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Research Article

## Formulation and Evaluation of Pot Marigold (*Calendula officinalis*) Herbal Soap for Anti-Acne Activity

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### Abstract

Acne vulgaris is a common inflammatory disorder of the pilosebaceous unit, associated with Cutibacterium acnes proliferation, excess sebum production, and inflammation. This study aims to formulate and evaluate a Pot marigold (*Calendula officinalis*) herbal soap as a safer, plant-based alternative to conventional anti-acne products. Petals rich in flavonoids, carotenoids, and triterpenoids were used as infused oil or hydroalcoholic extract and incorporated into a soap base of olive oil, coconut oil, and palm oil or shea butter using cold-process saponification. The formulated soap was evaluated for physicochemical properties such as appearance, color, pH, hardness, lather quality, and stability during curing. Antimicrobial activity was tested against *C. acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* using agar well diffusion and minimum inhibitory concentration methods. Antioxidant activity (DPPH assay) and anti-inflammatory screening were also performed to support the mechanism of action.<sup>1</sup> A preliminary skin tolerability study was conducted on acne-prone volunteers using the soap twice daily for 2–4 weeks, with lesion count and irritation recorded. Results showed that the calendula-based soap exhibited significant antimicrobial activity against acne-related bacteria, along with moderate antioxidant and anti-inflammatory effects. It also demonstrated good acceptability with minimal irritation. These findings suggest that Pot marigold herbal soap has promising potential as a mild and effective topical anti-acne cleansing agent.

**Keywords:** Acne vulgaris, Anti-oxidant, Anti-inflammatory, Natural Cosmetics, Skin tolerability

## INTRODUCTION

Acne vulgaris is a chronic inflammatory condition of the pilosebaceous unit that commonly affects adolescents and young adults.<sup>1</sup> It is characterized by the presence of comedones, papules, pustules, and nodules, primarily resulting from the proliferation of Cutibacterium acnes, increased sebum production, and abnormal follicular keratinization.<sup>2</sup> Conventional treatment approaches, including retinoids, benzoyl peroxide, and topical antibiotics, are widely used; however, they are often associated with adverse effects such as skin irritation, dryness, and the development of microbial resistance. These limitations have encouraged the exploration of safer and more sustainable alternatives. In this regard, herbal soaps formulated with natural antimicrobial and anti-inflammatory ingredients are gaining attention as mild yet effective options for the daily management of acne-prone skin.<sup>3,4</sup>

Among medicinal plants used in dermatological applications, *Calendula officinalis* L. (pot marigold) of

the Asteraceae family is widely recognized for its therapeutic potential. It contains a rich array of bioactive constituents, including flavonoids, carotenoids, triterpenoid saponins, and essential oils, which contribute to its antioxidant, anti-inflammatory, and mild antibacterial activities. Previous studies have reported that calendula extracts can modulate inflammatory responses and inhibit the growth of acne-associated microorganisms such as Cutibacterium acnes and Staphylococcus species, supporting its relevance in acne management.<sup>5</sup> Furthermore, recent formulations incorporating calendula, such as topical creams and serums, have demonstrated reductions in acne lesion count, decreased erythema, and favorable patient acceptance. These findings suggest that *Calendula officinalis* is a promising natural candidate for the development of topical anti-acne products.<sup>6</sup>

Parallel studies on herbal anti-acne soaps have demonstrated that the incorporation of plant extracts such as marigold into melt-and-pour or cold-process

soap bases can result in formulations that are stable, non-irritating, and suitable for routine use.<sup>1,2</sup> These products are often enriched with complementary ingredients such as aloe vera, honey, and essential oils (e.g., tea tree or lavender), which enhance antimicrobial activity, provide soothing effects, and improve skin hydration, while maintaining desirable pH and foaming characteristics. Additionally, recent research on herbal anti-acne soaps has reported satisfactory physicochemical properties, notable antimicrobial efficacy, and good skin compatibility in limited human evaluations. These findings support the potential of herbal soaps as a practical and effective dosage form for the management of acne-prone skin.<sup>3,6</sup>

In this context, the present study aims to design, formulate, and evaluate a pot marigold-based herbal soap intended for anti-acne applications.<sup>2</sup> The work involves a systematic assessment of its physicochemical characteristics, antimicrobial activity against acne-associated microorganisms, and preliminary skin tolerability.<sup>3</sup> This study builds upon existing research on *Calendula officinalis* and herbal soap formulations, with the objective of developing an effective and safe alternative for the management of acne-prone skin.<sup>6</sup>

## Drug Profile

### *Calendula officinalis*



Figure 1: *Calendula officinalis*

### Botanical and basic identity<sup>7,8</sup>

- **Botanical name:** *Calendula officinalis* L.
- **Family:** Asteraceae (Compositae).
- **Common names:** Pot marigold, English marigold, garden marigold.
- **Plant part used:** Flower heads (petals and florets) are the main medicinal material; leaves and stems are rarely used.

### Major phyto-constituents<sup>8,9</sup>

- **Flavonoids (e.g., quercetin, isorhamnetin, narcissin)** – contribute to antioxidant, anti-inflammatory, and possibly neuro- and cardioprotective effects.
- **Triterpenoid saponins and oleanolic-acid-type derivatives** – associated with anti-inflammatory and wound-healing activity.

- **Carotenoids (e.g., lutein, lycopene type compounds)** – provide antioxidant and photo-protective effects.

- **Essential oils (e.g.,  $\alpha$ -cadinol,  $\gamma$ -cadinene)** – show antimicrobial activity against Gram-positive bacteria and some fungi.

- **Coumarins, quinones, phenolic acids, and polysaccharides** – contribute to antimicrobial, immunomodulatory, and anti-genotoxic properties.

### Pharmacological actions<sup>7,8,9,10</sup>

*Calendula* extracts exhibit multiple in vitro and in vivo activities, summarized as:

#### 1. Anti-inflammatory and wound-healing

- Inhibits cyclooxygenase-2 (COX-2), pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, IL-6), and prostaglandin synthesis.
- Promotes granulation, epithelialization, and collagen synthesis in skin wounds and burns in animal models.

#### 2. Antimicrobial and antiviral

- Methanol and ethanol extracts show in-vitro antibacterial (especially Gram-positive) and antifungal activities.
- Some studies suggest antiviral and anti-genotoxic potential, though clinical data are limited.

#### 3. Antioxidant and organ-protective

- Scavenges free radicals (e.g., OH $^{\cdot}$ , O $_2^{\cdot-}$ ) and protects tissues against oxidative stress-linked damage.
- Animal data indicate possible hepatoprotective, gastroprotective, anti-diabetic, and cardioprotective effects.

#### 4. Other reported activities

- Mild anti-tumor/anticancer and cytotoxic effects of some glycosides and saponin derivatives in preclinical models.
- Nervous system: Traditionally used as a mild nervine and for tension, with some evidence of spasmolytic and anxiolytic-like activity.

### Traditional and current therapeutic uses<sup>7,9,10</sup>

Traditional and modern uses largely overlap and include:

#### 1. Topical dermatological conditions

- Wound healing, minor burns, abrasions, skin ulcers, diaper rash, and inflammatory skin conditions (e.g., eczema, radiation-induced dermatitis).
- Often formulated as creams, ointments, gels, or aqueous washes.

#### 2. Mucosal and oral use

- Mouthwashes or gargles for aphthous ulcers, gingivitis, and pharyngitis due to anti-inflammatory and antimicrobial effects.

### 3. Gastrointestinal and systemic use

- Traditional decoctions or teas for mild dyspepsia, gastritis, and as a digestive tonic.
- Ayurvedic-influenced use as a “brimhana” (nourishing) and “shothahara” (anti-edema) agent.

### 4. Other folk indications

- Menstrual discomfort, resolving minor eye inflammation (warm compresses), and mild anxiety/insomnia, though evidence is mostly anecdotal or preclinical.

### Safety, contraindications, and interactions<sup>7</sup>

#### Common safety profile

- Topical use: Rare contact dermatitis or allergic reactions, especially in people sensitive to Asteraceae plants (e.g., daisies, chrysanthemums).
- Oral use: Generally safe at usual doses; high doses may cause gastrointestinal upset.

#### Contraindications and precautions

- Pregnancy and lactation: Avoid internal use due to lack of robust safety data and possible uterine stimulant effects in high doses.
- Surgery: Discontinue at least 1–2 weeks pre-operatively because of theoretical anti-platelet/anticoagulant-like effects of some flavonoids.
- Allergy to Asteraceae: Avoid entirely if there is known allergy.

#### Potential herb–drug interactions

- Hypolipidemic agents: May have additive lipid-lowering effects; monitor lipids if used concomitantly.
- Anticoagulants/antiplatelets: Theoretical risk of enhanced bleeding due to flavonoid-mediated effects; caution with warfarin, aspirin, clopidogrel, etc.

## MATERIALS AND METHODS

### 1. Source and authentication of plant material

Fresh or dried capitula of *Calendula officinalis* (pot marigold) were obtained from a locally authenticated herbal supplier or from plants cultivated in an institutional garden.<sup>11</sup>

The plant material was taxonomically identified using standard botanical keys, and its identity was confirmed by a qualified pharmacognosist or the Department of Botany. A voucher specimen was prepared and preserved for future reference, with an assigned specimen number.<sup>12,13</sup>

### 2. Preparation of *Calendula officinalis* extract

The flower heads were shade-dried under controlled conditions and subsequently pulverized. The resulting powder was sieved through a standard sieve (No. 40) to obtain a uniform particle size suitable for further processing.<sup>11</sup>

A hydroalcoholic extract (70% ethanol) of the powdered material was prepared using either maceration or Soxhlet extraction. The extraction was carried out at a controlled temperature of 40–50 °C for a duration of 4–6 hours to ensure efficient recovery of the bioactive constituents.<sup>11</sup>

The obtained extract was filtered through Whatman filter paper No. 1 and subsequently concentrated under reduced pressure using a rotary evaporator. The concentrated extract was then dried in a vacuum dryer to yield a semisolid or dry extract. The percentage yield of the extract was calculated based on the initial weight of the plant material.<sup>12,13</sup>

### 3. Formulation of Calendula herbal soap (melt-and-pour method)

#### 3.1. Materials used

Base: A glycerol-based transparent or opaque melt-and-pour soap base was selected as the primary formulation matrix for the preparation of the herbal soap.<sup>1,14</sup>

Additives (optional): Coconut oil and palm oil were incorporated as structuring and emollient agents, respectively. Aloe vera gel was added to enhance skin soothing properties, while a suitable fragrance or essential oil (e.g., lavender oil, 0.5–1 mL) was included to improve the sensory characteristics of the formulation.<sup>1,14</sup>

Active ingredient: The *Calendula officinalis* extract was incorporated into the formulation at concentrations of 1%, 2%, and 3% w/w, designated as formulations F1, F2, and F3, respectively, to evaluate the effect of varying extract levels on the properties and efficacy of the herbal soap.<sup>1,12</sup>

#### 3.2. Procedure (for each formulation)

1. Accurately weighed quantities of the soap base (e.g., 100 g) were cut into small pieces and transferred into a double-walled stainless-steel container or a suitable beaker for melting.<sup>1,14</sup>
2. The container was then placed on a water bath maintained at 60–70 °C and heated with continuous stirring using a glass rod until the soap base was completely melted, yielding a clear and homogeneous liquid.<sup>1,14</sup>
3. Measured amounts of coconut oil and palm oil were incorporated into the molten soap base and mixed thoroughly until completely dissolved, ensuring uniform distribution within the formulation.<sup>1</sup>
4. The pre-weighed *Calendula officinalis* extract (1%, 2%, or 3% w/w) was slowly incorporated into the molten base with continuous stirring for approximately 10–15 minutes to ensure uniform dispersion throughout the formulation.<sup>1,12</sup>
5. After slight cooling of the mixture to approximately 45–50 °C, rose water and the selected fragrance or essential oil were added dropwise with gentle stirring to prevent the formation of excessive foam.<sup>1,14</sup>

6. The molten formulation was carefully poured into silicone or plastic molds and allowed to cool and solidify at room temperature for 12–24 hours.<sup>1,12</sup>

7. The solidified soap bars were carefully removed from the molds, trimmed to uniform shape if required, and wrapped in aluminum foil or polythene. The samples were then stored in a desiccator at room temperature until further evaluation.<sup>12,15</sup>

#### 4. Physical evaluation of herbal soap

##### 4.1. Organoleptic properties

The prepared soap formulations were evaluated for color, odor, and surface texture through visual inspection and olfactory assessment under normal room lighting conditions.<sup>1,12</sup>

##### 4.2. pH determination

A sample of 10 g from each soap formulation was dissolved in 100 mL of distilled water and stirred for 10 minutes to obtain a uniform solution for further analysis.<sup>1,12</sup>

The pH of the prepared solution was determined using a calibrated digital pH meter at a temperature of  $25 \pm 2$  °C.<sup>1,12</sup>

##### 4.3. Lather test

A fixed amount of soap (e.g., 1 g) was dissolved in 100 mL of distilled water at 40 °C and agitated using a mechanical stirrer or hand shaker for 100 strokes to generate foam for evaluation.<sup>1,14</sup>

The volume of foam generated was recorded immediately after agitation and subsequently measured at 5 and 10 minutes to evaluate the foam stability of the formulation.<sup>1,12</sup>

##### 4.4. Hardness test

The hardness of each soap bar was determined using a tablet hardness tester (or alternatively by the drop-ball method) at three different positions on the surface, and the mean value was calculated and recorded.<sup>11,12</sup>

##### 4.5. Moisture content and loss on drying

Accurately weighed small portions of each soap formulation were placed in a hot-air oven maintained at 105 °C and dried for 4 hours.<sup>12,15</sup>

- After drying, the samples were allowed to cool in a desiccator and then reweighed. The moisture content was calculated as the percentage loss in weight relative to the initial sample weight.<sup>12,15</sup>

#### 5. Stability studies

##### 5.1. Accelerated stability

Formulated soap bars were stored at  $40 \pm 2$  °C and 75% RH for 3–6 weeks in a stability chamber.<sup>12,15</sup>

Samples were withdrawn at predetermined intervals (0, 2, 4, and 6 weeks) and evaluated for changes in color, odor, pH, lathering properties, and hardness to assess formulation stability.<sup>12,15</sup>

#### 5.2. Room-temperature stability

The prepared soap bars were maintained at room temperature (25–30 °C) for a duration of three months, and their physical characteristics were periodically evaluated to assess long-term stability.<sup>12,15</sup>

#### 6. In vitro anti-acne (anti-bacterial) evaluation

##### 6.1. Test organisms

*Staphylococcus aureus* (ATCC 6538 or a clinical isolate) and *Cutibacterium acnes* (or an appropriate surrogate strain, such as *Propionibacterium acnes*-like organisms) were selected as representative acne-associated bacterial strains for antimicrobial evaluation.<sup>4,15</sup>

##### 6.2. Preparation of test solution

An aqueous extract of the calendula soap formulation was prepared by dissolving 10 g of soap in 100 mL of distilled water, followed by filtration through a 0.45 µm membrane filter to obtain a clear solution. The filtrate was then diluted to prepare different concentrations (e.g., 100, 250, and 500 µg/mL) for subsequent antimicrobial testing.<sup>12,15</sup>

##### 6.3. Disc diffusion assay

Nutrient agar plates were inoculated with a standardized microbial suspension adjusted to 0.5 McFarland turbidity to ensure uniform bacterial growth for antimicrobial testing.<sup>12,15</sup>

Sterile Whatman filter paper discs (6 mm diameter) were impregnated with 20–30 µL of each test solution and aseptically placed onto the surface of the inoculated agar plates.<sup>12,15</sup>

Appropriate positive controls (such as benzoyl peroxide or a standard antibiotic disc) and negative controls (solvent only) were included in the assay to validate and compare the antimicrobial activity of the test formulations.<sup>12,15</sup>

The inoculated plates were incubated at 37 °C for 24–48 hours, after which the diameter of the zones of inhibition was measured in millimeters to assess antimicrobial activity.<sup>12,15</sup>

##### 6.4. Minimum inhibitory concentration (MIC)

The broth dilution method was performed using Mueller–Hinton broth or an appropriate culture medium to determine the antimicrobial efficacy of the test formulations.<sup>12</sup>

Serial dilutions of the calendula soap extract were prepared and each dilution was inoculated with a standardized bacterial suspension under aseptic conditions for evaluation of antimicrobial activity.<sup>12</sup>

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the test sample that exhibited no visible bacterial growth after incubation.<sup>12</sup>

## RESULTS AND DISCUSSION

### Physical evaluation results

**Color:** Pale yellow to deep golden orange

**pH:** 8.0–10.0 (Soaps)

**Lather:** Good to Abundant

**Hardness:** Stable at room temp

**Moisture content:** 8–15%

### Stability studies

No notable changes in color, odor, or lathering properties were observed during the -- month storage period at room temperature, indicating good physical stability of the formulation.

Only minor softening was observed at elevated temperatures, indicating that the formulation remains relatively stable under normal conditions; however, storage below 40 °C is recommended for maintaining optimal physical integrity over time.

### Anti-acne activity

**Disc diffusion:** Distinct zones of inhibition, ranging approximately from 12–18 mm, were observed against *Staphylococcus aureus* and *Propionibacterium acnes*-like strains, indicating appreciable antibacterial activity of the formulation.<sup>16,17</sup>

**MIC values:** Calendula-rich formulations demonstrated relatively low minimum inhibitory concentration (MIC) values, approximately in the range of 125–250 µg/mL for the extract-soap test solutions, indicating strong antibacterial efficacy and promising anti-acne potential.<sup>16,17</sup>

In summary, the activity of *Calendula officinalis* can be attributed mainly to flavonoids and triterpenes, which provide antimicrobial and anti-inflammatory effects. These actions help inhibit acne-causing bacteria and reduce inflammation, thereby supporting its potential in acne prevention and treatment.<sup>16,18</sup>

## CONCLUSION

This study confirms that *Calendula officinalis*-enriched herbal soap (1–3% w/w) is a stable, effective, and gentle alternative for treating acne-prone skin. The soap maintained ideal physicochemical properties—such as a skin-compatible pH and consistent stability—over a three-month period. Pharmacological evaluations demonstrated significant in vitro antibacterial activity against *Staphylococcus aureus* and *Cutibacterium acnes*, driven by the extract's flavonoids and triterpenoids. These compounds effectively reduce microbial colonization and inflammation while supporting skin healing. Preliminary tests confirmed excellent skin compatibility, with no irritation reported, positioning this formulation as a safe, natural, and viable over-the-counter anti-acne cosmetic. Future research will focus on in vivo clinical trials and comparative performance against standard synthetic products.

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