Formulation and Assessment of Punica External Gel for Gallic Acid Composition and Anti-microbial Research

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Abstract

Soxhlet was used to extract the evaporated, crushed the Punica granatum peel with water acting as a solvent. Topical formulation gels containing different combinations of polymers and Punica aqueous extract were assessed for their antibacterial effectiveness, viscosity, spread ability, gallic acid concentration (as determined by HPLC), and physicochemical characteristics. The gel was effectively prepared and assessed for both the gallic acid content (3.5%) and the medicinal properties of the formulation. The in-house Punica gel had the strongest antimicrobial action against B. subtilis, P. aeruginosa, K. pneumoniae, and A. nigeria, while the gel also exhibited outstanding antibacterial and antifungal activity.

Key words: HPLC, gallic acid, gel, antimicrobial agent, and Punica granatum

OVERVIEW: The dried rind of the flower is what makes up Punica granatum L. (pomegranate), a member of the Punicaceae family. Pomegranates have a wide range of potential medical applications, including as the prevention and treatment of cancer, their anti-inflammatory and antioxidant properties, their ability to guard against ultraviolet (UV) radiation, and their ability to prevent chronic periodontitis 1–3. Many tannins and phenolic

acids, including gallic acid, ferulic acid, and catechuic acid, are found in the plant; nevertheless, gallic acid is the most abundant.4. It was discovered that the tannins in the fruit rind extract have antiviral, antifungal, antibacterial, and anthelmintic properties5,6. Wound healing occurs naturally. The human body has a unique system for addressing the open wound, but the bacterial infection linked to the wound is the more concerning issue7,8. Therefore, a good, effective topical antibacterial medication is needed for wound healing. Overuse of synthetic antimicrobial agents has been linked to adverse effects and antibiotic resistance. Natural therapies like herbal medicines are becoming more popular as people return to the traditional methods of therapy. The most convenient and patient-friendly dosing form is gel.in the pharmaceutical industry. Drugs are combined with a semi-rigid polymer framework to create gels. Gels have high aesthetic value, are readily spreadable, and are non-sticky9. Therefore, our goal was to create a topical gel with powerful and popular punica. extract, as well as an assessment of its antimicrobial (antibacterial and antifungal) properties and gallic acid concentration (active ingredient).

(A)Punica Granatum Plant

(B)Punica Granatum leaf and Fruit

(C)Punica Granatum leaf and Fruit

(D)Punica Granatum leaf

(E)Punica Granatum Flowe

(F)Punica Granatum Fruit

Procedure And Materials

Chemicals and solvents

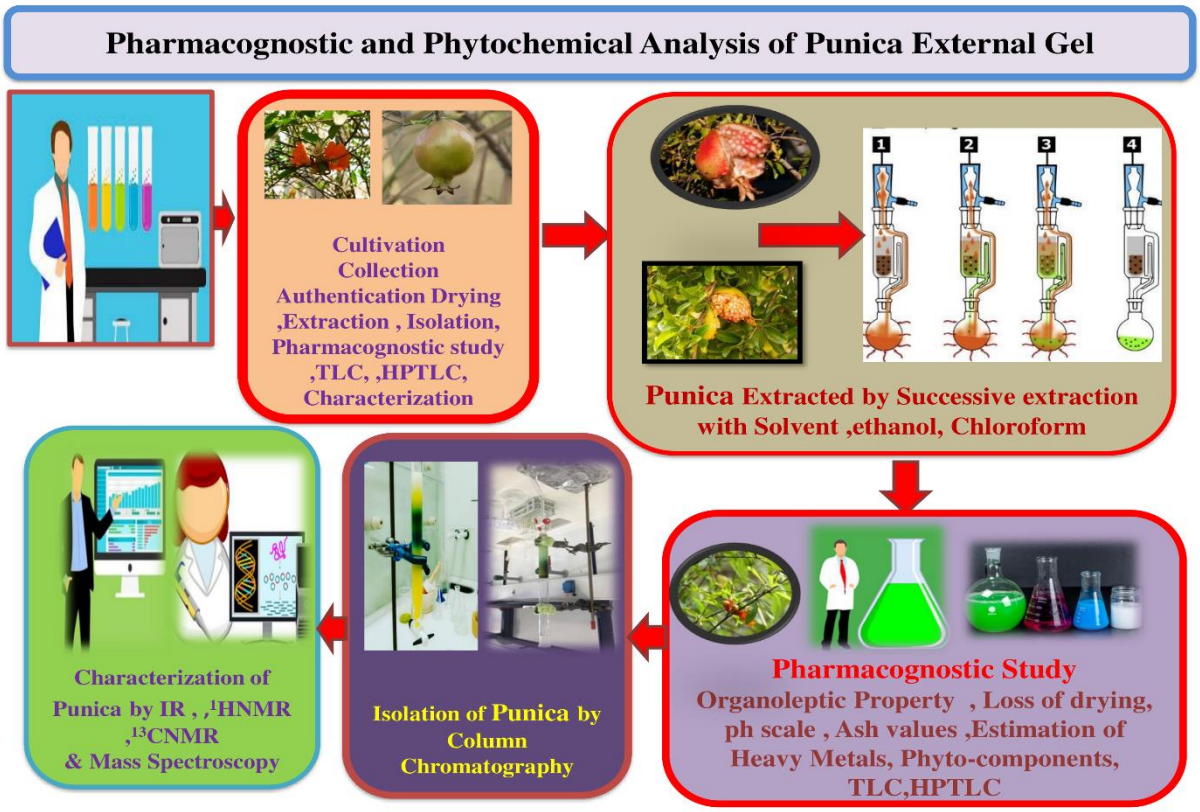
We bought the powdered peel of Punica granatum from Bionic Enterprises in Lucknow. Every chemical and reagent that was employed was of analytical and HPLC quality. Reference standards for gallic acid were acquired from Bionic Enterprises in Lucknow.

Microbes

Gram-negative organisms including Pseudomonas aeruginosa, the bacteria Klebsiella pneumoniae, and Escherichia coli, as well as gram-positive species like Staphylococcus aureus, and Bacillus subtilis, among others were used in the antibacterial investigation. Aspergillus niger and Candida albicans were employed in the antifungal investigation**.**

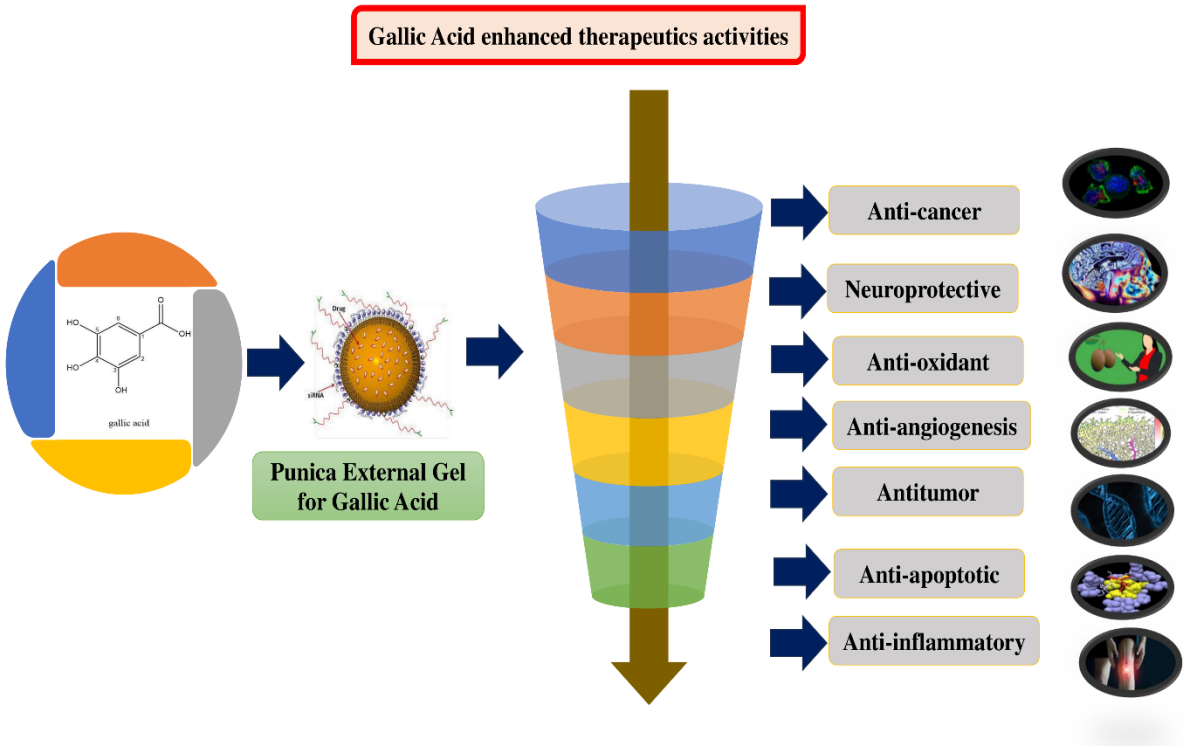
Making the extracts

The powdered peel of Punica granatum was extracted using the Soxhlet extraction technique at a 1:6 ratio. After filtering, the aqueous extract was dried and evaporated.



Formulation advancement [10-12]

Various gels were created using different amounts of polymer (Carbopol 940P). Overnight, distilled water was used to soak a weighed quantity of Carbopol 940P. After precisely weighing the bioactive extract, it was diluted in distilled water. With constant, steady stirring, The soaking Carbopol was gradually mixed with the solution. Care was taken to prevent air from being trapped during stirring. The gel was then neutralized with 50% triethanolamine, and the pH was brought to 7.3–7.5. Methyl paraben was added as a preservative after Glycerol was used because of its cooling properties.

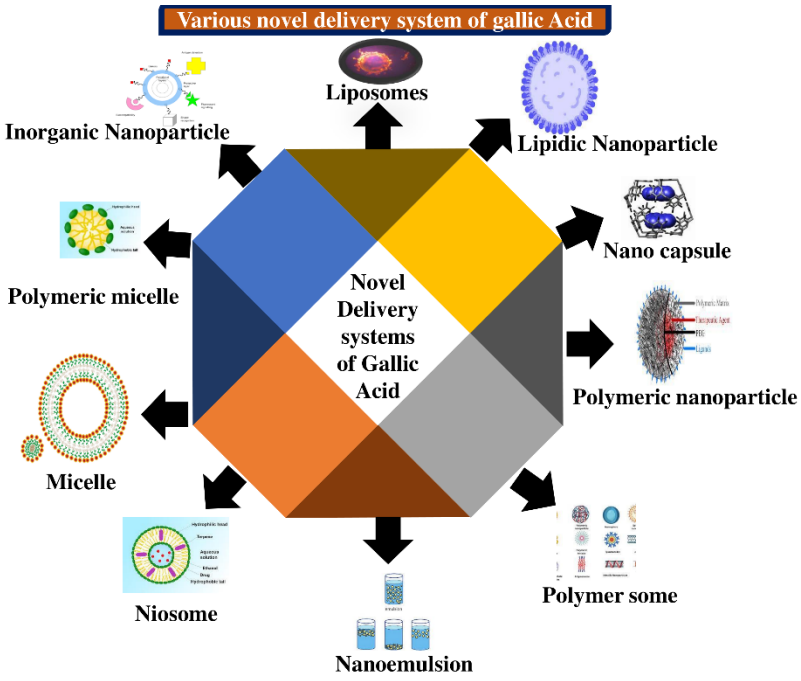


Assessment of gel[10-12]

A number of preliminary criteria, including appearance, colour, pH, and spread ability, were assessed for the formulations. Further tests, including Gallic acid, transport investigation, main cutaneous sensitivity the index, and viscous concentration, were performed on the formulation that satisfied these criteria. Uniformity Visual examination was used to assess the formulated gels' homogeneity, appearance, and colour. A pH meter was used to measure the formed gel's pH.

Table 1: Formulation change

Materials	**Code of composition**			
	A1	A2	A3	A4
Aqueous extract	5%	5%	5%	5%
Carbopol940	1.5%	2%	2.5%	1%
Glycerine	20%	20%	20%	20%
50% triethanolamine	pH7.3-7.5	pH7.3-7.5	pH7.3-7.5	pH7.3-7.5
Methyl paraben	q.s	q.s	q.s	q.s
Water q.s	100gm	100gm	100gm	100gm



Capability to Spread

The duration in seconds it takes for two slides to separate from the gel when positioned between each other and subjected to a specific force is known as spreadability. A specific the slides made from glass were compressed by applying a certain amount of pressure. them into a consistent thickness after the extra sample was sandwiched between the two glass slides. The The amount of time required to divide each of the images were recorded when a 70 g weight was applied. The formula S = ML/T was used to determine spread ability, where M is the weight attached to the higher slide, L is the length of the glass slides, and T is the time required to separate the slides.

Table 2: Formulated gel A3 evaluation variables

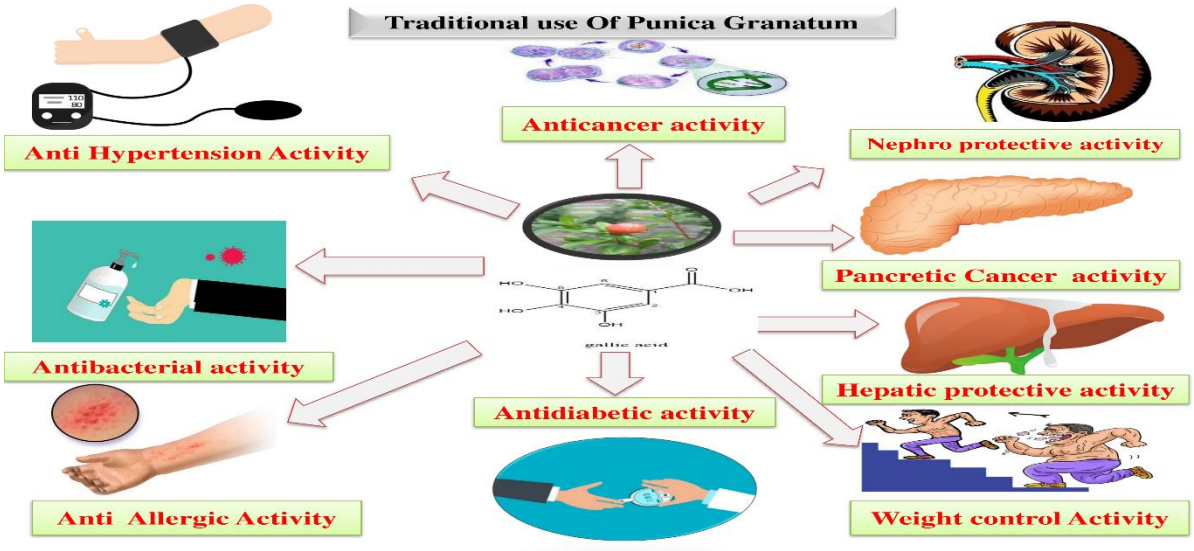
Specifications	The findings
Appearance	Smooth, clear, transparent
Color	Pale yellow
PH	7.3
Viscosity	5607 ±153 cps
Spread ability	5.01secs

The viscosity

A Brookfield viscometer was used to measure the formulations' viscosity (DV-I PRIME, USA).

Table 3: Antimicrobial analysis of formulated gel A3 and Punica water-soluble extract

Living Things	Inhibition zone (mm) Volume of Aqueous Extract Gel applied (µl)			
	5	10	15	30
S. aureus	21	25	27	26
B. subtilis	13	15	18	18
P. aeruginosa	15	18	22	23
K. Pneumonia	13	16	18	19
E. coli	19	24	27	26
Candida *albicans*	10	12	15	17
Aspergillus *Niger*	15	19	23	25



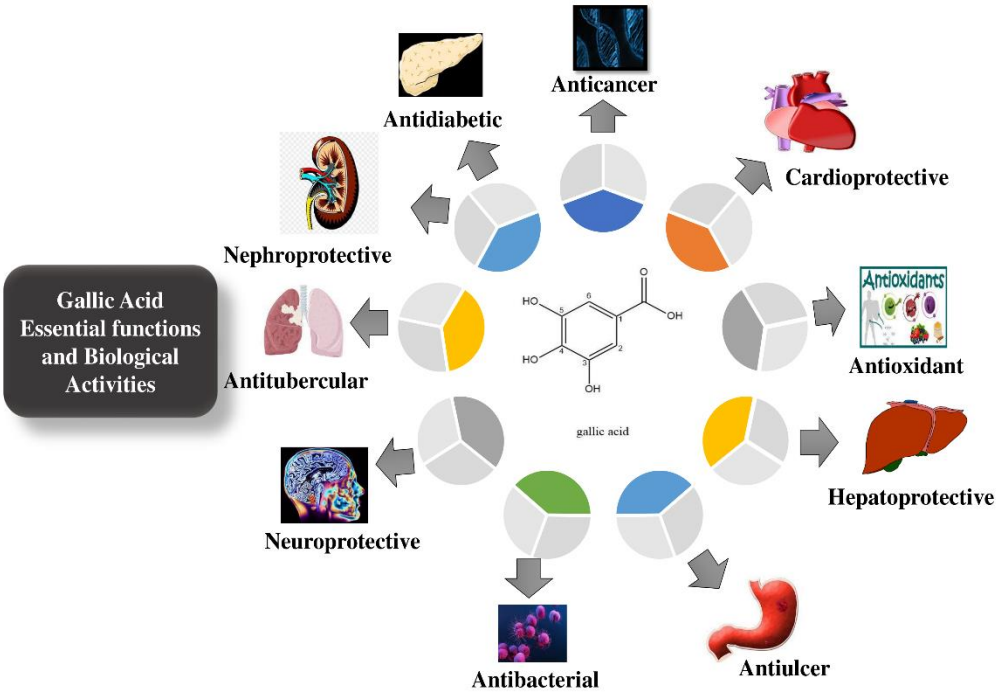
Index of primary cutaneous inflammation (PDII)

In this test, after applying the previously prepared gel to the face and body, within four hours, any reversible skin damage is monitored. One can classify the formulation in two categories: irritant or harmless based on its PDII score.

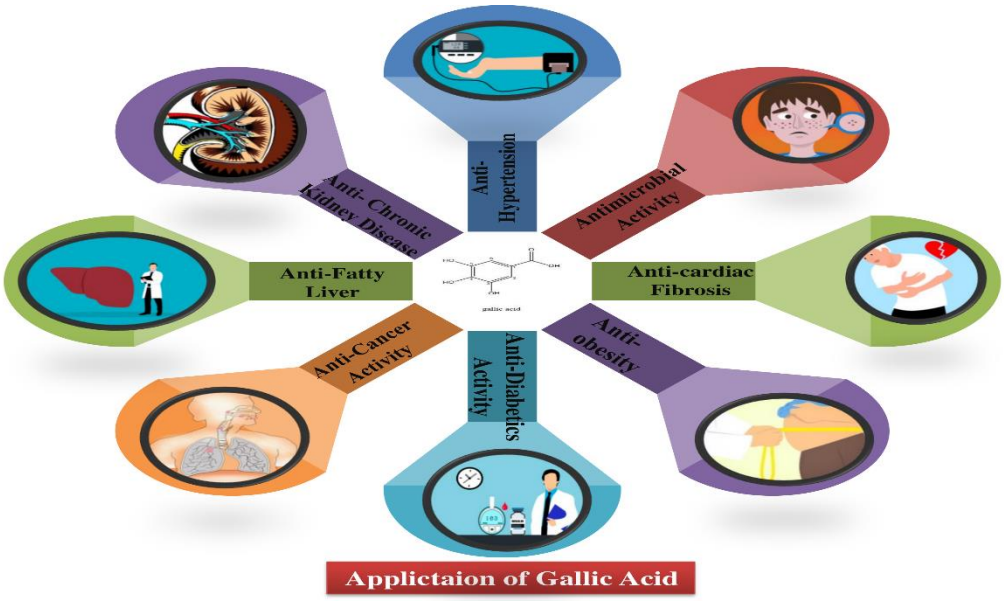
Frans Diffusion study I in vitro

In vitro Sigma Chemicals provided the cellulose membrane (0.45µm) that was used for the Frans diffusion investigation. A cellulose membrane that had been steeped in the buffering of phosphate 5.5 pH discharge solution during the previous night was covered with a sample of 1g of the preparation. At predetermined intervals, aliquots of three milliliters each were removed from the release medium while the shafts were rotating at 50 rpm. Equal amounts of new release media were used in lieu of the withdrawn samples. HPLC was used to analyze the samples, and the calibration curve that had been previously created was used to calculate the gallic acid content. The average of three conclusions was represented by each data point. Four hours were spent recording in vitro release investigations.

Research on antimicrobial agents

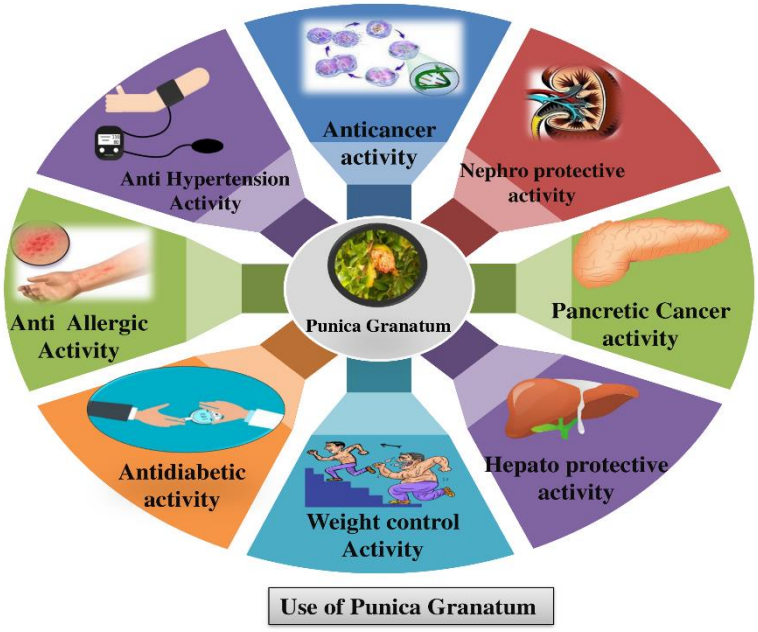


Test sample preparation (aqueous extract and gel) One milliliter of water was used to dissolve 100 mg of punica aqueous extract to create a test sample. One gram of punica gel was extracted into one milliliter of water to create a sample. Five, ten, and twenty microliters of the extract sample were used in the investigations. Extract samples containing 0.5, 1, 1.5, 2, and 2.5 mg were evaluated; the gel sample used the most effective extract concentration.



Assay for antibacterial

The agar well diffusion method was used to assess the antibacterial activity in vitro. The media utilized was nutrient agar. The bacterial culture was injected onto sterile agar for 48 hours at 37°C. A sterile borer was used to bore wells, and prepared gels were then inserted into them. To allow the extracts to pre-diffusion into the agar, plates were refrigerated for two hours. The plates were then incubated at 37°C for 24 hours.



Assay for antifungals

The agar well diffusion method was used to assess in-vitro antifungal activity. The medium was potato dextrose agar. The bacterial culture was injected onto sterile agar for 48 hours at 37°C. A sterile borer was used to bore wells, and prepared gels were then inserted into them. To allow the extracts to pre-diffusion into the agar, plates were refrigerated for two hours. The plates were then incubated at 37°C for 24 hours.

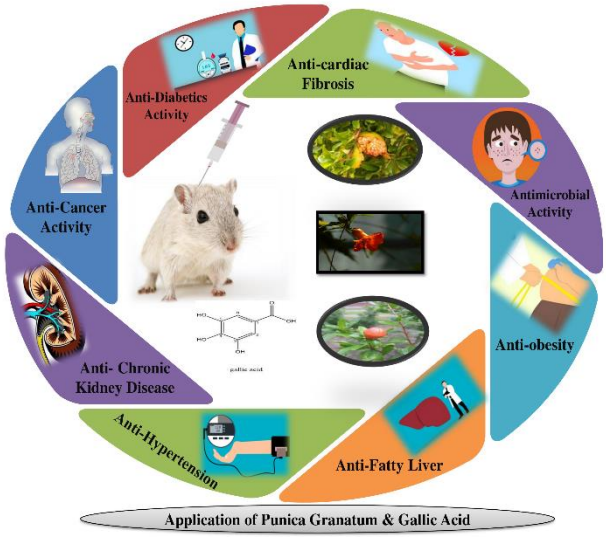


Table 4: Gallic Acid Chromatographic Condition.

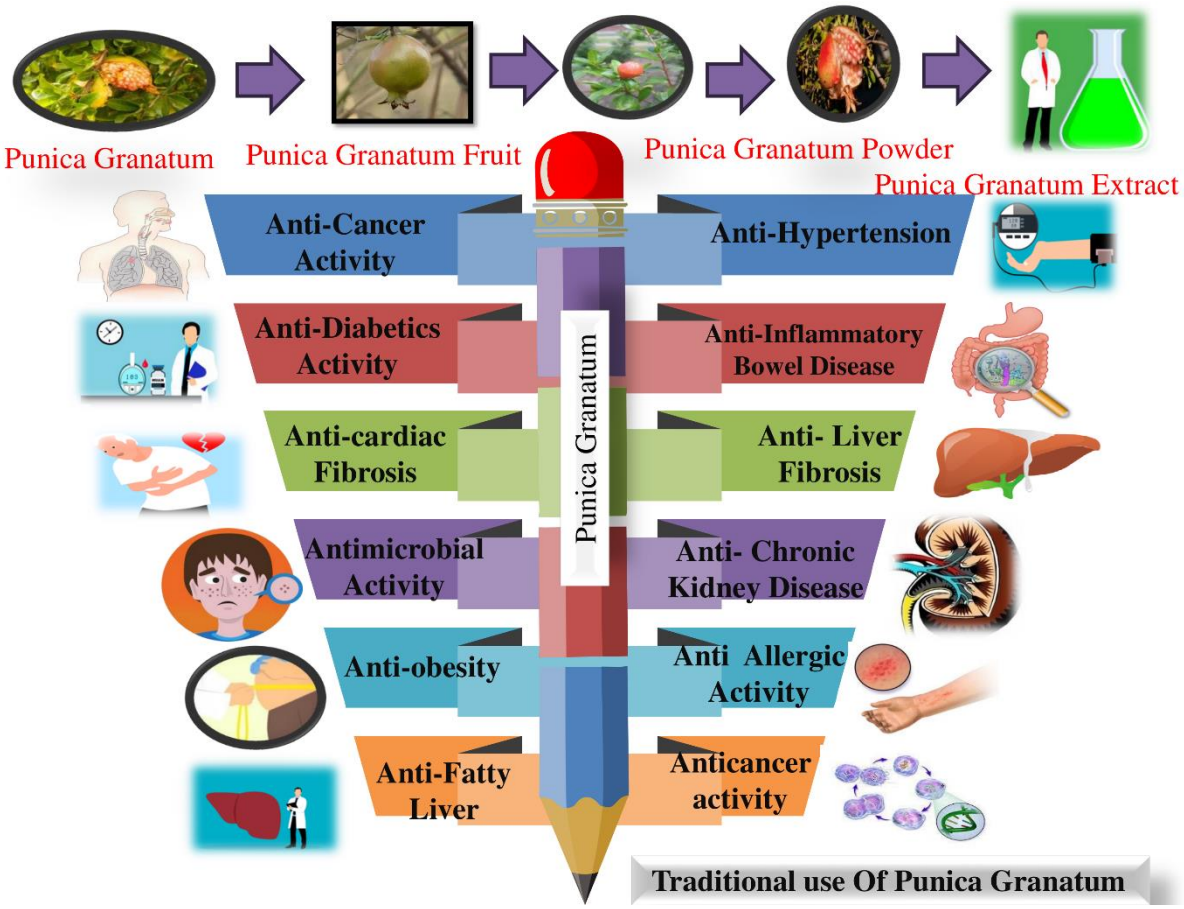
The value of the parameter.	Ideal circumstances
Mobile Phase	Water: methanol (80 :20 %v/v)
Stationary Phase	Phenomenex Luna C18 (4.6 x 250mm, 5µ particle size)
Wavelength	272 nm
Run time	10 min
Injection Volume	20 µL
Temperature	Ambient
Mode of Operation	Isocratic elution

HPLC-based quantitative evaluation of Punica gel and aqueous extract

Getting standard solutions ready Reference gallic acid standard stock solutions (1 mg/ml) were made in methanol. By appropriately diluting the stock solutions with methanol, working solutions of gallic acid were created. Every solution was made from scratch before analysis.

Fingerprinting using HPLC

The The Shimadzu Corporation LC 2010the HT chromatograph has a UV-VIS detector, a high throughput auto samples, and a quaternary low pressure grade unit pump, was used to conduct isocratic RP-HPLC. A 150 mm × 4.6 mm, i.e., 5 µm particle size, Phenomenex –c18 reverse-phase analytical column was employed. The ezchrom program was used to examine the data. An isocratic elution using a 70:30 v/v ratio of water to methanol was performed. The flow rate was 1 milliliter per minute. The optimal wavelength for recording the fingerprint chromatograms was 272 nm. The retention durations in the extract and gel chromatograms were compared to those of the reference standard gallic acid peak in order to identify the peaks in HPLC fingerprints.

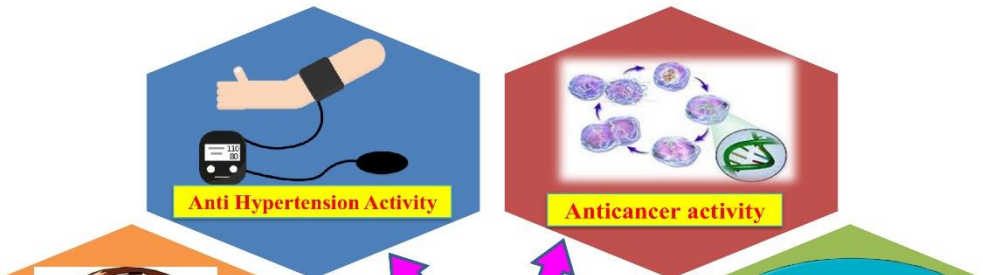


HPLC-based quantitative evaluation of Punica gel and aqueous extract

Parameters for calibration Under ideal circumstances, the HPLC technique was used to assess various quantities of the reference component (gallic acid). In order to demonstrate linear regression correlation, the investigation was carried out in triplicate, At 272 nm, the median area of peak response to various levels were measured.

OUTCOMES AND CONVERSATION

A gel containing varying concentrations of polymer and excipients was created using the aqueous extract of powdered pomegranate peel (Table 1). Out of the four distinct formulations, Gel C did not exhibit any significant changes in characteristics such as color, consistency, or Odor, and not one phase divergence occurred over the course of the study (Table 2). Gel C was therefore taken into consideration for additional assessment and antimicrobial research.



Frans Diffusion study in vitro

Figure 1 shows the punica topical gel C's in-vitro release profile. After four hours, 58.65% of the gallic acid was released, and within five hours, 96.9% of the gallic acid was released. This indicates that the gallic acid release was extremely rapid. Three duplicates of the research were conducted.

Antimicrobial analysis of gel and extract

Five, concentrations of the aqueous extract were shown to have antibacterial properties. Concentrations of 0.5–1.5 mg were determined to be the most effective among them. Therefore, a dosage of 30 mg was investigated for gel. Table 3 provides a description of the specific observations. Every sample was examined three times.

Optimization of HPLC settings for fingerprinting and HPLC analysis for gallic acid content

The HPLC separation parameters, including the isocratic program and mobile phase selection, were optimized. In order to get Different portable phases with different gradients were screened in order to achieve a baseline separation of gallic acid in a relatively short analysis time for the HPLC quantification and a reliable the colours with the majority of features at satisfactory precision and consistency for the HPLC fingerprinting. Lastly, for the herb's qualitative research and HPLC the identification of fingerprints an isocratic elution was performed using

methanol: water (70:30 v/v) as the mobile phase at a flow rate of 1 ml/min. Table 4 lists all of the optimized settings.

HPLC-based quantitative evaluation of punica gel and aqueous extract

Parameters for calibration

The reference standard was dissolved in methanol to create standard stock solutions with a gallic acid content of 1 mg/mL. The gallic acid reference standard used for calibration has a Methanol dosage range: 0.5–5 µg/µl. For the given range of the gallic acid values, the Pearson correlation factor was 0.99, indicating a strong relationship with the calibration equation. The devised reverse phase HPLC technique was used to quantitatively quantify the punica aqueous extract and gel. The mean amount of gallic acid in each sample was ascertained by triple analysis. A water-soluble extract of the gallic acid and within the company solution were determined to have respective contents of 3.4% and 3.5%.

RESULTS

The Punica gel produced in-house shown excellent antibacterial properties. The gel was effectively prepared and assessed for both the gallic acid content (3.5%) and the medicinal characteristics of the formulation. The HPLC method of measuring the gel's gallic acid concentration provided strong analytical evidence for the herbal gel. In the modern period, appropriate phytochemical analysis is required. Therefore, with more thorough research and a relatively low formulation and evaluation effort, this strong antibacterial herbal gel may become a commercial product.

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