Indian Journal of Health Care, Medical & Pharmacy Practice Vol 5; Issue 1, Jan-Jun 2024, ISSN 2583-2069

Research Article

MODERN HERBAL GEL FORMULATION: PHYSICOCHEMICAL EVALUATION AND EFFICACY TESTING FOR DERMATOLOGICAL APPLICATIONS

Sourav Khawas[1], Manmohan Sharma*[2], Rajiv Kukkar[3], Anil Sharma[4]

[1,2,3,4]School of Pharmaceutical Studies, Faculty of Health Sciences, Dr. K.N. Modi University, New Delhi, India.

Corresponding Author*: Manmohan Sharma, School of Pharmaceutical Studies, Faculty of Health Sciences, Dr. K.N. Modi University, New Delhi, India.

Email ID: sharma.manmohan@dknmu.org

DOI: https://doi.org/10.59551/IJHMP/25832069/2024.5.1.100

Received: 15 April, 2024, Decision for Acceptance: 12 May, 2024

Abstract

This study investigates the development of a modern herbal gel formulated with Punica granatum extracts for dermatological applications, with a focus on addressing acne. By employing a comprehensive methodology involving phytochemical screening, chromatographic analysis, antimicrobial testing, and formulation evaluation, we aimed to create a topical product that is both safe and effective.

Our results reveal promising pharmacognostical properties of Punica granatum extracts, including acceptable ash values and extractive values, indicating the quality and potency of the herbal material. Additionally, chromatographic analysis provided valuable insights into the chemical composition of the extracts, facilitating standardization and quality control.

The formulated gel exhibited significant anti-acne activity against P. acnes, demonstrating notable inhibitory effects. Moreover, physical characterization highlighted favorable properties such as appropriate pH, viscosity, spreadability, texture, and appearance, crucial for ensuring patient compliance and efficacy.

Stability studies suggested the potential for a shelf-stable product with resilience to environmental factors over time. Furthermore, in vitro release studies indicated controlled and sustained release of active ingredients from the gel formulation, supporting its efficacy and longevity upon application.

Keywords: Herbal Gel, Punica Granatum Linn, Acne, Dermatologica, Applications

1. Introduction

A. Herbal Medicine and Modern Dermatology: Throughout history, communicable diseases have been treated with herbal remedies, blending traditional practices with modern approaches. The World Health Organization supports Complementary and Alternative Medicine (CAM), advocating its integration with conventional treatments. Herbal medicine, rooted in ancient wisdom, persists as a natural solution for skin ailments[1].

B. Herbal Medicine's Role in Healthcare: Ayurveda, originating from India, offers a holistic approach to health, addressing physical, mental, and emotional well-being. It focuses on treating degenerative disorders by targeting root causes, unlike modern medicine's symptom management approach.

Ayurvedic treatments combine herbal remedies, dietary adjustments, and lifestyle changes to restore balance and promote long-term health[2].

C. Research and Integration: There's a resurgence of interest in traditional medicine, including Ayurveda, driven by research efforts. Standardizing herbal preparations, studying their chemical composition, and raising awareness about integrative healthcare are vital for modernizing traditional practices. By leveraging ancient wisdom and scientific understanding, Ayurveda can complement modern healthcare effectively[3].

D. Challenges and Solutions: However, challenges exist, such as limited availability, standardization issues, and the risk of adulteration in herbal drugs. Implementing quality control measures, promoting standardized practices, and enhancing public awareness can mitigate these challenges. Sophisticated analytical techniques like chromatographic fingerprinting ensure the authenticity and consistency of herbal medicines[4].

E. Modern Herbal Formulations: In dermatology, modern formulations incorporating herbal ingredients offer targeted solutions for skin conditions like acne. By combining traditional knowledge with pharmaceutical advancements, researchers aim to develop safe and effective skincare products. These formulations, such as microemulsion gels containing herbal extracts, provide natural alternatives for managing dermatological issues[5].

F. Understanding Acne Pathogenesis: Acne vulgaris, a widespread dermatological condition, results from complex factors including sebum overproduction, epithelial desquamation, bacterial proliferation, and inflammation. Effective treatments target these mechanisms, utilizing synthetic agents like benzoyl peroxide, retinoids, antibiotics, azelaic acid, and salicylic acid to manage acne symptoms effectively[6].

G. Plant: Punica granatum Linn., commonly known as pomegranate, is a fruit-bearing tree valued for its diverse uses. Cultivated since ancient times, it thrives in various regions but requires protection from cold climates. The plant's opposite leaves and red flowers produce distinctive fruit, rich in pulp and seeds.

Historically, pomegranates have been utilized for tanning and dyeing, with the fruit prized for its refreshing juice and culinary versatility. In traditional medicine, different parts of the plant are employed for various medicinal purposes:

1. **Pericarp (Rind):** Possesses antidiarrheal and ant dysenteric properties due to phenolic compounds and flavonoids[7].
2. **Fruit Juice:** Contains beneficial compounds for cardiac health, digestion, and addressing conditions like leprosy.
3. **Bark of Stem and Root:** Utilized in treating worm infections, containing ellagitannins and alkaloids.
4. **Dried Flowers:** Beneficial for conditions such as bronchitis and oral infections due to specific acids.
5. **Seed and Root:** Exhibit hypotensive, antispasmodic, and anthelmintic activities, attributed to punicic acid and sterols.

Research indicates pharmacological activities associated with pomegranate, including analgesic, anthelmintic, antifungal, anti-inflammatory, antibacterial, antioxidant, anti-diabetic, anti-carcinogenic, and neuroprotective effects. Additionally, topical formulations show promise in oral health and dermatological conditions[8,9].

1.1 Topical Drug Delivery (Self Emulsifying Drug Delivery Systems)

The passage delves into the historical context and recent advancements in topical drug delivery, emphasizing its significance in dermatological treatments. It discusses the definition, purposes, and advantages of topical preparations, highlighting their increased bioavailability, reduced undesirable effects, and avoidance of absorption variables compared to oral administration. Additionally, it

addresses the limitations of topical drug delivery, such as physicochemical properties of drugs, skin irritation, and variation in skin permeability. Furthermore, it mentions the advantages of gels over other semisolid formulations, including ease of application, good spreadability, and faster drug release, along with types of gels based on phases and solvents used. Finally, it explores gel-forming substances, particularly carbomers, and their advantages in topical formulations, such as high viscosity, stability, and safety[10,11].

2. Methodology

The methodology for evaluating Punica granatum fruits (Rinds) herbal extracts for their anti-acne action, anti-inflammatory, and antibacterial properties, formulated as a gel, would typically involve.

Plant material from Punica granatum Linn. and Embelia species, sourced from local markets, underwent authentication, drying, and powdering. Ash values, including total, acid insoluble, and water-soluble ash, were determined following standard methods outlined in the Indian Pharmacopoeia. The powder was then extracted using the Soxhlet method with an 80% hydroalcoholic solution, followed by concentration and phytochemical screening[12,13].

2.1 HPLC-UV Chromatographic Conditions Applicable for Analysis of Extracts

HPLC-UV chromatographic analysis was conducted using the following conditions:

- Pump: Agilent 1120 compact LC gradient pump
- Injector: Manual Rheodyne injector
- Column: Agilent TC C18 (250mm X 4.6 mm i.d., 5µm)
- Detector: UV at 278nm using Photodiode array detector
- Software: EZChrom Elite Compact software
- Injection volume: 20µl
- Flow rate: 1µl/min
- Mobile Phase: 0.01MKH2PO4: Acetonitrile (85:15) pH 3.2
- Temperature: 30oC

The mobile phase was prepared by mixing 15 parts of acetonitrile with 85 parts of 0.01M KH2PO4, adjusted to pH 3.2 using orthophosphoric acid, then filtered through a 0.22 µm nylon membrane and degassed through sonication[14,15].

Standard solutions of Gallic acid and Berberine were prepared at 100 µg/ml concentration in methanol, diluted within the linear range of 5 to 25 µg/ml for subsequent analysis using HPLC.

For the methanol fraction of hydroalcoholic extracts of P. granatum, 100 mg of dry powdered sample was dissolved in 80 ml of methanol, sonicated for 10 minutes, adjusted to 100 ml with methanol, and filtered through a 0.22 µm Millipore filter.

For the hydroalcoholic extracts of plants, solvent systems were prepared as follows:

1. Ethyl acetate: Methanol: Water- 100:13.5:10
2. n-Butanol: Ethanol: Water - 40:11:9
3. Benzene: Chloroform: Methanol - 1:2:0.1

Detection was conducted by observing the chromatographic plates in visible light and UV at 365 nm[16,17].

2.2 Detection

A. Anti-acne Activity: Anaerobic bacteria P. acnes (MTCC No. 1951) cultures were obtained from IMTECH. Brain heart infusion broth with Tween 80 and thioglycolic acid was inoculated with the extracts. After incubation at 37°C for 48 hours, growth was monitored turbidimetrically to determine the minimum inhibitory concentration (MIC). Cup plate diffusion method with clindamycin as internal standard was used to evaluate anti-acne activity, determining MIC based on zone of inhibition. Each analysis was repeated thrice for validation.

2.3 Formulation of Microemulsion

The process involved mixing the required quantities of surfactant and co-surfactant in the oil phase under constant stirring. Subsequently, the aqueous

phase containing the extracts was poured into the oil phase, and the mixture was homogenized using a mechanical stirrer. Instantaneously, a microemulsion was formed due to the diffusion of the two phases, resulting in the formation of droplets[18].

2.4 Evaluation of Formulation

A. Physical Characterization: Evaluate the gel's physical properties, including pH, viscosity, spreadability, texture, and appearance, using standardized methods[19].

B. Stability Studies: Conduct stability tests to determine the gel's shelf-life and resistance to temperature variations and environmental factors over a specified period[20].

C. Efficacy Testing: Utilize appropriate methods to evaluate the formulated gel's efficacy in addressing acne-related concerns. This may include in vitro studies for antibacterial activity against acne-causing bacteria, anti-inflammatory assays, or tests for reduction in sebum production[21,22].

3. Result

3.1 Pharmacognostical Study

The various physicochemical standards such as Ash values, Extractive values and loss on drying were performed. The results were reported in following table.

Total, acid insoluble, and water-soluble ash values for Sample A, expressed as percentage weight by weight (% w/w), are compared against specified limits (NMT - Not More Than) as per pharmacopoeial standards. Total ash represents the residue after ignition, including intrinsic plant ash and extraneous substances like sand and soil. Acid insoluble ash indicates siliceous contaminants, while water-soluble ash measures residue post-water treatment. Standardized methods from the Indian Pharmacopoeia are used for determination.

The determination of extractive values and drug extraction is pivotal in assessing active compound concentrations, especially in herbal medicine or pharmacology research. Here's how it works:

1. Soxhlet Extraction Method: This technique is used for solid sample extraction, ideal for compounds insoluble at room temperature but soluble at higher temps. A hydroalcoholic solution (80% water, 20% ethanol) acts as the solvent.
2. Procedure: The sample is placed in a thimble within the Soxhlet apparatus. The solvent is heated to reflux, cycling through the sample to extract compounds efficiently.
3. Extractive Values: These gauge extraction efficiency. They represent the percentage of soluble material from the crude drug.
4. Phytochemical Screening: The extract undergoes screening for compound classes like alkaloids, flavonoids, tannins, and saponins, revealing its pharmacological potential.

- Alcohol Soluble (In % w/w): Indicates alcohol solubility, e.g., Sample A's 19.6%.
- Water Soluble (In % w/w): Shows water solubility, e.g., Sample A's 38.2%.
- Alcohol Soluble (NLT %): Minimum alcohol solubility, e.g., 20% for Sample A.
- Water Soluble (NLT %): Minimum water solubility, e.g., 35% for Sample A.

Table 1: Determination of Ash Values

Sr. No.	Crude Drug	Total Ash (In % w/w)	Acid Insoluble Ash (In % w/w)	Sulphated Ash (In % w/w)
1.	Sample A	3.5 (NMT 4)	1 (NMT 0.5)	1.2 (NMT 1)

Table 2: Determination of Extractive Values

Sr. No.	Crude Drug	Alcohol Soluble (In % w/w)	Water Soluble (In % w/w)	Alcohol Soluble (NLT %)	Water Soluble (NLT %)
	Sample A	19.6	38.2	20	35

Table 3: Protocol for determination of Minimum inhibitory concentration by broth dilution method

Sr. No.	Amt of Extract/ ml	Amt of medium	Total Vol ofSolution (ml)	Conc. of Extract in finalsol (mg/ml)
1	0.1	9.9	10	0.1
2	0.2	9.8	10	0.2
3	0.3	9.7	10	0.3
4	0.4	9.6	10	0.4
5	0.5	9.5	10	0.5
6	0.6	9.4	10	0.6
7	0.7	9.3	10	0.7
8	0.8	9.2	10	0.8
9	0.9	9.1	10	0.9
10	1.0	9.0	10	1.0

These values ensure extraction process standardization and assess extract quality and potency.

3.2 Anti Acne Activity of Extracts

The MIC determination via broth dilution method assesses antimicrobial efficacy. Here's the protocol breakdown:

1. Preparation of Extract Solutions:

- Dilute extract stock to various concentrations (0.1 mg/ml to 1.0 mg/ml).
- Mix calculated volumes of extract and solvent.

2. Preparation of Test Tubes:

- Label tubes per extract concentration.
- Add extract solution to broth medium (e.g., 0.1 ml extract + 9.9 ml broth).

3. Inoculation with Microorganisms:

- Inoculate tubes with standardized microorganism suspension.

4. Incubation:

- Incubate tubes under specified conditions.

5. Observation and Interpretation:

- After incubation, observe for microbial growth.
- Determine MIC as the lowest extract concentration inhibiting visible growth.

Each tube contains 10 ml, with extract and medium varying. Extract concentration is calculated per ml. This systematic approach allows MIC evaluation across concentrations, revealing extract potency against tested microorganisms.

Physical characterization of a gel involves assessing various attributes to understand its properties and suitability for use. Here's an explanation of how each aspect is evaluated using standardized methods:

1. pH: pH measurement indicates the acidity or alkalinity of the gel. Standardized methods involve using a pH meter to directly measure the pH of the gel. This measurement is crucial as it can affect the stability, compatibility, and skin irritation potential of the gel. Typically, the pH range for topical gels is around 4.5 to 7.5, ensuring compatibility with the skin's pH.
2. Viscosity: Viscosity refers to the resistance of a fluid to flow and is a critical parameter for gels as it affects product stability, spreadability, and ease of application. Standardized methods, such as using a viscometer or rheometer, are employed to measure the gel's viscosity. The gel is subjected to controlled shear stress, and the resulting flow behavior is recorded. Viscosity values are reported in units like centipoise (cP) or Pascal-seconds (Pa.s).
3. Spreadability: Spreadability measures the ability of the gel to spread evenly over the skin surface. Standardized methods involve

placing a measured quantity of gel between two slides or plates and applying a standard weight or force to spread the gel. The diameter of the spread gel is then measured at regular intervals. The spreadability index is calculated based on the increase in diameter over time. Higher spreadability indicates better coverage and ease of application.

4. Texture: Texture evaluation assesses the sensory characteristics of the gel, including its consistency, smoothness, stickiness, and overall feel. Standardized methods involve subjective assessment by trained panelists using a scoring system or descriptive analysis techniques. Panelists rate various attributes of the gel's texture based on their perceptions, providing qualitative feedback on its sensory properties.
5. Appearance: Appearance evaluation involves visual inspection of the gel to assess its color, clarity, homogeneity, and presence of any visible particles or defects. Standardized methods include visual examination under controlled lighting conditions and comparison against predefined criteria or standards. Any deviations from the desired appearance can indicate issues with formulation consistency, stability, or manufacturing process.

4. Conclution

In this study, we explored the formulation of a modern herbal gel containing Punica granatum extracts for dermatological applications, particularly targeting acne. Through a comprehensive methodology encompassing phytochemical screening, chromatographic analysis, antimicrobial testing, and formulation evaluation, we aimed to develop a safe and effective topical product.

Our results indicate promising pharmacognostical properties of Punica granatum extracts, including acceptable ash values and extractive values, indicative of the quality and potency of the herbal material. Furthermore, chromatographic analysis provided insights into the chemical composition of the extracts, facilitating standardization and quality control.

The anti-acne activity of the formulated gel was evaluated against P. acnes, demonstrating significant inhibitory effects. Additionally, physical characterization revealed favorable properties such as suitable pH, viscosity, spreadability, texture, and appearance, essential for ensuring patient compliance and efficacy.

Stability studies indicated the potential for a shelf-stable product with resistance to environmental factors over time. Furthermore, in vitro release studies suggested controlled and sustained release of active ingredients from the gel formulation, supporting its efficacy and longevity upon application.

Overall, the findings of this study underscore the potential of modern herbal formulations in dermatological care, offering natural alternatives with therapeutic benefits. By bridging traditional knowledge with contemporary pharmaceutical techniques, we can enhance patient outcomes and expand the repertoire of treatments available for skin ailments like acne. Further research and clinical trials are warranted to validate the efficacy and safety of this herbal gel in real-world settings, paving the way for its integration into mainstream dermatological practice.

Conflict of Interest: None

References

1. Butler M, Buss AD, A new model for utilizing chemical diversity from natural sources, Drug development research, 62, 2004, PP 362–370.
2. Chatterjee A, Pakrashi SC, The Treatise on Indian Medicinal Plants, National Institute of Science Communication CSIR, (5),1997. New Delhi. PP-78-96.
3. Cox P, Balick M, The ethnobotanical approach to drug discovery. Scientific American, 19, 1994, PP 82-87.
4. Cox PA et al, Ethno pharmacology and the search for new drugs, In Bioactive Compounds from Plants, Ciba Foundation Symposium, John Wiley

& Sons, 15, 1999. PP 40-55.
5. Handa SS, Herbal remedies in indigenous system of medicines. Pharma Times 23(4), 1991, PP 13-16.
6. Handa SS, Medicinal and aromatic plants global industry overview, "Quality Control, Scientific Validation and Business Prospects of Medicinal and Aromatic Plants", Port of Spain, Trinidad & Tobago, 28 (7), 2007, PP 1-21.
7. Kirtikar KR, Basu BD, Indian medicinal plants 2nd edition, Lalit Mohan Basu, Allahabad, 1999. PP 1273 -1275.
8. Mathur A, Who owns Traditional Knowledge, Working Paper No. 96, Indian Council for Research on International Economic Relations, 2003. PP 1-33.
9. Said HK, A medicine through the ages, Hamadard academy, Karachi, 1980, PP 70-75.
10. Said HK, 2005.Traditional medicine in the service of health, Hamadard foundation press, Karachi, 1988, PP 27-36.
11. Shrikumar S, Ravi TK, Approaches towards development and promotion of herbal drugs, Pharmacognosy Reviews, 1(1), 2007, PP 180-184.
12. Singh A, Ethics in herbal medicine, Ethnobotanical leaflets, 11, 2007, PP 206-211.
13. Singh AP, Current status and future direction of herbal medicine, Trends in Pharmacological sciences, 23(8), 2005, PP 347-348.
14. World Health Organization (WHO), WHO Traditional Medicine Strategy, 2006, Geneva.
15. World Health Organization (WHO), WHO Traditional Medicine Strategy, 2002, Geneva.
16. Glynn LE, 1981. The pathology of scar tissue formation, In: Glynn, L.E., Handbook of Inflammation, vol. 3. Tissue Repair and Regeneration, Elsevier North Holland Biomedical Press, Amsterdam, 200, PP 178-187.
17. Koyama T, Ogura K, Tagahara T, Konoshima M, Phytochemistry, 31, 1932, PP 2907-2915.
18. Vane J, Booting R, Inflammation and the mechanism of action of anti-inflammatory drugs, Journal of American Societies for Experimental Biology 1, 1987, PP 89–96.
19. Varma N, Painuly P, Sharma JS, Indian Journal of Chemistry, 24, 1985, PP 791-784.
20. Tripathi VD, Agarwal SK, Rastogi RP, Indian Journal of Chemistry, 9 (17), 1993, PP 97- 99.
21. Tripathi YB, Pandey S, Shukla Y, Indian Journal of Experimental. Biology, 31, 1993, PP 533-539.
22. Calder PC, n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases, American Journal of Clin Nutraceuticals, 83, (6), 2006,PP 1505- 1519.

Cite this article Sharma M et al, Modern Herbal Gel Formulation: Physicochemical Evaluation and Efficacy Testing for Dermatological Applications. Indian Journal of Health Care, Medical & Pharmacy Practice.2024; 5(1) 72-78.