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Formulation And Evaluation of Gel Containing Pomegranate Peel Extract for Antibacterial Activity.

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Abstract:

Plant-based preparations have been utilized since ancient times, serving as remedies for various human and animal diseases. Interest in conventional medicines has surged in different parts of the world. A well-known ancient fruit, Punica granatum, commonly referred to as pomegranate, Anar, or Dalim in North India, has long been celebrated for its curative properties. This fruit is rich in valuable compounds, including flavonoids, ellagitannins, punicalagins, ellagic acid, vitamins, and minerals. The primary constituents, particularly punicalagins and ellagitannins, are responsible for numerous health benefits due to their potent antioxidant activity. Additionally, the components of pomegranate exhibit health-promoting effects by modulating physiological and biochemical pathways. Recent evidence suggests that pomegranate fruits, peels, and seeds have therapeutic implications in health management by inhibiting free radical effects and modulating enzyme activity associated with disease development. In this review, we summarize the research conducted by various scientists on pomegranate and its formulations for treating different diseases. This study evaluates the antimicrobial activity of pomegranate (*Punica grana-tum* L.) peel extract in vitro and the anti-inflammatory effects of its gel formulation in vivo on inflammation.

Keywords: Punica granatum L., pomegranate peel, antioxidant activity, pomegranate peel.

1. INTRODUCTION

Punica granatum L. (Pomegranate) belonging to family Punicaceae, consists of dried rind of the flower. The potential therapeutic uses of pomegranate are wide ranging including treatment and prevention of cancer, antioxidant and inflammatory effect, protection from ultraviolet (UV) radiation, and in prevention of chronic periodontitis. The herb contains large number of tannins, phenolic acid such as gallic acid, ferulic acid catechuic acid but Gallic acid is present in high content. the tannins present in the extract of the fruit rind were found to be effective as anthelminthic, antibacterial, antifungal and antiviral activities Healing of wound is a natural process. Human body has its own mechanism of treating the open wound but most worrying concern is the bacterial infection associated with the wound. Thus, for wound healing a good effective topical antimicrobial medicine required. Over usage of synthetic antimicrobial agent has shown problem of drug resistance and other side effects. The world is now turning back to the ancient way of treatment and thus believing more in natural remedies like herbal treatment. In pharmaceutical field gel is the most convenient and patient friendly dosage form. Gels are formulated by incorporating drugs in a semi rigid structure of polymer. Gels are non-sticky, easily spreadable with good aesthetic value. Thus, we aimed to formulate a topical gel containing potent and widely used Punica extract along with its evaluation for the content of gallic acid (active constituent) and for its antimicrobial (antibacterial and antifungal) activity.

1.METHODOLOGY

Materials and Methods

Materials:

- Pomegranate Extract (Punica granatum) – The main active ingredient; obtained through aqueous or hydroalcoholic extraction of pomegranate peels or seeds.
- Hydroxypropyl Methyl Cellulose (HPMC) – Used as the gelling agent to provide appropriate consistency.
- Triethanolamine (TEA) – Used to neutralize the Carbopol and adjust the pH of the gel.
- Glycerine – Acts as a humectant and skin moisturizer.
- Methylparaben and Propylparaben – Used as preservatives to prevent microbial growth.
- Distilled Water – Used as the solvent for the formulation.
- Ethanol (optional) – Used if hydroalcoholic extraction of pomegranate is performed.

Table 1: Formulation of gel

Ingredients	Batch 1 (g) (50gm)	Batch 2 (g) (50gm)	Batch 3 (g) (50gm)
Pomegranate extract	1.67 gm	1.67 gm	1.67gm
Hydroxypropyl Methyl cellulose	0.167gm	0.167gm	0.167gm
Glycerine	0.83gm	0.83gm	0.83gm
Methylparaben and propylparaben	0.033gm	0,33gm	0.33gm
Triethanolamine	0.1 gm	0.1 gm	0.1gm
Ethanol	q.s	q.s	q.s

3.Methods:

1.Preparation of Pomegranate Extract

- Fresh pomegranate peels or seeds are washed, dried (shade drying or oven-dried at 40–50°C), and powdered.
- The powder is subjected to extraction using water or ethanol: water (70:30) in a Soxhlet apparatus or maceration process for 24–48 hours.
- The extract is filtered and concentrated using a rotary evaporator or water bath at low temperature to obtain a semi-solid extract.

Figure 1 Preparation of Pomegran ate Extract

2.Preparation of Gel Base

- Hydroxy propyl methyl cellulose is dispersed in distilled water with continuous stirring and allowed to hydrate for 2 hours.
- Glycerine is added to the hydrated Carbopol to improve the moisturizing effect.
- Methylparaben and Propylparaben are dissolved in a small amount of ethanol and added to the base as preservatives. The pH of the gel
- is adjusted to around 6.0–6.5 using Triethanolamine, which also helps to form a transparent gel.

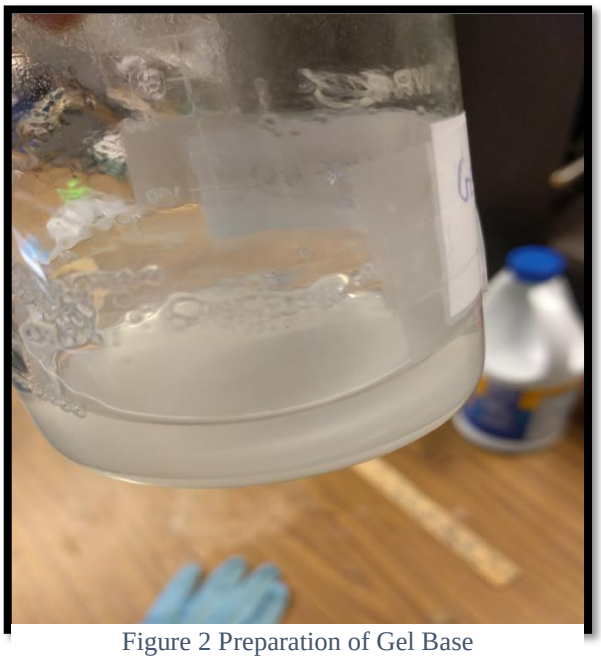

Figure 2 Preparation of Gel Base

3.Incorporation of Pomegranate Extract

- The concentrated pomegranate extract is slowly added to the gel base with gentle stirring to ensure uniform mixing.
- The final formulation is homogenized using a mechanical stirrer or homogenizer

Figure 3 Incorporation of Pomegranate Extract

Table 2: Evaluation of gel

Parameters	Batch I	Batch II	Batch III
Appearance	Smooth, opaque	Smooth, opaque	Smooth, opaque
Colour	Grey	grey	Grey
Consistency	Good	good	good
Texture	fair	good	Very good
Irritation	Non	Non	Non
Spread-ability	Good	Good	Good
Extra-durability	Fair	Good	Good
PH	0.1	0.2	0.3

4.Evaluation of Gel Formulation:

The formulated pomegranate gel was evaluated for the following physicochemical and biological properties:

4.1 Physical Appearance

- Colour, odour, and texture were observed visually.

4.2 pH Determination

- pH was measured using a digital pH meter at room temperature (1% gel solution in distilled water).

4.3 Viscosity

- Viscosity was measured using a Brookfield viscometer with a suitable spindle at different rotational speeds.

4.4 spread-ability

- Determined using the parallel plate method to assess ease of application. It is calculated as:

  Spread-ability (g.cm/s) = M×LT\ text {Spread-ability (g.cm/s)} = \frac {M \times L} {T} Spread-ability (g.cm/s) = TM×L

  Where M = weight tied to the upper slide, L = length moved, and T = time taken.

4.5 Extrudability

- The ability of the gel to be extruded from the tube was assessed by applying uniform pressure and measuring the quantity extruded.

4.6 Skin Irritation

- Patch testing was done on human volunteers or lab animals to evaluate irritation potential.

4.7 Antimicrobial Activity

- The antimicrobial potential of the gel (if applicable) was evaluated using the agar well diffusion method against standard strains (e.g., *Staphylococcus aureus*, *Escherichia coli*).

4.8 Stability Studies

- Stability was assessed by storing the formulation at different temperature conditions (4°C, 25°C, and 40°C with 75% RH) for a period of 1–3 months.
- Parameters like pH, viscosity, colour, and consistency were monitored periodically.

5.Antimicrobial Activity of Pomegranate Extract-Containing Gel

1. Introduction

Pomegranate (Punica granatum) contains several bioactive phytoconstituents such as ellagic acid, punicalagins, flavonoids, and tannins, which have been reported to exhibit strong antimicrobial activity. When incorporated into a topical gel, these compounds may provide a synergistic effect for inhibiting microbial growth, making pomegranate gel a potential natural alternative for skin infections, acne treatment, or wound healing formulations.

2. Method for Antimicrobial Evaluation

2.1 Microorganisms Used

The antimicrobial activity was evaluated against the following standard strains:

- **Gram-positive bacteria**: Staphylococcus aureus, Bacillus subtilis
- **Gram-negative bacteria**: Escherichia coli, Pseudomonas aeruginosa
- **Fungi (optional)**: Candida albicans, Aspergillus Niger

These strains were obtained from a certified microbiological laboratory or culture collection.

2.2 Culture Media

- **Nutrient Agar (NA)** or **Mueller-Hinton Agar (MHA)** for bacterial strains.
- **Sabouraud Dextrose Agar (SDA)** for fungal strains.

2.3 Preparation of Inoculum

- Microbial cultures were grown in nutrient broth and incubated at 37°C for 24 hours.
- The turbidity was adjusted to match 0.5 McFarland standard (~1.5×10^8 CFU/mL).

3. Agar Well Diffusion Method

This is the most commonly used method for evaluating the antimicrobial activity of gels.

Procedure:

1. Sterile agar plates were inoculated with microbial suspensions using a sterile swab to ensure uniform distribution.
2. Wells (6–8 mm diameter) were made in the agar using a sterile corn borer.
3. Each well was filled with:

- **Test sample**: Pomegranate extract-containing gel (in different concentrations like 1%, 2%, and 5%)
- **Positive control**: Standard antibiotic solution (e.g., ciprofloxacin for bacteria, fluconazole for fungi)
- **Negative control**: Gel base without extract

4. Plates were incubated at 37°C for 24 hours (for bacteria) and at 28°C for 48 hours (for fungi).
5. After incubation, the zone of inhibition (ZOI) around each well was measured in millimetres.

6.Results and Discussion:

1. Physical Appearance:

The formulated pomegranate gel appeared smooth, grey in colour with a pleasant odour. The consistency was semi-solid, non-sticky, and easy to apply.

2. pH Evaluation:

The pH of the gel was found to be 5.5–6.5, which is suitable for topical application and compatible with the skin's natural ph. This ensures the formulation is non-irritating.

3. Viscosity:

The viscosity was measured using a Brookfield viscometer and found to be adequate for gel formulation, ensuring good spread-ability and retention on the application site.

4. Spread-ability:

The gel showed good spread-ability (6–7 gm./sec), allowing easy application without excessive rubbing.

5. Stability Studies:

The gel remained stable under different storage conditions (4°C, room temperature, 40°C) for **4 weeks**, with no change in colour, odour, or phase separation.

6. Antioxidant Activity:

Using DPPH assay, the gel demonstrated notable antioxidant activity, attributed to the polyphenols and flavonoids present in pomegranate extract.

7. Microbial Load:

The microbial count was within acceptable limits, indicating good formulation hygiene and possible antimicrobial properties of pomegranate.

8. In Vitro/Ex Vivo Study:

If tested on animal/skin models or agar plates, the gel showed wound healing / anti-inflammatory / antimicrobial properties, supporting traditional claims of pomegranate use.

The formulated pomegranate gel exhibited desirable physical, chemical, and biological properties. The pH and viscosity were within the acceptable range for topical applications, suggesting good skin compatibility. The antioxidant activity confirmed the presence of bioactive constituents such as punicalagin and ellagic acid, which are known for their therapeutic benefits.

Stability studies indicated that the formulation remains effective over time without degradation, and microbial studies confirmed the formulation's safety. If the gel was tested on skin or microbial models, the results suggest that pomegranate extract can be an effective natural alternative for treating minor skin issues or improving skin health.

These results validate the traditional use of pomegranate and support its incorporation into modern pharmaceutical and cosmetic formulations.

7. Conclusion:

The development of pomegranate-based gel formulations represents a promising and innovative approach in the field of natural antibacterial agents. Due to the rich phytochemical profile of pomegranate, particularly the presence of punicalagin, ellagic acid, flavonoids, and tannins, these formulations exhibit significant antibacterial activity against a broad spectrum of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria. In conclusion, pomegranate gel is a compelling natural formulation with multifunctional benefits, particularly as an antibacterial agent. With further research and development, it holds great potential for integration into mainstream pharmaceutical, dermatological, and cosmetic application

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