 100 mL) to prepare the solution. Pour a fixed volume of the prepared solution (e.g., 10 mL) into a graduated cylinder. Close the test tube with the stopper and shake it vigorously for a fixed time (e.g., 25–30 seconds) to generate foam. Place the cylinder on a flat surface immediately after shaking. Measure the total height of the foam.

5)Foam stability test

Let the cylinder sit undisturbed and record the foam height at specific time intervals (e.g., 1, 2, 5 minutes) to assess foam stability. The decrease in foam height over time indicates the stability of the foam. The foam can be stable in the presence of foaming agent solution containing surface active agent will produce stable foams

6)Foam retention

Pour a measured amount (e.g., 10 mL) of the solution into the graduated cylinder. Close the cylinder with a stopper and shake it vigorously for a fixed time (e.g., 30 seconds). Immediately after shaking, place the cylinder on a flat surface and record the initial foam height (in cm or mL). Allow the foam to settle undisturbed and record the foam height at fixed intervals (e.g., 1, 2, 5 minutes).
Calculate Foam Retention:

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

$$\text{Foam Retention (\%)} = \left(\frac{\text{Foam Height at Time (t)}}{\text{Initial Foam Height}} \right) X100$$

7)Anti-bacterial activity

The antibacterial activity of the samples was tested against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Overnight-grown bacterial cultures were evenly spread on Mueller-Hinton agar plates using sterile swabs. The agar well diffusion method was employed, where 50 µl of each sample and 5 µl of chlorhexidine (used as the standard) were added to their respective wells. The plates were incubated at 37°C overnight, and the inhibition zones were measured to assess the antibacterial efficacy.

8)Paper spreadability

To check how easily the soap spreads on paper. Use a fixed quantity of the test substance (e.g., 0.5 mL for liquid sample). Place the sample at the centre or a corner, depending on your testing requirements. Let the substance spread naturally for a set time (e.g., 1 minute).
Measure the diameter of the spread liquid using a ruler.

IV. RESULT AND DISCUSSION

Figure.9: Formulated F1 Paper soap

Figure.10: Formulated F2 paper soap

A) Evaluation of essential oil of Ocimumbasilicum L.

1.Organoleptic characteristics

Sl. No	Parameter	Result
1	Colour	Pale yellow
2	Odour	Warm herbaceous aroma
3	Texture	Oily

Table.3: Organoleptic characteristicsof Ocimumbasilicum L.

2.Chemical test for volatile oil
Ocimumbasilicum L. essential oil on treatment with alcoholic solution of Sudan III develops red colour. It indicates the presence of volatile oils.

Figure.11: Formation of red colour

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

3.Chemical tests for determinaton of eugenol in O.basilicum essential oil.[15]

- Marquis test

To 1 ml volatile oil add Marquis Reagent. The reagent is prepared by adding 8-10 drops (approx. 0.25 ml) of 37% formaldehyde solution to 10 ml concentrated sulfuric acid. The colour obtained is deep red, indicating the presence of eugenol.

Figure.12: Appearance of deep red colour

- Ferric chloride test

A few drops of volatile oil add ethanol followed by 5% ferric chloride solution. It resulted in formation of light green colour.

Figure.13:Formation of light green colour

4.Thin Layer Chromatography of O. basilicum L. essential oil [15]
Stationary phase – Silica gel
Mobile phase - Hexane: Ethyl acetate (9:1)
Spraying agent – Vanillin Sulphuric Acid
Reference standard- Clove oil

Figure.14: TLC of O.basilicum L. essential oil

Rf = Distance travelled by the spot /Distance travelled by the solvent front
Distance travelled by spot = 3.5 cm
Distance travelled by solvent front = 5.3 cm
Rf = 3.5 / 5.3 = 0.66
Retention factor of eugenol was already found to be 0.66.

5. Antibacterial activity of tulsi oil

By literature survey it is observed that tulsi oil had antibacterial activity towards numerous pathogenic bacterias. The oil at concentrations of 4.50% and 2.25%absolutely inhibited the increase of Staphylococcus aureus (along with MRSA) and Escherichia coli, at the same time as the identical concentrations partly inhibited the increase of Pseudomonas aeruginosa. Of fiftyfour compounds recognized in Tulsi leaves, flower spikes, or volatile oil, 3 are proposed to be accountable for this activity, camphor, eucalyptol and eugenol. Since S. aureus (along with MRSA), P. aeruginosa and E. coli are main pathogens responsible for skin and gentle tissue infections, Tulsi volatile oil may be a crucial topical antimicrobial agent for the control of skin infections resulting from the above organisms. [16,17,18,19,20]

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

B) Evaluation of water soluble paper

1.Solubility test

Figure.15: Checking paper solubility

Water soluble paper	Time taken to solubilize (sec)
Sample	2 sec

Table.4: Determination of paper solubility

2.pH

Figure.16: Measuring pH of paper

Solution of water soluble paper	pH Value
Sample	7.87

Table.5: Determination of paper pH

C) Evaluation of antibacterial herbal paper soap

1. Organoleptic parameters

The prepared herbal paper soap was evaluated for its colour, odour and texture. Both F1 and F2 showed light orange colour with characteristic odour. Results were as shown in table 9.

Formulation	Colour	Odour	Texture
F1	Light orange	Herbaceous	Soft
F2	Light orange	Herbaceous	Soft

Table.6: Organoleptic characteristicsof herbal paper soap

2.Size and Shape of herbal paper soap

Formulation	Size	Shape
F1	4 x 3	Rectangle
F2	4 x 3	Rectangle

Table.7: Determining size and shape of herbal paper soap

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

3.pH [21]
pH studies were performed and the results were as tabulated below. pH of the prepared formulations was found to be above 8.

Figure.17: Determining pH of F1

Figure.18: Determining pH of F2

Formulation Code	pH value
F1	8.02
F2	8.06

Table.8: pH of herbal paper soap

4.Foam height
Foam height was carried out using stoppered test tubes. Foam height of F1 was found to be 5.12 and foam height of F2 was 5.7.


Figure.19: Foam height of F1

F1	Initial foam height(cm)
Sample 1	5.2
Sample 2	6.0
Sample 3	5.4
Sample 4	6.2
Average	5.12

Table.9: Determination of foam height (F1)

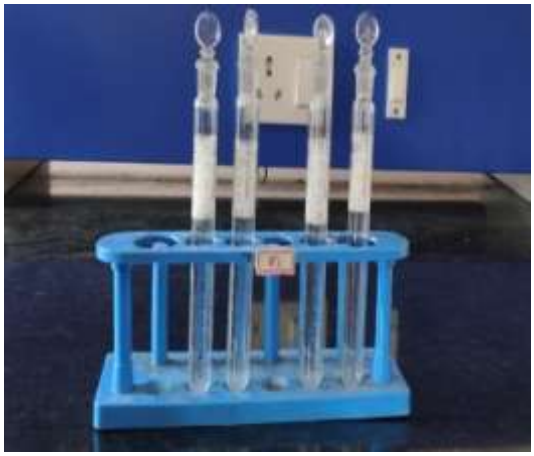
Figure.20: Foam height of F2

F2	Initial foam height(cm)
Sample 1	5.2
Sample 2	4.9
Sample 3	5.4
Sample 4	5.0
Average	5.7

Table.10: Determination of foam height (F2)

5.Foam stability
Foam stability was measured at 1,2,5 min. F2 paper soap exhibited comparatively better foam stability.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

F1	Foam height (cm)		
	1 min	2 min	5 min
Sample 1	5.0	4.8	4.0
Sample 2	5.4	5.0	4.5
Sample 3	5.0	4.2	4.0
Sample 4	5.2	4.9	4.2
F2	Foam height (cm)		
	1 min	2 min	5 min
Sample 1	4.8	4.3	3.8
Sample 2	4.6	4.0	3.6
Sample 3	5.0	4.4	4.0
Sample 4	4.2	3.9	3.6

Table.11: Determination of foam stability.

6.Foam retention

Retention of foam at 1,2,5 min was measured. F2 showed better foam retention.

F1	Foam retention (%)		
	1 min	2 min	5 min
Sample 1	96	92	76
Sample 2	90	83	75
Sample 3	92	77	74
Sample 4	83	79	67

Table.12: Determination of foam retention(F1).

F2	Foam retention (%)		
	1 min	2 min	5 min
Sample 1	92	82	73
Sample 2	93	81	73
Sample 3	92	81	74
Sample 4	84	78	72

Table.13: Determination of foam retention(F2).

7.Antibacterial activity [23,24,25]

F2 paper soap had significant antibacterial efficacy against S.aureus, E.coli, P.aeruginosa.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

Figure. 21: Antibacterial activity of samples against S. aureus

Figure. 22: Antibacterial activity of samples against E. coli

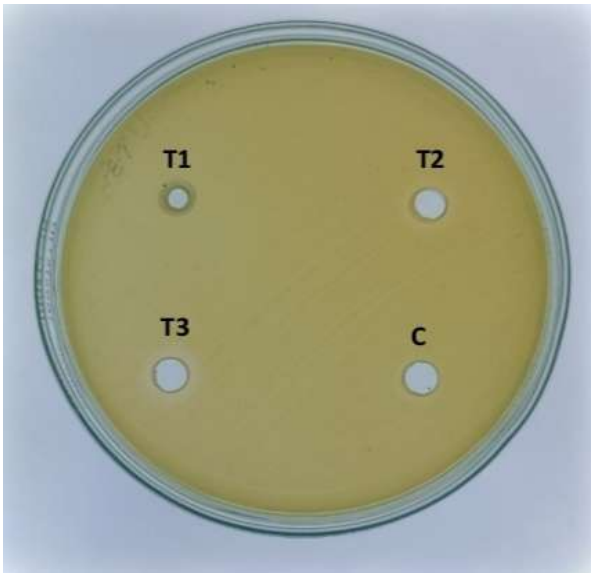

Figure. 23: Antibacterial activity of samples against P. aeruginosa

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 1 Jan - Feb 2025, pp: 1034-1047 www.ijprajournal.com ISSN: 2456-4494

Formulation	Anti-bacterial activity as indicated by zone dimensions in cm		
	E. coli	S. aureus	P. aeruginosa
F1	1.3	0.6	0.5
F2	1.4	0.8	0.7

Table.17: Determination of antibacterial efficacy

8.Paper spreadability

F2 liquid soap was observed to have better spreadability on paper.

Figure.24: Spreadability of F1

Figure.25: Spreadability of F2

Formulation	Spreadability (cm)
F1	1.40
F2	2.33

Table.15: Determination of paper spreadability

V. CONCLUSION

In this study, antibacterial herbal paper soap made from Ocimumbasilicum L and evaluated. F2 was found to be the optimized formulation. A thorough literature analysis is the first step in the study, highlighting earlier research on paper soap compositions that included the use of natural ingredients like orange peel extract and eucalyptus oil. Developing a solution that meets customer needs. In summary, the study effectively illustrates herbal paper soap's potential as a useful hygiene solution. The results highlight the potential of using regional herbs to create environment friendly and sustainable personal care products. In addition to opening up new avenues for investigation into herbal compositions, this study promotes the fusion of ancient wisdom with contemporary scientific methods. Finally, the effective creation of antibacterial herbal paper soap is a step toward the promotion of natural substitutes in the personal care sector, in line with the expanding consumer trend of sustainability and health consciousness, promotes hygiene while being eco-friendly and easy to use was the goal. The process entails utilizing a Clevenger equipment to extract essential oils from Ocimumbasilicum L. and then creating the paper soap. The product's quality and efficacy are guaranteed by evaluating a number of physicochemical characteristics, including spreadability, solubility and antibacterial potency. Herbal paper soap has strong antibacterial qualities, according to the results, which makes it a good substitute for conventional paper soaps.

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