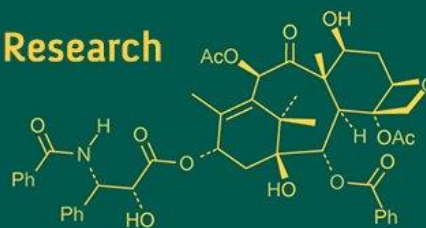


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Formulation and evaluation of an anti-aging topical emulsion utilizing pomegranate (*punica granatum*) seed oil: A comprehensive review

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Abstract

Skin aging is a complex biological process characterized by the degradation of collagen, reduced elasticity, and increased oxidative stress, which results in wrinkles and sagging. As consumer demand shifts toward safer, non-toxic alternatives to synthetic skincare, herbal cosmetics have emerged as a significant area of research in dermatological care. Pomegranate (*Punica granatum*) seed oil (PSO), rich in punicic acid and polyphenols, is highly regarded for its potent antioxidant, anti-inflammatory, and skin-rejuvenating properties. This review highlights the critical role of PSO in anti-aging applications, particularly in stimulating keratinocyte proliferation and providing protection against UV-induced photo aging. The article discusses the formulation techniques specifically oil-in-water (O/W) emulsions and optimization strategies for creating stable, nutrient-rich, and effective anti-aging creams containing PSO. Finally, the review summarizes standard evaluation methods, including pH assessment, viscosity, spread ability, stability studies, and *in vitro/in vivo* assays, which demonstrate that PSO-based formulations are safe, stable, and effective in enhancing skin moisture and reducing the appearance of wrinkles.

Keywords: Skin aging, herbal cosmetics, pomegranate seed oil, anti-aging, formulation, evaluation

Introduction

Skin, the body's largest organ, serves as a dynamic biological barrier that protects against environmental insults while maintaining internal homeostasis (Proksch *et al.*, 2008; Wang *et al.*, 2020) [46, 64]. Skin aging is a complex and multifactorial biological process characterized by progressive deterioration in physiological function and structural integrity, leading to reduced elasticity, increased fragility, dryness, and the formation of deep rhytides or wrinkles (Farage *et al.*, 2008; Hussien *et al.*, 2025) [14, 24]. The pathophysiology of skin aging is broadly classified into intrinsic and extrinsic aging processes. Intrinsic aging, also referred to as chronological aging, is a genetically regulated and inevitable phenomenon associated with dermal fibroblast senescence, reduced collagen synthesis, and slower keratinocyte turnover (Makrantonaki and Zouboulis, 2007). In contrast, extrinsic aging is primarily driven by environmental factors such as ultraviolet (UV) radiation, atmospheric pollution, smoking, and lifestyle-related stressors (Kammeyer and Luiten, 2015; Suvedi *et al.*, 2025) [30, 59]. Although these mechanisms differ in origin, both pathways converge through excessive generation of reactive oxygen species (ROS), resulting in oxidative stress and chronic inflammation (Pillai *et al.*, 2005; Wang *et al.*, 2025) [45, 65].

At the cellular level, elevated ROS production overwhelms the endogenous antioxidant defense system of the skin, triggering multiple pro-inflammatory signaling pathways that stimulate the expression of matrix metalloproteinases (MMPs), enzymes responsible for extracellular matrix (ECM) degradation (Fisher *et al.*, 2002) [16]. Increased MMP activity particularly affects type I and type III collagen fibers, leading to collagen fragmentation, dermal matrix disorganization, and loss of skin resilience (Quan *et al.*, 2009) [49]. As elastin fibers become fragmented and disordered, the skin progressively loses its tensile strength and

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elasticity, contributing to wrinkle formation and sagging (Baumann, 2007 [8]; Hussen *et al.*, 2025) [24]. Recent studies further emphasize that oxidative stress alters collagen homeostasis, enhances inflammatory cytokine release, and accelerates premature skin aging (Costa *et al.*, 2022; Wang *et al.*, 2025) [10, 65].

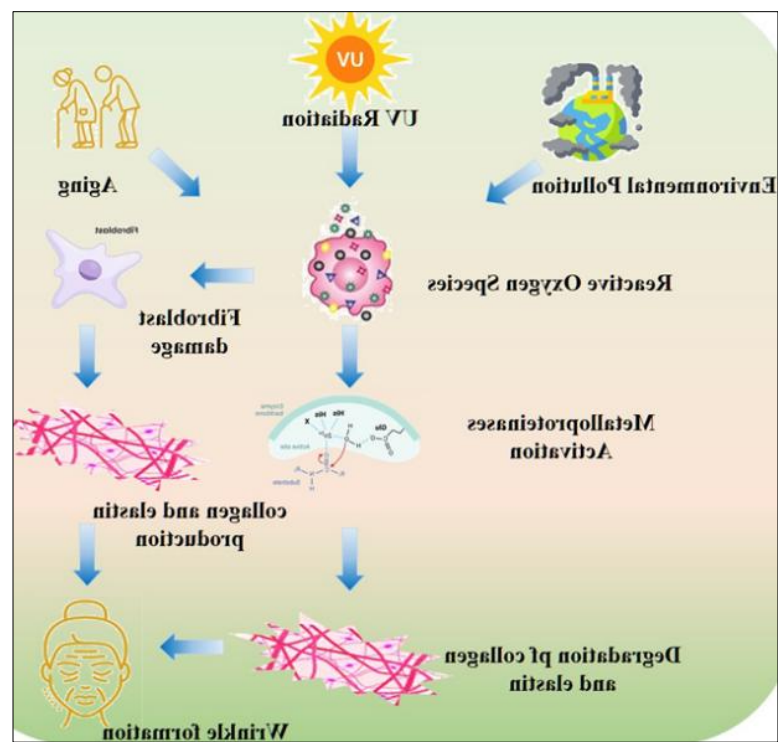


Fig 1: Mechanism of Skin Aging and Role of Oxidative Stress

In response to increasing concerns regarding premature skin aging, the dermatological and cosmetic industries have focused extensively on advanced anti-aging formulations. Although synthetic retinoids and peptides have traditionally dominated the anti-aging market, recent consumer preference has shifted toward plant-based and herbal cosmetics due to their improved biocompatibility, antioxidant potential, and lower incidence of adverse effects (Mukherjee *et al.*, 2011) [42]. Natural phytochemicals including polyphenols, flavonoids, carotenoids, and essential fatty acids have demonstrated substantial protective effects against oxidative damage, inflammation, collagen degradation, and photodamage (Nowak-Perlak *et al.*, 2025; Hussen *et al.*, 2025) [24, 43]. Recent clinical evidence also suggests that topical botanical formulations significantly improve skin hydration, elasticity, and overall skin appearance,

Highlighting their growing relevance in modern cosmeceutical research (Burlando *et al.*, 2024) [9].

Pomegranate (punica granatum): an overview

Punica granatum L., commonly known as pomegranate, is a deciduous shrub or small tree that has been revered for millennia for its high nutritional and medicinal value (Jurenka, 2008) [29]. Native to the regions spanning from modern-day Iran to northern India, it is now widely cultivated in tropical and subtropical regions worldwide, including the Mediterranean, China, and the United States (Viuda-Martos *et al.*, 2010; Mohammadi *et al.*, 2023) [39, 40, 63]. Historically considered the “fruit of paradise,” it is one of the oldest known fruits and serves as a vital natural resource for diverse therapeutic interventions (Lansky and Newman, 2007) [35].

Botanical classification

Table 1: Botanical Classification

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Lythraceae
Genus	Punica
Species	<i>Punica granatum</i> L.

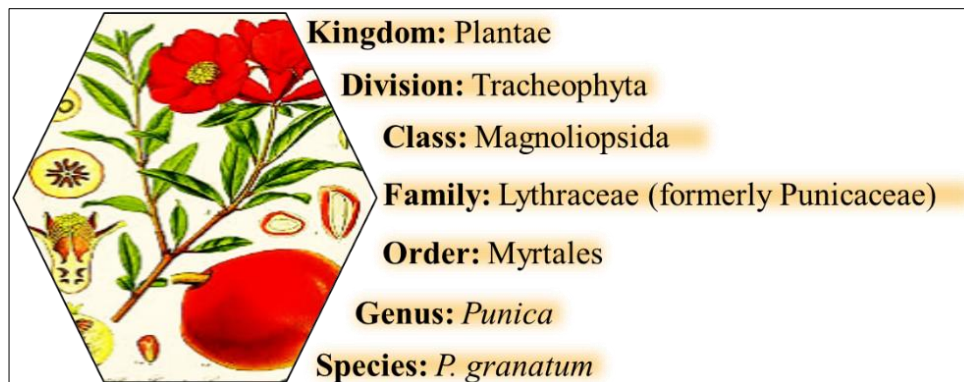


Fig 2: Botanical Morphology of Pomegranate

Plant parts and their phytochemical profiles

Medicinal value is distributed across various compartments of the plant, with each part offering a unique array of bioactive metabolites (Viuda-Martos *et al.*, 2010) [63].

Seed and seed oil: seeds comprise roughly 10-20% of the total fruit weight. They are a dense source of total lipids, with pomegranate seed oil (PSO) containing between 12% and 20% of the seed weight (Lansky and Newman, 2007; Verardo *et al.*, 2014) [35, 62].

Peel (pericarp): The inedible rind accounts for approximately 43-50% of the fruit's total weight. It is exceptionally rich in hydrolyzable tannins, specifically punicalagins and ellagic acid, which contribute to its potent antioxidant capacity (Elfalleh *et al.*, 2012; Gullón *et al.*, 2016) [13, 20].

Juice and arils: The arils (edible portion) consist of juice (approx. 78%) and seeds (22%). Pomegranate juice is recognized for its high content of anthocyanins (giving it its red hue), vitamin C, and potassium (Jurenka, 2008; Mphahlele *et al.*, 2014) [29, 41].

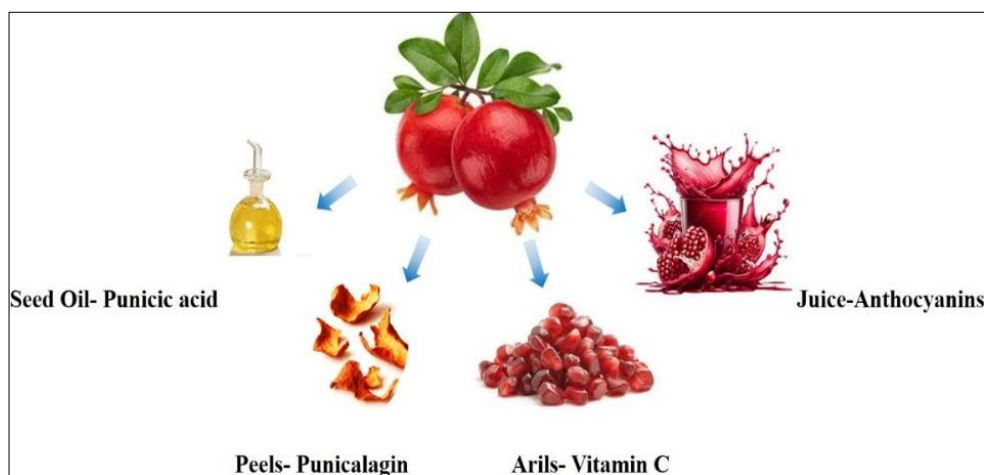


Fig 3: Distribution of Bioactive Compounds in Different Plant Parts

Traditional and ethno medicinal uses

Pomegranate has a storied history in holistic medicine systems including Ayurveda, Traditional Chinese Medicine (TCM), and Unani (Jurenka, 2008; Sharma *et al.*, 2023) [29, 55, 56].

Gastrointestinal disorders: Traditionally, the bark, peel, and juice have been used to treat diarrhea, dysentery, and intestinal parasites. In Ayurvedic medicine, the fruit is considered a "stomachic" and is used to manage acidity and abdominal distension (Lansky and Newman, 2007) [35].

Oral health: In several cultures, powdered rinds are utilized in tooth powders and mouthwashes to treat gingivitis, dental plaque, and mouth ulcers due to their strong antibacterial properties (Ismail *et al.*, 2012) [27].

Wound and skin care: In the Iranian system of medicine, extracts are applied to cuts, wounds, and edemas. In TCM, it is valued for its astringent nature to address skin inflammations (Viuda-Martos *et al.*, 2010) [63].

Reproductive health: Traditional records suggest the use of seeds for managing menstrual pain and preventing miscarriage (Jurenka, 2008; Sharma *et al.*, 2023) [29, 55, 56].

Pomegranate seed oil and punicic acid (pa)

Chemical composition of pso

A significant focus of recent research is the unique chemical composition of the seed's lipid fraction (Verardo *et al.*, 2014) [62].

Punicic acid content: Pomegranate seed oil is characterized by an exceptionally high concentration of punicic acid (~64-85%), a rare omega-5 conjugated triene isomer of (alpha) linolenic acid (Melo *et al.*, 2014; Almoraie *et al.*, 2025) [5, 38].

Biological activity: PA is considered the "rising star" of pomegranate nutraceuticals due to its multifaceted biological effects (Almoraie *et al.*, 2025) [5].

Anticarcinogenic: It has been shown to induce apoptosis and inhibit the proliferation of breast, prostate, and colon cancer cells (Lansky and Newman, 2007; Rocha *et al.*, 2012) [35].

Metabolic regulation: PA improves insulin sensitivity, aids in obesity management by reducing visceral adipose tissue, and helps normalize lipid profiles (Melo *et al.*, 2014; Almoraie *et al.*, 2025) [5, 38]. **Anti-inflammatory:** It inhibits the production of pro-inflammatory cytokines like (TNF-

alpha) and (IL-6), potentially serving as a therapeutic agent for inflammatory bowel diseases and cardiovascular protection (Juhaimi *et al.*, 2017; Kobylinska *et al.*, 2026) [4, 28].

Neuroprotection: Emerging studies indicate that PA may reduce (beta) amyloid deposits and neuroinflammation, showing promise for Alzheimer’s and Parkinson’s disease research (Almoraie *et al.*, 2025) [5].

Punica granatum is a high-value medicinal plant where the seed oil, primarily through the action of punicic acid, provides significant anti-inflammatory, anticarcinogenic, and metabolic health benefits (Verardo *et al.*, 2014; Kobylinska *et al.*, 2026) [62].

Pomegranate Seed Oil (PSO) is a highly valued, nutrient-dense oil extracted from the seeds of *Punica granatum* L., typically containing 74-85% conjugated linolenic acids (CLnA). It is increasingly used as a nutraceutical and in cosmetics due to its potent antioxidant, anti-inflammatory, and antimicrobial properties. The oil’s therapeutic effects are derived from a synergistic interaction among its unique fatty acid profile, tocopherols, and polyphenols (Kobylinska *et al.*, 2026; Verardo *et al.*, 2014) [62].

Phytochemical composition of pomegranate seed oil

- **Punicic acid (omega-5 fatty acid):** As the primary bioactive component, punicic acid (often 70-80%+ of the oil) is a conjugated isomer of linolenic acid. It is a rare polyunsaturated fatty acid known for its unique ability to modulate lipid metabolism and inhibit tumor growth (Almoraie *et al.*, 2025) [5].
- **Polyphenols:** Although more concentrated in the peel and juice, PSO contains significant levels of phenolic compounds, specifically Gallic acid, catechin, vanillic acid, and ellagic acid. These act as secondary metabolites that contribute to the oil’s overall stability and antioxidant capacity (Zielińska *et al.*, 2022) [66, 67].
- **Flavonoids:** Pomegranate seeds contain various flavonoids (including catechin and quercetin), which are known for their strong scavenging ability against reactive oxygen species (ROS) (Afshari *et al.*, 2016) [2].
- **Tocopherols (vitamin e):** PSO is rich in tocopherols (360-392 mg/100g), with (gamma) tocopherol being the most abundant (accounting for up to 95% of total tocopherols). These compounds act as powerful antioxidants to protect the oil from degradation and

provide vitamin E to the body (Melo *et al.*, 2014; Kobylinska *et al.*, 2026; Juhaimi *et al.*, 2017; Iriti *et al.*, 2023) [4, 26, 28, 38].

Biological and pharmacological activities

- **Antioxidant:** The combination of punicic acid and tocopherols neutralizes free radicals and reduces oxidative stress, protecting cell membranes from lipid peroxidation (Almoraie *et al.*, 2025) [5].
- **Anti-inflammatory:** Punicic acid inhibits pro-inflammatory markers (such as TNF-(alpha), IL-6, and NF (kappa) B. This makes it effective in managing chronic inflammatory conditions like obesity-related inflammation, arthritis, and skin conditions (Juhaimi *et al.*, 2017) [4, 28].
- **Anti-microbial:** PSO and its components (specifically phenolic compounds) exhibit antimicrobial activity by inhibiting fungal and bacterial growth (e.g., *S. aureus*) on the skin, contributing to its use in treating acne or minor infections (Kobylinska *et al.*, 2026).

Neutralizing free radicals and protecting against oxidative stress

Pomegranate seed oil is particularly adept at mitigating oxidative stress, a condition linked to chronic diseases, by multiple mechanisms (Verardo *et al.*, 2014; Hamouda and Felemban 2023; Almoraie *et al.*, 2025) [5, 22, 62].
Scavenging Free Radicals: The punicic acid’s unique conjugated double bonds are highly effective at neutralizing reactive oxygen species (ROS).
Enhancing Endogenous Enzymes: PSO increases the activity of natural antioxidant enzymes in the body, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase.
Inhibiting Lipid Peroxidation: It protects membrane lipids from damage caused by peroxy radicals, which is crucial for preventing atherosclerosis and cell aging.
Synergistic Action: The high levels of (gamma) tocopherol work in synergy with punicic acid to provide a robust defense against damage at both the cellular and tissue levels. (Verardo *et al.*, 2014; Hamouda and Felemban 2023; Almoraie *et al.*, 2025) [5, 22, 62]. The high punicic acid content (above 70-80%) makes PSO a "super-food" for addressing metabolic syndrome, including the reduction of triglycerides and improvement of insulin sensitivity. (Almoraie *et al.*, 2025; Melo *et al.*, 2014) [5, 38].

Table 2: Phytochemical Composition of PSO

Phytochemical Fraction	Primary Active Compound	Key Dermatological Mechanism	References
Conjugated PUFA (~65-85%)	Punicic Acid (Omega-5)	Down-regulates pro-inflammatory eicosanoid pathways and reduces oxidative stress	Verardo <i>et al.</i> , 2014; Almoraie <i>et al.</i> , 2025 [5, 62]
Tocopherols	γ-Tocopherol	Protects cell membranes from lipid peroxidation and enhances antioxidant defense	Melo <i>et al.</i> , 2014; Kobylinska <i>et al.</i> , 2026 [38]
Phytoestrogens	Genistein / Daidzein	Stimulates endogenous collagen and hyaluronic acid production, improving skin elasticity	Mukherjee <i>et al.</i> , 2011; Hussen <i>et al.</i> , 2025 [24, 42]
Free Phenolics	Ellagic Acid	Inhibits copper-dependent tyrosinase activity and protects against UV-induced photodamage	Gullón <i>et al.</i> , 2016; Burlando <i>et al.</i> , 2024 [9, 20]

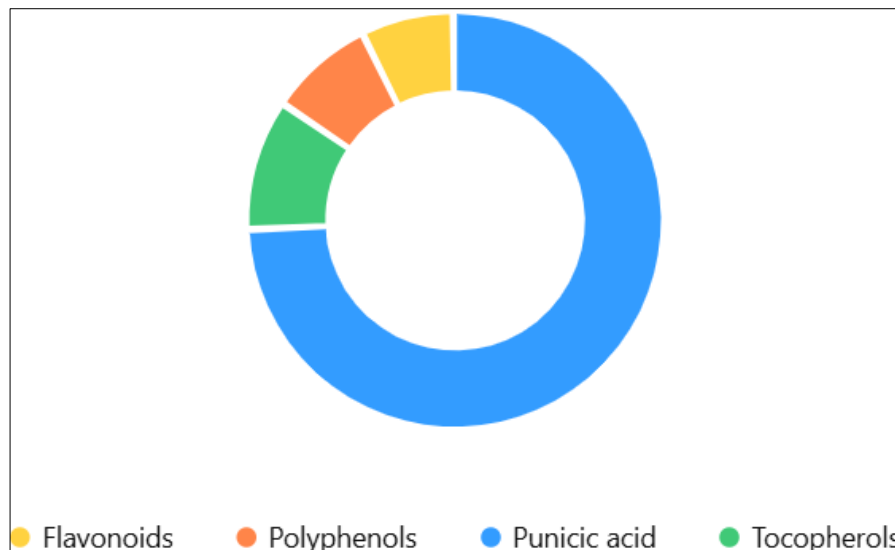


Fig 4: Major Bioactive Components of PSO

Mechanism of anti-aging action

Antioxidant activity

PSO intercepts cellular oxidative cascades through electron donation. Its high concentrations of punicic acid and γ -tocopherol effectively neutralize hydroxyl, superoxide, and peroxy free radicals (Verardo *et al.*, 2014; Kobylinska *et al.*, 2026) [62]. By limiting cellular oxidative stress, PSO prevents the activation of the downstream MAPK/AP-1 pathway, suppressing the expression of collagen-degrading MMPs (Fisher *et al.*, 2002; Pillai *et al.*, 2005) [16, 45].

Collagen synthesis and skin elasticity

PSO stimulates anti-aging pathways through a dual mechanism. While its phenolic components inhibit the enzymatic breakdown of type I procollagen by down-regulating MMP-1 (Quan *et al.*, 2009; Burlando *et al.*, 2024) [9, 49], its phytoestrogenic components interact with dermal fibroblast estrogen receptors. This interaction up-regulates the expression of transforming growth factor-beta (TGF-

β), which increases endogenous collagen deposition and elastin cross-linking to restore skin elasticity (Mukherjee *et al.*, 2011; Hussien *et al.*, 2025) [24, 42].

Anti-inflammatory effects and epidermal thickening

Chronic, low-grade structural inflammation often termed "inflammaging" is a primary driver of dermal degradation (Franceschi *et al.*, 2018; Wang *et al.*, 2025) [65]. Punicic acid acts as a potent inhibitor of cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) pathways, reducing the conversion of arachidonic acid into pro-inflammatory mediators such as prostaglandin E₂ (PGE₂) (Juhaimi *et al.*, 2017; Almoraie *et al.*, 2025) [4, 5, 28]. Concurrently, research shows that topical PSO selectively induces keratinocyte proliferation in the stratum basale, significantly increasing epidermal thickness and reinforcing the skin's physical barrier (Syed *et al.*, 2006; Burlando *et al.*, 2024) [9].

Photoprotection

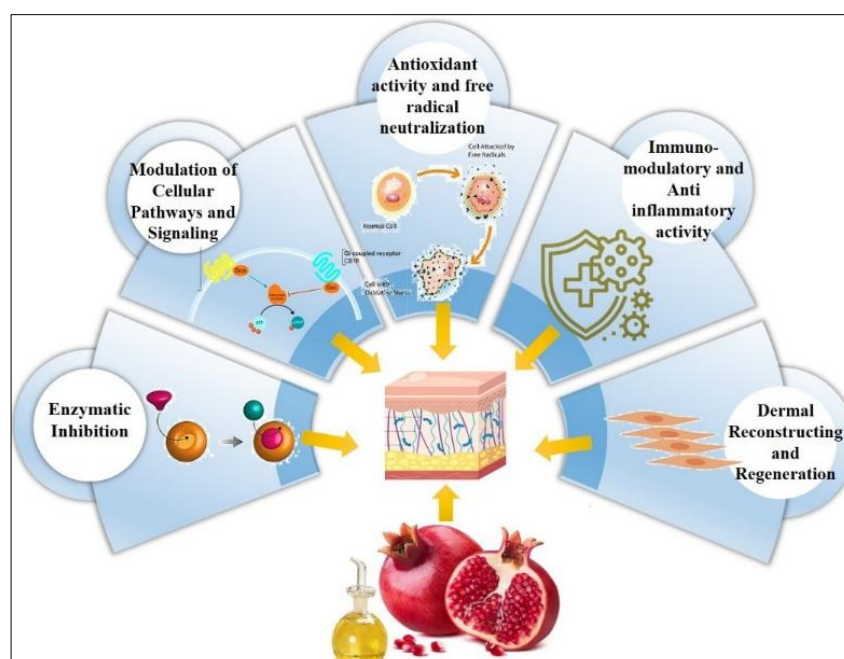


Fig 5: Mechanism of Anti-Aging Action of PSO

PSO functions as a natural photoprotective agent against solar ultraviolet radiation. It attenuates UVB-induced intracellular signaling pathways, reducing erythema,

Preventing UV-driven lipid oxidation, and mitigating photo carcinogenesis (Afaq *et al.*, 2005; Kammeyer and Luiten, 2015; Wang *et al.*, 2025) ^[1, 30, 65].

Table 3: Mechanism of Anti-aging Action

Mechanism	Target	Effect	References
Antioxidant	ROS	Reduces oxidative stress	Verardo <i>et al.</i> , 2014 ^[62] ;
Anti-inflammatory	COX-2, TNF- α	Reduces inflammaging	Juhaimi <i>et al.</i> , 2017; Wang <i>et al.</i> , 2025 ^[4, 28, 65]
Collagen synthesis	TGF- β pathway	Improves elasticity	Quan <i>et al.</i> , 2009; Hussen <i>et al.</i> , 2025 ^[24, 49]
Photoprotection	UVB-induced ROS	Prevents photoaging	Afaq <i>et al.</i> , 2005; Burlando <i>et al.</i> , 2024 ^[1, 9]

Role of pomegranate seed oil in cosmeceuticals

Topical delivery formats

Due to its molecular weight and lipophilic profile, PSO is highly versatile and can be formulated into various cosmeceutical vehicles (Mukherjee *et al.*, 2011; Burlando *et al.*, 2024) ^[9, 42]

- **Serums:** Concentrated anhydrous lipid delivery systems designed for rapid transepidermal penetration (Draelos, 2012; Kobylinska *et al.*, 2026) ^[11].
- **Lotions:** Lower-viscosity fluids suited for broad body coverage (Baki and Alexander, 2015) ^[7].
- **Emulsified creams:** Biphasic systems that provide optimal skin delivery by sustaining active compounds on the skin surface while delivering immediate barrier hydration (Aulton, 2018; Burlando *et al.*, 2024) ^[6, 9].

Dermatological benefits

- **Hydration dynamics:** The fatty acids in PSO integrate into the damaged stratum corneum lipid bilayer, repairing lipid gaps and restoring natural moisturizing factor (NMF) retention capacity (Proksch *et al.*, 2008; Verardo *et al.*, 2014) ^[46, 62].
- **Barrier Repair:** PSO accelerates healing in compromised or scaling skin barriers by promoting the cellular synthesis of essential ceramides and keratinocyte differentiation structures (Syed *et al.*, 2006; Wang *et al.*, 2025) ^[65].
- **Dermal re-densification:** The synergistic interaction of puniceic acid, tocopherols, and phytoestrogens increases dermal fiber density, clinically reducing wrinkle depth and improving skin volume (Mukherjee *et al.*, 2011; Hussen *et al.*, 2025) ^[24, 42].

Formulation of an anti-aging cream

Selection of emulsion system

An Oil-in-Water (O/W) emulsion system is generally preferred for anti-aging cosmeceutical creams. This structural design encloses the oil droplets within a continuous aqueous phase, ensuring a non-greasy texture, rapid skin absorption, and superior patient compliance compared to heavy, occlusive Water-in-Oil (W/O) bases (Aulton, 2018; Baki and Alexander, 2015; Burlando *et al.*, 2024) ^[6, 7, 9].

Functional ingredient selection

Developing a stable, dermatologically compatible O/W emulsion requires a precise balance of excipients (Lachman *et al.*, 2013; Aulton, 2018) ^[6, 34]

- **Oil phase (active & matrix):** *Punica granatum* seed oil serves as the primary active botanical oil. It is blended with solid fatty acids, such as stearic acid (an emulsifying/structuring agent) and cetyl alcohol (a co-emulsifier and consistency factor), to optimize the formulation's physical matrix (Rowe *et al.*, 2009; Baki and Alexander, 2015) ^[7, 52].
- **Aqueous phase:** Deionized, purified water serves as the continuous vehicle (Aulton, 2018) ^[6].
- **Humectant:** Glycerin or propylene glycol is added to the aqueous phase to draw moisture into the stratum corneum and prevent product desiccation (Draelos, 2012; Proksch *et al.*, 2008) ^[11, 46].
- **Preservative system:** Antimicrobial agents, such as methylparaben and propylparaben, are added to prevent microbial proliferation across both phases (Rowe *et al.*, 2009; USP, 2023) ^[52].
- **Stabilizers:** Polymeric rheology modifiers, such as Carbopol 940, can be introduced to enhance long-term structural stability and prevent phase separation (Baki and Alexander, 2015; Aulton, 2018) ^[6, 7].

Table 4: Ingredients for Formulation of Anti-aging Cream

Ingredient	Function	References
Pomegranate seed oil	Active ingredient	Verardo <i>et al.</i> , 2014; Kobylinska <i>et al.</i> , 2026 ^[62]
Stearic acid	Emulsifier	Rowe <i>et al.</i> , 2009; Baki and Alexander, 2015 ^[7, 52]
Cetyl alcohol	Consistency enhancer	Aulton, 2018; Rowe <i>et al.</i> , 2009 ^[6, 52]
Glycerin	Humectant	Proksch <i>et al.</i> , 2008; Draelos, 2012 ^[11, 46]
Carbopol 940	Stabilizer	Baki and Alexander, 2015; Aulton, 2018 ^[6, 7]
Methyl paraben	Preservative	USP, 2023; Rowe <i>et al.</i> , 2009 ^[52]

Processing and preparation method

The formulation is produced via an optimized high-temperature emulsification process (Aulton, 2018; Lachman *et al.*, 2013) [6, 34]

- **Phase separation and heating:** The oil phase components (PSO, stearic acid, cetyl alcohol) are combined in a vessel and heated to 70-75°C until completely liquefied. Concurrently, the aqueous phase components (water, glycerin, water-soluble preservatives) are combined and heated to the same temperature range (Baki and Alexander, 2015; Rowe *et al.*, 2009) [7, 52].

- **Phase blending:** The heated aqueous phase is added slowly to the heated oil phase under continuous, high-shear mechanical agitation. This high-energy mixing shears the internal lipid phase into sub-micron droplets (Aulton, 2018; Sinko, 2011) [6, 57].
- **Cooling and homogenization:** Mechanical stirring is maintained as the system cools to room temperature (~25°C). Thermolabile additives, including natural fragrances or specialized antioxidants, are introduced during the cooling phase at approximately 40°C to 45°C maintain chemical stability (Lachman *et al.*, 2013; Baki and Alexander, 2015) [7, 34].

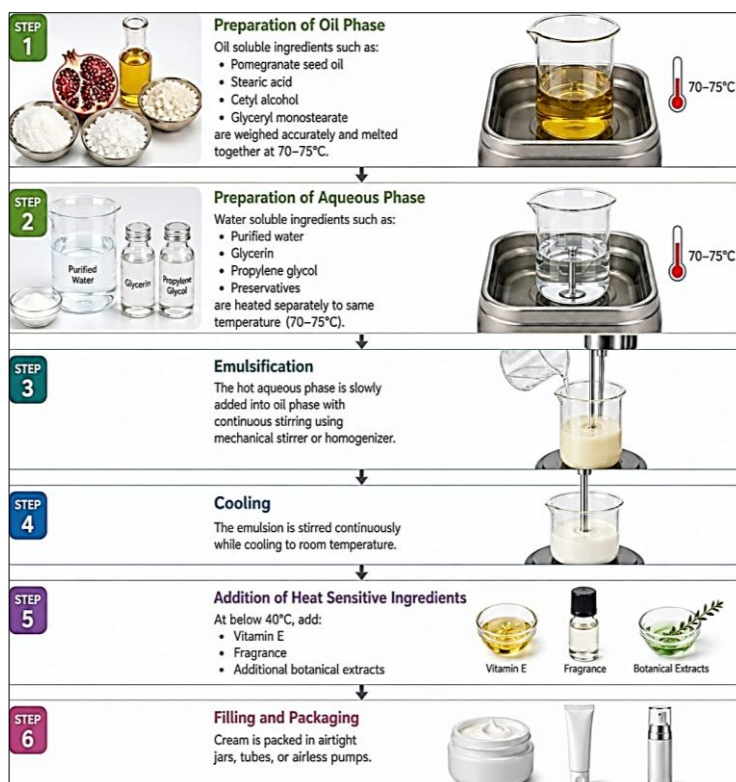


Fig 6: Method for Preparation

Evaluation parameters of cream

To transition from laboratory scale to industrial processing, the formulated PSO anti-aging cream must satisfy rigorous pharmaceutical standards (ICH Q1A (R2), 2003; Aulton, 2018) [6].

Physical evaluation

- **Color & Odor:** Visual and olfactory testing must confirm an appealing, uniform off-white to pale cream color with a characteristic mild, pleasant scent (Baki and Alexander, 2015; Lachman *et al.*, 2013) [7, 34].
- **Homogeneity:** Visual inspection and microscopic analysis must demonstrate consistent particle distribution without structural grittiness, lipid agglomeration, or phase separation (Aulton, 2018; Sinko, 2011) [6, 57].

PH determination

The pH of the topical cream must align with the acid mantle of human skin to minimize irritation and protect the skin

barrier. Formulations should maintain a target pH range between 5.0 and 6.5, measured using a calibrated digital pH probe inserted directly into a 10% w/v aqueous dilution of the cream (Proksch *et al.*, 2008; USP, 2023) [46].

Viscosity and rheological profiles

Topical emulsions must exhibit non-Newtonian pseudo plastic (shear-thinning) flow behavior. Viscosity is quantified using a Brookfield viscometer at controlled temperatures (25°C). High viscosity at rest prevents product sedimentation during storage, while decreased viscosity under shear force allows smooth topical application (Martin, 2011; Baki and Alexander, 2015) [7, 37].

Spreadability kinetics:

Spreadability dictates user experience and ensures the uniform delivery of active ingredients across the skin surface. It is measured using two glass plates: a specified weight (e.g., 100g) is applied to the cream sample sandwiched between the plates, and the resulting spread diameter is recorded over a set timeframe,

calculated using the formula (Garg *et al.*, 2002; Aulton, 2018) [6, 18]:

$$S = \frac{M \times L}{T}$$

Where: S= Spreadability (g.cm/s), M=Weight tied to upper plate, L= Length of glass slide, T= Time taken

Accelerated stability protocols

To project commercial shelf-life, formulations undergo strict International Council for Harmonisation (ICH) stability testing. Samples are stored under varying stress conditions (ICH Q1A (R2), 2003; USP, 2023)

- Refrigerated: 4°C±2°C
- Ambient: 25 °C±2 °C / 60%±5% RH
- Accelerated Stress: 40°C±2°C / 75%±5% RH

Table 5: Evaluation Parameters

Parameter	Method	Acceptance Criteria	References
pH	Digital pH meter	5.0-6.5	Proksch <i>et al.</i> , 2008; USP, 2023 [46]
Viscosity	Brookfield viscometer	Uniform	Martin, 2011; Baki and Alexander, 2015 [7, 37]
Spreadability	Glass slide method	Good spread	Garg <i>et al.</i> , 2002; Aulton, 2018 [6, 18]
Stability	ICH testing	No phase separation	ICH Q1A(R2), 2003; Lachman <i>et al.</i> , 2013 [34]

Recent advances in nano-cosmeceuticals

Conventional macro-emulsions often fail to cross the stratum corneum due to large droplet morphology and low active-ingredient bioavailability. Advanced nanotechnology-based delivery architectures overcome these physiological limitations (Puglia *et al.*, 2018; Souto *et al.*, 2020) [58].

Nanoemulsions

Nanoemulsions are structurally distinct lipid dispersions featuring a sub-micron droplet diameter (typically 20-200 nm). Nanonization increases the relative surface area of the active droplet, allowing deeper fluidization of the stratum corneum lipids and maximizing the flux of punicic acid across cellular membranes. Pomegranate seed oil nanoemulsions demonstrate improved physical clarity, absolute resistance to gravity-induced sedimentation, and enhanced baseline antioxidant capacity against UVB-induced spongiosis (Khurana *et al.*, 2023; Burlando *et al.*, 2024) [9, 31, 32].

Nanocapsules

Nano capsules represent vesicular reservoir systems containing a distinct polymeric shell (composed of biodegradable aliphatic polyesters such as poly-lactic-co-glycolic acid - PLGA) wrapped around a core of liquid phototherapeutic oil. This encapsulation isolates unstable, highly volatile components from external atmospheric moisture and oxygen, providing sustained, controlled ingredient release (Fathi *et al.*, 2018; Souto *et al.*, 2020) [15].

Synergistic model application: a prominent advanced model features Resveratrol-loaded pomegranate seed oil nanocapsules. While resveratrol typically exhibits poor water solubility and extreme light sensitivity, its encapsulation within a protective PSO lipid core shields the molecule from light degradation. Simultaneously, the high skin permeability of PSO enhances the dermal absorption of resveratrol, yielding a powerful synergistic effect that increases intracellular glutathione (GSH) and suppresses

Testing is conducted over 30, 60, and 90 days to monitor for signs of chemical breakdown, syneresis, or phase cracking. Centrifugation testing (e.g., 3000 rpm for 30 minutes) is also performed to verify resistance to phase separation under mechanical stress (Lachman *et al.*, 2013; Aulton, 2018) [6, 34].

Dermal irritation and biocompatibility

Safety is validated via *in vivo* animal models (e.g., healthy Albino Wistar rats) or through human patch testing. The formulation is applied to a shaved skin site and monitored over 24 to 72 hours for signs of erythema, edema, or local hypersensitivity reactions, confirming its safety for long-term clinical use (Draize *et al.*, 1944; OECD Guideline 404, 2021; Burlando *et al.*, 2024) [9, 12].

skin-aging enzyme activity more effectively than isolated treatments (Puglia *et al.*, 2018; Khurana *et al.*, 2023) [31, 32].

Hydrogel formulations

Hydrogels consist of three-dimensional, cross-linked hydrophilic polymer networks capable of swelling and holding large volumes of water. When nanocapsules or nanoemulsions are loaded into a secondary hydrogel base (forming a "nanogel" network), the formulation exhibits quasi-plastic rheological properties, maintaining a skin-compatible pH and reducing trans-epidermal water loss (TEWL). *In vivo* histopathology confirms that these hydrogels decrease UV-induced epidermal hyperplasia and structural matrix damage, proving highly effective for target-specific photoaging treatments (Khurana *et al.*, 2023; Wang *et al.*, 2025) [31, 32, 65].

Challenges and limitations

Despite the strong clinical efficacy profile of PSO, transitioning the ingredient into large-scale commercial cosmeceutical production presents several critical challenges (Souto *et al.*, 2020; Burlando *et al.*, 2024) [9, 58]

- **Stability of Natural Oils:** Unrefined vegetable oils exhibit physical variability across different harvests. Seasonal parameters modify the baseline lipid profile, which directly complicates batch-to-batch reproducibility during industrial manufacturing (Verardo *et al.*, 2014; Kobylinska *et al.*, 2026) [62].
- **Oxidation Vulnerability:** Due to its molecular structure containing three conjugated double bonds, punicic acid is highly sensitive to autoxidation when exposed to atmospheric oxygen, ambient light, or high thermal ranges. Lipid peroxidation can lead to rancidity, the generation of irritating aldehydes, a dramatic loss of anti-aging efficacy, and formula discoloration (Melo *et al.*, 2014; Almoraie *et al.*, 2025) [5, 38].
- **High Cost of Extraction:** Isolating pure, unrefined oil from pomegranates requires intensive extraction

processing. Traditional solvent extraction yields chemical residue risks, whereas cold-expression and supercritical carbon dioxide (scCO_2) fluid extraction maintain absolute purity but demand high initial capital expenditure and specialized infrastructure (Goula and Lazarides, 2015) [19].

- **Standardization Deficits:** Establishing uniform international quality control guidelines remains difficult. Regulatory challenges persist in standardizing the minimum therapeutic concentrations of punicic acid, limiting pesticide residues, and establishing standard biomarker thresholds across diverse international suppliers (Sagar *et al.*, 2022; Burlando *et al.*, 2024) [9].

Future perspectives

The future evolution of PSO-driven dermatological applications will focus on three key integration domains (Souto *et al.*, 2020; Wang *et al.*, 2025) [58, 65]

- **Advanced nanotechnology-based delivery systems:** Future formulations will increasingly utilize structured lipid nanocarriers (SLNs), nanostructured lipid carriers (NLCs), and ethosomes. These nanocarriers can carry both lipophilic molecules (like punicic acid) and hydrophilic botanical actives simultaneously, protecting active ingredients from chemical breakdown and allowing deeper dermal penetration (Gupta *et al.*, 2019; Khurana *et al.*, 2023) [21, 31, 32].
- **Rigorous clinical evaluation protocols:** Pre-clinical testing must transition toward extensive, multi-centered, double-blind, placebo-controlled human clinical trials. Studies should utilize advanced, non-invasive bioengineering techniques such as high-frequency ultrasound and optical coherence tomography to quantitatively validate long-term safety, cellular toxicity profiles, and in-depth wrinkle reduction metrics over extended application periods (Burlando *et al.*, 2024; Hussen *et al.*, 2025) [9, 24].
- **Integration with personalized skincare platforms:** The integration of digital diagnostic technology allows anti-aging products to be adapted to individual needs. Future cosmetic platforms can match specific consumer biomarkers such as genetic variations in collagen synthesis or sebum production with customized ratios of PSO nanocapsules, maximizing therapeutic efficacy while minimizing individual dermal reactivity (Wang *et al.*, 2025) [65].

Conclusion

Punica granatum seed oil stands as a premier botanical active for advanced anti-aging formulations. Its high content of punicic acid, combined with protective tocopherols and structural phytoestrogens, effectively targets the cellular mechanisms of skin aging by neutralizing free radicals, reducing chronic inflammation, and preserving dermal collagen integrity. When formulated into an optimized oil-in-water emulsion or advanced nanostructured delivery systems, it provides stable physicochemical properties, excellent spreadability, and a safe biocompatibility profile. Integrating cold-pressed PSO into modern cosmetic systems offers a scientifically validated, sustainable pathway for the development of high-performance, natural anti-aging therapies.

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Conflict of interest

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Author contributions

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