

## Formulation and Evaluation of Polyherbal anti-bacterial Soap for treating Impetigo

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### Abstract

Impetigo is one of the easily transmissible through the external skin infection of bacteria, majorly caused by *Staphylococcus aureus*. The increasing incidence of antibiotic resistance and adverse effects of synthetic soaps has encouraged the development of herbal alternatives. The present study aimed to formulate and evaluate a polyherbal antibacterial soap using selected medicinal plants with proven antimicrobial properties. The active pharmaceutical ingredients included *Manilkara zapota*, *Annona reticulata*, and *Ocimum sanctum* while the antimicrobial potential of these plants has been reported individually, their combined antibacterial efficacy in a polyherbal soap formulation has not been previously explored. Natural surfactants such as *Sapindus mukorossi* (Reetha) and *Acacia arabica* (Babool) were incorporated. *Butea monosperma* (Palash) flower extract was used as a natural colorant along with suitable excipients to prepare the polyherbal soap. The polyherbal soap was prepared by cold process method and evaluated for antibacterial activity by agar disc diffusion test against *Staphylococcus aureus* and compared with a marketed herbal soap medimix sandal and eladi oil. The results demonstrated that F2 showed the highest zone of inhibition (13 mm) among all four formulations, indicating superior and effective antibacterial activity of the formulated polyherbal soap even at lower concentrations. The findings suggest that the developed polyherbal soap could serve as a safe, effective and economical alternative for the management of impetigo bacterial skin infection.

**Keywords** Polyherbal soap, Impetigo, *Staphylococcus aureus*, Agar disc diffusion method, Antibacterial activity



**Fig. 1** Experimental design of polyherbal soap

## 1 Introduction

Impetigo is one of the common, highly spreadable superficial bacterial skin infection which predominantly effects infants and young children, particularly those aged 2 to 5 years. It is more prevalent in warm and humid climates and considered a vital public health concern due to its rapid transmission and major recurrence rates. This condition contributes to morbidity in pediatric populations on healthcare systems worldwide [17]. It is primarily caused by *Staphylococcus aureus* and less frequently *Streptococcus pyogenes*. The infection occurs when these pathogens spread into the epidermis through minor skin breaches such as insect bites, cuts, pre-existing dermatoses or abrasions. *Staphylococcus aureus* produces exfoliative toxins that disrupt desmosomal proteins, leading to the loss of cell-to-cell adhesion in the superficial layers of the skin. This results in intra-epidermal blister formation, particularly in bullous impetigo, while non-bullous impetigo arises from a localized inflammatory response to bacterial colonization [1]. Clinically, impetigo presents as erythematous macules that progress to pustules or vesicles and later on rupture to form a honey-colored crusts. The lesions are usually painless but may be mildly pruritic. Impetigo commonly affects exposed areas such as the face, nose, mouth, hands, and legs. Based on clinical presentation, impetigo is classified into bullous and non-bullous types. Non-bullous impetigo is the most prevalent form and typically occurs on intact skin, whereas bullous impetigo is characterized by flaccid, fluid-filled blisters and is

more frequently observed in neonates and young children. The disease may also be categorized as primary impetigo, developing on healthy skin, secondary impetigo is occurring at sites of skin injury or underlying dermatological conditions [12]. The standard treatment of impetigo includes topical antibiotics such as Mupirocin and Fusidic acid for localized infections, while systemic antibiotics are prescribed for extensive or severe cases. Additionally, prolonged antibiotic use may lead to adverse effects and increased risk of recurrence. Herbal medicines have gained global attention due to their safety, affordability and therapeutic efficacy. Medicinal plants possess diverse active phytoconstituents such as alkaloids, tannin, flavonoids, and phenolics that possess antibacterial, anti-inflammatory and wound-healing activity. In recent years, herbal cosmetics have emerged as an effective approach for managing skin disorders without the side effects associated with synthetic formulations. *Manilkara zapota* is known for its antimicrobial and antioxidant properties [23]. *Annona reticulata* possesses significant antibacterial and anti-inflammatory activity [24]. *Ocimum sanctum* is a well-established medicinal plant with potent antimicrobial and immunomodulatory effects [24]. Natural surfactants such as *Sapindus mukorossi* and *Acacia arabica* not only enhance cleansing action but also contribute to antibacterial activity [13,16]. *Butea monosperma* flowers are traditionally used as natural colorants and possess anti-inflammatory and antioxidant activity [23]. The phytochemical compounds of *Manilkara zapota* possess Alkaloids, Tannins, Terpenoids, Glycosides, Saponins and Flavonoids have been widely reported as antimicrobial activity by methanolic extract of Leaves and Bark of *Manilkara zapota* [20]. The ethanolic, methanolic extract and petroleum ether of Fruit and Leaf of *Annona reticulata* extracts against the bacterial strains are attributed due to phenolic and polyphenolic compounds [22]. Essential oil from leaves of *Ocimum sanctum* which contains Eugenol, Methyl eugenol, Caryophyllene, Carvacrol are mainly responsible for different antimicrobial activities [16]. 2 Flavonoids of Orientin and Vicenin of aqueous and methanolic leaf extract are responsible for active against antibacterial activity [2]. The current study focus on the formulation of a polyherbal antibacterial soap using selected medicinal plants and natural excipients, followed by evaluation of its antibacterial activity was analyzed against *Staphylococcus aureus* through the agar well diffusion technique (Fig. 1). The formulated soap was compared with a marketed herbal soap of medimix Sandal and Eladi oil soap to assess its effectiveness in the management of impetigo [15].

## **2 Study materials and methods**

### **2.1 Drugs, chemicals and solvents**

Coconut oil, castor oil, sodium hydroxide, glycerin, Propylene glycol, sorbitol, stearic acid, phenoxyethanol, distilled water, methanol. was procured from Taranadh scientific and chemicals, Hanamkonda, Warangal, Telangana, India. A marketed herbal soap (Medimix Sandal and Eladi oil) was procured from a local pharmacy and used as a standard.

### **2.2 Collection of plant (botanical) material**

The fresh plant leaves of *Manilkara zapota*, *Annona reticulata* and *Ocimum sanctum* were collected from Narsampet, Warangal, Telangana, India. The plant (botanical) material was authenticated by Dr.M. Mustafa, Professor of Botany, Kakatiya university, WGL. A voucher specimen of the plants with numbers KU/UCPSC/65, KU/UCPSC/66, KU/UCPSC/67 respectively were kept in the herbarium of Pharmacognosy and Phytochemistry department in university college of pharmaceutical sciences, Kakatiya university, Warangal. Pods of *Sapindus mukorossi* (Reetha) *Acacia nilotica* (Babool) and flowers *Butea monospermic* (Palash) were collected.

### **2.3 Preparation of Plant Extracts**

The fresh leaves of *Manilkara zapota* (140 grams), *Annona reticulata* (210 grams), and *Ocimum sanctum* (132 grams) were collected and cleaned thoroughly with running tap water, then it is shade dried and separately in to coarsely powdered. This coarsely powdered material was extracted with methanol by a maceration method for a week and then filtered. The obtained filtrate was evaporated under vacuum to obtain dark green extract. The dried pods of *Sapindus mukorossi* (10 grams) and *Acacia nilotica* (10 grams) was prepared by aqueous extract by maceration method and it is used as a natural surfactant. The natural colorant was isolated from flowers of *Butea monosperma* by Soxhlet method. The coloring pigment (reddish-orange) produced by this flower is butrin [13].

### **2.3 Formulation of Polyherbal Soap**

The polyherbal soap was prepared listed in table 1 by the cold process method. The cold process saponification method with mild heating refers to a soap-making technique in which oils are gently heated for melting and then reacted with sodium hydroxide, allowing saponification to occur naturally during the curing period.

The lye solution was made by carefully dissolving sodium hydroxide (NaOH) in distilled water using a clean glass beaker. It is essential to always add sodium hydroxide to

water slowly and never add water to sodium hydroxide, as the reaction is highly exothermic and can cause splashing. The solution was stirred gently until the sodium hydroxide was completely dissolved. The solution was kept at room temperature to cool down approximately 30–40 °C before further use.

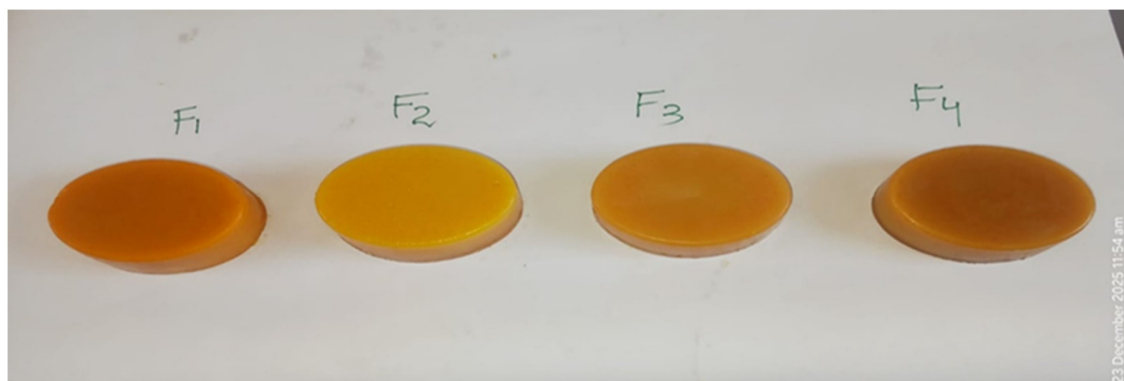
The oil phase was prepared by combining coconut oil, castor oil along with stearic acid in a glass beaker. The mixture was heated until all components were formed a uniform liquid. The temperature of oil phase was maintained at around 40 °C to ensure proper mixing with the lye solution. Once both the oil phase and the lye solution reached approximately the same temperature (around 40 °C), the lye solution was gradually poured into the mixture while stirring continuously. Slow and steady stirring was maintained to promote uniform mixing and to initiate the saponification process. If the mixture became excessively hot, glycerin propylene glycol and sorbitol were added to help regulate the temperature and improve the texture of the soap base. plant extracts were incorporated into the mixture. Add natural surfactants, preservatives, natural colorants, and fragrance were added. Stirring was continued until the mixture thickened stage, characterized by a pudding-like consistency.

The prepared soap mixture was poured into clean silicone moulds and gently tapped to remove any trapped air bubbles. The mold was left undisturbed at room temperature to allow the soap to harden. After initial setting, the soap was allowed to cure for approximately two weeks. During the curing period, excess moisture evaporates, the saponification process completes fully, and the soap achieves optimal hardness, mildness, and performance characteristics. Once curing was complete, the soap bars (Fig. 2) were unmolded and stored in a dry place until further use [13].

**Table 1** Formulation of Polyherbal anti-bacterial Soap for treating Impetigo

S.No.	Ingredients	Category	F1	F2	F3	F4
1.	<i>Manilkara zapota</i> (mg)	A.P. I	2.5	5	7.5	10
2.	<i>Annona reticulata</i> (mg)	A.P. I	2.5	5	7.5	10
3.	<i>Ocimum sanctum</i> (mg)	A.P. I	2.5	5	7.5	10
4.	Coconut oil (ml)	Cleansing & lather	20	20	20	20
5.	Castor oil (ml)	Transparency & lather	12	12	12	12
6.	Sodium hydroxide(gm)	Alkali	7	7.5	8	8.5
7.	Distilled water (ml)	Solvent	14	15	16	17
8.	Glycerin (ml)	Humectant	8	8	8	8
9.	Propylene glycol (ml)	Solvent	10	10	10	10

10.	Sorbitol (ml)	Thickener & Humectant	0.5	0.5	0.5	0.5
11.	Stearic acid (gm)	Hardening agent	2.2	2.3	2.4	2.5
12.	<i>Acacia arabica</i> (ml)	Natural Surfactant	-	-	2	3
13.	<i>Sapindus mukorossi</i> (ml)	Natural Surfactant	2	3	-	0
14.	<i>Butea monosperma</i> (mg)	Natural colorant	25	25	25	25
15.	<i>Santalum album oil</i> (ml)	Fragrance	qs	qs	qs	qs
16.	Phenoxyethanol (ml)	Preservative	0.5	0.5	0.5	0.5



**Fig. 2** Formulation of Polyherbal anti-bacterial Soap for treating Impetigo

### 3 Quality control evaluation of formulated soap

#### Physicochemical parameters of formulated formulation

The formulated polyherbal soap was evaluated for physicochemical parameters such as appearance (size and shape), color, odor, homogeneity, consistency, texture, irritation test, after feel, washability, lather quality done by using a standard procedure to ensure suitability for topical application was reported in table 2.

#### pH measurement

The prepared polyherbal soap sample was mixed with distilled water in a 1:10 proportion. The pH of the solution was then measured by a pre-calibrated digital pH meter, and the final value was recorded as the average of three separate readings [6].

#### Foam height and Foam retention time

To evaluate foaming ability, 0.5 g of the formulated polyherbal soap was dissolved in 50 ml of distilled water in a 100 ml measuring cylinder. The cylinder was shaken 25

times to generate foam, and the height of the foam produced was measured. Foam retention time, defined as the duration for which the foam remained stable, was also recorded. [6].

**Lathering ability** Rub the Formulated polyherbal soap in your palms for 30 seconds, trapped with the soap and water mixture, forming bubbles and foam.

### **Total fatty matter**

10 g of the prepared polyherbal soap was dissolved in 150 ml distilled water. The soap solution was mixed with 20% sulphuric acid (20 ml concentrated H<sub>2</sub>SO<sub>4</sub> diluted with water to 100 ml) and heated on a water bath at 40°C until a clear solution was obtained. Fatty acids separated at the surface, and 7 g of beeswax was added to solidify them. The mixture was reheated at 40°C until a solid cake formed, which was subsequently collected and weighed [6].

$\% \text{ Total Fatty Matter} = (\text{Weight of dry cake} - \text{Weight of wax}) \text{ in gm} / \text{weight of soap in gm} \times 100.$

### **Moisture content**

The moisture content of the polyherbal soap was determined to estimate the amount of water present in the sample. A clean, dry china dish was first weighed. Then approximately 5 g of the soap sample was placed in the dish and kept in an oven at 100–115 °C for about 2 hours. After heating, the dish was kept aside, allowed to cool and weighed again. The loss in weight after heating indicated moisture present in the soap sample [24].

$\% \text{ Moisture Content} = (\text{Initial weight} - \text{final weight}) \text{ in gm} / \text{final weight} \times 100.$

### **Alcohol insoluble matter**

5 g of prepared polyherbal soap was added in a conical flask then treated with 50 mL of warm ethanol to facilitate dissolution. The resulting solution was filtered, by using tarred filter paper. Subsequently heated at 105°C (1 hour). The dried residue was then collected and its weight was recorded [21].

$\% \text{ Alcohol insoluble matter} = \text{Weight of residue in gm} / \text{Weight of soap in gm} \times 100$

### **Saponification value**

2 g of soap sample was taken in a conical flask and 25 mL of 0.5 N KOH solution was added. The mixture was refluxed at about 55 °C for 30 minutes on a hot water bath with continuous stirring. After cooling to room temperature, 2–3 drops of phenolphthalein indicator was added. Then excess alkali was titrated with standardized 0.5 N HCl until the pink color disappears. A blank determination was performed similarly, and saponification value was calculated by standard formula [6].

*Saponification value* = Titrant value of Blank (ml) - Titrant value of Sample (ml) x Normality of KOH x equivalent of KOH (56.1)/Weight of soap in gm.

### **Free Alkali content**

5 g of polyherbal soap was taken in a conical flask and 50 mL of neutralized alcohol was added. The mixture was heated under reflux on a water bath at 50–60 °C for 30 min. later cooling, 1 mL of phenolphthalein indicator was added. The solution was titrated with 0.01 N hydrochloric acid until the end point was reached. [19].

*% Free Alkali* = Volume of HCL (ml) x Normality of HCL x 40 X 100 / Weight of soap in gm x 1000.

**Accelerated stability studies** are designed to increase the rate of chemical or physical degradation of a drug substance or product using exaggerated, high-stress conditions. stability was carried out for a month at temperatures 40 ± 2°C. The stability data for four different formulations (F1 to F4) under three various storage conditions. Room Temperature (25 ± 2°C): Assumed to be the initial condition. Accelerated stability (40 ± 2°C; RH 70 ± 5): This condition stimulates long-term storage at an elevated temperature and humidity, accelerating potential degradation. Stored in freezer (4± 2°C): This condition tests the formulations stability at very low temperatures.

### **Invitro Antibacterial Activity of polyherbal soap**

The Test organism of *Staphylococcus aureus* (ATCC 25923 / clinical isolate) was obtained from the Department of Microbiology, Kakatiya university, Warangal, India. The antibacterial activity of the test sample was analyzed using the agar disc diffusion technique. A pure culture of *Staphylococcus aureus* was grown in nutrient broth and incubated at 37 °C for 18–24 hrs. Sterile agar plates were inoculated using swabbing the bacterial suspension. Sterile Whatman No. 1 filter paper discs (6 mm) were

impregnated with test sample then placed on inoculated agar surface. A standard soap as Medimix Sandal and Eladi oil soap was used as a positive test, while discs impregnated with distilled water used as a negative control. plates were incubated at 37°C for 24 hours, zones of inhibition was measured in millimeters (mm). All formulation of polyherbal soaps was performed to triplicate, then results were expressed as mean standard deviation [15,3].

#### 4 Results and discussion

The formulated polyherbal soaps (F1–F4) was evaluated for physicochemical parameters to assess their quality, safety and found to be satisfactory.

**Table 2:** Physicochemical parameters of formulated polyherbal soaps (F1–F4)

S.No.	Parameters	F1	F2	F3	F4
1.	Appearance	Solid	Solid	Solid	Solid
2.	Size (Width x height)	7cm x 2cm	7cm x 2cm	7cm x 2cm	7cm x 2cm
3.	Shape	Oval	Oval	Oval	Oval
4.	Color	Vibrant orange	Golden yellow	Light orange	Dark orange
5.	Odor	Pleasant	Pleasant	Pleasant	Pleasant
6.	Homogeneity	Uniform	Uniform	Uniform	Uniform
7.	Consistency	Good	Good	Good	Good
8.	Texture	Smooth	Smooth	Smooth	Smooth
9.	Skin Irritation test	No signs	No signs	No signs	No signs
10.	After feel	Soft & non-greasy	Soft & non-greasy	Soft & non-greasy	Soft & non-greasy
11.	Washability	Easy	Easy	Easy	Easy
12.	Lather quality	Creamy & stable	Creamy & stable	Creamy & stable	Creamy & stable

#### Evaluation of pH

Soap has an alkaline pH because it is produced by the saponification of fats or oils with bases such as sodium hydroxide, which enables effective removal of grease and dirt. The pH values of the formulated polyherbal soaps were found to be 8.13 (F1),

8.29 (F2), 8.52 (F3), and 8.64 (F4). According to the Bureau of Indian Standards (BIS), ISI specification IS 2888:2004 and IS 13498:1997 for toilet soaps state that the pH of soap solution should be in the range of 8.0–10.5 when tested under standard conditions. A high pH in soap formulations increases alkalinity, which can disrupt the acid mantle of the skin, leading to excessive removal of natural lipids from the stratum corneum. This results in skin dryness, irritation, erythema, and itching. A low pH in soap formulations may reduce the cleansing efficiency of the soap. Which may result in reduced foaming, poor washability, and instability of the formulation [21]. In the present study, all polyherbal soap formulations exhibited pH values within the ISI-specified range, indicating acceptable skin compatibility.

### **Foam height and Foam retention**

Foam height is the measurement of lather produced by soap, indicating its foaming ability. It is used to evaluate cleansing performance and quality of the soap formulation. According to ISI Specification IS 13498, the acceptable Foam height range for toilet soap is 4–6 cm, which indicates satisfactory foaming ability suitable for cleansing purposes. The variation in foam height among all the formulation can be attributed to the type and concentration of natural surfactants used. Formulations F1 and F2, which contained *Sapindus mukorossi* extract as the primary foaming agent, showed comparatively higher foam heights. Reetha is rich in Triterpenoid saponins (10%-11.5%), which are known for their excellent surface-active and foam-forming properties [7]. An increase in Reetha concentration in F2 resulted in the highest foam height (5.8 cm), indicating enhanced foaming efficiency. In formulations F3 and F4 were prepared using *Acacia arabica* extract as a natural surfactant system. These formulations exhibited relatively lower foam heights when compared to F1 and F2; however, the values remained within the acceptable ISI range of 4–6 cm. The moderate foaming observed in F3 and F4 may be due to the presence of emollient-rich (carbohydrate group) Babool pods, which tends to reduce excessive foam formation while improving skin conditioning properties like soften and moisturize [8]. Overall, the results indicate that Reetha-based formulations (F1 and F2) provide higher foaming ability than Babool-based formulations (F3 and F4). Foam retention time is used to evaluate the stability and persistence foam produced by soap formulations. It reflects the ability of the soap to maintain lather for a sufficient duration, which is directly associated with effective cleansing action, consumer acceptability. In

the study, the foam retention time of the four developed polyherbal soap formulations (F1–F4) ranged between 16.07 and 19.45 minutes, indicating good foam stability. According to ISI Specification IS 13498, the acceptable Foam retention time range for toilet soap is 8-20mins. Formulations F1 and F2, containing *Sapindus mukorossi* extract, exhibited longer foam retention times, demonstrating their superior natural surfactant activity due to the presence of saponins. Formulations F3 and F4, prepared using *Acacia arabica* extract, showed comparatively lower but acceptable foam retention times. Overall, the foam retention time results indicate that all four formulations possess satisfactory foam stability for toilet soap applications.

### **Lathering ability**

The prepared polyherbal soaps are recorded the time required to produce the stable foam during the hand washing. The F1 was recorded lather formation at 28 seconds, F2 at 25 seconds, F3 at 45 seconds, while in F4 lather formed in 37 seconds. Overall, based on the observed lathering times, the Reetha-based soap (F1, F2) demonstrates superior and quicker lathering ability compared to the Babool-based soap (F3, F4).

### **Total Fatty Matter (TFM)**

It is a quality assessing parameter for soaps, indicating the total amount of fatty substances present and directly reflecting cleansing efficiency, mildness, and moisturizing properties [10]. According to Indian Standards (IS 2888), toilet soaps are categorized into different grades based on TFM content: Grade 1 needs  $\geq 76\%$  TFM, Grade 2  $\geq 70\%$  TFM, and Grade 3  $\geq 60\%$  TFM. Higher TFM values are generally associated with superior soap quality, better skin compatibility, reduced irritation and it is important for therapeutic soaps intended for skin infections as Impetigo. The TFM content of all four polyherbal soap formulations was 71.2%(F1), 72.4%(F2), 70.8%(F3), and 70.4%(F4) indicating good quality and suitability for dermatological use. Among all the formulations, F2 showed the highest TFM value (72.4%), suggesting a higher concentration of fatty acids and better emollient properties. This enhanced fatty content may contribute to improved skin hydration and barrier repair, which is particularly beneficial in impetigo where the skin barrier is compromised.

### **Moisture Content**

It plays a Vital role in evaluating its hardness, shelf-life, lathering property and microbial stability. Excess moisture in soap can lead to softness, reduced durability and an increased susceptibility to microbial contamination. It significantly influences its physical, chemical and microbiological stability Very low moisture content can make the soap brittle and difficult to handle. Maintaining moisture content within a standard range ensures the product remains stable during storage and use. The moisture content observed for formulations of 11.2%(F1), 12.8%(F2), 13.6%(F3), and 14.4% (F4). According to the Bureau of Indian Standards (BIS), IS 722:1994, the moisture content of toilet soaps should generally not exceed 14–16%. The moisture content observed for formulations F1 to F4 falls within the acceptable range for soaps, indicating good formulation stability and suitability for prolonged shelf-life.

### **Alcohol Insoluble Matter**

Alcohol insoluble matter represents the fraction of soap that does not dissolve in alcohol and mainly consists of fillers, pigments, insoluble inorganic matter, and plant-derived residues. The alcohol-insoluble matter was found to be 5%(F1), 5.8%(F2), 6.4%(F3), and 7.6%(F4). According to Indian Standards (IS 2888: Toilet Soap Specification), the alcohol insoluble matter should not exceed 10% w/w. Alcohol insoluble matter are essential to ensure good lathering, cleansing efficiency, and acceptable properties of the soap. Formulation F1 exhibited the lowest alcohol insoluble matter (5.0%), suggesting a higher proportion of alcohol-soluble soap components and minimal insoluble additives. A gradual increase in alcohol insoluble matter was observed from F1 to F4. Formulation F4 showed the highest alcohol insoluble matter (7.6%), which may be due to the greater incorporation of herbal constituents. The values ranged from 5.0% to 7.6%, indicating acceptable quality and purity of the formulations. The alcohol insoluble matter values of all polyherbal soap formulations were found to be within ISI limits. This confirms that the prepared soaps possess acceptable quality, purity, and uniformity, making them suitable for topical use.

### **Saponification value**

The number of mg of KOH is needed to completely saponify 1 g of fat or oil. According to ISI (BIS) specifications for toilet soap, the acceptable saponification value generally ranges between 160–200 mg KOH/g, depending on the type of oils used. Too high saponification value (>200) leads to strong cleansing action and too low saponification

value (<160) leads to poor cleansing action and less lather [9]. The saponification values were 182.32, 185.13, 175.31, and 168.3 mg KOH/g for F1, F2, F3, and F4, respectively. Among all the formulations, F2 exhibited the highest saponification value 185.13 mg KOH/g, indicating the presence of short-chain fatty acids, which contribute to better cleansing action. The variation in saponification values among all four formulations is due to type of oils used and higher concentration of polyherbal extracts. Overall, the results suggest that all formulations possess acceptable saponification values and suitable for topical use.

### Free alkali content

Free alkali content is the amount of unreacted alkali (such as sodium hydroxide) remaining after the saponification process. It is an important quality parameter because excess free alkali can cause skin irritation, dryness and reduced safety of the soap [23]. The percent free alkali of all the formulations was 0.0096%(F1), 0.0100%(F2), 0.0104%(F3), 0.0120% (F4). According to IS 5572:1978 (Toilet Soaps), the acceptable limit for percent free alkali in soap is  $\leq 0.5\%$ . This indicates that the soap formulations are well saponified, with minimal residual alkali, which is desirable for skin-friendly soap. Formulation F1 showed the lowest percent free alkali (0.0096%) indicating almost complete saponification. Formulation F4 had the highest percent free alkali (0.012%), still within acceptable limits for cosmetic use. Low percent free alkali ensures minimal irritation and suitability for skin application and it is safe for regular use in table 5.

### Accelerated stability studies

Stability testing is important to verify that the product is stable throughout its shelf life. The stability was carried out for 4 weeks at desired temperatures. The table 3 presents stability data for four different formulations (F1 to F4) under three different storage conditions.

**Table 3:** Stability of the formulation

#### Stability studies condition at Room Temperature ( $25 \pm 2^\circ\text{C}$ )

Formulation	Color	Odor	Texture	Foam stability	Skin irritation
F1	No	Pleasant	Smooth	Stable	Nil

F2	No	Pleasant	Smooth	Stable	Nil
F3	No	Pleasant	Smooth	Stable	Nil
F4	No	Pleasant	Smooth	Stable	Nil

#### Stability studies condition at Accelerated stability (40 ± 2°C; RH 70 ± 5)

Formulation	Color	Odor	Appearance	Foam stability
F1	No change	Pleasant	Slightly melted at 40°C	Stable
F2	No change	Pleasant	Slightly melted at 40°C	Stable
F3	Slight fading	Mild	Partial melted at 40°C	Stable
F4	Slight fading	Mild	Partial melted at 40°C	Stable

#### Stability studies condition at Stored in freezer (4± 2°C)

Formulation	Color	Odor	Appearance	Skin irritation
F1	No change	Pleasant	Slightly hard	Nil
F2	No change	Pleasant	Slightly hard	Nil
F3	No change	Pleasant	Hard	Nil
F4	No change	Pleasant	Hard	Nil

#### Invitro Antibacterial Activity of polyherbal soap

Test samples are prepared by 1% w/v formulated soap; 1g of prepared polyherbal soap in 10 ml distilled water.

**Table 4:** Antibacterial activity of polyherbal soap formulation against *staphylococcus aureus*

Formulation	Test sample	Inhibition zone mm
F1	Soap 1	8 mm
F2	Soap 2	13 mm
F3	Soap 3	11mm
F4	Soap 4	10 mm
Medimix soap	Positive test	14 mm
Distilled water	Negative test	3 mm



**Fig. 3** Antibacterial Activity of Polyherbal Soap Formulation by agar disc diffusion method.

The antibacterial activity of the prepared polyherbal soap formulations (Soap 1–Soap 4) using the agar disc diffusion assay and compared with a marketed herbal soap (Medimix sandal and eladi oil soap). The zones of inhibition observed were 8 mm (Soap 1), 13 mm (Soap 2), 11 mm (Soap 3), and 10 mm (Soap 4) in table 4. Among all the formulations, Soap 2 exhibited the highest antibacterial activity. The superior activity of Soap 2 (13 mm) is due to optimized concentration and combined effect of the APIs from methanolic leaf extracts of *Manilkara zapota*, *Annona reticulata* and *Ocimum sanctum* along with *Sapindus mukorossi* (Reetha), which functions as a natural surfactant with inherent antimicrobial properties [7]. The results suggest a possible synergistic interaction among the phytoconstituents, enhancing antibacterial efficacy. Soap 2 emerged as the most effective formulation, showing antibacterial activity indicating a promising natural topical antibacterial agent for the management of skin infections such as impetigo. Table 5 shows all the four evaluated tests of polyherbal soaps.

**Table 5:** Evaluation tests of all four formulated polyherbal soaps

Evaluation test	F1	F2	F3	F4	According to BIS,ISI Specification
pH measurement	8.13 pH	8.29 pH	8.52 pH	8.64 pH	8.0–10.5 pH
Foam height	5.3 cm	5.8 cm	4.0 cm	4.3 cm	4–6 cm

Foam retention time	19min 04sec	19min 45 sec	16min 07 sec	17min 29sec	<b>8-20mins</b>
Total fatty matter	71.2%	72.4%	70.8%	70.4%	<b>Grade1≥ 76%</b> <b>Grade2≥ 70%</b> <b>Grade3≥ 60%</b>
Moisture content	11.2%	12.8%	13.6%	14.4%	<b>14–16%</b>
Alcohol insoluble matter	5%	5.8%	6.4%	7.6%	<b>10% w/w</b>
Saponification value	182.32 KOH/ mg	185.13K OH/ mg	175.31 KOH/ mg	168.3 KOH/ mg	<b>160–200</b> <b>KOH/mg</b>
Percentage Free Alkali	0.0096%	0.0100%	0.010%	0.012%	<b>≤0.5%</b>

## Conclusion

The study was successfully performed to formulate and evaluate a polyherbal antibacterial soap intended for the management of impetigo. Various polyherbal soap formulations were prepared and evaluated for their physicochemical parameters, stability and antibacterial activity. All formulated soaps showed acceptable organoleptic properties like color, size, shape, odor, texture and appearance indicating good patient acceptability. The physicochemical evaluation parameters including pH, foam height, foam retention, moisture content, saponification value, free alkali content, TFM and cleansing ability were found to be within acceptable limits confirming the suitability of the formulations for topical application on the skin. The antibacterial activity of the formulated soaps was assessed using the agar well diffusion method against *Staphylococcus aureus*, the primary causative organism of impetigo. Among all the formulations tested, Soap-2 exhibited the highest antibacterial activity, showing a zone of inhibition of 13 mm and it can be considered as a promising, safe and effective topical herbal preparation for the management of impetigo. However, further studies such as clinical dermatology trials are recommended to establish its efficacy and safety. Overall, this research supports the potential use of polyherbal soaps as an alternative to synthetic antibacterial agents in the treatment of bacterial skin infections like impetigo.

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