RESEARCH ARTICLE

Formulation and Evaluation of *Punica granatum* L Seed Oil-loaded Cosmetic Cream: Its *In-vitro* Antioxidant Activity

Shrishail M. Ghurghure*, Atul A. Phatak

Department of Pharmaceutics, PES Modern College of Pharmacy, Pune, Maharastra, India

Received: 28th July, 2022; Revised: 12th September, 2022; Accepted: 03rd March, 2023; Available Online: 25th June, 2023

ABSTRACT

Cosmetic creams for the antioxidant activity of *Punica granutum L* seed oil (PSO) were formulated in different concentrations of oil in F1 to F6 formulations of cosmetic creams and their physicochemical properties were evaluated. There are several different pharmacological actions attributed to *P. granutum* L seed oil. The prepared cream was evaluated its properties like pH, resistance to flow, spreadability size, and surface charge value, and *ex-vivo* release study were performed. All results are found within the limit and it complies with the specification as per requirements. Good *in-vitro* DPPH scavenging activity was observed from the optimized batches throughout the preparation. To determine the stability profile, cosmetic creams containing PSO (Batches F1 through F6) were exposed to a three-month accelerated stability study in accordance with ICH recommendations. From this study it was concluded that prepared batches used for various treatments of skin disorders.

Keywords: *Punica granutum*, Seed oil, pH, Viscosity, Stability, Antioxidant.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.2.08

How to cite this article: Ghurghure SM, Phatak AA. Formulation and Evaluation of *Punica granatum* L Seed Oil-loaded Cosmetic Cream: Its *In-vitro* Antioxidant Activity. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):289-293.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cosmetics are products that contain herbs in their raw or extracted form.[1] Rigveda, Yajurveda, Ayurveda, Unani, and Homeopathy all provide inspiration for the concept of skin care cosmetics.[2,3] In the modern era, herbal knowledge and experience are combined with advanced cosmetic technology to create a safe and elegant beauty product acceptable to a wider range of people.[4] Essentially, it is beauty created by nature and perfected by technology.[5]

Creams are semisolid emulsions designed for topical application for localized effects. They can be applied to the skin, eyes, or used nasally, vaginally, or rectally for therapeutic or protective action or cosmetic function.[6,7] Traditional medicine is more affordable, accessible, and all-encompassing than Western medicine. Hence the World Health Organization (WHO) and the United States have both advocated for its use in underdeveloped nations.[8,9]

Many times, the treatment for skin diseases must be continued for an extended period of time. A safe and effective herbal skin cream is required to treat various skin diseases such as wounds, acne vulgaris, cracks, psoriasis, and other types of skin diseases. Although various creams are being considered for skin disease, the rate of tissue regeneration appears to be limited. After careful consideration of etiology and numerous conventional and unconventional treatments.[10] Rigveda, Yajurveda, Ayurveda, Unani, and Homeopathy all have foundational ideas on skincare that date back thousands of years. In the modern era, herbal knowledge and experience are being combined with advanced cosmetic technology to create a new product.[11]

MATERIALS AND METHOD

PSO bought products from a local ayurvedic market in Solapur. The following items were purchased: stearic acid, glyceryl monostearate, cetyl alcohol, carbopol 940, methyl paraben, methyl propyl paraben, propylene glycol, and triethanolamine.

Preparation of Cream with PSO Oil

Prepare the polymeric blend in water, then add glycerin and gel base, and finally add the PSO oil mix until a homogeneous dispersion is obtained. After that, add preservatives. Figure 1, Table 1, represents the preparation and composition methods.

Cosmetic Cream with PSO Formulation Characterization

PSO's acid value

Backeet AH and Stenlake JB[12] described a procedure for determining the acid value of *Punica granutum* L seed oil. As 10 gm cream was dissolved in 50 mL of mixture solvent and slowly heated with a reflux condenser until the cream was dissolved. After cooling, 0.1N NaOH was added and the mixture, using phenolphthalein as an indicator, was titrated.

*Author for Correspondence: shrishail.ghurghure@gamil.com

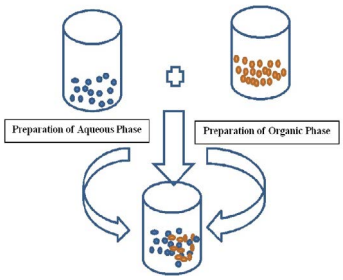

Figure 1: Method of Preparation Herbal Cosmetic Cream

The acid value was calculated using the following formula:

$$Acid\ value = \frac{Burette\ Reading\ X\ (0.1\ molarity\ of\ alkali) \times 5.61}{Weight\ of\ cream\ sample\ in\ gm}$$

Saponification value

R Manavalan and C Ramasamy[13] determined the saponification value of an oil sample. For 1 hour, 2 mL oil sample was refluxed with 0.5N alc. KOH mixture. The solution should then be cooled before adding 2 to 3 drops of phenolphthalein as an indicator, titrated against 0.5 N HCL. Formula 13 was used to calculate the saponification value.

$$\text{Saponification Value} = \frac{Volume\ of\ titate\ in\ ml - Volume\ of\ titrant\ in\ ml\ X\ 0.02805\ X\ Normality\ of\ HCL}{Weight\ of\ Sample\ X\ 0.5}$$

Alkaloid content of PSO Oil[15]

The following alkaloid test was performed for the identification of alkaloid contents (Table 2).

Refractive index

The refractive index of oil was observed under the optical Abbe's Refractometer.[16]

Evaluation of Cosmetic Cream Containing PSO[17,18]

Organoleptic properties

The color, smell, and overall appearance of each of the six different prepared formulations (F1–F6) were recorded.

Table 2: Alkaloid content of pso oil

Test	*Inferences*
Dragendroff's	+
Mayer's	+
Hanger's	+
Wagner's	+

pH of the cream

All formulated preparations were tested for pH using a digital pH meter [Equip-tronics -EQ-610] and the results are shown in Table 3

Resistance to Flow

The viscosity was measured using a Brookfield DV-II + viscometer equipped with an LV-4 spindle. The modified formula was poured into the adaptor of the viscometer, and the rotational speed was elevated from 5 to 60 rpm.[17]

Spreadability studies[19]

The parallel plate method was used to determine the spreadability of cosmetic cream. Two glass slides were chosen for this method. One gram of cream was weighed and spread across a glass slide, and then additional glass slides were stacked on top of the cream. The slide was weighed with 1-gm of cream, and a thin coating of cream was smeared across the surface. After the load was taken off, the diameter of the crack was measured, and the time it took to open the glass slide was noted.

$$Spreadability = \frac{Weight\ of\ sample\ X\ Length\ of\ glass\ slide}{Time\ \ required\ to\ spread\ the\ sample}$$

Particle size

Using SAGLO SOFT-SGL Micro-Imaging software version 2, the average particle size and size distribution of different batches of cosmetic cream were found. The Malvern particle size analyzer was used to evaluate the optimized batch F6.[21]

Table 1: Cosmetic cream composition includes PSO formulations F1 through F6

Sr. No.	*Ingredients*	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*
1	Punica seed oil (mL)	1	2	3	4	5	6
2	Steric Acid (g)	4	4	4	4	4	4
3	Glyceryl Monostearate (g)	2	2	2	2	2	2
4	Cetyl alcohol (g)	4	4	4	4	4	4
5	HPMC (g)	0.4	0.4	0.4	0.4	0.4	0.4
6	Glycerin (mL)	3	3	3	3	3	3
7	Tween 80 (mL)	2.5	2.5	2.5	2.5	2.5	2.5
8	Carbopol (g)	0.3	0.3	0.3	0.3	0.3	0.3
9	Propylene glycol (mL)	1	1	1	1	1	1
10	Propyl paraben (g)	0.3	0.3	0.3	0.3	0.3	0.3
11	Methyl paraben (g)	0.3	0.3	0.3	0.3	0.3	0.3
12	Almond oil	1–mL added in each formulation					
13	Triethanolamine	Quantity sufficient in all the formulation					
14	water	Quantity sufficient in all the formulation					

Drug Content[22]

The drug dose was far below the maximum level. The amount of the drug released following incorporation should be measured. So, a UV-vis spectrophotometer was used to figure out how much drug was in the sample. With constant stirring, 0.1 mL of the emulsion was dissolved in 10 mL of distilled water. The solution was filtered with what-man filter paper. More dilutions are done until the right concentration is reached. Then, a UV-spectrophotometer-2201 is used to measure the concentration at 273 nm against a blank reagent of distilled water.

The drug content was calculated using the formula

$$Drug\ content = \frac{Analyzed\ content}{Therotical\ content}\ X\ 100$$

Globule size and zeta potential

Using a method called photon correlation spectroscopy the average globule size (GS) and surface charge (ZP) of cream were found using the Malvern Instrument Zetasizer ZS 90 (UK). At a 90° angle in 10 mm cells, the mean diameter was found to be 25°. The zeta potential was a great way to figure out how stable a colloidal system was because it showed how the electrical charges on the surface of the particles worked. A method called electrophoretic light scattering was used to figure it out. All globule size and zeta potential measurements were done at 25°C using disposable polystyrene cells, disposable 5 plain folded capillary zeta cells, and suitable dilutions of all samples with the original dispersion medium.

In-vitro release study

The *in-vitro* diffusion study was conducted in a cellophane membrane-modified diffusion cell. The membrane was doused in phosphate buffer pH 7.4 overnight, and then it was carefully clamped to one end of the hollow glass tube in the dialysis cell (2.3 cm diameter; 4–16 cm^2 area). The cream was then put on the dialysis membrane in an even layer. As the receptor compartment, there was a beaker with 80 mL of phosphate buffer in it. Both the donor compartment and the receptor compartment were kept close to each other. This whole thing was managed to keep on a magnetic stirrer, as well as the solution on the receptor side was stirred constantly with a magnetic bead at a temperature of 372°C. At the right times, a 5 mL sample was taken out and replaced with the same amount of new dissolution media.

The samples were looked at with a UV spectrophotometer at -max = 271 nm, and the total amount of drug released was calculated. Both the average and the standard deviation were found.

Antioxidant activity

The DPPH radical scavenging method, which again is based on the stable 1, 1-diphenyl-2-picryl hydrazyl, was used to figure out how well pomegranate oil worked as an antioxidant (DPPH). The reference standard was ascorbic acid, and the control was a DPPH solution in methanol. 20 to 120 g/mL of different concentrations of pomegranate oil were mixed with 1- mL of a 0.1 mM DPPH solution in methanol and left to sit for 30 minutes. It was checked how much the samples absorbed light at 517 nm.

The Inhibition Percentage was calculated as follows:

$$\%DPPH\ Radial\ Inhibition = \frac{Control - Symbol\ X\ 100}{Control}$$

Stability Studies[23]

To find out how stable the drug and formulation are stability studies were done according to ICH guidelines. Stability tests were done in line with ICH guidelines, and samples were looked at for their physical parameters and viscosity.

RESULTS AND DISCUSSION

- Acid Value: 0.66 and 0.63 mg KOH/g of oil were found to be the acid values of pomegranate seed oil. Acid value is a measure of the amount of free fatty acids in food and is usually a sign that it has gone bad. It can have no more than 3 mg KOH per gram of oil.
- Saponification Value Both: 185 and 181 mg KOH/g were found to be the saponification values of pomegranate seed oil. Because the saponification value shows the average molecular weight of fatty acids in oil fractions, chain length and lipid molecular weight are inversely related.

Alkaloids content

The pure PSO oil is tested for a number of different alkaloids, and the findings are tabulated here in Table 3.

RI of PSO

The RI value of PSO was observed under the optical Abbe's Refractometer, the refractive index is observed at 1.514 at a temperature of 37°C as shown in Figure 2.

Evaluation of Cosmetic Cream containing PSO

Organoleptic properties

All prepared formulation F1 to F6 was observed visually. They appear for their color, odor, and taste is given in following as in Table 4.

pH value

The pH of all cosmetic cream formulations containing PSO F1 to F6 was measured; the results were within the range of 5.4 to 6.2, indicating a neutral pH. The value is shown in Table 5.

Resistance to flow

A Brookfield viscometer set on spindle 61 at 60 rpm was used to measure the shear resistance of each batch. All measurements were done in triplicate for cosmetic batches F1–F6 with values ranging from 232 to 734 cps, and the average is reported in Table 5.

Table 3: Identification test for Alkaloids in pure PSO oil

Test	*Inference*
Dragendroff's Reagent	Confirmed
Mayer's Reagent	Confirmed
Hager's Reagent	Confirmed
Wagner's Reagent	Confirmed

Spreadability studies

The spreadability of all prepared formulations F1 to F6 was evaluated. The results are within the range of 56.7 to 120 g.cm/s. Table 5 summarizes the outcomes..

Average particle size

With the help of SAGLO SOFT-SGL Micro-Imaging software version-2, the sizes of batches F1 through F6 of the cosmetic cream containing PSO were calculated. Table 5 displays the results, which were found to be between 3.106 and 6353 nm. Figure 3 depicts the typical size distribution of particles.

Drug content

The percent content of drug for all cosmetic cream F1 to F6 was determined, and the results ranged from 75 to 94%. Figure 5 depicts the results of all batches.

Globule size

Average globule size ranged from 3.106 to 6353 µm. In terms of cosmetic cream stability, the optimized F6 batch, including PSO oil has a zeta potential of F6= -36.15 mV, which points towards a moderate level of stability. Figure 5 displays a surface plot and the average globule size.

Ex-Vivo drug release studies

In-vitro were performed for prepared cosmetic cream containing PSO batches F1 to 12 was found to be 75 to 98.41% given after 8 hours. Batch F6 showed the height drug release rate 98.41% *ex-vivo* release profile is given in Figure 6.

In-vitro antioxidant activity

In the microtiter plate, 100L of F6, F12 (1-mg/ mL) batches were taken. 0.1% methanolic DPPH (100 L) was applied to the samples and incubated in the dark for 30 minutes. The samples had a discoloration parameter. Purple to yellow and pale pink were categorized as strong and weak positive. The sample plates were read at 490 nm using an ELISA plate reader. DPPH (1, 1-Diphenyl-2, Picryl-Hydroxyl) free radicals were used to demonstrate F6 batch free radical scavenging activity (George *et al*., 1996). The following equation was used to calculate radical scavenging activity, which is shown in Table 6:

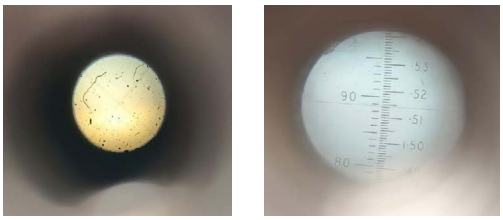

Figure 2: The refractive index of PSO

Table 3: Morphological features and thermal stability for pomegranate seed oil cosmetic cream

Sr. No	*Properties*	*Batches*	*Inference*
1.	Appearance	F1 to F6	Semisolid
2.	Color	F1 to F6	Batch 1 to 3 Faint color, 4 to 6 dark green color
3.	Odor	F1 to F6	Characteristic

Table 5: Properties of batch F1 to F6

Formulation	*pH*	*Viscosity (cps)*	*Spreadability (g.cm/s)*	*Particle size in µm*	*Drug Content (%)*
F1	5.7	472.7	56.7	5.011	75
F2	5.6	734	75	6.353	79
F3	5.7	453	73.3	3.106	83
F4	6.0	333.7	120	3.351	87
F5	6.2	598.7	120	4.711	91
F6	6.2	232	83	4.276	94

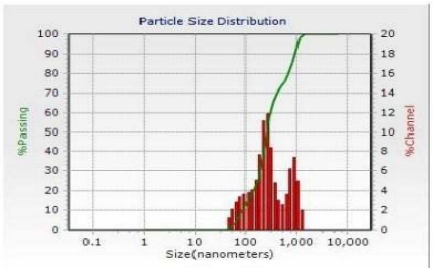

Figure 3: PSD of optimized batch F6

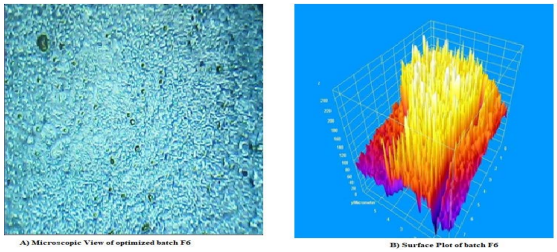

Figure 4: Particle surface area as a function of size for an optimized batch of F6 cosmetic cream containing PSO, seen using a microscope.

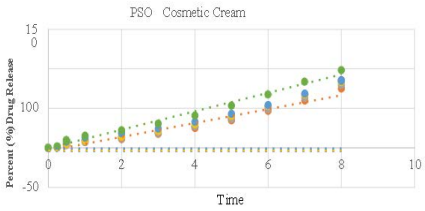

Figure 5: Release Studies of cosmetic cream batches F1 to F6

Table 6: Antioxidant activity of optimized batch F6

S. No	*Compounds (1-mg/ mL)*	*ABS (OD)*	*Mean*	*Percentage of DPPH radical scavenging*
1	Control	3.252		
		3.241	3.22	-
		3.195		
2	Std. Ascorbic acid	1.516		
		1.550	1.53	52.48
		1.541		
3	Cosmetic Cream batch F6	2.629		
		2.585	2.60	19.38
		2.597		

Table 7: Accelerated stability studies of cosmetic cream F1 to F6

Stability Parameters	*F 1*	*F 2*	*F 3*	*F 4*	*F 5*	*F 6*
Physical Nature	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Color	White	White	White	White	White	White
Thermal Stability at RH 65% and 35ºC	All batches are stable.					
Degradation of product	To our knowledge, no observable shifts have occurred..					

Stability studies

Thermal short-term accelerated stability testing of produced cosmetic cream comprising PSO oil F1 through F6 was done at room temperature in accordance with ICH recommendations. The humidity chamber ensured that the cream in the bottle remained at a consistent temperature. The sample was stored for two months. Physical properties were determined for the samples. Table 7 displays the results.[24]

CONCLUSION

In the present study, we developed a cosmetic cream with the *P. granutum L* seed oil-based preparation for various treatments of skin disorders. The PSO oil contains various alkaloids, glycosides, and tannin, as active key components. The prepared formulation shows good antioxidant activity; therefore, it is used to the treatment of skin disorders.

ACKNOWLEDGMENT

Dr. P.D. Chaudhari, Principal of the PES Modern College of Pharmacy in Yamunanagar Nigdi, Pune-411044, has inspired the authors during this research.

REFERENCES

1. Mahawar V, Patidar K, Joshi N. Development and Evaluation of herbal Antiaging Cream Formulation containing Annona squamosa leaf extract. Asian Journal of Pharmaceutical and clinical research. 2018;2;5:546.
2. Gavarkar P, Thorat S, Adnaik R, Mohite S, Magdum C. Characterization and formulation of skin cream seed oil extracted from cucumins melo. Schlor research library der pharmacia lettre.2016;8:90-3.
3. Mohiuddin A. k. Skin Care Creams: Formulation and use. OSP journal of clinical trials.2019;1: 2-22.
4. Dash A, Singh S, Justin T. Semi-solid dosage forms in pharmaceutics- basic principles and application to pharmacy practice, published by Academic Press.2013.
5. Swarbrick J, Rubino J, Rubino O.P. Course Dispersion in Remington: The Science and Practice of Pharmacy, published by Lippincott Williams & Wilkins, 2006; 1.
6. Matangi S.P, Arana S, Mamidi Gulshan M.D, Raghavamma S.T, Nadendla R.R. Formulation and Evaluation of Anti-aging Poly Herbal Cream. International Journal of Pharmaceutical Sciences Review and Research. 2014; 24(2):133-136. Available from: www.globelonline research.net Pal A, Soni M, Patidar K. Formulation and Evaluation of Poly-Herbal Cream.
7. International Journal of Pharmaceutical and Biological.2014; 5: 67-71.
8. Patel RP, Kamani R. Formulation Optimization and Evaluation of Mometasone furoate cream. Journal of Pharmacy Research.2009; 10(1):1565-1569.
9. Awal A, Kalra M, Raut A. Preparation and Evaluation of Polyherbal Cosmetic Cream. Der Pharmacia Lettre. 2013;5(1):83-88 Available online at www.scholarsresearchlibrary.com
10. Sherrow V. For Appearance Sake: The Historical encyclopedia of good Looks Beauty, and Grooming. Orxy press. 1995; 4:238–39.
11. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th edition-Part one by CBS Publisher New Delhi.146-147.
12. Manavalan R, Ramasamy C. Textbook of Physical Pharmaceutics. 9th Reprint, Pharma Med Press Publication. 96-97.
13. Jamshiya S. Formulation and Evaluation of Herbal skin Cream for Wound Healing (Doctoral dissertation, RVS College of Pharmaceutical Sciences, Coimbatore). Available from: http://repository-tnmgrmu.ac.in
14. Kaur S. Wound healing potential of medicinal plants with their screening models: A comprehensive review. Journal of drug delivery and therapeutics. 2016;6(1):56-66.
15. Mittal A, Sardana S. Herbal boon for wounds. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5:1-12.
16. Tiwari V.K. Burn wound: How it differs from other wounds. Indian journal of plastic surgery: official publication of the Association of Plastic Surgeons of India.2012;45(2):364.
17. Bathsa L.J, Arakareddy B, Kezia K. Formulation of Anti-bacterial Cream from *Plantoxalis Corniculata* and its Evaluation. International Journal of Current Pharmaceutical Research. 2020;12:40-44.
18. Chavan P, Kalshetti M, Navindgikar N. Formulation and Evaluation of Polyherbal Cream. International Journal of Current Research Pharmaceutical Research.2020; 12(4):75-77.
19. Himaja N. Formulation and evaluation of herbal cream from *Azadirachta Indica* ethanolic extract. International Journal of Pharmaceutical Science. 2017;1(1):23-26
20. Mali A, Karekar P, Yadav A.V. Formulation and Evaluation of Multipurpose Herbal Cream. International Journal of Science and Research. 2015;4(11):1495-1498.
21. Mishra A.P, Saklam S, Luigi M, Tiwari P. Formulation and Evaluation of Herbal Antioxidant Face Cream of *Nardostachys jatamansi* collected from Indian Himalayan region. Asian Topical Journal of Tropical Biomedicine. 2014;4(2):S679-S682.
22. Matangi S.P, Aruna S, Mamidi, G, Raghavamma S.T, Nadendla R.R. Formulation and Evaluation of Anti-aging Poly Herbal Cream. International Journal of Pharmaceutical Science Review and Research. 2014; 24(2):133-136.
23. Singh M, Sharma S, Kokhra S.L. Preparation and Evaluation of Herbal Cosmetic Cream. Pharmacology online. 2011;2 (1):1258-1264.
24. Arshan A, Nath B. Formulation and in-vitro Evaluation of Poly-herbal Anti-ageing Face Cream of *Coriandrum sativum* and Rose hip oil. International Journal of Current Pharmaceutical Research. 2017; 9(4):75-78.