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Research Article

Design and Characterization of *Punica granutum* Seed Oil-loaded Cream: Assessment of *In vitro* Antimicrobial Potential

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ABSTRACT

The present investigation was started with aim to formulate and assess the antimicrobial potential of *Punica granatum* seed oil cream. The *P. granatum* seed oil cream was formulated using glyceryl monostearate and other lipids like stearic acid as well as cetyl alcohol. The formulated cream was then evaluated with respect to physicochemical properties and *in vitro* antimicrobial activity against *Escherichia coli*. The formulated oil loaded cream showed acceptable physicochemical properties like pH, spreadability, type of emulsion and viscosity which confer its suitability for topical application. In addition to this, the *in vitro* antimicrobial potential of oil loaded cream against *E. coli* was also found to be acceptable. Thus, formulated cream could be promising alternative for topical application of *P. granatum* seed oil with better antimicrobial potential.

Introduction

Pomegranate (*Punica granatum* L.) is well-known edible fruit having well recognized medicinal values.[1] Pomegranate fruit is well known for its antibacterial and anti-inflammatory potentials.[2] The fruit contains 22% of seeds which is generally waste products of processed pomegranate fruits.[3] However these seeds has great interest of presence of high quality oil in it. Pomegranate seed oil contains 65 to 85% conjugated fatty acids mainly punicic acid in conjugation with linolenic acid.[4] The seed oil, also contains rich proportion of γ-tocopherol, phytosterols, phospholipids and triterpenes.[5] The pomegranate seed oil and fruit extract has proved to be an effective antimicrobial agent. In addition to this, it reveals profound cell growth inhibition effect in skin and breast cancers.[6]

Oraibi *et al.*,[7] assessed antimicrobial potential of aqueous as well as alcoholic extracts of *P. granatum* peel against various species of microorganisms. Both extracts showed maximum antibacterial potential against *Staphylococcus aureus* and minimum antibacterial potential against *Pseudomonas aeruginosa*. Abdollahzadeh *et al.*,[8] investigated antibacterial and antifungal activities of *Punica granatum* peel extract against oral pathogens. The formulated extract exhibited maximum antibacterial activity against *Staphylococcus epidermis* and acceptable antifungal potential against *Candida albicans*. Kaur *et al.*,[9] successfully designed copper nanoparticles using *Punica granatum* peel extract. *P. granatum* peel extract was used as reducing agent to synthesize the copper nanoparticles and assessed for *in vitro* antimicrobial potential against opportunistic microorganisms. The formulated nanoparticles exhibited acceptable antimicrobial potential *Micrococcus luteus*, *P. aeruginosa*, *Salmonella enterica* and *E. aerogenes*. Janani *et al.*,[10] analyzed antimicrobial potentials of *P. granatum* peel and seed extracts against

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various microbial species causing infection in oral cavity. Both aqueous and alcoholic extracts showed good antimicrobial activities. Fathi *et al.*,[3] have successfully designed *P. granatum* seed oil loaded lipid nanoparticles and assessed antimicrobial potential. The formulated oil loaded nanoparticles showed acceptable physicochemical properties and morphology. In addition to this, the oil loaded lipid nanoparticles revealed enhanced *in vitro* antimicrobial activity than *P. granatum* seed oil emulsion. Thus, nanoparticles could be promising carrier for delivery of *P. granatum* seed oil.

Numerous landmark studies have highlighted the antimicrobial potential of *P. granatum* peel extract.[11] However very limited investigations were planned to investigate antimicrobial activity of *P. granatum* seed oil. Thus, it is stringent need to assess antimicrobial potential and to design suitable drug delivery system for ease of application of seed oil.

Topical drug formulations are used for local treatment of various types of skin disorders but are also interesting for systemic delivery. The major advantage of topical drug delivery is avoidance of the hepatic first-pass metabolism, allowing local skin treatment, and decreasing the risks of side effects from some drug substances. Emulsions in the form of creams and lotions are the most accepted systems for topical delivery as they have good cosmetic properties and are suitable for delivery of hydrophobic substances.[12]

Thus present investigation has planned with aim to design *P. granatum* seed oil loaded cream and evaluate antimicrobial potential of the same. The seed oil loaded cream was formulate by emulsification method using three different lipids and assessed for physicochemical properties as well as *in vitro* antimicrobial potential. The formulated cream revealed acceptable physicochemical properties and profound antimicrobial activity. Thus, seed oil cream is acceptable alternative for treatment of topical microbial infections. However *in vivo* efficacy study using suitable animal model need to be carried out to measure *in vivo* performance of formulated cream.

Material and Method

Material

P. granatum oil purchased from local Ayurvedic Market. Solapur, India. Almond oil purchased from Rogan Badam. HPMC -K4M, cetyl alcohol, Carbopol 940, Tween 80, glyceryl monosterate (GMS), methyl Paraben were obtained from SD Fine Chemical Ltd. Mumbai, India. All other reagents, solvents and chemicals were analytical grade and purchased locally.

Formulation of cream

The *P. granatum* oil loaded cream was formulated using emulsification method.[13] Briefly, the cetyl alcohol, glyceryl monostearate, stearic acid were mixed and melted by heating in water bath at 70°C and calculated amount of *P. granatum* oil and almond oil were mixed to form oil phase (Table 1). The weighed quantity of carbopol 934 and other water-soluble ingredients were dissolved in water with continuous stirring using a mechanical stirrer and temperature of water is maintained at 70˚C. The oil phase was then mixed with aqueous phase with continuous stirring and pH of the formulation adjusted using triethanolamine.[14]

Evaluation of *Pomegranate seed* oil loaded cream

Assessment of Physicochemical Properties of Oil and Oil Loaded Cream

Physicochemical properties of *Pomegranate* seed oil like colour, odor, texture, refractive index, appearance, density,

Table 1: Formulation *P. granatum* oil loaded cream

S. no.	*Ingredients*	*F1 (% w/w)*	*F2 (% w/w)*	*F3 (% w/w)*	*F4 (% w/w)*
1	*Pomegranate seed* oil	3	5	3	5
2	Almond oil	3	5	3	5
3	Glyceryl monostearate	2	3	4	5
4	Cetyl alcohol	4	-	-	4
5	Stearic acid	4	-	-	4
6	Propylene glycol	0.5	1	1.5	2
7	Honey	4	-	-	4
8	Tween 20	1.05	2.1	1.05	2.1
9	Glycerin	1.5	3	1.5	3
10	HPMC-K4M	0.6	-	-	0.6
11	Carbopol 940	0.3	0.4	0.5	0.6
12	Propyl paraben	0.3	0.3	0.3	0.3
13	Methyl paraben	0.3	0.3	0.3	0.3
14	Triethanolamine	q.s.	q.s.	q.s.	q.s.
15	Water	q.s.	q.s.	q.s.	q.s.

acid value, saponification value, iodine value were assessed as per procedure mentioned in official publication like Indian Pharmacopoeia. In addition to this, the formulated oil loaded cream was assessed with respect to appearance, color, odor, texture, consistency, greasiness, homogeneity and grittiness.

pH Determination

The pH of oil loaded cream was determined using a digital pH meter.[15] The pH meter electrode was prewashed with distilled water and dipped in the formulation and the pH was recorded at ambient conditions. The ideal range of pH for topical preparation is 6.0–7.5.

Spreadability

Spreadability of oil loaded cream was assessed using spreadability apparatus.[16] The apparatus consisted of two glass slides, one which is movable and another one is fixed onto the wooden board, tied to a thread which is passed over a pulley, it carries weight. The 1 gm of the cream was placed between the two slides. On the upper slide, 100 gm was placed for 1 to 2 mins to remove entrapped air between the slides and to provide a uniform film of the cream. Later on, top slide was subjected to a pull by attaching 5 gm weight to slide. The time required for the top slide to travel premarked 6.5 cm distance was noted and spreadability of cream was calculated using following equation.

$$S = (M \times L)/T$$

Where, S is spreadability of cream, M is weight attached to top slide in 'gm', L is the length of slide in 'cm' and T is the time required for slide to travel fixed distance.

Viscosity

The viscosity of the formulated oil loaded cream was measured using Brookfield viscometer. The cream was rotated at 100 rpm using spindle S63, S64 the corresponding dial readings were noted.[13]

Type of Emulsion

The type of emulsion was determined using dilution test. The 2.5 mL of water was taken in the test tube and 1 gm of formulated cream was added into it with continuous shaking. The dispersion was then observed for distribution of phases. The separation of phases indicates W/O type emulsion. Where even distribution of phases indicates O/W type emulsion.[13]

Assessment of Skin Irritancy Potential

The skin irritation potential of formulated oil loaded cream was assessed on dorsal left-hand surface.[17] Briefly, the area of 1 cm^2 was marked on dorsal left-hand and 0.5 gm formulated cream was applied on marked area. The irritation potential was measured by periodically checking the skin surface for sign of any erythema, edema and irritancy up to 24 hrs and reported.

Stability Study

The stability of formulated oil loaded cream was measured according to ICH guidelines (Q1A R2). Briefly, the well packed cream was placed in a stability chamber under a controlled temperature at 30 ± 2˚C and 65% RH for three months. After prescribed storage period the cream was evaluated with respect to physiological and organoleptic parameters.

Assessment of In vitro Antimicrobial Potential

In-vitro antimicrobial potential of formulated cream against *Escherichia coli* was evaluated using agar well diffusion technique. Muller-Hinton agar was used as an microbial growth medium. The sterile agar was inoculated with the *E. coli* in petri plate and the wells were bored by using a sterile borer. The each batch of formulated oil loaded cream was dispersed is concentration was adjusted to 1000 ppm. 100 µL of resulting dispersion was placed well and plates were incubated at 37°C for 24 hrs. The antimicrobial potential of cream was assessed by measuring zone of microbial growth inhibition after 24 hrs.

Result and Discussion

Formulation of *Pomegranate Seed* Oil Loaded Cream

The cream containing *Pomegranate seed* oil and almond oil was formulated in four different batches (F1 to F4). The lipids like glyceryl monostearate, cetyl alcohol and stearic acid were used for formulation of oil loaded cream. The amount of these three lipids were varied to formulate four different batches of oil loaded cream.

Evaluation of *Pomegranate Seed* Oil Loaded Cream

Physicochemical Properties of Oil and Oil Loaded Cream

Pomegranate seed oil was evaluated with respect to physical properties like color, odor, texture, appearance, specific gravity and refractive index and results are highlighted in Table 2. All physical properties were found to be acceptable as per Indian Pharmacopoeia.

All batches (F1 to F4) of formulated oil loaded cream were assessed for physicochemical properties like homogeneity, grittiness, greasiness and consistency. All the prepared batches F1 to F4 showed light buff color with pleasant odor. In addition to this, all cream batches were homogenous with absence of grittiness and greasiness which revealed its suitability for topical application (Table 3). The consistency of F3 and F4 was found to be very good compared to F1 and F2. This may be due to increase in percentage of lipid in F3 and F4.

Determination of pH of Formulated Cream

The ideal pH range of the cream is 5.5 to 7.2. In present oil loaded cream, the triethanolamine was used to adjust

the pH. The pH of the formulated oil loaded cream was maintained in the range of 6.0 to 6.5 and highlighted in Table 4. The pH values all batches of formulated oil loaded cream were found to be suitable for topical application and compatible with skin.

Table 2: Physicochemical properties of *Pomegranate seed* oil

Sr, No	*Parameters*	*Pomegranate seed oil*
1	Colour	Yellow
2	Odor	Characteristics
3	Texture	Clear
4	Appearance	Clear liquid
5	Specific gravity(g/mL)	0.934
6	Refractive Index	1.4630

Table 3: Physicochemical properties of oil loaded cream

Parameters	*F1*	*F2*	*F3*	*F4*
Homogeneity	Yes	Yes	Yes	Yes
Grittiness	No	No	No	No
Greasiness	No	No	No	No
Consistency	++	++	+++	+++

Good:++, very good:+++

Assessment of Viscosity of Formulated Cream

Brookfield viscometer was used to measure viscosity of formulated oil loaded cream. The viscosity of all formulated batches of cream was in the range of 16900 to 46700 cps (Table 4). It is found that, the viscosity of cream was increase on increasing the concentration of lipid in the cream. This could be due to increasing total concentration of lipids in cream.

Spreadability and Type of Emulsion

The spreadability plays key role in overall performance of topically applied semisolids. The formulated oil loaded cream revealed spreadability in the range of 66.75 to 87.26 gcm/s, which found to be suitable for topical application (Table 4). The formulation batches F3 and F4 showed good spreadability which could be due to presence of more concentration of glyceryl monostearate in these formations. The dilution test confirmed that all formulations were O/W cream.

Table 4: Physicochemical properties of formulated oil loaded cream at different storage period

Batches	*parameters*	*0 month*	*1 month*	*2 month*	*3 month*
F1	Colour	Buff	Buff	Buff	Buff
	Texture	Very smooth	Very smooth	Smooth	Slight sticky
	pH	6.15	5.98	6.21	6.41
	Spreadability(gcm/s)	85.36	84.52	78.53	72.25
	Viscosity (cps)	24500	22500	21500	20500
	Appearance	+++	++	++	+
F2	Colour	Buff	Buff	Buff	Buff
	Texture	Very smooth	Very smooth	Smooth	Slight sticky
	pH	6.25	6.38	6.46	6.74
	Spreadability(gcm/s)	87.26	85.14	79.86	74.32
	Viscosity	16900	15800	13500	11200
	Appearance	+++	++	++	+
F3	Colour	Buff	Buff	Buff	Buff
	Texture	Very smooth	Very smooth	Smooth	smooth
	pH	6.35	6.23	6.42	6.59
	Spreadability (gcm/s)	66.75	65.52	65.22	65.12
	Viscosity (cps)	34700	34600	34500	34500
	Appearance	+++	+++	++	++
F4	Colour	Buff	Buff	Buff	Buff
	Texture	Very smooth	Very smooth	Smooth	Smooth
	pH	6.29	6.41	6.49	6.55
	Spreadability(gcm/s)	66.86	66.56	66.45	66.35
	Viscosity(cps)	46700	46000	45500	46600
	Appearance	+++	+++	+++	++

Very good:+++, good:++, poor:+

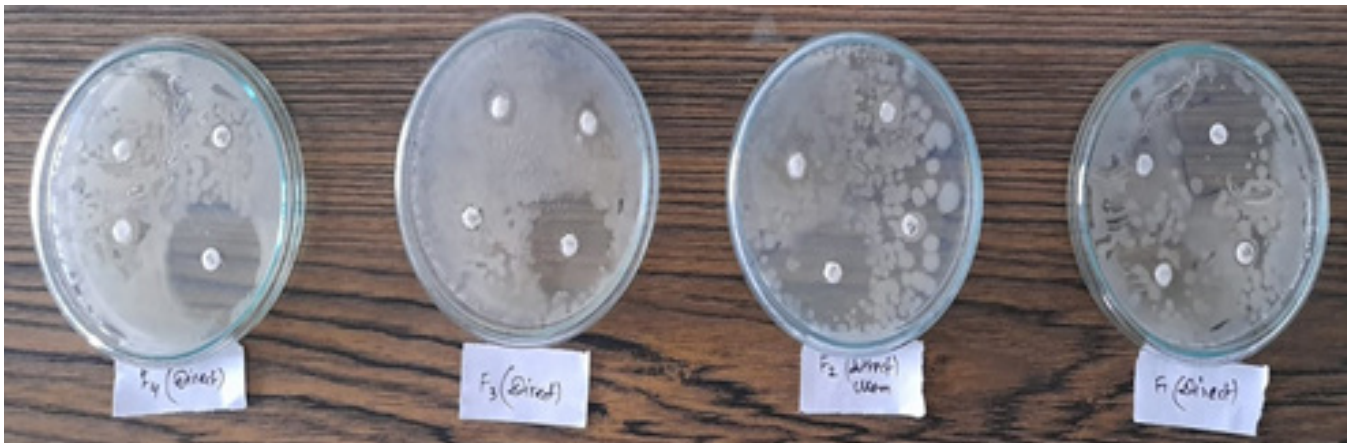

Fig. 1: *In-vitro* antibacterial activity of oil loaded cream

Table 5: Zone of microbial growth inhibition on oil loaded cream treatment

Formulation Code	*Zone of inhibition (mm)*
F1	12
F2	14
F3	13
F4	16

Irritancy Test

When the formulations were applied to the dorsal surface of the hand in the specified area and observed for erythema, edema, and irritancy up to 24 hrs. None of the formulations showed any sign of edema, inflammation and irritation, redness. The results of the study proved safety of the formulated cream.

Assessment of In-vitro Antimicrobial Potential

The *in vitro* antimicrobial potential of formulated oil loaded cream was assessed against *E. coli* using agar well diffusion technique.[3] The zones of microbial growth inhibition on treatment of various batches oil loaded cream are highlighted in Table 5. The oil loaded cream revealed increase in antimicrobial potential with increase in amount of oil from F1 to F4 (Fig.1). The cream batch F4 showed maximum antimicrobial potential with 16 mm diameter of microbial growth inhibition. This could be due to presence of maximum proportion of oil in F4 to kill *E. coli*.

Conclusion

P. granatum seed oil has been used for many years in medicine for various therapeutic purposes. The aim of present study was formulate *P. granatum* seed oil loaded cream for ease of topical application of oil. The formulated oil revealed acceptable physicochemical properties with minimal irritation potential to skin. In addition to this, the formulated cream showed good antimicrobial activity against *E. coli.* Thus, topical cream could be promising carrier for topical application of *P. granatum* seed oil with good antimicrobial potential.

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