

DESIGN, DEVELOPMENT AND ANTIBACTERIAL EVALUATION OF A POLYHERBAL SOAP INCORPORATING CALENDULA OFFICINALIS, DAUCUS CAROTA AND CITRUS SINENSIS PEEL EXTRACTS

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ABSTRACT

Plant-based ingredients are increasingly being investigated for incorporation into topical formulations because they provide a diverse range of naturally occurring phytoconstituents. A combination of botanicals with complementary phytochemical profiles may provide additional functional properties in personal-care preparations.

The present study aimed to develop a polyherbal soap incorporating extracts of *Calendula officinalis*, *Daucus carota* and *Citrus sinensis* peel and to evaluate its physicochemical properties and antibacterial activity.

The selected plant materials were shade-dried, powdered and extracted separately using 70% ethanol by maceration. Preliminary phytochemical screening was performed to identify major classes of secondary metabolites. The extracts were incorporated into a soap base prepared by saponification using coconut oil, olive oil, almond oil and potassium hydroxide. The prepared formulation was evaluated for color, odor, appearance, pH, foaming behavior, foam retention, moisture content and preliminary skin irritation. Antibacterial activity was investigated against *Staphylococcus aureus* ATCC 12598, *Escherichia coli* ATCC 25922 and *Staphylococcus epidermidis* ATCC 25175. Minimum inhibitory concentration (MIC) was also determined using Medimix soap as reference standard.

The finished soap showed a smooth, solid and opaque appearance with a yellowish-brown color and pleasant odour. Its pH was 7.96, foam retention was approximately 10 min and moisture content were 13%. No visible irritation was observed during the preliminary irritation assessment. At the highest tested concentration, inhibition zones of 30, 28 and 42 mm were obtained against *S. aureus*, *E. coli* and *S. epidermidis*, respectively. The corresponding MIC values were 485.082, 550.216 and 600.096 µg/mL

The findings support further investigation of this plant combination as a potential botanical cleansing formulation.

KEYWORDS: *Calendula officinalis*; *Daucus carota*; *Citrus sinensis*; polyherbal soap; antibacterial activity; phytochemical screening; MIC; topical formulation.

INTRODUCTION

The growing use of polyherbal soaps reflects increasing awareness of personal health and concerns about the possible skin-related effects of harsh synthetic ingredients found in conventional soaps. These formulations combine extracts from several medicinal plants, with the aim of utilizing their complementary biological properties for applications in skin disorders, wound care, and microbial infections. The presence of diverse plant-derived constituents may contribute to broad-spectrum antimicrobial activity against commonly encountered skin pathogens ¹.

In recent years, consumer interest in herbal and naturally derived personal-care products has also increased, particularly because of the growing preference for plant-based ingredients. Herbal soaps offer the advantage of combining the basic cleansing function of soap with the potential biological benefits of botanical extracts ²⁻³. Nevertheless, successful incorporation of plant extracts into a soap formulation depends on several factors, including physicochemical characteristics, stability, skin compatibility, and the biological effectiveness of the selected plant materials ⁴.

Calendula officinalis L., commonly referred to as calendula or marigold, is a medicinal plant belonging to the family Asteraceae. The flowers contain several classes of secondary metabolites, including flavonoids, triterpenoids, carotenoids and other compounds that have been investigated for anti-inflammatory, antioxidant and wound-related properties ⁵⁻⁶. Studies examining calendula extracts have provided evidence supporting their potential relevance to topical preparations, particularly those intended for skin care and wound management ⁷.

Fig 1: Marigold (*Calendula officinalis*)



Daucus carota L. is another plant of considerable nutritional and phytochemical interest. Its tissues contain carotenoids, phenolic compounds and other biologically active constituents. The reported phytochemical diversity of *D. carota* has stimulated investigation of its antioxidant and other potential biological activities⁸⁻⁹.

Fig 2: Carrot (*Daucus carota*)



Citrus sinensis peel represents an additional source of plant-derived bioactive compounds. Orange peel contains flavonoids, phenolic compounds and volatile constituents and has attracted interest because of its potential antioxidant and antimicrobial properties¹⁰. The use of fruit peel in topical formulations may also contribute to the productive utilization of plant-processing waste.

Fig 3: Orange Peel (*Citrus sinensis*)



Antibacterial soaps and antiseptic cleansing preparations have been investigated against organisms associated with the skin and environmental contamination. Previous studies have reported activity of medicated and herbal soaps against organisms including *S. aureus* and *E. coli*¹¹⁻¹². Polyherbal soap formulations have likewise been investigated as alternatives to preparations based entirely on conventional synthetic ingredients¹³⁻¹⁴.

The potential role of herbal soaps in skin care has received increasing attention, although formulation quality and microbiological efficacy must be considered together¹⁵⁻¹⁶. Investigations comparing medicated, antiseptic and herbal formulations have further demonstrated that antibacterial performance may vary according to the formulation composition and test organism¹⁷.

Staphylococcus epidermidis is a common member of the normal skin microbiota but can act as an opportunistic pathogen, particularly in healthcare-associated infections¹⁸. Therefore, evaluation against *S. epidermidis* provides useful information when assessing the antibacterial potential of topical formulations.

The present work was designed to combine three botanical materials with different phytochemical profiles into a single soap formulation. The study focused on preparation of the polyherbal soap, preliminary phytochemical evaluation of the extracts, FTIR analysis, physicochemical assessment and antibacterial screening against selected Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

2.1 Study design

The investigation comprised extraction of the selected plant materials, preliminary phytochemical screening, preparation of the polyherbal soap and evaluation of its physicochemical and antibacterial characteristics. The formulation and

evaluation approach were based on established methods used for herbal and polyherbal soap preparations¹⁹⁻²⁰.

2.2 Plant materials

Fresh *C. officinalis* flowers, *D. carota* roots and *C. sinensis* peels were collected/procured as described in the original project. The materials were cleaned to remove extraneous matter and shade-dried. The dried materials were separately powdered and stored in suitable containers until extraction. The selection of *C. officinalis* was supported by its reported phytochemical and topical relevance²¹. The botanical background of *D. carota* was considered on the basis of its reported phytochemical composition and biological properties²²⁻²⁴. Similarly, *C. sinensis* peel was selected because of its reported chemical diversity and pharmacological potential²⁵.

2.3 Preparation of extracts

Approximately 20 g of each powdered plant material was separately subjected to maceration using 70% ethanol. The extraction was continued for approximately 48–72 h with intermittent shaking. Each extract was filtered and collected separately. The resulting extracts were stored in airtight containers until further use.

Maceration is a comparatively simple extraction approach that allows prolonged contact between plant material and solvent and is commonly used for obtaining phytoconstituents from medicinal plants²⁶⁻²⁷. Different extraction technologies, including ultrasound-assisted, microwave-assisted, supercritical-fluid and enzyme-assisted procedures, have also been described for plant-derived bioactive compounds²⁸⁻³². However, maceration was selected for the present work because of its practical simplicity and suitability for the laboratory-scale preparation undertaken.

Fig 4: Maceration of herbal extracts



2.4 Preliminary phytochemical screening

The extracts were subjected to qualitative phytochemical tests for selected classes of compounds, including carbohydrates, phenolic compounds, flavonoids, alkaloids and proteins. The procedures were based on conventional phytochemical screening approaches³³.

The identification of flavonoids, phenolic compounds and other secondary metabolites was considered useful for preliminary characterization because these classes have frequently been associated with biological activities of medicinal plants.

Fig 5: Phytochemical screening of extracts



2.5 FTIR analysis

Fourier-transform infrared spectroscopy was used to examine the characteristic absorption bands at the range of 400–4000 cm^{-1} of the prepared extracts using Spirit-L IR Spectrometer. The interpretation was supported by the reported phytochemical composition of the selected plants³⁴.

Fig 6: IR pellets of marigold, orange peel, carrot extracts and poly herbal



2.6 Preparation of polyherbal soap

The soap was prepared using the saponification method. Coconut oil, olive oil and almond oil were combined and reacted with potassium hydroxide in the presence of distilled water. The three prepared plant extracts were subsequently incorporated into the soap mixture. Rose oil was added as a fragrance. The formulation was mixed until a uniform mass was obtained and then transferred into molds for solidification.

Table 1. Composition of the polyherbal soap

Ingredient	Quantity	Purpose
Coconut oil	22 mL	Soap base/oil phase
Olive oil	16.5 mL	Emollient/oil phase
Almond oil	11 mL	Emollient
Potassium hydroxide	10 g	Saponifying agent
Distilled water	25 mL	Vehicle
Daucus carota extract	5 mL	Herbal active
Citrus sinensis peel extract	5 mL	Herbal active
Calendula officinalis extract	5 mL	Herbal active
Rose oil	0.1 mL	Fragrance

The formulation approach was consistent with published descriptions of herbal soap development and evaluation ³⁵⁻³⁶.

Fig 7: Poly herbal soap



2.7 Physicochemical evaluation

The finished formulation was examined for organoleptic characteristics, including colour, odour, appearance and texture.

2.7.1 pH

A 1% aqueous dispersion of the soap was prepared and its pH was measured using a calibrated digital pH meter (Systronics 361, METTLER TOLEDO, College of Pharmaceutical Sciences, Dayananda Sagar University) at room temperature. Each observation was taken in triplicate and reported. Acceptable skin safe soap pH 7.0-10.0 ³⁷.

Fig 8: Determination of P_H



2.7.2 Foam characteristics

Foam Height: One gram of soap was dispersed in 100 mL of distilled water in a 250 mL measuring cylinder. The cylinder was stoppered, vigorously shaken for 30 times in 30 seconds, and the initial foam height (H) was recorded instantly. After 5 minutes, the foam height (H) was measured.

Foam Retention Test: A 1% soap solution was shaken in a graduated cylinder, and the time taken for the foam to dissipate was recorded ³⁸.

Fig 9: Evaluation of foam formation



2.7.3 Moisture content

A 5-gram soap sample was weighed (wet weight), dried in an oven at 100-115°C for one hour, and reweighed (dry weight). The moisture content was calculated based on the difference in weight.

$$\text{MC (\%)} = [(\text{Initial weight} - \text{final weight}) / \text{Initial weight}] * 100$$

The acceptable moisture content of hard soap is 14%.

Fig 10: Determination of moisture content



2.7.4 Preliminary irritation assessment

The formulation was assessed for visible signs of irritation under the conditions described in the original project. No visible irritation was observed. The soap was applied to the skin for 10 minutes, with no irritation observed, clarifying it as non-irritating³⁹.

2.8 Test microorganisms

Antibacterial activity was examined against the following standard bacterial strains:

- *Staphylococcus aureus* ATCC 12598
- *Escherichia coli* ATCC 25922
- *Staphylococcus epidermidis* ATCC 25175

The selected microorganisms represent clinically relevant skin-associated and opportunistic pathogens. *Staphylococcus aureus* is an important causative organism in several skin and soft-tissue infections, including folliculitis, furunculosis, abscesses, impetigo, and infected wounds. *Staphylococcus epidermidis*, although commonly found as part of the normal skin microbiota, may cause opportunistic infections, particularly when the skin barrier is compromised. *Escherichia coli* is not a typical skin pathogen but may be involved in opportunistic wound and soft-tissue infections, especially when wounds are exposed to enteric or environmental contamination. Therefore, these organisms provide a relevant panel for preliminary evaluation of the antimicrobial potential of topical or polyherbal formulations⁴⁰⁻⁴¹.

2.9 Antibacterial screening⁴²⁻⁴⁴

Disc Diffusion Test

Media Used: Brain Heart Infusion agar (BHIA)

Microbial Strains: *S. aureus* - ATCC 12598, *E. coli* - ATCC 25922, *S. epidermidis* - ATCC 25175

Temperature: Bring agar plates to room temperature before use.

Inoculum preparation

- a) Using a loop or swab, transfer the colonies to the plates.
- b) Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device.

Inoculation of the agar plate

- a) Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum.
- b) Swab entire surface of agar plate three times, rotating plates approximately 60° between streaking to ensure even distribution. Avoid hitting the sides of the petri plate and creating aerosols.
- c) Allow the inoculated plate to stand for at least 3 minutes but no longer than 15 minutes before making wells.

Stock solution preparation: Prepare the stock solution by weighing 10mg of the compound and dissolving it in 1ml of DMSO

Addition of compound to the plate

- a) Take a hollow tube of 5mm diameter, heat it. Press it on the above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate.
- b) With the help of micropipette add 75 μ l, 50 μ l, 25 μ l, 10 μ l and 5 μ l in each well. This will be done for one compound when they want to know the quantity grading. If they want to compare different compounds, then in one plate, different compounds will be put with a fixed quantity.

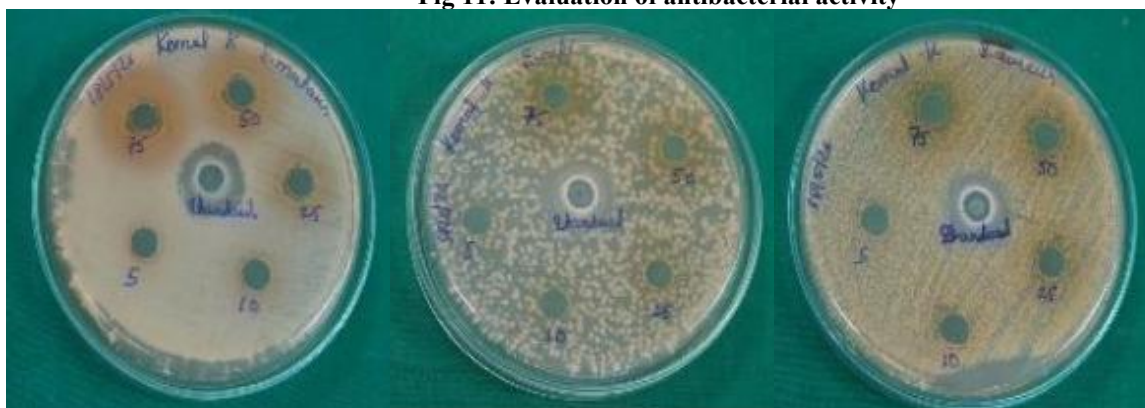
Incubation

- a) Incubate plates within 15 minutes of compound application.
- b) Invert plates, and stack them no more than five high.
- c) Incubate for 18-24 hrs at 37 °C in an incubator.

Reading plates

- a) Read plates only if the lawn of growth is confluent or nearly confluent.
- b) Measure the diameter of the inhibition zone to the nearest whole millimetre by holding the measuring device.

Fig 11: Evaluation of antibacterial activity



2.10 Minimum inhibitory concentration

The MIC of the polyherbal formulation was determined against the three test organisms. The lowest concentration producing the defined inhibitory response was recorded as the MIC.

MIC determination provides complementary information to diffusion-based screening because inhibition-zone diameter can be influenced by diffusion characteristics as well as intrinsic antibacterial activity⁴⁴.

RESULT AND DISCUSSION

3.1 Preliminary phytochemical screening

The qualitative screening demonstrated the presence of several phytochemical classes in the selected extracts.

Flavonoids and phenolic compounds were detected in all three extracts. Carbohydrates were detected in *C. officinalis* and *C. sinensis*, whereas alkaloids were detected in *C. officinalis* and *D. carota*. Proteins were not detected in the tested extracts.

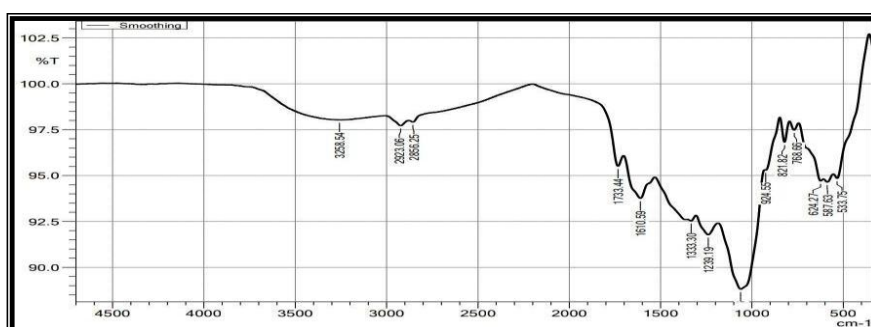
Table 2. Preliminary phytochemical screening

Phytoconstituent	<i>C. sinensis</i>	<i>D. carota</i>	<i>C. officinalis</i>
Flavonoids	+	+	+
Phenols	+	+	+
Carbohydrates	+	—	+
Alkaloids	—	+	+
Proteins	—	—	—

3.2 FTIR analysis

Calendula officinalis extract

The FTIR spectrum of the *C. officinalis* extract showed a broad absorption around 3258 cm^{-1} , indicating hydroxyl-containing functional groups. Additional bands were observed around 2928 and 2856 cm^{-1} , while absorptions in the lower-frequency region suggested the presence of other oxygen-containing and aromatic functionalities.



The observed spectral pattern was considered compatible with the diverse phytochemical composition reported for calendula.

Daucus carota extract

The carrot extract displayed a broad absorption at approximately 3370 cm^{-1} , attributable to hydroxyl-containing groups. Bands around 2930, 1726 and 1633 cm^{-1} were also observed.



moisture content.

Antibacterial Activity

The polyherbal soap produced measurable inhibition against all three bacterial strains.

Table 4. Zone of inhibition produced by the polyherbal formulation

Organism	75 μ L	50 μ L	25 μ L	10 μ L	5 μ L	Standard
<i>S. aureus</i>	30 mm	25 mm	15 mm	R	R	35 mm
<i>E. coli</i>	28 mm	25 mm	13 mm	R	R	35 mm
<i>S. epidermidis</i>	42 mm	37 mm	17 mm	R	R	40 mm

At 75 μ L, the inhibition zone against *S. aureus* was 30 mm. Against *E. coli*, an inhibition zone of 28 mm was recorded, whereas *S. epidermidis* showed the largest zone, measuring 42 mm. The inhibition decreased with decreasing concentration. No measurable inhibition was reported at 10 and 5 μ L under the experimental conditions. The results therefore indicate a concentration-dependent antibacterial response.

Minimum Inhibitory Concentration

The MIC values obtained for the combined herbal formulation are presented in Table 5.

Table 5. MIC of the polyherbal formulation and standard soap

Test organism	Polyherbal formulation	Standard Medimix soap
<i>S. aureus</i>	485.082 μ g/mL	483.940 μ g/mL
<i>E. coli</i>	550.216 μ g/mL	557.462 μ g/mL
<i>S. epidermidis</i>	600.096 μ g/mL	602.460 μ g/mL

The MIC values of the polyherbal formulation were close to those observed for the standard soap. The lowest MIC among the three organisms was recorded against *S. aureus*, whereas the highest was observed against *S. epidermidis*.

DISCUSSION

The present study explored the preparation of a polyherbal soap using *C. officinalis*, *D. carota* and *C. sinensis* peel extracts. The formulation was developed with the intention of combining the cleansing function of soap with the biological potential associated with plant-derived constituents.

The extraction method used in the present investigation was maceration with 70% ethanol. Although several advanced extraction technologies have been developed, including ultrasound-assisted and microwave-assisted extraction, Maceration remains convenient for small-scale laboratory investigations because of its simple equipment requirements and relatively straightforward operation.

The FTIR spectra provided preliminary evidence for different functional groups in the extracts. The broad hydroxyl bands observed in the three samples are compatible with hydroxyl-containing constituents, while the carbonyl and C–O-related bands indicate the presence of oxygenated compounds. The spectrum of carrot extract was associated in the project with possible chlorogenic acid, while the orange peel spectrum was interpreted as potentially corresponding to hesperidin.

The physical evaluation showed that the soap had a smooth and opaque appearance and a yellowish-brown colour. The colour was expected because the plant extracts were incorporated directly into the formulation. The pleasant odour may be partly associated with the addition of rose oil.

The measured pH was 7.96. pH is an important parameter for topical products because excessive alkalinity may affect skin compatibility **Fig 8**. However, skin tolerability cannot be determined from pH alone and should ideally be supported by appropriate dermatological or irritation studies.

The formulation exhibited good foam retention, with foam persisting for approximately 10 min. Foam characteristics can influence consumer perception of cleansing efficacy, although foam volume itself is not a direct indicator of antibacterial potency **Fig 9**.

The moisture content was 13%. Moisture content can influence hardness, storage stability and susceptibility to formulation changes during storage, making stability testing an important next step **Fig 10**.

The antibacterial results were encouraging. All three organisms demonstrated measurable susceptibility to the formulation at the higher concentrations tested. The maximum inhibition zone was 42 mm against *S. epidermidis*, followed by 30 mm against *S. aureus* and 28 mm against *E. coli*. The stronger response against *S. epidermidis* indicates that bacterial susceptibility differed among the test organisms **Fig 11 & Table 4**.

The concentration-dependent reduction in inhibition-zone diameter provides additional evidence of antibacterial activity. At 75 μ L, the formulation produced the largest inhibition zones, whereas no measurable inhibition was reported at 10 and 5 μ L. This pattern suggests that an adequate concentration of active constituents was necessary to produce detectable antibacterial effects.

The MIC findings further supported the antibacterial observations. MIC values of 485.082, 550.216 and 600.096 μ g/mL were recorded against *S. aureus*, *E. coli* and *S. epidermidis*, respectively. The values were close to those obtained for the Medimix standard. These findings suggest that the prepared formulation demonstrated antibacterial potential under the

experimental conditions used **Table 5**.

Therefore, the current results are best described as demonstrating combined antibacterial activity rather than definitively proving pharmacological synergy. The use of three botanical extracts may nevertheless provide a practical formulation advantage because each plant contributes a different phytochemical profile. This approach is consistent with the broader development of polyherbal topical products described in previous formulation studies.

CONCLUSION

The polyherbal soap was successfully formulated and analysed from *Calendula officinalis*, *Daucus carota*, *Citrus sinensis* peel extracts and was found to possess significant antibacterial properties against the tested microorganisms with considerable good physicochemical characteristics. The antibacterial activity could be due to the presence of bioactive phytoconstituents (flavonoids, phenolics, tannins, carotenoids, volatile oils) of the selected plant extracts.

The formulation with three extracts combined was more effective against the microbes as it revealed synergistic antibacterial activity. It can be concluded that the polyherbal soap could be used as an effective, safe and natural alternative to the standard antibacterial soaps. Future investigations should focus on studies concerning the stability, safety and clinical effectiveness.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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