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Formulation, Evaluation, and Antimicrobial Investigation of a Polyherbal Soap against Skin Pathogens

Adita Barua
University of Science and Technology Chittagong https://orcid.org/0009-0005-7857-966X
Rukyea Yeasmin Mysha
University of Science and Technology Chittagong https://orcid.org/0009-0000-6488-7550
Usra Tabassum Chowdhury
University of Science and Technology Chittagong https://orcid.org/0009-0005-9956-4459
Rupkatha Chowdhury
University of Science and Technology Chittagong https://orcid.org/0009-0006-4841-4992
Bristi Dey
University of Science and Technology Chittagong https://orcid.org/0009-0006-2480-1824
Ms. Joushan Ara
University of Science and Technology Chittagong
Aysha Ahmed Khan
Institute of Applied Health Sciences Hospital https://orcid.org/0000-0003-4101-2035
Pair Ahmed Jiko
Chittagong University of Engineering & Technology https://orcid.org/0009-0008-2375-9729
Arif Ahmed
arif.ahmed@ustc.ac.bd
University of Science and Technology Chittagong https://orcid.org/0000-0002-8424-0305

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Abstract

Bachground:

Azadirachta indica (Neem), *Carica papaya* (Papaya), *Curcuma longa* (Turmeric), *Citrus sinensis* (Orange Peel), *Tagetes erecta* (Marigold), and *Alternanthera philoxeroides* (Alligator Weed) are medicinal known for their antimicrobial and antifungal properties. Although these plants have been individually reported to antimicrobial activity, limited studies have investigated their combined efficacy in polyherbal soap formulations.

Objectives

This study aimed to evaluate the antimicrobial activity of selected medicinal plants and formulate an antimicrobial polyherbal soap.

Methods

Aqueous extracts of the selected plant materials were prepared using the maceration method and incorporated into five soap formulations (F1–F5). The formulations were evaluated for physicochemical properties, including weight variation, pH, solubility, foamability, foam retention, organoleptic characteristics, and skin irritation. Antimicrobial activity was determined against *Staphylococcus aureus*, *Escherichia coli*, and *Candida* species using the agar well diffusion method. Among the five formulations, F1 and F3 were selected as the most effective based on their antimicrobial performance. F1 exhibited the highest activity with a zone of inhibition of 13 mm against *S. aureus*, while F3 showed zones of inhibition of 10 mm, 9 mm, and 8 mm against *S. aureus*, *Candida* species, and *E. coli*, respectively. All formulations showed acceptable physicochemical characteristics, with pH values ranging from 5.6 to 6.5, good foam stability, and complete solubility in water. No signs of skin irritation were observed in mice.

Conclusions

The selected polyherbal formulations demonstrated promising antimicrobial activity and favorable physicochemical properties, indicating their potential as safe, eco-friendly, and effective antimicrobial cleansing products.

1. INTRODUCTION

Skin infections are among the most common health problems worldwide and are caused by a variety of pathogenic microorganisms, including bacteria and fungi. These infections can have a major effect on human health and quality of life, ranging from minor superficial ailments to chronic and recurrent illnesses[(1)]. Although synthetic antimicrobial soaps and disinfectants are widely used to prevent and control skin infections, prolonged use of such products may contribute to microbial resistance and skin irritation due to the presence of synthetic preservatives, artificial fragrances, petrochemicals, and other

harsh chemicals[2]. Consequently, there is a growing interest in developing safer, natural, and environmentally friendly alternatives for personal hygiene and skin care.

Medicinal plants have been used in traditional medicine for centuries and continue to serve as an important source of therapeutic agents. Numerous studies have reported that medicinal plants possess antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory activities. These biological properties are largely attributed to bioactive phytochemicals such as flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, and essential oils[3, 4]. Owing to their effectiveness, affordability, availability, and relatively fewer adverse effects, medicinal plants are increasingly being explored as natural antimicrobial agents.

Bangladesh possesses a rich diversity of medicinal plants that have long been utilized in Ayurveda, Unani, and traditional folk medicine. Many indigenous and cultivated medicinal plants are widely used for the treatment of wounds, skin diseases, infections, inflammation, and other health conditions[5]. Therefore, medicinal plants represent a valuable resource for the development of novel herbal formulations with potential therapeutic benefits.

Neem or *Azadirachta indica* A. Juss., is one of the most researched medicinal plants and a member of the Meliaceae family. Neem's antibacterial, antifungal, antiviral, anti-inflammatory, and wound-healing qualities are attributed to physiologically active substances like azadirachtin, nimbin, and nimbidol[6]. Neem leaves have historically been used to heal wonds,ulcers,eczema,skin infections, and other dermatological conditions[7].

Alkaloids, flavonoids, saponins, and phenolic chemicals are abundant in *Carica papaya L. (Papaya*), which belongs to the Caricaceae family [8]. The plant's various sections have shown anti-inflammatory, antioxidant, and antibacterial properties. Papaya has been used for many years to treat microbiological infections, skin conditions, and wounds[9].

Turmeric or *Curcuma longa* L. (Turmeric), is a member of the Zingiberaceae familyand is well known for its therapeutic properties[10]. Turmeric's main bioactive component, curcumin, has broad-spectrum antibacterial, antioxidant, anti-inflammatory, and wound-healing properties[11]. Traditional medicine has made considerable use of turmeric to treat infections and skin conditions.

Orange peel or *Citrus sinensis* (L.) Osbeck (Orange Peel), belong to the Rutaceae family, has important antibacterial and antioxidant qualities due to its flavonoids, limonoids, and essential oils[12]. Similarly, the Asteraceae family's *Tagetes erecta* L. (Marigold) contains flavonoids, carotenoids, and other phytochemicals that have antibacterial, anti-inflammatory, and wound-healing properties[13]. *Alternanthera philoxeroides* (Mart.) Griseb. (Alligator Weed), a member of the Amaranthaceae family, is traditionally utilized for a variety of therapeutic uses and has also been shown to have antibacterial and antioxidant qualities[14].

While neem, papaya, turmeric, orange peel, marigold, and alligator weed have all been studied separately for their antibacterial properties, few research have assessed their combined effectiveness in polyherbal soap compositions. Moreover, the creation and assessment of herbal soaps with many extracts from medicinal plants have been the subject of very few investigations. The synergistic antibacterial activities of these particular plants when combined into a single product are not well supported by the literature currently available. Combining many medicinal plants with various bioactive components was thought to increase antibacterial effectiveness through synergistic interactions[15] and together offer better defense against common skin infections than single plant extracts.

Thus, the goal of the current study was to create and assess polyherbal antimicrobial soaps using specific extracts from medicinal plants five formulations (F1–F5) were created and assessed. The agar well diffusion method was used to assess the formulations' antibacterial efficacy against *Staphylococcus aureus*, *Escherichia coli*, and *Candida*. Furthermore, physicochemical factors such as pH, solubility, foamability, foam retention, organoleptic qualities, and skin irritation potential were evaluated to determine the most effective and acceptable polyherbal soap formulation.

2. METHOD AND MATERIALS

2.1 Study design & setting

Formulating and evaluating a polyherbal antibacterial soap using particular extracts from medicinal plants was the aim of the current experimental laboratory study. The antibacterial efficacy, skin compatibility and physicochemical properties of five different formulations (F1-F5) were evaluated.Skin safety was assessed using in vivo skin irritation experiments in mice, while antibacterial activity was measured in vitro. The research was conducted in the Department of Pharmacy at the University of Science and Technology Chittagong (USTC) in Chattogram, Bangladesh. The Department of Pharmacy conducted the polyherbal soap's formulation and physicochemical analysis, and standard microbiological laboratory equipment was used for the antibacterial activity investigations.

2.2 Collection of plant materials and authentication

Fresh flowers of Tagetes erecta (marigold), fresh rhizomes of Curcuma longa (turmeric), fresh peels of Citrus sinensis (orange), and fresh leaves of Azadirachta indica (neem), Carica papaya (papaya), and Alternanthera philoxeroides (alligator weed) were gathered from various parts of Chattogram, Bangladesh. Dr. Mohammad Harunur-Rashid of the Department of Botany at the University of Chittagong in Bangladesh verified the authenticity of the gathered plant materials. The polyherbal antimicrobial soap was formulated after extracts were prepared using the verified plant sources

Table 1
Pharmacognostical Profile of Active Ingredients

S. No.	Name	Biological Source	Part Used	Chemical Constituents	Uses
1	Neem (*Azadirachta indica*)	Leaves of *A. indica*	Leaves	Azadirachtin, Nimbin, Quercetin, Nimbidin	Antibacterial, antifungal, anti-inflammatory, anti-acne
2	Papaya (*Carica papaya*)	Fruit/Leaves of *C. papaya*	Pulp, Leaves	Papain, Flavonoids, Alkaloids, Vitamins A, C	Exfoliating, antimicrobial, antioxidant
3	Orange Peel (*Citrus sinensis*)	Fruit peel of *C. sinensis*	Peel	Limonene, Flavonoids, Vitamin C, Phenolics	Antioxidant, antibacterial, skin brightening
4	Marigold (*Tagetes erecta*)	Flowers of *T. erecta*	Flower	Flavonoids, Carotenoids, Triterpenoids	Wound healing, antiseptic, anti-inflammatory
5	Turmeric (*Curcuma longa*)	Rhizome of *C. longa*	Rhizome	Curcumin, Turmerone, Flavonoids	Antimicrobial, antioxidant, skin soothing
6	Alligator weed (*Alternanthera philoxeroides*)	Whole plant	Whole plant	Alkaloids, Flavonoids, Tannins, Saponins	Antimicrobial, anti-inflammatory, wound healing
7	Coconut Oil (*Cocos nucifera*)	Kernel of coconut	Oil	Lauric acid, Capric acid, Vitamin E	Cleansing, moisturizing, antimicrobial
8	Honey	Produced by *Apis* species	Whole	Sugars, Enzymes, Phenolics	Moisturizing, antimicrobial, soothing
9	Vitamin E	Natural plant oils	Oil extract	Tocopherols	Antioxidant, skin protection

Source: Neem- *Azadirachta indica* A. Juss. [16], *Carica papaya* L. (Papaya)[17], *Citrus sinensis* (L.) Osbeck (Orange Peel)[18], Marigold (*Tagetes erecta*)[19], Turmeric (*Curcuma longa*)[20], Alligator Weed (*Alternanthera philoxeroides*)[21], Coconut Oil (*Cocos nucifera*)[22], Honey[23], Vitamin E[24]

2.3 Preparation of extracts

The collected plant materials, including leaves of *Azadirachta indica*, *Carica papaya*, and *Alternanthera philoxeroides*, rhizomes of *Curcuma longa*, peels of *Citrus sinensis*, and flowers of *Tagetes erecta*, were thoroughly washed with running water to remove dirt and foreign materials. The plant materials were

shade-dried and subsequently ground into fine powder using a mechanical grinder. The powdered materials were stored in airtight containers until extraction.

Aqueous extracts were prepared by the maceration method. Briefly, 130 g of each powdered plant material was soaked in distilled water (1:10 ratio) and kept at room temperature for five days with occasional stirring to facilitate the extraction of bioactive constituents. After the extraction period, the mixtures were filtered first through muslin cloth and then through Whatman No. 1 filter paper to remove plant residues. After proper drying the obtained extract were collected and stored in airtight containers at 2–8°C until further use in the formulation of polyherbal antimicrobial soaps.

2.4 Formulation of polyherbal soap

This experiment was carried out to prepare polyherbal antimicrobial soaps. First, all raw materials were weighed in accordance with the dosages specified in Table 2. Next, the glycerin soap base was weighed and placed in a 55–60°C water bath to melt. After the soap base melted completely, the temperature was lowered to approximately 40°C[25]. According to the formulation design, the necessary amounts of aqueous plant extracts—*Azadirachta indica, Carica papaya, Curcuma longa, Citrus sinensis, Tagetes erecta*, and *Alternanthera philoxeroides*—were added to the melted soap base[26]. After adding coconut oil, aloe vera gel, honey, and vitamin E, the mixture was constantly swirled with a glass rod until a uniform blend was achieved[27].

To avoid creating air bubbles, the mixture was gently poured into soap molds after being allowed to cool for a few minutes. The molds were left undisturbed for five to ten minutes before being chilled at 2 to 8°C to allow for complete solidification[25]. After a day, the soaps were removed from the molds and stored appropriately until further examination[28]. Using various plant extract combinations and concentrations, five polyherbal soap compositions (F1–F5) were created[29].

Table 2
Composition of polyherbal antimicrobial soap formulations

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
Glycerin soap base (g)	10.3	10.3	10.3	10.3	10.3
Coconut oil (mL)	0.9	0.9	0.9	0.9	0.9
Vitamin E (ml)	0.5	0.5	0.5	0.5	0.5
Honey (ml)	0.5	0.5	0.5	0.5	0.5
Aloe vera (ml)	0.8	0.8	0.8	0.8	0.8
Turmeric extract (g)	1.0	–	1.0	2.0	1.1
Neem extract (g)	2.0	2.0	1.0	–	1.1
Papaya extract (g)	2.0	1.0	1.0	–	1.1
Marigold extract (g)	1.0	2.0	2.0	1.5	1.1
Alternanthera philoxeroides extract (g)	1.0	–	–	2.0	1.5
Orange peel extract (g)	–	2.0	2.0	1.5	1.5
Total weight (g)	**20**	**20**	**20**	**20**	**20**

2.5 Animal Handling

Healthy Swiss albino mice were bred in the animal facility of the Department of Pharmacy Practice, Department of Pharmacy, USTC. The healthy mice weighed between 30–35 grams at the commencement of the experiment. The animals were all housed in the Animal House of the Department of Pharmacy, USTC, at an ambient temperature of 22 ± 5°C and a relative humidity of 50–70%. Light was provided for 12 hours to avoid circadian rhythm disturbance. Standard mice pellets and filtered water were given ad libitum throughout experimental time. The experimental protocol was approved by the Ethics Committee on Animal Research, Department of Pharmacy, USTC.

2.6 Evaluation of Herbal Soap

2.6.1 Physical Evaluation

2.6.1.1 Appearance and odor

The formulated soaps were evaluated for appearance, color, texture, and odor by visual inspection and sensory observation.

2.6.1.2 Weight variation

Weight variation was determined by individually weighing each soap formulation using a digital balance.

2.6.1.3 pH determination

The pH of the formulated soaps was determined using universal indicator paper.

2.6.1.4 Solubility test

Solubility was evaluated by dissolving 5 g of soap in 50 mL of distilled water and observing the extent of dissolution.

2.6.2 Foaming Ability and Stability

The cylinder shaking method was used to calculate the foaming capacity[25]. In a graduated cylinder, a predetermined volume of soap solution was shaken, and the initial foam height was noted. Foam height was measured at intervals of five, fifteen, and twenty-five minutes in order to evaluate foam stability.

2.6.3 Test for Skin Irritation (In Vivo)

A total 18 mice were used in skin irritation tests to assess the six test compositions' dermal safety (n = 3 per group)[26]. Soap samples were applied after a little portion of the dorsal skin was shaved. The test sites were checked for erythema, edema, or irritation at intervals of one, two, four, and twenty-four hours.

2.7 Determination of antimicrobial activity of formulated herbal soap-(MAAM)

The antimicrobial activity of the formulated polyherbal soap formulations was evaluated using the agar well diffusion method. *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were used as the test microorganisms. Mueller–Hinton Agar (MHA) was used for bacterial cultures, whereas Potato Dextrose Agar (PDA) was used for the culture of *Candida albicans*.

3. RESULT

3.1. Physical Evaluation of Polyherbal Soap

The physical characteristics like color, odor, texture, homogeneity and surface appearance of the formulated polyherbal soap were evaluated. All formulations exhibited a light brown color, a mild herbal odor, smooth texture, and uniform consistency without any visible phase separation. No cracks, fissures, or other surface defects were observed. In contrast, the soap base (F6) showed a yellowish-white appearance with an oily odor. The results of the physical evaluation are presented in Table 3 and Table 4.

Table 3
Physical characteristics of the formulated polyherbal soap formulations

Formulation	Color	Odor	Texture	Homogeneity	Surface appearance
F1	Light brown	Mild herbal	Smooth	Uniform	No visible cracks or defects
F2	Light brown	Mild herbal	Smooth	Uniform	No visible cracks or defects
F3	Light brown	Mild herbal	Smooth	Uniform	No visible cracks or defects
F4	Light brown	Mild herbal	Smooth	Uniform	No visible cracks or defects
F5	Light brown	Mild herbal	Smooth	Uniform	No visible cracks or defects
F6 (Base)	Yellowish white	Oily	Smooth	Uniform	No visible cracks or defects

Table 4
Evaluation parameters of the formulated polyherbal soap formulations

Formulation	Weight (g)	pH	Foam (mL)	Foam retention at 25 min (%)	Solubility (Normal water)	Solubility (Hot water)	Skin irritation
F1	20.7425	6.5	90	93.44	Soluble	Soluble	NIL
F2	20.7101	6.1	90	93.44	Soluble	Soluble	NIL
F3	20.6536	6.0	90	93.44	Soluble	Soluble	NIL
F4	20.4378	5.6	90	90.25	Soluble	Soluble	NIL
F5	20.2736	6.5	90	93.44	Soluble	Soluble	NIL

3.2. Antimicrobial Activity of Polyherbal Soap Formulations

The method of diffusion of agar wells was used to assess the antibacterial activity of the prepared polyherbal soaps against *Staphylococcus aureus, Escherichia coli, and Candida folliculitis.* After incubation, the zone of inhibition (ZOI) surrounding each well was measured to assess the antimicrobial efficacy. The positive control for comparison was a conventional antibacterial solution.

Table 5
Zone of inhibition of polyherbal soap formulations against tested microorganisms

Sample	*S. aureus* (mm)	*C. folliculitis* (mm)	*E. coli* (mm)
Standard	12	10	10
F1	13	8	Intermediate sensitive
F2	0	0	0
F3	10	9	8
F4	0	0	7
F5	Intermediate sensitive	0	0
F6 (Base)	0	0	0

Zones of inhibition (Table 5) against *S. aureus, C. folliculitis, and E. coli* were 12 mm, 10 mm, and 10 mm, respectively, in the normal preparation. Among the tested formulations, F1 demonstrated the highest antimicrobial activity, producing a zone of inhibition of 13 mm against *S. aureus* and 8 mm *against C. folliculitis*, while showing intermediate sensitivity against *E. coli*. F3 also exhibited notable antimicrobial activity with inhibition zones of 10 mm, 9 mm, and 8 mm against *S. aureus, C. folliculitis, and E. coli*, respectively.

F2, on the other hand, minimum antibacterial action against the microbes that were examined. F4 showed a 7 mm inhibitory zone against *E. coli* but was ineffective against *S.aureus and C. folliculitis.*F5 exhibited an intermediate sensitivity to *S.aureus* and no effectiveness against the other microorganisms. While the soap base (F6) showed no inhibitory action, the integrated plant extract were in charge of the observed antibacterial activities.

3.2.1. Comparison of Formulations Based on Antimicrobial Performance

The antibacterial activity of F1 and F3 was superior to that of the other five formulations.F1 showed the most activity, particularly against *S.aureus*, F3 had broad spectrum action against all tested pathogens. The synergistic effects of the added herbal extracts—neem, turmeric, papaya, marigold, orange peel, and *Alternanthera philoxeroides*—may be responsible for these formulations improved antibacterial activity. The results show that certain polyherbal combinations have promising antibacterial potential and may be further developed as natural antimicrobial cleansing solutions, despite the mild observed zones of inhibition.

4. DISCUSSION

Because of growing worries about germ resistance and the negative consequences of synthetic antimicrobial agents, herbal antimicrobial soaps have drawn a lot of attention as natural substitutes for traditional cleaning solutions[32]. Flavonoids, phenolics, tannins, alkaloids, and essential oils are just a few of the many bioactive substances found in medicinal plants that support their antibacterial, antioxidant, and anti-inflammatory properties[33, 34]. Maintaining skin hygiene and preventing microbial infections may be made more efficient and environmentally friendly by adding such plant-derived chemicals to soap compositions[34].

The present study was undertaken to formulate and evaluate a polyherbal antimicrobial soap using selected medicinal plants, namely *Azadirachta indica*, *Carica papaya*, *Curcuma longa*, *Tagetes erecta*, *Citrus sinensis*, and *Alternanthera philoxeroides*. Five formulations (F1–F5) were prepared and assessed for their physicochemical characteristics, antimicrobial activity, and skin compatibility. The results demonstrated that all formulations possessed acceptable physical properties and exhibited varying degrees of antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida* spp.

According to earlier reports, the chosen plants have strong antibacterial properties. The well-known antibacterial and antifungal properties of *A. indica* (neem) are ascribed to bioactive components such azadirachtin, nimbin, and nimbidol. Alkaloids, flavonoids, and papain found in *C. papaya* have antibacterial and wound-healing qualities. Curcumin, a substance with broad-spectrum antibacterial and anti-inflammatory properties, is abundant in *C. longa*, or turmeric. While *C. sinensis* peel includes phenolic compounds and essential oils that prevent microbial growth, *T. erecta* has flavonoids and carotenoids with antibacterial activity. *A. philoxeroides* has also been shown to have antioxidant and antibacterial properties. Therefore, it was anticipated that adding these therapeutic plants to a single formulation would increase the soap's antibacterial effectiveness.

Out of the five tested batches, F1 and F3 were the most effective formulations, according to the antimicrobial evaluation. With a zone of inhibition of 13 mm, F1 showed the greatest activity against *S. aureus*, whereas F3 displayed zones of inhibition of 10 mm against *S. aureus*, 9 mm against *Candida spp*., and 8 mm against *E. coli*. F4 and F5, on the other hand, showed minimal inhibitory effects, while F2 showed no discernible antibacterial action. The findings showed that the antibacterial efficacy of the formulations was significantly impacted by the combination and quantity of plant extracts used. Previous research has demonstrated comparable outcomes, with herbal medicines comprising citrus, turmeric, and neem extracts exhibiting higher antibacterial action against common skin diseases[35].

The enhanced performance of F1 and F3 could be attributed to the synergistic interaction between the phytoconstituents in the combined plant extracts[36, 37]. Synergism is the result of multiple bioactive chemicals working together to have a greater antibacterial effect than when they work alone. The addition of flavonoids, phenolics, terpenoids, alkaloids, and essential oils from different plant sources may have contributed to the formulations' increased inhibition of microbial growth.

The physicochemical analysis showed that the soaps' quality was satisfactory. With weights ranging from 20.27 g to 20.74 g, the variation results showed good uniformity among all formulations, indicating consistent molding and mixing procedures. The pH values, which varied from 5.6 to 6.5, are thought to be appropriate for topical administration with little chance of irritation because they are near the natural pH of human skin [30]. Herbal soaps that are commercially acceptable have been reported to have similar pH ranges.

Consumer perception of soap products is greatly influenced by foam retention and foamability[38]. All formulations showed excellent cleaning efficacy and stability after 25 minutes, maintaining over 90% of their foam volume and producing a reasonable foam value[38].The slight drop in foam retention seen in F4maybe related to variations in the integrated extract's phytochemical interaction.

According to solubility research, all formulations readily dissolved in water, showing sufficient saponification and uniform dispersion of herbal elements throughout the soap matrix. Good solubility promotes both the release of bioactive compounds during use and an efficient cleaning effect.

Throughout the observation period,The study found no evidence of erythema, edema or irritation in the mice. This results confirm the acceptability of the chosen plant extracts for cosmetic and personal care and show that the produced polyherbal soaps are safe for topical use[39].

Overall, the stydy's findings show that polyherbal soap formulations particularly F1 and F3, exhibit potential antibacterial activity, acceptable physicochemical characteristics, and skin compatibility. The result show that combining extracts from therapeutic plants can results in safe effective and eco-friendly antimicrobial washing products. Further study involving stability testing, extract concentration optimization, and testing against a broader range of microorganisms maybe beneficial for the development of commercially viable polyherbal antimicrobial soaps.

5. CONCLUSION

The antimicrobial activity of five polyherbal soap formulations (F1–F5) containing aqueous extracts of *Azadirachta indica*, *Carica papaya*, *Curcuma longa*, *Citrus sinensis*, *Tagetes erecta*, and *Alternanthera philoxeroides* was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida* species. Among the tested formulations, F1 and F3 exhibited superior antimicrobial activity, with F1 showing the highest inhibition against *S. aureus* and F3 demonstrating activity against all tested microorganisms. The formulated soaps possessed acceptable physicochemical characteristics, including suitable pH, satisfactory foamability and foam retention, good solubility, uniform appearance, and absence of skin irritation in mice. The enhanced antimicrobial performance observed in F1 and F3 may be attributed to the synergistic effects of the combined medicinal plant extracts. In conclusion, the selected polyherbal formulations demonstrated promising antimicrobial activity together with favorable physicochemical properties, indicating their potential for the development of safe, effective, and eco-friendly antimicrobial

cleansing products. For the formulated polyherbal soap to be produced and sold on a large scale, additional standardization, stability research, and safety assessments would be helpful.

Declarations

Author contribution

Conceptualization, Ahmed, A. and Mysha, R. Y.; **Methodology**, B, Adita, Ahmed, A.; **Validation**, Ahmed, A. and Mysha, R. Y.; **Formal Analysis**, B, Adita, Mysha, R. Y. and Ara, J.; **Investigation**, Dey, B. Jiko, P.A. & Khan, A.A.; **Resources**, B, Adita.; **Data curation**, Chowdhury, U.T., Chowdhury, R.; **Writing – original draft preparation**, B, Adita., Chowdhury, R.; **Writing – review and editing**, Mysha, R. Y. and Jiko, P.A.; **Supervision**, Ahmed, A.; Ara, J. and Mysha, R. Y.

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Ethics approval and Consent to Participate

The proposal of the study was submitted and approved by the ethical review committee of the Department of Pharmacy, Faculty of Pharmaceutical Science and Technology, University of Science and Technology Chittagong (USTC) before the commencement of the study. A letter of clearance was obtained with reference number IERC/USTC/26/011, dated on 02nd February 2026. In addition, all possible steps were taken to avoid animal suffering during the experiment. However, no consent was needed for this study.

Data Sharing Statement

The majority of the data are included in the manuscript. Further data can be found from the corresponding author based on reasonable request.

Disclosure

The authors declare that they have no conflicts of interest for this work.

References

1. Urban K, Chu S, Scheufele C, Giesey RL, Mehrmal S, Uppal P et al (2020) The global, regional, and national burden of fungal skin diseases in 195 countries and territories: A cross-sectional analysis

from the Global Burden of Disease Study 2017. JAAD Int 2:22–27. 10.1016/j.jdin.2020.10.003 PubMed PMID: 34409349; PubMed Central PMCID: PMC8362308

2. Caioni G, Benedetti E, Perugini M, Amorena M, Merola C (2023) Personal Care Products as a Contributing Factor to Antimicrobial Resistance: Current State and Novel Approach to Investigation. Antibiotics 12(4):724. 10.3390/antibiotics12040724 PubMed PMID: 37107085; PubMed Central PMCID: PMC10135053
3. Alhodieb FS, Farid M, Sabir M, Nisa S, Sarwar S, Abbas S (2025) Exploring the bioactive compounds of Carica papaya leaves: phytol's role in combatting antibiotic-resistant bacteria. Front Cell Infect Microbiol 15:1564787. 10.3389/fcimb.2025.1564787 PubMed PMID: 40692687; PubMed Central PMCID: PMC12277969
4. Brar GS, Nandy SK, Sharma A, Siddiqui AJ, Sharma L (2025) Antimicrobial and Anti-Infective Potential of Herbal Creams in Dermatology: Efficacy, Safety, and Challenges in Skin Infection Management. Infect Drug Resist 18:6289–6311. 10.2147 /IDR.S565852 PubMed PMID: 41362433; PubMed Central PMCID: PMC12682302
5. Rahman AHMM, Asha NA (2021) A Survey of Medicinal Plants Used by Folk Medicinal Practitioners in Daulatpur Upazila of Kushtia District, Bangladesh. Res Plant Sci 9(1):1–6. 10.12691/plant-9-1-1
6. An Overview of Antibacterial and Antifungal effects of Azadirachta indica Crude Extract A Narrative Review – Biomedical and Pharmacology Journal [Internet]. [cited 2026 Jun 15]. Available from: https://biomedpharmajournal.org/vol15no1/an-overview-of-antibacterial-and-antifungal-effects-of-azadirachta-indica-crude-extract-a-narrative-review/
7. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U (2002) Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci 82(11):1336–1345
8. Ojimelukwe P, Eji C (2012) Chemical composition of leaves, fruit pulp and seeds in some Carica papaya (L) morphotypes. Int J Med Aromat Plants. ;2
9. Kong YR, Jong YX, Balakrishnan M, Bok ZK, Weng JKK, Tay KC et al (2021) Beneficial Role of Carica papaya Extracts and Phytochemicals on Oxidative Stress and Related Diseases: A Mini Review. Biology 10(4):287. 10.3390/biology10040287 PubMed PMID: 33916114; PubMed Central PMCID: PMC8066973
10. Fuloria S, Mehta J, Chandel A, Sekar M, Rani NNIM, Begum MY et al (2022) A Comprehensive Review on the Therapeutic Potential of Curcuma longa Linn. in Relation to its Major Active Constituent Curcumin. Front Pharmacol 13:820806. 10.3389/fphar.2022.820806 PubMed PMID: 35401176; PubMed Central PMCID: PMC8990857
11. Tejada S, Manayi A, Daglia M, Nabavi SF, Sureda A, Hajheydari Z et al (2016) Wound Healing Effects of Curcumin: A Short Review. Curr Pharm Biotechnol 17(11):1002–1007 doi:10.2174/1389201017666160721123109 PubMed PMID: 27640646
12. Dongre P, Doifode C, Choudhary S, Sharma N (2023) Botanical description, chemical composition, traditional uses and pharmacology of *Citrus sinensis*: An updated review. Pharmacol Res - Mod Chin

Med 8:100272. 10.1016/j.prmcm.2023.100272

13. Singh P, Mishra D, Singh S, THERAPEUTIC BENEFITS, AND PROCESSING OF MARIGOLD (TAGETES SPECIES) (2024) A REVIEW. Indian J Health Care Med Pharm Pract 5:148–166. 10.59551/IJHMP/25832069/2024.5.1.190
14. Bhattacherjee A, Ghosh T, Sil R, Datta A (2014) Isolation and characterisation of methanol-soluble fraction of Alternanthera philoxeroides (Mart.) - evaluation of their antioxidant, α-glucosidase inhibitory and antimicrobial activity in in vitro systems. Nat Prod Res 28(23):2199–2202. 10.1080/14786419.2014.930857 PubMed PMID: 24956019
15. Manwar GG A Review on Formulation and Evaluation of Polyherbal Soap. Int J Res Publ Rev 10.55248/GENGPI.4.1023.102712
16. Tufail T, Bader Ul Ain H, Ijaz A, Nasir MA, Ikram A, Noreen S et al (2025) Neem (Azadirachta indica): A Miracle Herb; Panacea for All Ailments. Food Sci Nutr 13(9):e70820. 10.1002/fsn370820 PubMed PMID: 40901661; PubMed Central PMCID: PMC12400164
17. Kong YR, Jong YX, Balakrishnan M, Bok ZK, Weng JKK, Tay KC et al (2021) Beneficial role of *Carica papaya* extracts and phytochemicals on oxidative stress and related diseases: a review. Biology (Basel) 10(4):287. 10.3390/biology10040287. PMID: 33916114; PMCID: PMC8066973
18. Al-Snafi AE (2023) Phytochemistry and biological activities of *Citrus sinensis* and *Citrus limon*: an update. J Herb Med 41:100737. 10.1016/j.hermed.2023.100737
19. Yuan H, Ma Q, Ye L, Piao G (2016) The Traditional Medicine and Modern Medicine from Natural Products. Molecules 21(5):559 doi:10.3390/molecules21050559 PubMed PMID: 27136524; PubMed Central PMCID: PMC6273146
20. Menon VP, Sudheer AR (2007) Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol 595:105–125. 10.1007/978-0-387-46401-5_3 PubMed PMID: 17569207
21. Benedetti P, Femminella M, Reali G, Steenhaut K (2021) Experimental Analysis of the Application of Serverless Computing to IoT Platforms. Sensors 21(3):928 doi:10.3390/s21030928 PubMed PMID: 33573209; PubMed Central PMCID: PMC7866535
22. DebMandal M, Mandal S (2011) Coconut (Cocos nucifera L.: Arecaceae): In health promotion and disease prevention. Asian Pac J Trop Med 4(3):241–247. 10.1016/S1995-7645(11)60078-3
23. Mandal MD, Mandal S (2011) Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 1(2):154–160. 10.1016/S2221-1691)60016-6 PubMed PMID: 23569748; PubMed Central PMCID: PMC3609166
24. Thiele JJ, Ekanayake-Mudiyanselage S Vitamin E in human skin: Organ-specific physiology and considerations for its use in dermatology. Mol Aspects Med. 2007;Vitamin E: An Overview of Major Research Directions28(5):646–67. 10.1016/j.mam.2007.06.001
25. Kumar A, N AVAK, FR KVTS (2025) Formulation and evaluation of polyherbal soap. J Pharm Biol Res 13(1):40–43. 10.30904/j.jpbr.2025.4847
26. Mendake RA, Amalkar SV, Hatwar PR, Bakal RL (2024) Formulation and Evaluation of Polyherbal Soap. J Drug Deliv Ther 14(11):99–104. 10.22270/jddt.v14.i11.6892

27. K SP GM (2025) Formulation and Evaluation of Polyherbal Soap. J Pharm Biol Res 13(1):40–43. 10.30904/j.jpbr.2025.4847
28. Mendake R, Amalkar S, Hatwar P, Bakal R (2024) Formulation and Evaluation of Polyherbal Soap. J Drug Deliv Ther 14:99–104. 10.22270/jddt.v14.i11.6892
29. Igbeneghu OA (2013) The Antimicrobial Assessment of Some Nigerian Herbal Soap. Afr J Tradit Complement Altern Med 10(6):513–518. 10.4314/ajtcam.v10i621 PubMed PMID: 24311879; PubMed Central PMCID: PMC3847394
30. Das S, Agarwal S, Samanta S, Kumari M, Das R (2024) Formulation and evaluation of herbal soap. J Pharmacogn Phytochem 13(4):14–19. 10.22271/phyto.2024.v13.i4a.14990
31. Acute skin Irritation-corrosion test. OECD 404 (2015) - IVAMI [Internet]. [cited 2026 Jun 15]. Available from: https://www.ivami.com/en/biocidal-activities-and-toxicology-with-disinfectants-25-accredited-tests/4810-b-irritation-corrosion-acute-dermal-b-epa-870-2500-oppts-1998-oecd-404-2015-directive-92-69-eec-of-31-july-1992-section-b-4-acute-skin-irritation-corrosion
32. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564–582. 10.1128/CMR.12.4.564 PubMed PMID: 10515903; PubMed Central PMCID: PMC88925
33. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MdelM, Segura-Carretero A et al (2018) Thymol, thyme, and other plant sources: Health and potential uses. Phytother Res 32(9):1688–1706. 10.1002/ptr.6109
34. Ghosh AK, Banerjee S, Mullick HI, Banerjee J (2011) Zingiber officinale: A natural gold. Int J Pharma Bio Sci 2:283–294
35. Gohane T, Gupta A, Patel M, Shewale V, Mahajan V, Salunke S et al (2025) Formulation and evaluation of herbal soap. Biochem Cell Arch 25:2351–2359. 10.51470/BCA.2025.25.2.2351
36. Wagner H, Ulrich-Merzenich G (2009) Synergy research: Approaching a new generation of phytopharmaceuticals. Phytomedicine 16(2):97–110. 10.1016/j.phymed.2008.12.018
37. Caesar LK, Cech NB (2019) Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat Prod Rep 36(6):869–888. 10.1039/c9np00011a PubMed PMID: 31187844; PubMed Central PMCID: PMC6820002
38. sonar S, Patil V, P.Koli D, Jaiswal A, G.Khond K, D.Ghogare T et al (2026) Preparation and Evaluation of Herbal Soap Films for Hygiene Applications. Int J Sci Strateg Manag Technol 02:1–9. 10.55041/ijsmt.v2i4.502
39. OECD (2015) Test 404: Acute Dermal Irritation/Corrosion. OECD Guidel Test Chem Sect 4. 10.1787/9789264242678-en