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Original Article

Investigation of antioxidant and aging-related enzymes' inhibitory activities of Jatuphalathika extract for application in skincare serum formulation

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Abstract

This study investigated the antioxidant and aging-related enzyme inhibitory activities of Jatuphalathika (JP), a formula combining *Terminalia chebula*, *Terminalia bellirica*, *Terminalia arjuna*, and *Phyllanthus emblica*. Aqueous (JPW) and ethanolic (JPE) extracts were prepared. In the DPPH assay, JPW and JPE exhibited free radical scavenging activity, with the half-maximal inhibitory concentrations (IC_{50}) of 49.94 ± 1.06 µg/mL and 304.62 ± 8.04 µg/mL, respectively. Their FRAP values were 0.24 ± 0.06 mmol Fe^{2+}/g (JPW) and 0.18 ± 0.05 mmol Fe^{2+}/g (JPE). Both extracts showed mild-to-moderate inhibitory activity against tyrosinase, elastase, and collagenase. JPW was chosen to formulate a serum, containing 3.33% (w/w) gallic acid and 2.68% (w/w) ellagic acid based on high-performance liquid chromatography. Among seven formulations, F7 was optimal; it maintained its stability best at 27 ± 2°C. These findings suggest the potential of the JP extract for cosmetic applications, particularly in anti-aging products, with room-temperature storage recommended for optimal stability.

Keywords: Jatuphalathika, antioxidant, age-related enzymes, formulation, stability testing

1. Introduction

Aging is a progressive decline in physiological processes, contributing to age-related diseases, including cardiovascular, pulmonary, and neurodegenerative diseases, as well as skin disorders. Skin aging is influenced by extrinsic factors (such as ultraviolet [UV] radiation, pollution, and alcohol and tobacco use) and intrinsic factors (e.g., genetics). As the skin ages, it becomes more susceptible to wrinkles, pigmentation changes, and loss of firmness, often impacting emotional and social well-being (Costa, Magalhães, & Di Stasi, 2022). Oxidative stress plays a significant role in skin aging through the increased formation of reactive oxygen species (ROS) and a decline in the body's antioxidant defenses, implying the importance of antioxidants in skincare (Papaccio, D'Arino, Caputo, & Bellei, 2022). Natural antioxidants, such as those found in *Phyllanthus emblica* and gallic acid, have shown potential to combat oxidative damage. Excessive melanin production, leading to conditions such as melasma, is regulated by tyrosinase. Natural extracts that inhibit tyrosinase can help reduce pigmentation and maintain

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youthful skin tone (Pillaiyar, Manickam, & Namasivayam, 2017). Additionally, collagenase and elastase break down collagen and elastin, accelerating skin aging. Targeting these enzymes can help retain skin firmness and elasticity, addressing wrinkles and other age-related changes.

Jatuphalathika (JP) is an herbal preparation composed of dried fruits from *Terminalia chebula*, *Terminalia bellirica*, *Terminalia arjuna*, and *Phyllanthus emblica*. Traditionally, JP has been utilized to promote health, for anti-aging, and to treat aging-related conditions (Department of Medical Sciences, 2021). JP extracts exhibit antioxidant properties, effectively scavenging free radicals and chelating metals (Chusri, Keawmanee, & Sudkhaw, 2016). Each component contributes specific antioxidant actions: *T. chebula* has metal-chelating capabilities, *T. bellirica* displays ferric-reducing power, and both *P. emblica* and *T. arjuna* have strong free radical scavenging abilities.

The JP formula contains phytochemicals such as alkaloids, phenols, saponins, and tannins, including gallic and ellagic acids. These compounds align with those found in Triphala, a traditional blend recognized for its antioxidative and anti-melanogenic properties (Kondawar, Kamble, & Mali, 2011; Mahajan, Sawant, Pandita, Machale, & Pai, 2012). Gallic acid, in particular, is a potent antioxidant and tyrosinase inhibitor (Kim, 2007). While research on the anti-collagenase and anti-elastase effects of JP is limited, JP aqueous extracts have shown safety in acute toxicity studies, making them promising candidates for skincare formulations (Rinthong, Mingmalairak, & Tantisira, 2016).

Serums are popular in skincare due to their concentrated active ingredients and rapid skin absorption, ideal for targeting specific concerns. They are lightweight, non-greasy, and deliver high levels of essential nutrients for skin health (Budiasih *et al.*, 2018). This study aimed to evaluate the antioxidant and enzyme-inhibiting activities of the JP extract, develop a JP-enriched skincare serum, and assess the stability of the optimal formulation.

2. Materials and Methods

2.1 Materials

Dried fruits of *T. chebula*, *T. bellirica*, *T. arjuna*, and *P. emblica* were purchased from a local herbal shop in Phitsanulok, Thailand. Dried herbs and their powders were characterized by the Department of Medical Sciences Ministry of Public Health, Nonthaburi, Thailand.

Iron(III) chloride hexahydrate, iron(II) sulfate, aceto nitrile, and ethanol were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetra methyl chroman-2-carboxylic acid (Trolox), *N*-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA), *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (SANA), porcine pancreatic elastase (PPE), collagenase, tyrosinase, l-DOPA, gallic acid, kojic acid, and ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All chemicals in the formulation were purchased from Chemipan Corporation Co., Ltd. (Bangkok, Thailand).

2.2 Preparation of JP herbal extract

Dried fruits of *T. chebula*, *T. bellirica*, *T. arjuna*, and *P. emblica* were powdered and combined in a 1:1:1:1 ratio to create the Jatuphalathika (JP) formula. For the aqueous extract (JPW), 240 g of JP powder was extracted by decoction in 1000 mL of distilled water at 70–80°C for 1 h. For the ethanolic extract (JPE), JP powder was macerated in 95% ethanol (1:3 ratio) for 7 days. After filtration, the JPE extract was concentrated, oven-dried (40°C), and stored. A JP solution in distilled water was prepared using <1% dimethyl sulfoxide (DMSO).

2.3 DPPH scavenging assay

The antioxidant activity of the JP extract was assessed using the DPPH radical scavenging assay (Blois, 1958). A 0.2 mM DPPH solution in ethanol was prepared, and the JP extract samples (50–500 μg/mL) were diluted with ethanol. A 50-μL sample solution was mixed with 100 μL of DPPH solution in 96-well plates, shaken, and incubated in the dark for 30 min at room temperature. Absorbance (Abs) was measured at 517 nm using a microplate reader (Eon, Bio-Tek, USA). Ascorbic acid served as the positive control. Free radical inhibition was calculated as follows:

$$\%\text{Inhibition} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{blank}}] \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control, which contained all of the reagents but no sample; $\text{Abs}_{\text{sample}}$ is the absorbance of the sample; and $\text{Abs}_{\text{blank}}$ is the absorbance of the blank, which contained the sample but no reagents. The percentage inhibition was plotted against the concentration, and linear regression determined the concentration needed for 50% DPPH radical inhibition (IC_{50}). The Trolox equivalent antioxidant capacity (TEAC) was calculated and is presented as nanomoles of Trolox equivalents (TE) per gram of sample.

2.4 Ferric reducing antioxidant power (FRAP) assay

The ferric-reducing ability of the JP extract was assessed by a colorimetric assay (Benzie & Strain, 1996). The JP extract (30–600 μg/mL) was diluted with acetate buffer, and 30 μL of the sample was combined with 270 μL of the FRAP reagent (300 mM acetate buffer [pH 3.6], 20 mM $FeCl_3$, and 10 mM TPTZ in HCl at a 10:1:1 ratio). After incubation for 30 min in the dark at room temperature, the reduction of Fe^{3+} (colorless) to Fe^{2+} (blue) was measured at 595 nm using a microplate reader. The absorbance was plotted against $FeSO_4$ concentrations (0.2–1.2 mM), and the antioxidant capacity is reported as millimoles of Fe^{2+} equivalents per gram of extract.

2.5 Anti-tyrosinase activity assay

The tyrosinase inhibitory activity was evaluated with the Dopachrome assay (Zhang, Hu, Hou, & Wang, 2009). The substrate was 0.8 mM l-DOPA in phosphate buffer (pH 6.8). The JP extract was prepared at 4–12 mg/mL in

distilled water (total volume of 40 µL) and mixed with 40 µL of l-DOPA and 80 µL phosphate buffer, then incubated for 10 min at room temperature in the dark. After that, 40 µL of tyrosinase in phosphate buffer (125 units/mL) was added. Then, the mixture was incubated again for 20 min at room temperature in the dark. The absorbance of the reaction mixture was determined using a 96-well microplate reader at 492 nm, with kojic acid and ascorbic acid as the positive controls. The percentage of the tyrosinase activity inhibition was calculated according to the equation:

$$\%\text{Inhibition} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{blank}}] \times 100$$

The percentage of inhibition was plotted against the concentration. The IC_{50} was determined by linear regression analysis.

2.6 Anti-elastase activity assay

The elastase inhibition activity of the JP extract was measured using a modified spectrophotometric method (Laothaweerungsawat, Sirithunyalug, & Chaiyana, 2020). Extract solutions (50–400 µg/mL), PPE (5 µg/mL), and SANA (0.18 mg/mL) were prepared in 200 mM Tris-HCl buffer (pH 8.0). In a 96-well plate, 160 µL of extract solution was combined with 20 µL of elastase and incubated for 20 min. Then, 20 µL of SANA was added and incubated for 60 min. Absorbance was measured at 410 nm using a 96-well microplate reader, with epigallocatechin gallate (EGCG) as a positive control. The anti-elastase activity percentage and IC_{50} were calculated similarly to the anti-tyrosinase assay (see section 2.5).

2.7 Anti-collagenase activity assay

The anti-collagenase activity of the JP extract was evaluated via a modified spectrophotometric method (Thring, Hili, & Naughton, 2009). Extracts (50–400 µg/mL), collagenase (0.25 mg/mL), and FALGPA (0.8 mM) were prepared in 50 mM Tricine buffer (pH 7.5). In a 96-well plate, 160 µL of extract solution was mixed with 20 µL of collagenase and incubated for 20 min, followed by the addition of 20 µL of FALGPA to initiate the reaction. EGCG (20–200 µg/mL) was used as a positive control. Absorbance was measured at 340 nm using a 96-well microplate reader, and the percentage of inhibition and IC_{50} were calculated similarly to the anti-tyrosinase assay (see section 2.5).

2.8 High-performance liquid chromatography (HPLC) analysis of the gallic acid and ellagic acid contents

The gallic and ellagic acid contents in the JPW extract were analyzed using HPLC with an LC-20AT pump, SPD-20A UV detector, and SIL-10ADVP sample injector (Shimadzu, Kyoto, Japan). Separations used a C18 column (250 mm × 4.6 mm, 5 µm; ACE®, Aberdeen, Scotland). The mobile phase was a 90:10 water:acetonitrile mix with 1% orthophosphoric acid (pH 3.0) for gallic acid, and a 60:40 methanol:water mix with 0.2% phosphoric acid (pH 3.0) for ellagic acid. The flow rate was 1 mL/min. The UV detector was set to 210 nm for gallic acid and 254 nm for ellagic acid. Gallic acid (2–10 µg/mL), ellagic acid (0.1–100 µg/mL), or the JPW extract was dissolved in the mobile phase, filtered through a 0.45 mm membrane filter, and injected into the system. Gallic acid and ellagic acids were identified by comparing the retention times of the reference standard peaks and quantified using the calibration curve of pure standards ($R^2 = 0.999$).

2.9 Formulation of JP serum

Seven serum formulations (F1–F7) were prepared by varying the concentrations of the JPW extract and thickening agents. The serum base was prepared by mixing propylene glycol 5, butylene glycol 3, glycerin 2, and hydroxyethyl cellulose (HEC) 0.8% (w/w) in distilled water. For F1–F3, the JPW extract, Sakura extract, and vitamin B3 were dissolved in distilled water, and mixed with the serum base, sodium hyaluronate, rose water, and Microcare PHC. The formulation was adjusted to pH 5.5 using triethanolamine. For F4–F7, carboxymethyl cellulose (CMC) mucilage and the serum base were optimized. The optimal formulation was chosen for stability evaluation.

2.10 Stability evaluation

The F7 formulation was stored in glass bottles at 2–8°C, room temperature (27 ± 2°C), or 45°C for 3 months, or subjected to six cycles of accelerated heating-cooling (4°C for 24 h followed by 45°C for 24 h). Physical stability (color, odor, and precipitation) was observed, viscosity was measured with a Brookfield DV-III viscometer (AMETEK, Pennsylvania, USA), and pH was measured with a pH meter (Mettler-Toledo International Inc., Ohio, USA). Microbiological stability was assessed using the spread plate technique on nutrient agar to detect microbial contamination. The results were compared with those of freshly prepared formulation.

2.11 Statistical analysis

All experiments were conducted in triplicate (n = 3). One-way analysis of variance was used to detect statistically significant differences ($p < 0.05$). The data are presented as the mean ± standard deviation (SD).

3. Results

3.1 Preparation of the JP extract

The JP formula, composed of dried fruits from *T. chebula*, *T. bellirica*, *T. arjuna*, and *P. emblica*, was extracted using distilled water (JPW) and ethanol (JPE), yielding 19.52% (w/w) and 10.07% (w/w) of dry herb, respectively. Both extracts exhibited a viscous consistency and a deep brown color.

3.2 Antioxidant activity assay

The antioxidant activity of the JP extract was assessed using the DPPH and FRAP assays. In the DPPH assay, the JPW extract showed higher activity than the JPE extract, with an IC_{50} of 49.94 ± 1.06 µg/mL and 304.62 ± 8.04

µg/mL, respectively. Moreover, the TEAC was higher for the JPW extract (0.10 ± 0.04 mmol TE/g) than for the JPE extract (0.02 ± 0.01 mmol TE/g). For the FRAP assay, the JPW showed a value of 0.24 ± 0.06 mmol Fe^{2+}/g and the JPE extract showed a value of 0.18 ± 0.05 mmol Fe^{2+}/g. Both extracts exhibited lower antioxidant activity than ascorbic acid (Table 1).

3.3 Aging-related enzyme inhibition assays

Table 2 shows the results for the enzyme inhibition assays. Both extracts exhibited similar tyrosinase inhibition, with an IC_{50} of 3.04 ± 0.36 mg/mL for the JPW extract and 2.85 ± 0.47 mg/mL for the JPE extract. Of note, both extracts were less potent than ascorbic and kojic acids, which showed IC_{50} values of 0.26 ± 0.03 mg/mL and 0.18 ± 0.07 mg/mL, respectively. Both extracts exhibited anti-elastase activity, with an IC_{50} of 2.45 ± 0.64 mg/mL for the JPW extract and 2.34 ± 0.32 mg/mL for the JPE extract. Finally, the extracts demonstrated significant collagenase inhibition, with an IC_{50} of 1.94 ± 0.46 mg/mL for the JPW extract and 1.77 ± 0.17 mg/mL for the JPE extract.

3.4 HPLC analysis of the gallic and ellagic acid contents in the JPW extract

HPLC analysis confirmed the presence of gallic and ellagic acids in JP. Calibration curves for these compounds showed excellent linearity ($R^2 \geq 0.999$), enabling quantification in the JPW extract based on derived regression equations. The retention time was 3.6 min for gallic acid and 4.6 min for ellagic acid, consistent with their standard peaks (Figure 1). The JPW extract contained 3.33% (w/w) gallic acid and 2.68% (w/w) ellagic acid, based on dry herb weight.

3.5 Formulation of JP serum

Seven JPW extract–based serum formulations (F1–F7) were prepared for skin nourishment. The initial F1 formulation included 2% (w/w) JPW extract and presented a strong herbal scent and dark color. Reducing the concentration of the JPW extract to 1.0% (F2) and 0.5% (F3) improved the odor and color. For the F4–F6 formulations, the viscosity was adjusted with CMC mucilage. The F7 formulation had an increased serum base content, which allowed for a better consistency. The final F7 formulation included vitamin B3 for skin brightening; Sakura extract as a co-antioxidant; and moisturizers such as propylene glycol, butylene glycol, glycerin, and sodium hyaluronate. HEC and CMC served as thickening agents, while phenoxyethanol and chlorphenesin preserved the product, with triethanolamine used to adjust the pH. The optimized F7 formulation was a clear, reddish-brown serum with a mild herbal scent, a pH of 5.48 ± 0.03, and low viscosity (9.23 ± 0.42 cP). It had a light, non-greasy texture that hydrated the skin without staining, as detailed in Table 3.

3.6 Stability testing

The F7 formulation underwent stability testing at 2–8°C, room temperature (27 ± 2°C), and 45°C for 3 months, and an accelerated heating-cooling cycle. Throughout testing, the serum's scent remained unchanged. While the viscosity decreased slightly, the change was not statistically significant. The pH remained within the optimal range for skin, although

Table 1. The antioxidant and anti-tyrosinase activities of the Jatuphalathika aqueous and ethanolic (JPW and JPE, respectively) extracts and the positive control

Test sample	DPPH assay		FRAP assay (mM Fe^{2+}/g)
	IC_{50} (µg/mL)	TEAC (mmol TE/g extract)	
JPW	9.94 ± 1.06[c]	0.10 ± 0.04[b]	0.24 ± 0.06[b]
JPE	304.62 ± 8.04[a,b,c]	0.02 ± 0.01[a,b]	0.18 ± 0.05[b]
Ascorbic acid	10.70 ± 0.52	0.47 ± 0.09	30.06 ± 1.23
Trolox	1.25 ± 0.36	-	-

a, b, and c indicate statistically significant differences ($p < 0.05$) when compared with JPE, ascorbic acid, and trolox, respectively. DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric acid reducing power; IC_{50}, half-maximal inhibitory concentration; TEAC, Trolox equivalent antioxidant capacity.

Table 2. Aging-related enzyme inhibitory activities of the Jatuphalathika aqueous and ethanolic (JPW and JPE, respectively) extracts and the positive controls

Test sample	IC_{50} (mg/mL)		
	Anti-tyrosinase activity	Anti-elastase activity	Anti-collagenase activity
JPW	3.04 ± 0.36[a,b]	2.45 ± 0.64[c]	1.94 ± 0.46[c]
JPE	2.85 ± 0.47[a,b]	2.34 ± 0.32[c]	1.77 ± 0.17[c]
Ascorbic acid	0.26 ± 0.03	-	-
Kojic acid	0.18 ± 0.07	-	-
EGCG	-	0.12 ± 0.01	0.50 ± 0.01

a, b, and c indicate statistically significant differences ($p < 0.05$) when compared with ascorbic acid, kojic acid, and epigallocatechin gallate (EGCG), respectively. IC_{50}, half-maximal inhibitory concentration

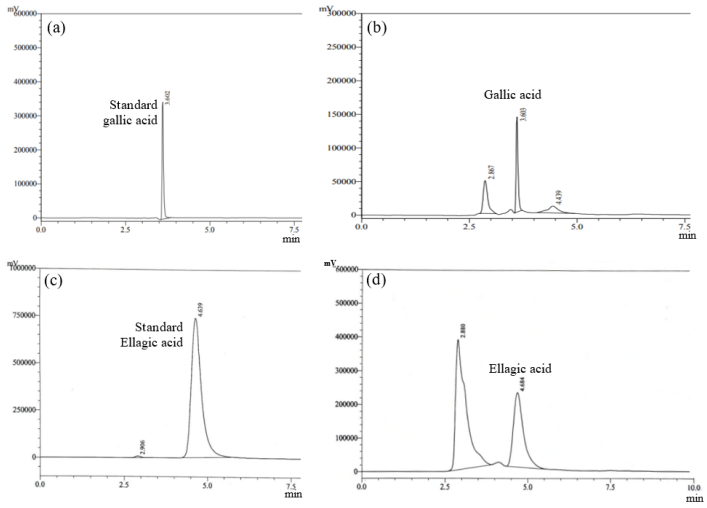

Figure 1. High-performance liquid chromatogram of (a) standard gallic acid (6 µg/mL) with a retention time (Rt) of 3.6 min, (b) gallic acid in the JPW extract (100 µg/mL), (c) standard ellagic acid (100 µg/mL) with an Rt at 4.6 min, and (d) ellagic acid in the JPW extract (1 mg/mL)

Table 3. The ingredients used in the F7 serum formulation

Ingredient	% (w/w)
Serum base	73.0
1% Sodium hyaluronate	5.0
Sodium carboxymethyl cellulose	0.6
Distilled water	16.2
Vitamin B3	2.0
Sakura extract	2.0
Jatuphalathika aqueous (JPW) extract	0.5
Microcare PHC®	0.6
Triethanolamine to adjust pH to 5.5	0.1

it became slightly more acidic over time. There was a slight darkening of the color, and a reddish-brown precipitate was observed under the heating-cooling cycle and after storage for 3 months at 45°C. No microbial growth was detected throughout the study period. Figure 2 illustrates the physical changes, and Table 4 summarizes the stability outcomes.

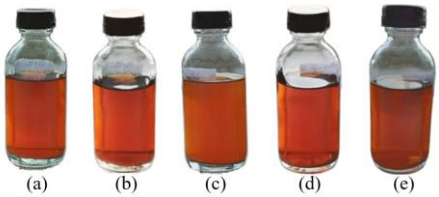

Figure 2. Physical appearance of the F7 serum formulation: (a) the freshly prepared formulation; (b) after exposure to six cycles of accelerated heating-cooling cycling; and after storage for 3 months at (c) 2–8°C, (d) room temperature (27 ± 2°C), and (e) 45°C

4. Discussion

Natural phytochemicals are widely utilized in cosmetics due to their diverse and generally safe bioactive compounds. Antioxidant properties are commonly evaluated through several free radical scavenging activities. In the present study, the DPPH and FRAP assays were used to assess the antioxidant potential of the JP extracts. The DPPH assay measures antioxidant activity by measuring the reduction of purple DPPH to yellow DPPH through hydrogen donation (Costa *et al.*, 2022). In the FRAP assay, antioxidants reduce ferric-tripyridyl triazine (Fe^{3+}-TPTZ) to a blue ferrous form (Fe^{2+}-TPTZ) (Benzie & Strain, 1996). We found that the JPW extract demonstrated strong DPPH scavenging ability, while the JPE extract showed moderate activity. However, both extracts showed a FRAP value indicating mild antioxidant activity. These findings are consistent with previous studies of JP aqueous extracts, with an IC_{50} of 0.031 ± 0.02 mg/mL for DPPH radical scavenging and a FRAP value of 23.07 ± 1.84 mM Fe^{2+}/mg extract (Chusri *et al.*, 2016). Some variations may be due to differences in herbal sources or assay methods.

The enzymes tyrosinase, elastase, and collagenase play crucial roles in skin aging. Tyrosinase is a key enzyme in melanin synthesis, and its inhibition is often targeted in skin-lightening products to reduce hyperpigmentation. In this study, the anti-tyrosinase activity was assessed using the Dopachrome method. The red-brown dopachrome formed through the oxidation of the substrate l-DOPA was quantified spectrophotometrically (Zhang *et al.*, 2009). While both the JPW and JPE extracts demonstrated some inhibitory effect on tyrosinase, their potency was lower than those of kojic and ascorbic acids. To optimize their potential in skin-lightening formulations, combining them with more potent agents could be a promising strategy.

Elastase, an enzyme responsible for the degradation of elastin, contributes to skin aging. Inhibiting elastase offers a promising strategy for preserving skin firmness and preventing wrinkle formation. In this study, anti-elastase activity was assessed using SANA as the substrate. PPE hydrolyzes SANA, resulting in *N*-succinyl-(Ala)3 and *p*-nitroaniline. The amount of yellow-colored *p*-nitroaniline produced was quantified spectrophotometrically. The JPW and JPE extracts exhibited mild anti-elastase activity, similar to most Thai medicinal plant extracts showing minimal or negligible elastase inhibition (Chaikhong *et al.*, 2023). These

Table 4. Stability assessment of the F7 serum formulation

Characteristic	Freshly prepared formulation	Heating-cooling cycle	Formulation stored for 3 months at various temperature		
			Room temperature	2–8°C	45°C
Color	Reddish-brown	Reddish-brown (darker)	Reddish-brown	Reddish-brown	Reddish-brown (darker)
Odor	Light herbal scent	Light herbal scent	Light herbal scent	Light herbal scent	Light herbal scent
Precipitation	No	Yes	No	No	Yes
Viscosity (cP)	9.23 ± 0.42	8.84 ± 0.40	8.70 ± 0.03	8.84 ± 0.04	8.69 ± 0.03
pH	5.48 ± 0.03	5.34 ± 0.07	5.00 ± 0.05*	5.09 ± 0.01*	4.89 ± 0.01*
Microbial growth	No	No	No	No	No

The data are presented as the mean ± standard deviation of triplicate experiments. *$p < 0.05$ compared to freshly prepared formulation.

findings suggest that combining them with more potent elastase inhibitors could enhance their anti-aging potential.

Collagenase is an enzyme that degrades collagen, which plays an essential role in maintaining skin structure and elasticity. This study assessed the anti-collagenase activity of JP extracts using FALGPA as a substrate in a reaction with collagenase. The product was measured spectrophoto metrically, with EGCG as the positive control. Both JP extracts exhibited moderate anti-collagenase activity, with the JPE extract being slightly more potent. These results suggest potential applications of JP extracts in formulations aimed at preserving skin integrity and combating age-related collagen degradation. Although studies specifically evaluating the anti-elastase and anti-collagenase activities of JP are limited, some relevant findings of their components have been reported. Satardekar and Deodhar (2010) demonstrated that *T. chebula* fruit extract inhibited hyaluronidase and elastase, and promoted fibroblast proliferation and collagen synthesis. On the other hand, Mathen, Thergaonkar, Teredesai, Soman, and Peter (2014) reported variable anti-elastase results, possibly due to extraction or assay differences. Moreover, *P. emblica* extract has shown strong collagenase inhibition: Chanvorachote *et al.* (2010) observed 78.67% inhibition at 1 mg/mL, while Pientaweeratch, Panapisal, and Tansirikongkol (2016) presented matrix metalloproteinase 1 (MMP-1), MMP-2, and elastase inhibition by gallic acid. These findings suggest that JP extracts, particularly *T. chebula* and *P. emblica*, may offer anti-aging benefits.

The JP formula contains various phytochemicals, including alkaloids, phenols, saponins, and tannins, with key bioactive compounds such as gallic, ellagic, and chebulagic acids (Department of Medical Sciences, 2021; Wuttidharm mavej, 2004). Its profile closely resembles the traditional Triphala formulation, which includes *T. chebula*, *T. bellirica*, and *P. emblica*, known for its high gallic acid content and notable ellagic, chebulagic, and chebulinic acid contents (Kondawar *et al.*, 2011; Mahajan *et al.*, 2012).

Gallic acid, a well-known antioxidant, demonstrates strong efficacy in DPPH, FRAP, and oxygen radical absorbance capacity (ORAC) assays via single-electron transfer (Velderrain-Rodríguez *et al.*, 2018). It also shows anti-tyrosinase activity in B16 melanoma cells ($IC_{50} = 3.59 \times 10^{-6}$ M) (Kim, 2007). Studies suggest that its effects are enhanced by synergistic interactions with other phytochemicals in extracts (Corradi, De Souza, Sande, & Takahashi, 2018). Moreover, gallic and ellagic acids act as natural elastase and collagenase inhibitors, valuable for skin care. Gallic acid exhibits collagenase inhibition (IC_{50} = 2.07 mM) (Barla *et al.*, 2009), while ellagic acid shows potent elastase inhibition (IC_{50} = 1.44 mg/mL) (Xing, Yang, & Cao, 2016) and helps prevent UV-B-induced collagen degradation (Bae *et al.*, 2010), supporting anti-aging benefits.

The JPW extract was selected to prepare serum formulations due to its strong antioxidant activity and ability to inhibit enzymes relevant to skin aging. Biological variations in the JP extracts arise from solvent polarity and extraction method: water extracts more hydrophilic compounds, while ethanol captures a wider range of compounds. The decoction can degrade heat-sensitive compounds, whereas cold maceration preserves them. HPLC confirmed gallic and ellagic acids in the JPW extract, known for antioxidant and anti-aging benefits. Hot-water extraction likely increased the gallic acid content, thus enhancing the antioxidant activity. Although enzyme inhibition by the JPW extract was slightly lower than the controls, a 0.5% (w/w) concentration in the serum exceeded the IC_{50} for DPPH radical scavenging and inhibition of aging-related enzymes. This is considered sufficient to achieve the desired effects, supporting skin elasticity and youthfulness with regular application.

Among the seven serum formulations with the JPW extract, we chose F7 due to its optimal balance of efficacy and appeal, featuring a light, non-greasy texture and a reddish-brown color. The inclusion of humectants, moisturizers, and thickening agents improved hydration and viscosity, while preservatives effectively prevented microbial growth. The stability evaluation confirmed that the F7 formulation retained its physical and chemical properties under various storage conditions, with only slight decreases in viscosity and pH at high temperatures. A minor reddish-brown precipitate appeared at elevated temperatures, likely from ingredient interactions, but overall stability and preservative effectiveness remained satisfactory, ensuring product quality.

5. Conclusions

Our research highlights JP as a promising natural ingredient for anti-aging skincare products. The JPW extract showed potent antioxidant activity and inhibited aging-related enzymes *in vitro*, probably due to the presence of gallic and ellagic acids. The F7 serum formulation demonstrated desirable properties and stability under ambient conditions. However, additional long-term stability studies are needed to assess its shelf life. Clinical trials are also essential to evaluate

its efficacy in addressing aging and pigmentation, as well as consumer acceptance.

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