Formulation and Evaluation of Antimicrobial Potential of Herbal Cream Containing Moringa Leaf and Pomegranate Peel

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Abstract: **Moringa oleifera and pomegranate peel is considered a versatile plant. It has been shown that antimicrobial properties, anti-inflammatory, anti-oxidant properties exist exist in leaves, stem bark, root bark, cotyledon seeds, and seed coats. These antimicrobial properties are believed to be due to secondary metabolites such as alkaloids, flavonoids, glycosides, terpenoids, and sterols. Acne is a common skin condition`belived to be caused by the build-up of dead skin cells, bacteria, and dried sebum that blocks the hairs follicles in the skin. This study aims to evaluate the anti-microbial activity of hydroalcoholic extract of moringa oleifera leaves and pomegranate peel with concentration of 5%, 10%,15% and 20% formulated into topical cream preparations against Staphylococus epidermidis. The moringa leaves powder were assemble in soxhlet apparatus for extraction for 24 hrs in 70% ethanol and pomegranate peel powder were assemble in soxhlet apparatus for extraction for 48 hrs in 50% ethanol. Four extract concentration were prepared: 5%, 10%, 15% and 20%. The cream was physically evaluated for its organoleptic characteristics, homogeneity, pH, and adhesion. Also the anti- microbial activity tests of moringa leaves and pomegranate peel extracts revealed that formulas 1,2,3 and 4 had inhibition zones between 6.12mm, 7.98 mm, and 8.35 mm, respectively. In conclusion, the formulations containing 5%, 10%, 15% and 20% moringa leaf and pomegranate peel extract showed anti- microbial activity against the test organism and normal range for organoleptic, homogeneity and viscocity test, pH and adhesion tests.**

Key words: **secondary metabolites anti-microbial, anti-inflammatory, anti-oxidant, anti-aging, organoleptic characteristics, inhibition zones.**

INTRODUCTION

Moringa Oleifera

Moringa oleifera belonging to the family Moringaceae is a tree distributed throughout India. It is commonly called as drumstick tree which is widely used in culinary and herbal medicine preparations. *Moringa oleifera* is considered a versatile plant Numerous studies have investigated the cosmeceutical properties of medicinal plants that grow in various climatic zones. Phenolic-rich plantextracts can be formulated into cosmetic cream products, significantlyenhancing their commercial value. It is possible to prepare herbalextracts from various plant parts and integrate them into different skincare cosmetic creams, lotions and ointments.*Moringa oleifera* leaves are particularly rich in phytochemicals such as flavonoids, phenolic acids, and tannins, which contribute to its therapeutic potential. Previous studies have demonstrated the efficacy of *Moringa oleifera* extracts in inhibiting bacterial growth and reducing inflammation, suggesting its potential application in acne treatment. However, the formulation of an effective and user-friendly topical delivery system remains a challenge.

Fig.1: *Moringa oleifera* leaf

Moringa oleifera, a fast-growing tree, typically reaches 10-12 meters in height, featuring a spreading crown, feathery tripinnate leaves, and fragrant, yellowish-white flowers in drooping clusters. Its fruits are three-sided pods, and seeds are dark brown with papery wings. [1]

Table 1: Conventional use of moringa oliefera [2]

Part of moringa tree	Conventional uses
leaves	Used as vegetables; rich in vitamins,minerals and amino acids.

flowers	Consumed as vegetables; used for traditional medicinal purpose.
Fresh pods	Eaten as vegetables; use in various culinary dishes.
seeds	Extracted oil used for cooking, cosmetics, and lubrication; seeds used for water purification.
Bark	Traditional medicine for treating ailments such as a diabetes, cardiovascular disease and inflammation.
roots	Used in traditional medicines; possess antibiotics and antifungal properties.
gum	Employed as a thickening agent in foods; used in traditional medicines.
Whole tree	Enhances food security, nutrition and rural development.
Livestock feed	Leaves and pods used as feed; improves animal health and productivity.

Mechanism of Action of *Moringa oleifera*

- Antimicrobial Activity: Moringa extracts have been shown to have antimicrobial properties, which can help in reducing the proliferation of acne-causing bacteria like Propioni bacterium acnes.
- Anti-Inflammatory Properties: The anti-inflammatory compounds in Moringa can reduce redness and swelling associated with acne.
- Antioxidant Effects: Antioxidants in Moringa help to neutralize free radicals, reducing oxidative stress and preventing skin damage.
- Moisturizing and Healing: The hydrogel base often contains moisturizing agents that help keep the skin hydrated and promote healing. [3]

Pomegranate

Pomegranate, belonging to the Punica L. genus, Punicaceae family, originated in Iran, India, China, and the Mediterranean region in 3000 B.C In general, the contents of bioactive compounds in peels tend tobe higher than in edible parts. It is also worth notingthat complex bioactive compounds in pomegranate peel oftenexist in the form of a mixture, so the synergistic effect of different compounds can produce a variety of physiological activities. Numerous phytochemicals, including catechins, flavonoids,tannins, gallic acids, ellagic acid, and anthocyanins, have beenassociated with the medicinal potential of pomegranate peel.

Fig.2: Pomegranate peel

Pomegranate plants are shrubs or small trees with evergreen leaves, and the fruit is a berry with a leathery, red rind, containing numerous arils (fleshy seeds) and a juicy, edible pulp.

Mechanism of Action

Polyphenols: These compounds, including tannins, flavonoids, and anthocyanins, are the primary drivers of pomegranate peel's beneficial effects.

Antioxidant Activity: Polyphenols act as antioxidants by scavenging free radicals and preventing oxidative stress, which is a major factor in the development of various diseases.

Anti-inflammatory Effects: Pomegranate peel extracts have shown anti-inflammatory properties by modulating inflammatory pathways, potentially reducing inflammation in the body.

pomegranate peel, like ellagic acid and punicalagin, have demonstrated antibacterial and antifungal properties, potentially inhibiting the growth of harmful microorganisms.

Anticancer activity: Pomegranate peel extract has shown anticancer activity by inducing apoptosis and inhibiting cell proliferation. [4]

MATERIALS AND METHODS

EquipmentGlassware, Rotary evaporator, Ph meter, Viscometer, Incubator, Autoclave, Supporting tools.

Materials

Moringa leaf, Pomegranate peel, Beeswax, Liquid paraffin, Borax, Nutrition agar, Propyl paraben, 70% ethanol, Distilled water.[6]

Methods:

Extraction of moringa leaves :- Moringa oleifera powder was extracted using the soxhlet apparatus method with 70% alcohol as a solvent. 50 grams of moringa leaf powder was added to the soxhlet apparatus with 200 ml of 70% ethanol, stirred and sealed. The solution was left for 24 hrs and shaken occasionally three times.[6]

Fig 3: Soxhlet Method of Extraction (*Moringa)*

Extraction of pomegranate peel :- Pomegranate peel powder was extracted using the soxhlet method with 50% ethanol as the solvent. 50 grams of pomegranate peel powder was added to a soxhlet apparatus with 200 ml of 50% ethanol, stirred and sealed. The solution was left for 48hrs and shaken occasionally three times.[7]

Fig 4: Soxhlet Method of Extraction (Pomegranate)

Formulation design of anti acne cream with moringa oleifera leaves and pomegranate peel :

F1: Anti acne cream formula with 5% hydroalcoholic extract of moringa leaves and pomegranate peel.

F2: Anti acne cream formula with 10% hydroalcoholic extract of moringa leaves and pomegranate peel.

F3: Anti acne cream formula with 15% hydroalcoholic extract of moringa leaves and pomegranate peel.

F4: Anti acne cream formula with 20% hydroalcoholic extract of moringa leaves and pomegranate peel.[8]

Table 2: Cream formula of hydroalcoholic extract of moringa oliefera leaves and pomegranate peel.

Ingredient	F1(g)	F2(g)	F3(g)	F4(g)
Moringa leaves extract	1	1.5	2	2.5
Pomegranate peel extract	1	1.5	2	2.5
Bees wax	5	5	5	5
Liquid paraffin	7	7	7	7
Borax	2	2	2	2
Distilled water	6	6	6	6
Propylene paraben	0.02	0.02	0.02	0.02

The formulation of the anti- acne cream with *Moringaoliefera* leaves and pomegranate peel.

Prepration: melt bees wax in liquid paraffin(oil phase) in china disc and in another beaker melt borax in distilled water(water phase).

Mixing: slowly adding mixture of borax and distilled water (water phase) in a mixture of bees wax and liquid paraffin (oil phase).

Final step: adding the hydroalcoholic extract of moringa oleifera leaves and pomegranate peel to the water in oil cream and stirring until homogeneous.[9]

Fig 5: Final product

PHYSICAL EVALUATION OF ANTI-ACNE CREAM:

Organoleptic Test

This test involves describing the cream shape, color, smell, and texture.

pH Test

The pH test aimed to ensure the safety of the cream for skin application, as skin pH ranges from 4.5 to 6.5. The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50 ml of distilled water and its pH was measured. Initial pH values were 5.83 for Formula 1, 5.75 for Formula 2, and 5.72 for Formula 3, all within the acceptable range for skin pH, indicating their safety for use.

Viscosity Test

Viscosity measures the resistance of a liquid to flow. In the initial week, Formula 1 had a viscosity of 600 dPa·s (60 Pas), Formula 2 had 700 dPa·s (70 Pas), and Formula 3 had 800 dPa·s (80 Pas). However, Formulas 2 and 3 experienced decreased viscosity due to the slightly acidic pH of the Moringa leaves hydroalcoholic extract. Viscosity of the formulation was determined using Brookfield Viscometer at 100 rpm, using spindle no 7. [10]

Adhesion Test

The attachment test assesses how well the cream sticks to the skin after some time, influencing the arrival of dynamic substances.

Spreadability Test

Creams are expected to spread easily on the skin without requiring significant pressure, ensuring even distribution. Good spreadability typically ranges between 5 to 7 cm. In the initial week, Formula 1 demonstrated good spreadability with a value of 5.00 cm, while Formulas 2 and 3 did not meet the criteria, showing values of 4.70 cm and 4.50 cm, respectively.

Stability study

Acne cream was kept for two months at three different temperatures: 8°C, 27°C, and 40°C for the stability research.

Homogeneity

The formulations were tested for homogeneity by visual appearance and by touch. The cream sample (500 mg) was dispersed on a microscopic and examined under a microscope to determine if the cream preparation was homogeneous.

Irritancy test

The cream was applied to the specified area and time was noted. Irritancy, erythema, and edema, were checked if any for regular intervals up to 24 h and reported.[11]

RESULT

(A) PHYSIOCHEMICAL PARAMETER OF CREAM

Summarizes the initial week's physical evalution of the cream derived from[12]

Paramerter	Observation	F1	F2	F3
Organoleptic	colour	+	+	+
	smell	+	+	+
	consistency	++	+	++
Homogeneity	Homogen	Homogen	Homogen	Homogen
pH		6.2	6.2	6.2
Viscosity		600	700	800
Adhesive(sec)		6.36	4.43	3.78
Spreadability(cm)		5.00	4.70	4.50

Records the changes in organoleptic properties of the cream over a 4-week period[13]

Observation parameter	Formula	0	1	2	3	4
color	F1	Stable color	Stable color	Stable color	Stable color	Stable color
	F2	Stable color	Stable color	Slight change	Slight change	Slight change
	F3	Stable color	Stable color	Slightly change	Slightly change	Slightly change
smell	F1	consistent	consistent	consistent	consistent	consistent
	F2	consistent	consistent	consistent	consistent	consistent
	F3	consistent	consistent	consistent	consistent	consistent
consistency	F1	Homogen	Homogen	Homogen	Homogen	Homogen
	F2	Homogen	Homogen	Slightly	Slightly	Slightly

				varied	varied	varied
	F3	Homogen	Homogen	Slightly varied	Slightly varied	Slightly varied

(B) PHYTOCHEMICAL DETECTION

Phytochemical detection was carried out to know the group of active compounds into hydroalcoholic extract of Moringa leaves and pomegranate peel.[14]

Sr.no	Test	Observation	Inference	
			Moringa leaf extract	Pomegranate peel extract
1	Wagner's test	Brown precipitate	+	+
2	Saponin test	Formation of foam	+	+
3	Hager's test	Bright yellow precipitate	+	+
4	Ferric chloride test	Blue color	+	+
5	Benedict's test	Reddish brown color	+	+
6	Sodium hydroxide test	Shows precipitation	+	+
7	Ninhydrin test	Violet precipitate	-	+
8	Shinoda test	Yellow color	+	+

(C) MICROBIAL ASSAY OF CREAM

An antimicrobial assay for an anti-acne cream assesses its ability to inhibit or kill bacteria associated with acne, primarily Propionibacterium acnes (now known as Cutibacterium acnes). [15]

Antimicrobial activity of hydroalcoholic extract was determined by cup plate method

Sterilization of Petri plates and Nutrient agar in autoclave at 121°C for 15 min, Allow the sterile molten nutrient agar to cool and then inoculate test microorganism, Pour agar into the plates and allow to solidify then Place a sterile well on agar plate with the help of cork borer.

Add dilutions to the wells of test sample, Incubate the plates for 24-48 hours at appropriate temperature, Measure the zone of inhibition.[16]

Formula 1,2 & 3 in this study show inhibition zones measuring 6.12mm, 7.98mm and 8.35mm, respectively (figure.7). An inhibition zone diameter of 28.10mm was observed in the positive control, indicating significant inhibitory activity, while no inhibition zone was observed in negative control. At concentration of 5%, 10% & 15%, moringa hydroalcoholic extract was tested for its ability to inhibit e coli bacteria growth. Comparatively, formula 3 produced the highest inhibition zone with a 15% concentration of moringa extract compared to the other formulas.

Fig.6: Inhibition zone of control positive (A), Control negative, Formula 1 (F1), Formula 2(F2) and Formula 3 (F3) against E-coli bacteria.

DISCUSSION

Moringa leaves were maserated using polar solvent to extract polar molecule (saponin, tannin, and flavonoid). Hydroalcoholic extract of Moringa leaves were brownish-green, smells herbally, and has very viscous consistency. Sample were produced 135.96 g (22.66%) of extract.

Pomegranate peel extract was active and effective against the growth of tested microorganisms. Whereas, the inhibition zones ranged from 9.6 to 25.7 mm depend on type of microorganism. These

results provide evidence for the presence of antimicrobial phenolic compounds in PPE.

CONCLUSION

In conclusion, the moringa leaf and pomegranate peel extracts all had valuable components for use in cosmetics. The moringa leaf and pomegranate peel extract showed anti-inflammatory, anti-aging, and skin-brightening, and anti-oxidant properties. There has been evidence that moringa leaves and pomegranate peel extract is effective against Staphylococcus epidermidis when applied as a topical. Furthermore, the formulations containing 5%, 10%, and 15% moringa and pomegranate peel extract also showed moderate antibacterial activity, homogeneity, viscosity, pH, and adhesion tests were within normal limits. The combination cream preparation of Moringa leaf and pomegranate peel extract has a synergistic effect in inhibiting acne bacteria. Poly herbal anti acne cream was developed and characterized under stability study. A formulated polyherbal cream containing extract of moringa leaf and pomegranate peel, bees wax, liquid paraffin, borax, distilled water, propyl paraben and perfume. This formulation are more acceptable in belief that it is safer than synthetic others. Because of no more chemical are used in it except propyl paraben.

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