



## Formulation and Evaluation of Antimicrobial Herbal Soap

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### ABSTRACT

Bacterial skin infections are most common amongst people, requiring significant attention for treatment and also for maintaining healthy skin. Some herbal plant extracts and their oils were found to have antibacterial activity. The aim and objective of the present study are to formulate and evaluate anti-bacterial herbal soap using *Azadirachta indica*, *Ocimum tenuiflorum* oils. The antibacterial activity of the prepared formulations was tested using the agar well diffusion method against the organisms *Pseudomonas aeruginosa*, *Staphylococcus aureus* and, *Escherichia coli* and they exhibited a good anti-bacterial effect. The prepared formulations were evaluated for various physicochemical parameters for which good characteristics were observed. The easy availability of plants and their effectiveness helps manufacturers with cost-effective benefits and with less or no side effects.

**Keywords:** Herbal Soap, *Azadirachta indica*, *Ocimum tenuiflorum*, Cold process method, Agar well diffusion method.

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### INTRODUCTION

Soap is a mixture of sodium salts of various naturally occurring fatty acids. If the fatty acid salt has potassium rather than sodium, a softer lather is the result. Soap is produced by saponification or basic hydrolysis reaction of a fat or oil. Most commercial soaps contain chemicals that can be harmful to the skin and using a natural herbal soap can be a good alternative. Herbal soaps are made using natural herbs and ingredients that are healthier and beneficial for the skin and are less likely to cause any damaging effect<sup>1</sup>. Some of the natural soap manufacturers also use aromatherapy and herbal treatments to offer the best skin treatment solution for your skin. Made of rare herbs and 100% natural ingredients, herbal soaps are found to be highly beneficial for the skin. The herbs infused in these soaps have therapeutic and healing characteristics that offer specific benefits to the skin, such as nourishment, strength, healing, and moisturizing. These soaps also contain super fatty oils, Vitamin E, Aloe, and essential oils that are allied to the goodness of skin and overall health<sup>2</sup>.

Herbal soaps are also effective in curing different skin complaints. These soaps also contain glycerine, which is generally not used in commercial soaps. Glycerine helps in retaining the moisture in the skin thereby making these soaps for dry skin conditions. Herbal soap preparations are medicines or drugs which contain anti-bacterial & anti-

fungal agents which mainly uses parts of plants such as like leaves, stem, roots & fruits for treatment for an injury or disease or to achieve good health. These preparations possess anti-microbial properties and are administered topically and available to apply in various forms like creams, gels, soaps, solvent extracts, or ointment<sup>3</sup>. In the present study, *Azadirachta indica* and *Ocimum tenuiflorum* oils were used to prepare the anti-bacterial herbal soaps and their physicochemical characteristics were evaluated.

### MATERIALS AND METHODS

Sodium hydroxide was procured from Qualigens Fine chemicals, Mumbai. Neem oil, Tulasi oil, and Coconut oil were procured from the local market. Glycerine, Propylene Glycol, and Sodium lauryl sulfate were procured from Thermo fisher scientific India Pvt Ltd., Mumbai. Triethanolamine was procured from Loba Chemie Pvt Ltd., Mumbai. The other entire chemicals used were of analytical grade.

#### Procedure for Preparation of Anti-microbial Herbal Soap

##### Preparation of medicated soap

##### Cold process method

Two herbal soap formulations (F1&F2) were prepared by cold press method given in table no.1. Formulation F1 soap base was prepared by taking 72ml of coconut oil in 500ml of the beaker. It was placed on the water bath & heated to 70 °C. After that 29.5gms of sodium hydroxide was weighed and dissolved in 40ml of distilled water. Sodium hydroxide or Lye solution was heated to 70 °C & added to coconut oil which was maintained at the same temperature. The mixture was stirred slowly and removed from the water bath after the mixture was thickened; 2ml of Neem oil, 2ml of Tulasi oil and 1ml of jasmine oil, and 15



gm of SLS, and 0.2ml of amaranth solution were added to the above-thickened mixture. All the contents were stirred properly (vigorous stirring was avoided to reduce foam formation in the mixture) to get a uniform soap mixture. Finally, this soap mixture was poured into circular moulds having dimensions of 8.4cm diameter. The moulds were kept aside for 3-4 days for the solidification of soap<sup>4</sup>.

Formulation F2 soap base was prepared by taking 1.6gm sodium hydroxide and dissolved in distilled water and the solution was heated to 70 °C. In another beaker 18.75ml of Propylene glycol, 6.25ml of glycerine, 19ml Of ethanol, and 15gm of SLS were taken into 250ml beaker on the hot plate with a stir bar and heated the mixture to 60°C. Once this heat is attained 13.0gm of Stearic acid was added and

heated to 68°C and slowly added the lye solution with stirring until the mixture becomes transparent. Further required quantity of herbal oils like 2ml of Neem oil, 2ml of Tulasi oil, and 0.2ml of amaranth solution were mixed to the above mixture, and volume was made up to 100 ml by adding remaining distilled water. The solution was kept undisturbed for a 1 hour at 68°C and a few drops of essential oil (1ml of jasmine oil) was also added to impart aroma to the prepared soap and after 1 hour 5ml Triethanolamine (TEA) was added slowly and the soap solution was cooled to 62-64°C and finally, the soap mixture was poured into circular moulds having dimensions of 8.4cm diameter. The moulds were kept aside for 3-4 days for the solidification of soap.

**Table 1:** Formulation of Anti-microbial Herbal Soap

S. No	Ingredients	Formulations (100gm)		Uses
		F1	F2	
1	Sodium hydroxide	29.5 gm	1.6gm	Lye
2	Stearic acid	--	13gm	Hardening agent
3	Neem oil	2 ml	2 ml	Anti-bacterial agent
4	Tulasi oil	2ml	2 ml	Anti-bacterial & Anti-fungal agent
5	Coconut oil	72ml	--	Saponifying agent
6	Jasmine oil	1 ml	1 ml	Perfume
7	Glycerine	--	6.25 ml	Humectant
8	Propylene Glycol	--	18.75ml	Humectant
9	Sodium lauryl sulfate	15gm	15gm	Surfactant
10	Triethanolamine	--	5ml	pH Adjustifying agent
11	Amaranth solution	0.2ml	0.2ml	Colouring agent
12	Ethanol	--	19ml	Antimicrobial
13	Distilled water	Q.s.100ml	Q.s 100ml	Vehicle

### Evaluation Tests

Evaluation of physicochemical parameters of the prepared formulation, various physicochemical parameters which are mentioned below were performed to establish the quality of the prepared formulations.

- Determination of Organoleptic Characteristics:** Clarity and colour was checked by naked eyes against the white background, and the odour was smelled<sup>5</sup>.
- Size and shape Determination:** The soap diameter of the size of 8.4 cm, with a thickness of 2.6 cm, which is round-shaped, was chosen for the preparation of soap bars. This was chosen, as this size is ideal in regular usage to apply on the affected skin parts of the body<sup>6</sup>.
- Thickness determination:** The thickness was determined with the help of a screw gauge which is pre-calibrated. The thickness was measured, by observing the thickness at five different parts of the soap<sup>7</sup>.
- Weight determination:** The weight was determined by using a Digital weighing balance<sup>8</sup>.
- Foam Height:**<sup>23</sup> 0.5gm of the sample of soap was taken and dispersed in 25 ml of distilled water. Then, transferred it into 100 ml measuring cylinder; the volume was made up to 50 ml with water. 25 strokes were given and stand till aqueous volume was measured up to 50 ml and measured the foam height, above the aqueous volume<sup>9</sup>.
- Foam Retention:** Prepared the 25 ml of the 1% soap solution and transferred it into the 100 ml measuring cylinder. Then the cylinder was shaken 10 times. The volume of foam was recorded at one minute for 4 to 5 minutes<sup>10</sup>.
- pH TEST:** The pH test was performed for all the formulations. Each formulation of soap solution was dissolved in 20ml of distilled water and tested for pH with the help of a digital pH meter. The measurement of pH of all the formulations was done in the previously calibrated pH meter<sup>11</sup>.



**8. Alcohol insoluble matter:** 5gm of soap was taken in a conical flask and added 50ml warm ethanol and shaken vigorously to dissolve the soap. The solution was filtered through a tarred filter paper with 20ml of warm ethanol and dried at 105 °C for 1hr. The weight of the dried paper with residue was taken<sup>12</sup>.

$$\text{Formula: \% Alcohol insoluble matter} = \frac{\text{Wt. of residue}}{\text{wt. of sample}} \times 100$$

**9. High-temperature stability:** Liquid soap was allowed to stand at 50°C for 1 week. The stability of liquid soap was observed during this period. The sample which was homogenous and stable liquid after standing was indicated as stable and the sample in which the crystals were roughened and the sample in which precipitation was caused then liquid soap was said to be as unstable<sup>13</sup>.

**10. Anti-microbial test:** The prepared soap was subjected to antimicrobial screening by the agar well diffusion standard cup plate method. Organisms used were *E. coli*, *S. aureus*, and *P. aeruginosa*. One gram of soap was mixed with 5ml of sterile water<sup>14</sup>.

#### Evaluation of Prepared Herbal Soap Formulation for Antimicrobial Activity

The [agar -well diffusion] standard cup plate technique was used to determine the antimicrobial activity by using sabouraud's dextrose agar [Hi- media]. The melted media were seeded with the suspension of microorganisms and allowed to solidify. The formulations were aseptically transferred to the Hi-media in Petri-dish with the help of sterile forceps. The medicated soap was kept for incubation in an incubator at 30°C for 5-7 days<sup>15</sup>. Observation: The assessment of antimicrobial activity was based on the measurement of the diameter of the zone of inhibition in mm. The values were recorded and given in table no 3.

#### RESULTS AND DISCUSSIONS

The evaluation of anti-microbial herbal soap was performed successfully and tabulated in table no.2. The prepared herbal soap was shown in figure 1. The physicochemical parameters for herbal soap formulations F1 and F2 such as color, appearance, pH were determined. The formulations have a light pink color with an aromatic odor and had a good appearance as well as the pH was found to be in the range of 7.0-7.3. Healthy skin has a pH of 5.4 to 5.9 and the prepared formulations pH was found to be neutral in nature and doesn't cause any irritation or sensitization to the skin. Other parameters like foam height, foam retention were also performed and showed good results, the prepared soaps produced good lather i.e. 2.5-3.0 cm and retained on the skin for 3 minutes. Alcohol insoluble matter was also evaluated successfully which was found to be 15-18 %, indicates that the prepared soaps were free from non-soap ingredients and soft soaps were produced which improves the overall quality of the soap. The anti-microbial activities of herbal soaps were studied. The results obtained from the studies were shown photographically as well as in table no:

2. The zone of inhibition for F1 and F2 formulations were calculated. F2 formulation showed a maximum zone of inhibition than the F1 formulation. A significant result was obtained for F2 formulation against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*, which were found to be 15mm, 18mm, and 16 mm respectively due to the synergistic effect produced by the incorporation of alcohol in the F2 formulation, which showed significant zone of inhibition and acts effectively against bacteria on the skin and can be used to treat acne and bacterial infections on the skin. F2 formulation was found to be the best formulation due to good quality of the soap with a neutral pH, safe to use on the skin without any irritation to the skin and also it forms good lather and retains well on the skin. The prepared Soap had minimal matter insoluble in alcohol and the soap prepared was pure with minimal moisture content and with increased shelf life. The F2 formulation has high amounts of fatty acid (stearic acid) which imparts lubrication effect to the skin while washing, which was the basic criterion of good quality soap.



**Figure 1:** Prepared Anti-microbial Herbal soap

**Table 2:** Physicochemical Parameters of Herbal Soap Formulations

S.no	Parameters	Formulation F1	Formulation F2
1.	Colour	Light Pink	Light Pink
2.	Odor	Aromatic	Aromatic
3.	Appearance	Good	Good
4.	Size	8.4cm x2.6 cm	8.4cm x2.6 cm
5.	Shape	Round	Round
6.	Foam Height (cm)	2.5	3.0
7.	Foam Retention (Min)	3.0	3.0
8.	pH	7.3	7.0
9.	Alcohol insoluble matter (%)	18	15
10.	High-temperature stability	Soap melts above 45°C	Soap melts above 50°C

**Table 3:** Antimicrobial Test for Herbal Soaps

S.no	Formulation code	Zone of inhibition (mm)		
		Microorganisms		
		<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. coli</i>
1	F1	12	14	13
2	F2	15	18	16

**Figure 2:** Zone of Inhibition for F2 formulation

1. *P. aeruginosa*, 2. *S. aureus*, 3. *E. coli*

### SUMMARY AND CONCLUSION

In the present work, antimicrobial herbal soaps were prepared, with suitable size and shape, thickness, weight, and with good foam producing ability. Herbal soaps of neem and tulsi were prepared for their anti-bacterial activity for the treatment of pimples, acne and scars. Two different formulations F1 and F2 were prepared by cold press method and the formulations were characterized for different evaluation parameters like clarity, color, and odor, size, and shape, thickness, weight, pH in which they exhibited satisfactory results. The herbal soap showed a good appearance with pink color and with a pleasant aromatic smell and showed good anti-bacterial properties. Based on the study it can be concluded that herbal products can be effectively formulated in the form of medicated herbal soaps by using cold process technique with excellent anti-bacterial properties.

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