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Research Article

Formualtion and Evaluation of Punica Topical Gel for its Content of Gallic Acid and Anti-Microbial Study

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ABSTRACT

Dried, powdered peel of *Punica granatum* was extracted with aqueous as solvent using soxhlet. Topical formulation gels were formulated with different combination of polymers and aqueous extract of punica. These formulations were evaluated for their physicochemical parameters, viscosity, spreadability, gallic acid content (by HPLC) and antimicrobial activity. Gel was successfully formulated and evaluated for the pharmaceutical parameters of the formulation and also for the content of gallic acid (3.5%). The gel gave very well anti bacterial and anti fungal activity. The in-house punica gel showed most effective antimicrobial activity against *B. subtilis, P. aeruginosa, K. Pneumonia, A. niger*.

Keywords: *Punica granatum*, gel, anti-microbial agent, gallic acid, HPLC.

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[1-3]. 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[4]. The tannins present in the extract of the fruit rind were found to be effective as antihelmintic, antibacterial, antifungal and antiviral activities[5,6]. 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[7,8]. 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 esthetic value[9]. 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.

MATERIAL AND METHOD

Solvent and chemicals

The powdered of *Punica granatum* peel were purchased from Green pharmacy, Pune. All chemicals and reagents used were of analytical grade and HPLC grade. Gallic acid reference standard were purchased from Yucca Enterprises, Mumbai.

Microorganisms

For anti-bacterial study gram positive bacteria such as *Staphylococcus aureus, Bacillus subtilis* and gram negative bacteria like *Pseudomonas aeruginosa, Klebsiella Pneumonia, Escherchia coli* were used in the study. For anti-fungal study *Candida albicans* and *Aspergillus niger* were used in the study.

Preparation of extracts

Soxhlet extraction method was employed for extraction of *Punica granatum* peel powder in the ratio of 1:6. The aqueous extract was filtered, evaporated and dried.

Development of formulation[10-12]

Different gels were formulated with varying the percentage of polymer (Carbopol 940P) Weighed amount of Carbopol 940P was soaked in distilled water overnight. The bioactive extract was accurately weighed and dissolved in distilled water. The solution was slowly added to the soaked Carbopol with continuous slow stirring. Care was taken to prevent air entrapment during stirring. This was followed by neutralization of the gel by 50% tri-ethanolamine; pH was adjusted to 7.3-7.5. Glycerin was added for its cooling effect followed by addition of methyl paraben as preservatives.

Evaluation of gel[10-12]

Formulations were evaluated for various preliminary parameters such as appearance, color, pH and spreadability. The formulation which passed these parameters were subjected to further evaluation such as viscosity, primary dermal irritation index, diffusion study and gallic acid content.

Homogeneity

Homogeneity, appearance, colour of formulated gels was

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Table 1: Development of formulation

Contents	Formulation code			
	A	B	C	D
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

Table 2: Evaluation parameters of formulated gel C

Parameters	Observations
Appearance	Smooth, clear, transparent
Color	Pale yellow
PH	7.3
Viscosity	5607 ±154 cps
Spreadability	5.01secs

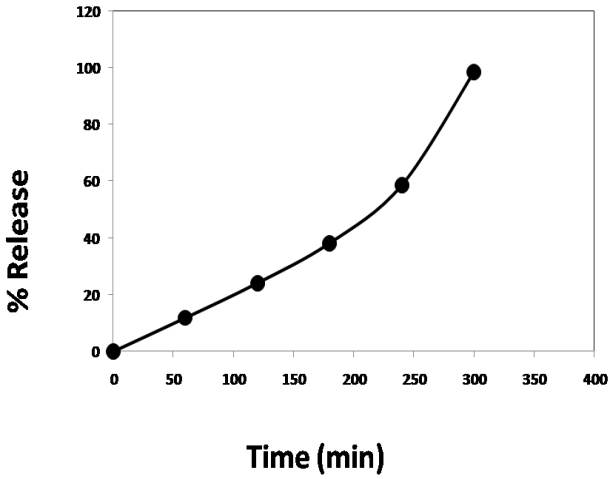

Figure 1: In-vitro Frans Diffusion study

Table 3: Anti-microbial study of punica aqueous extract and formulated gel C

Organisms	Zone of inhibition(mm) Volume added (µl)			
	Aqueous Extract			Gel
	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

examined by visual inspection.

pH

pH of formulated gel was determined by pH meter.

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides, and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spreadability was calculated using the formula S = ML/T, where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

Viscosity

The viscosity of the formulations was checked using a Brookfield Viscometer (DV-I PRIME, USA).

Primary Dermal irritation index (PDII)

This test is done by applying the formulated gel onto the skin and then observed for any reversible damage to the skin within 4 hrs. Based on their PDII score, the formulation can be graded as irritating or non-irritating.

In-vitro Frans Diffusion study

In-vitro Frans diffusion study was carried out by using Cellulose membrane (0.45µm, obtained from sigma chemicals). A sample of 1g of the preparation was spread on a cellulose membrane previously soaked overnight in the release medium (phosphate buffer pH 5.5.) The shafts were rotated at 50 rpm and aliquots each of 3 ml were withdrawn from the release medium at specified time intervals. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed by HPLC and the concentration of the gallic acid was determined from the previously constructed calibration curve. Each data point represented the average of three determinations. In vitro release studies were recorded for a four hour period.

Antimicrobial study

Preparation of test samples (Aqueous extract and gel)

Test sample of punica aqueous extract was prepared by dissolving 100mg in 1ml of water. Punica gel sample was prepared by extracting 1 gm of gel into 1ml of water. The studies were carried out on 5, 10, 20µl of extract sample. Extract sample were tested on 0.5, 1, 1.5, 2, 2.5mg and the effective highest concentration of extract was employed for gel sample.

Antibacterial assay

In-vitro antibacterial activity was evaluated using the agar well diffusion technique. Nutrient agar was used as the medium. The sterile agar was inoculated with the bacteria culture for 48 hrs, at 37°C. Wells were bored by using a sterile borer and formulated gels were place into them. Plates were kept for 2 hrs in the refrigerator to enable pre-diffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hrs) at 37°C.

Antifungal assay

In-vitro antifungal activity was evaluated using the agar well diffusion technique. Potato dextrose agar was used as

Table 4: Chromatographic Condition for Gallic acid.

Parameter	Optimized conditions
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

the medium. The sterile agar was inoculated with the bacteria culture for 48 hrs, at 37°C. Wells were bored by using a sterile borer and formulated gels were place into them. Plates were kept for 2 hrs in the refrigerator to enable pre-diffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hrs) at 37°C.

Quantitative analysis of punica aqueous extract and punica gel by HPLC

Preparation of standard solutions

Standard stock solutions (1 mg/ml) of reference gallic acid were prepared in methanol. Working solutions of gallic acid were prepared by appropriate dilutions of the stock solutions with methanol. All solutions were prepared freshly prior to analysis.

HPLC fingerprinting

Isocratic RP-HPLC was performed using shimadzu LC-2010HT chromatograph equipped with quaternary low pressure gradient unit pump, high throughout auto sampler and UV-VIS detector. A phenomenex –c18 reverse –phase analytical column (150 mm × 4.6 mm, i.d., 5 µm particle size) was used. the data were analyzed using the ezchrom software. an isocratic elution was carried out with water: methanol (70:30 v/v). flow rate was 1ml/min flow rate. the fingerprint chromatograms were recorded at an optimized wavelength of 272 nm. the peaks in hplc fingerprints were identified by comparing the retention times in the chromatograms of extract and gel with those of reference standard gallic acid peak.

Quantitative analysis of punica aqueous extract and punica gel by HPLC

Calibration parameters

Different concentrations of the reference compound (Gallic acid) were analyzed by HPLC method under the optimized conditions. The analysis was performed in triplicates and mean peak area responses to the concentrations were recorded at 272 nm to establish linear regression correlation.

RESULTS AND DISCUSSION

The aqueous extract of pomegranate peel powder was formulated in gel with different concentration of polymer with excipients (Table 1). Among four different formulation Gel C did not show any considerable change in characters like colour, odour, consistency and there was no phase separation during study (Table 2). Thus, gel C was considered for further evaluation and anti microbial studies.

In-vitro Frans Diffusion study

The In-Vitro release profile of punica topical gel C is represented in Figure 1. It was observed that the amount of gallic acid released after 4 hours was 58.65% and after the 4th hour gallic acid release was very fast with 96.9 % releasing within 5 hours. The study was carried out in triplicate.

Antimicrobial study of extract and gel

The antimicrobial study of aqueous extract was observed at five concentration**s.** Among them, 0.5-1.5mg concentrations were found to be significantly effective. Thus, for gel 30mg of dose was studied. The detailed observations are described in table 3. Each sample was analysed in triplicate.

HPLC analysis for determination of gallic acid content

Optimization of HPLC conditions for fingerprinting

The HPLC separation conditions, such as choice of mobile phase and isocratic program, were optimized. A number of mobile phases with different gradients were screened in order to obtain a reliable chromatogram with most peaks at acceptable resolution and balance for the HPLC fingerprinting and to obtain baseline separation of gallic acid in a relatively short analytical time for the HPLC quantitation**.** Finally, an isocratic elution was carried out with methanol: water (70:30 v/v) as the mobile phase, and 1ml/min flow rate for the HPLC fingerprinting and quantitative analyses of the herb. In table 4 all optimized parameters are mentioned.

Quantitative analysis of punica aqueous extract and punica gel by HPLC

Calibration parameters

Standard stock solutions were prepared by dissolving the reference standard in methanol to obtain a concentration of 1mg/mL for gallic acid. The concentration range of gallic acid reference standard used for calibration was 0.5-5 µg/µl in methanol, respectively. For the given range of concentration of gallic acid the correlation coefficient was 0.99 showing good correlation with calibration equation. Punica aqueous extract and gel of were quantitatively determined using the developed reverse phase HPLC method. Each sample was analyzed in triplicate to determine the mean content of gallic acid. The % content of gallic acid aqueous extract and in-house gel was found to be 3.4% and 3.5% respectively.

CONCLUSION

The in-house punica gel showed effective antimicrobial activity. Gel was successfully formulated and evaluated for the pharmaceutical parameters of the formulation and also for the content of gallic acid (3.5%). The quantification of gel by HPLC method for gallic acid content gave a good analytical support of the herbal gel. Appropriate phytochemical analysis is demand of current era. Thus, this effective antimicrobial herbal gel has a potential to be a commercial product with more extensive studies which has very minimal efforts to formulate and evaluate.

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