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Hydration evaluation of a controlled-release Herbal gel formulation containing *Imperata cylindrica* and *Portulaca oleracea* using an artificial skin model

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ABSTRACT

The common dermatological issue is skin dehydration caused by low moisture retention, damaging the skin barrier. Traditional methods of moisturizing formulations depend on human or animal studies, which raises ethical issues and expenses. The present study aims to develop and study a collagen-gelatin artificial skin model and the hydration efficacy of an herbal gel made with leaf extract from *Portulaca oleracea (purslane)* and root extract from *Imperata cylindrica* (cogon grass). Artificial skin membranes (F1 and F2) were made from collagen and gelatin respectively, with glycerine as a plasticizer and glutaraldehyde as a cross-linking agent. Among these, formulation F2 showed high structural integrity due to the increased concentration of gelatin and was taken as the optimized membrane. The optimized artificial skin demonstrated an average thickness of 1.08 mm, with pH of 6.8, indicating compatibility with physiological skin conditions. Dryness was successfully induced by treating the membrane with 1% sodium dodecyl sulfate for 30 minutes. Herbal gel formulations (G1, G2, and G3) were made with Carbopol as gelling agent and was tested for physicochemical conditions. Among three formulations, G3 had optimized performance, with high moisture uptake, reduced water loss, and prolonged moisture retention. Overall, the developed artificial skin model proved to be reliable, cost-effective, and ethically acceptable in vitro method for preliminary screening of topical moisturizing formulations.

Keywords: Artificial skin model, Controlled release, *Imperata cylindrica*, *Portulaca oleracea,* Skin hydration.

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INTRODUCTION

The most significant organ in humans and the first line of defence is the skin (1). Limiting water evaporation from the body is skin's primary functions (2). Adequate skin hydration is essential to preserve barrier integrity and prevent conditions like xerosis (3). Moisturizers make the skin smooth by increasing the water content. Depending on their mechanism, they may act as humectants or emollients to restore moisture balance (4). Hydrogels and organogels with their smooth texture, non-greasy feel, and compatibility with skin secretions make them ideal for topical use (5). The essential formulation ingredients are gelling agents, permeation enhancers, and preservatives (6). Herbal formulations derived from traditional medicine, containing plant-based bioactive compounds offer a promising approach (7). *Purslane (Portulaca oleracea)* an incredible herb with medicinal properties containing various phytochemicals have pharmacological effects, including anti-inflammatory, wound-healing properties (8). Cogon grass (*Imperata cylindrica*) is a perennial grass belonging to the family Poaceae, and the rhizome-root portion is used for skin infections (9).

Though the interest is growing on plant-based skincare products, the development of scientifically validated herbal moisturizing formulations remains limited. Many commercial products depend on synthetic materials to enhance their properties that may not provide sustained hydration or cause irritation on sensitive skin. Thus, Medicinal plants with traditional dermatological uses are gaining attention as strong alternatives. *Imperata cylindrica* and *Portulaca oleracea* are known for their hydrating and soothing properties, preparation of stable topical gel formulations with them and evaluating their moisturizing performance is not explored. Furthermore, reliable in vitro models simulating skin barrier disruption and allow testing of the hydrating efficacy of herbal formulations are still limited. This provides the need for the development and evaluation of plant-based gel formulations using suitable models.

MATERIALS AND METHOD

The root extract of *Imperata cylindrica* and Flavonoid-rich *Portulaca oleracea* leaf extract were utilized (8,9). Carbopol, was utilized as a gelling agent (10). Glycerol was a humectant to increase hydration (11). As a soothing foundation, aloe vera gel was utilized (12). As an antioxidant, vitamin E was utilized to stop oxidative deterioration (13). Phenoxyethanol as a preservative inhibits microbial contamination (14). Triethanolamine, a pH-adjusting and neutralizing agent (15). Formulation was done using purified water (16). Gelatin served as the matrix-forming polymer in constructing artificial skin model, while collagen was integrated to replicate dermal architecture (17). The protein matrix was preserved by cross-linking agent

glutaraldehyde (18), Glycerol was used as a plasticizer to improve the flexibility of the membrane. Sodium dodecyl sulfate was utilized to cause dryness **(19).** All materials used were of analytical grade.

METHOD

Imperata Cylindrica Root Aqueous Extract

The roots of *Imperata Cylindrica* were properly cleaned. One-gram was added to 100mL of distilled water, stirred for half an hour at room temperature and filtered through muslin cloth and Whatman filter paper. The clear filtrate was used as an aqueous extract.

Portulaca oleracea Aqueous Extract

Clean *Portulaca oleracea* leaves were chopped into small pieces for crushing and combined with distilled water and centrifuged. Aqueous extract was obtained from the clear supernatant**.**

Sodium Dodecyl Sulfate Solution

1% of sodium dodecyl sulfate solution was prepared.

Preparation of Artificial Skin

According to Table 1 gelatin was dissolved in distilled water and heated until it gets completely soluble. Collagen was separately dissolved in water to prevent from air bubbles and slowly added to the gelatin solution with constant stirring. Then it is cooled and glycerin was added to the solution. Two to three drops of glutaraldehyde was added, stirred thoroughly and poured onto the mold and placed in refrigerator for 24hrs. After gelation, the developed artificial skin patch was removed from the mold.

Table 1: Formulation of Artificial Skin

S. No	Ingredients	Quantity (F1)	(F2)
1	Gelatin	3.5 g	6.25 g
2	Collagen	1.0 g	1.0 g
3	Glutaraldehyde	0.1 ml	0.1 ml
4	Glycerol	3 ml	3 ml
5	Distilled Water	50 ml	50 ml

Preparation of Moisturizing Gel

Carbopol 940 was slowly dispersed in distilled water according to Table 2. with gentle stirring for 30 minutes. Glycerin and Aloe vera gel were added to the mixture with. Aqueous herbal extracts were added gradually. Vitamin E was incorporated and mixed thoroughly. The above prepared Aqueous phase mixture was slowly added to hydrated Carbopol dispersion. Continuous stirring was carried out to avoid lump formation. Triethanolamine was added dropwise for pH adjustment. Gel formation was observed upon neutralization. Phenoxyethanol is added as preservative. Final weight was adjusted with distilled water. Homogeneous gel was obtained.

Table 2: Formulation Of Herbal Moisturizing Gel

S. No	Ingredient	G1	G2	G3
1	Carbopol 940	0.5 g	0.8 g	1.2 g
2	Glycerin	1 ml	1.7 ml	2.5 ml
3	Aloe vera Gel	4 g	4 g	4 g
4	Cogon Grass Root Aqueous extract	5 ml	6.5 ml	8 ml
5	Purslane leaves extract	2.5 ml	4 ml	6.5 ml
6	Vitamin E	0.3 g	0.4 g	0.5 g
7	Phenoxyethanol	0.5 g	0.5 g	0.5 g
8	Triethanolamine	q. s	q. s	q. s
9	Distilled water	q. s	q. s	q. s

Induction of Dryness

Artificial skin patches were treated with 1 percent sodium dodecyl sulfate solution to disrupt the lipid structure and simulate dry skin conditions. The patches were exposed to few drops of SDS for 5 minutes and rinsed with distilled water to remove residual surfactant. After treatment, the membranes were stabilized for 30 minutes to obtain a dry skin–induced artificial skin model.

Herbal Gel Application

The prepared herbal gel was applied over the surface of the dry artificial skin patch and was allowed to remain in contact for a specified period before evaluation of moisturizing efficacy.

EVALUATIONS

Artificial Skin

Physical Appearance

The overall structural integrity, smoothness, translucency, and uniformity of the artificial skin patches were visually assessed (20).

Surface pH

The surface pH of the artificial skin patches was determined to evaluate compatibility with physiological skin pH. A few drops of distilled water were placed on the surface and allowed to equilibrate before measurement using a digital pH meter. The average of three readings was recorded (21).

In Vitro Diffusion Studies

Diffusion studies were carried out using a Franz diffusion cell apparatus. The artificial skin membrane was mounted between donor and receptor compartments. Samples were withdrawn at predetermined intervals and replaced with fresh diffusion medium to maintain sink conditions. This model is used for evaluating permeability across artificial membranes (22).

Thickness Measurement

Thickness of artificial skin patches was measured using a calibrated Vernier calliper at three different positions without compressing the membrane. The mean thickness was calculated. Uniform thickness ensures reproducible mechanical and diffusion characteristics in polymeric membranes (23).

Artificial Dry Skin

Moisture loss was determined using the gravimetric method by recording initial weight (W_1) and final dried weight (W_2) after treatment. Percentage moisture content was calculated. Gravimetric moisture analysis is used for evaluating water content in polymeric hydrogels (24).

Weight Variation Analysis

Weight variation before and after dryness induction was calculated to confirm successful moisture depletion.

Barrier Disruption Model

SDS disrupts stratum corneum lipids and impairs barrier function, thereby simulating dry and damaged skin conditions. This model is widely validated in dermatological research (25).

Rehydration

Following dryness induction, a uniform layer of the prepared herbal moisturizing gel was applied to artificial skin membranes and incubated at 25 ± 2°C and the weight was measured at intervals 30min,60min,120min,5hrs,10hrs and 12 hrs. After incubation, final weight (W_2) was recorded (24).

Evaluation of Herbal Moisturizing Gel

Physical Appearance

The gel was inspected for colour, uniformity and absence of phase separation or air bubbles. Organoleptic characteristics which is standard for topical semisolid formulations were assessed (26).

pH Measurement

One gram of gel was dispersed in 10 mL distilled water and analysed using a calibrated digital pH meter.

Spreadability

It was evaluated using the glass slide method by placing a fixed quantity of gel between two slides under a specified weight and measuring the diameter of spread (26).

Viscosity

Viscosity was determined using a Brookfield viscometer at controlled temperature (25 ± 1°C). Rheological evaluation determines the flow behaviour of semisolid formulations (27).

Phytochemical Screening

Molisch's Test

Performed to detect carbohydrates (28) in aqueous extracts of *Portulaca oleracea* and *Imperata cylindrica.*

Sodium Cobaltnitrite Test for detection of potassium salts in plant extracts (29).

RESULTS AND DISCUSSION

Evaluation Result:

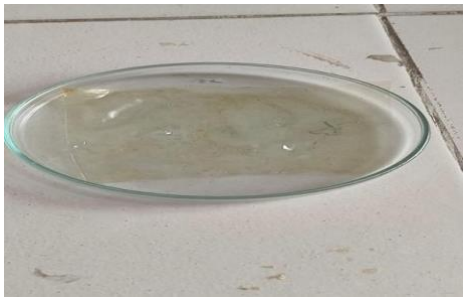

Figure 1: Appearance of artificial skin

Transparent uniformly smooth surface with good translucency a skin-like appearance with no visible cracks and air bubbles was observed.

Surface pH:

The Surface pH observed is 6.3 which lies within the range of human skin.

In Vitro Diffusion:

Figure 2: Diffusion of artificial skin

The artificial skin patch allowed controlled permeation of water across the membrane. No sudden or excessive permeation was observed, indicating effective barrier formation.

Thickness:

Table 3: Thickness results

Measurement Point	Thickness(mm)
T1	1.05
T2	1.10
T3	1.08
Average	**1.08mm**

Figure 3: Thickness of artificial skin

The average thickness of the artificial skin patch was found to be 1.08 mm in the acceptable range of 0.8–1.5 mm.

Moisture Content Reduction:

Moisture content (%) =Initial weight Final weight/initial weight x100

$$\frac{05 - 0.86}{0.86} \times 100$$

= 22.09%

Figure 4 Weight of the skin before and after SDS treatment

Weight loss = Initial weight − Final weight

Initial weight = 1.05 g

Final weight = 0.86 g

$$= 1.05 - 0.86$$

$$Weight\ loss = 0.19\ g$$

Results For Moisturizer:

Physical Appearance:

Figure 5: Moisturiser gel

The gel exhibited pale green to light brown colour with smooth and non-gritty texture with uniform consistency.

pH Measurement

Figure 6: pH of the gel

The pH of the formulation was found to be 6.88, which lies within the acceptable range (5.5–7.0)

Spreadability:

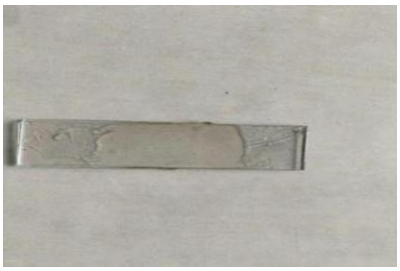

Figure 7: Spreadability

Formula:

M x L/T Where:

M = Weight tied to upper slide

L= Length moved by slide

T = Time

M=50g

L = 7.5cm

14 sec = 26.8g/sec

15 sec = 25g/sec

16 sec = 23.4g/sec

Good spread ability = 15-30

Avg = 25.1g

Table 4: Spreadability results

M	L	T	M*L/T
50	7.5	14	26.8
50	7.5	15	25
50	7.5	16	23.4

Viscosity:

Instrument: Brookfield Viscometer

Spindle: No. 64

Speed: 10 rpm

Temperature: 25 ± 1°C

Dial reading: 37 %

Factor (Spindle 64 at 10 rpm): 500

Calculation

Viscosity=Dial reading* Factor

=37*500

18,500cP

Figure 8: Measurement of viscosity using Brookfield viscometer

The observed dial reading was 37%, and the viscosity was calculated to be 18,500 cP at 25°C.

Rehydration

Initial weight (W_1): 0.86 g

Final weight (W_2): 1.04 g

% Moisture Regain=w2-w1/w1*100

=1.04-0.82/0.86*100

=20.93%

Figure 9: Dryness induced artificial skin Figure 10: After gel treatment

The percentage moisture regain of the artificial skin after treatment with the prepared gel was found to be 20.93%. This indicates moisture regain.

Purslane

Molisch's test: a violet ring is formed at the junction indicating presence of carbohydrates**.**

Figure 11: Molisch test

Cogon grass

Sodium Cobaltnitrite Test: It gives crystalline yellow precipitate indicating positive reaction

Figure 12: Sodium Cobaltnitrite Test

DISCUSSION

In order to evaluate the hydrating effectiveness of the herbal gel made with extracts, the current study focused on the development of an artificial collagen–gelatin skin model. Collagen and gelatin combine to form a semi-interpenetrating polymer network that can form hydrogen bonds with water molecules. This enables artificial skin to react dynamically to topical moisturizers.

The artificial skin model demonstrated uniform thickness, smooth surface texture, and mechanical stability. When evaluating hydrating agents, this stability is necessary because too much swelling or breakdown could affect the accuracy. The controlled degradation of the matrix supports its suitability for hydration and diffusion studies.

Hydration in the prepared skin model occurs primarily through water diffusion and retention within the polymeric network. Application of the herbal gel, water molecules penetrate leading to polymer chain relaxation and swelling similar to hydration in natural skin. Effective water absorption is confirmed by increase in weight and moisture content. The slow thickness rise indicates controlled swelling behaviour; thus, the model reacts to hydration formulations in a predictable manner.

Cogon grass root extract bioactive chemicals and mineral components help the skin matrix retain more water. In this study, the extract functioned as a humectant, facilitating the binding of water molecules to the collagen–gelatin network and to reduce water evaporation. Purslane leaf extract rich in mucilage required in skin hydration and barrier reinforcement. The hydration performance of the herbal gel was superior to that of the control and placebo formulations.

Advantages

Reproducibility, acceptability, cost-effectiveness and ease of handling are some benefits of the collagen-gelatin artificial skin model. This gives relevant data without biological variability or ethical issues. It adjusts factors like hydration level, composition and thickness. This model is an initial screening technique for topical herbal and synthetic drugs.

Limitations and Future scope

The complexity of skin is not entirely replicated by the artificial skin model, despite its benefits. Systemic absorption and irritation potential cannot be tested resulting the study to be indicative rather than conclusive.

To replicate the stratum corneum barrier, research on adding lipid components or cross-linking agents, clarified hydration mechanism by water vapor transmission should be done.

The findings translational value would also be strengthened by comparison studies using ex vivo human or animal skin models.

CONCLUSION

The present study successfully developed and characterized a collagen–gelatin based artificial skin model for evaluating the moisturizing efficacy of an herbal gel formulation. The prepared artificial skin membrane was compatible and had good structural integrity suitable as in vitro evaluation platform.

Dry skin conditions were induced using 1% SDS resulting in moisture reduction and weight variation, simulating disrupted xerotic skin.

Among the prepared herbal gel formulations, the optimized formulation exhibited desirable physicochemical properties ensuring ease of topical application and formulation stability. The customized herbal gel applied to artificial dry skin the moisture regained was 20.93%, indicating a notable hydration. The bioactive components especially polysaccharide and water-binding components improve moisture retention and barrier support. The produced artificial skin model is an affordable, repeatable, and moral substitute for initial testing of topical moisturizing compositions. The improved herbal gel formulation is used as natural treatment for dry skin. To improve translational applicability sophisticated barrier function analysis and clinical validation is recommended.

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