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Assessment of the Effect of Berberine on Metalloprotease Enzymes Inhibition and Antioxidant Activity: Possible Application in Skin Aging

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Abstract: Skin aging has been defined to enclose both intrinsic and extrinsic aging. Phytochemicals are frequently used for developing skin care formulations and could protect the skin's epidermal and dermal layers, consisting mainly of elastin and collagen, from UV radiation. Berberine is an isoquinoline alkaloid and a biologically active component from plant sources. Our objective was to assess Berberine's anti-aging capabilities by conducting elastase and collagenase enzyme inhibition and kinetic studies and to also evaluating its antioxidant capacity with three different methods. Furthermore, heat stability, pH and sun protection factor (SPF) of the formulated cream containing 1.5% berberine was evaluated. The elastase and collagenase IC_{50} values of berberine were estimated to be 47.54 and, 22.16 µg/mL respectively. Berberine was determined as an un-competitive inhibitor of elastase and collagenase. It scavenged DPPH and ABTS free radicals with IC_{50} values of 66.81 and 180.5 µg/mL respectively. 210.387 mg/L of berberine was equivalent in reducing power of 176 mg/L of ascorbic acid. SPF and pH value of cream containing berberine was found to be 12.3 and 5.62 respectively. In conclusion, these findings suggest that Berberine is a promising candidate for use as an active ingredient in cosmeceuticals, offering a natural approach to enhance skin health and reduce the visible signs of aging.

Keywords: Skin aging, Berberine, Collagenase, Elastase.

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1. INTRODUCTION

Skin aging has been defined to encompass both intrinsic and extrinsic aging. Intrinsic aging, also called chronologic aging, is influenced by internal physiological factors, while extrinsic aging is influenced by various external factors, including dietary factors, ultraviolet (UV) radiation, and environmental pollution. Exposure to UV radiation is most often referred to as photodamage or photoaging. UV radiation triggers a series of chemical reactions in the skin that ultimately lead to the development of photoaging. Continued exposure to solar radiation results in compromised repair of the skin's underlying structure. The accumulation of unrepaired cells and extracellular materials in the mended skin structure eventually manifests as wrinkles and sagging of the skin over time. Alterations in skin pigmentation are intricately linked to the process of photaging. Furthermore, a range of photo-induced skin cancers have been attributed to both short-term and prolonged exposure to sunlight (1,2).

Among the UV radiations, specifically UVB (290–320 nm) and UVA (320–400 nm), UVA predominantly exerts its influence on skin structure. Endogenous chromophores, also referred to as photosensitizers, such as retinal, porphyrins, bilirubin, melanin, and hemoglobin, in the presence of UVA and molecular oxygen, initiate destructive effects on skin biomolecules. These reactions lead to DNA damage and the subsequent formation of reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide, and superoxide (3).

There are specific pathways that contribute to Extracellular matrix remodeling followed by UVA irradiation. They enhance the degradation of structural proteins like collagen and elastin and impair their synthesis, leading to the loss of structural integrity and eventually skin aging. These pathways include Matrix metalloproteases activation

(MMPs) specially MMP1 (Collagenase-1), Mitogen activated protein kinase (MAPKs) particularly ERK (Extracellular signaling regulated kinase) and JNK (c-Jun N-terminal kinase), and Nuclear factor kappa B (NF-kB) pathways. UVA radiation induces oxidative stress and activates transcription factors like AP-1, which, in turn, leads to the upregulation of matrix metalloproteinases (MMPs) such as collagenase and elastase. The increased production and activity of these enzymes contribute to the degradation of collagen and elastin, resulting in the progressive breakdown of skin structure and elasticity over time (4,5).

Studies demonstrate that lifestyle changes can delay chronologic aging caused by internal factors, which occur naturally (6). It is also possible to delay photoaging by modifying external factors. Photoaging can be prevented and treated by applying cosmeceuticals with antioxidant properties (7). Cosmeceuticals are cosmetic products that contain active compounds, often blurring the line between cosmetics and pharmaceuticals (8). Antioxidants are frequently investigated for their potential to prevent photoaging due to their capacity to quench free radicals and suppress the expression and activity of MMPs (9). In addition to vitamins A, C, and E, natural antioxidants from plant resources such as flavonoids, alkaloids, and terpenes are applied in anti-aging skin products (10). They are continually studied for their beneficial effects on skin aging and their potential to protect the skin from harmful UV radiation. Numerous studies have shown that these plant secondary metabolites have the potential to inhibit the collagenase and elastase metalloproteases in the skin (11). In one of the studies conducted by K. Satyavani, the ethanol extract of Excoecaria agallocha L. was identified as a potent, non-competitive inhibitor of elastase and collagenase (12,13). In another study, Carica papaya seed water extract was found to be highly protective against oxidative stress induced by hydrogen peroxide (H_2O_2) in human skin Detroit 550 fibroblasts (13). Seo A. Seo also investigated the skin anti-aging effects of Carica papaya leaf in UVB-irradiated normal human dermal fibroblast cells and found that Carica papaya leaf, through its radical scavenging ability, can suppress AP1 signaling pathway, resulting in a decrease in the expression of collagenase and elastase enzymes (14). Maity et al. (15) reported Tagetes erecta Linn flower as an anti-wrinkle agent since it has been shown to effectively inhibit hyaluronidase, elastase, and MMP-1 in-vitro. The flavonoids and phenolic acids present in Pomegranate (Punica granatum L.), green tea (Camelliasinensis (L) Kuntze) and Rosemary Extract (Rosmarinus officinalis) have proven to exhibit powerful antioxidant properties and showed powerful protective effects against UV damage in human skin fibroblast cells (16-18). Many in-vivo studies also suggest that herbal compounds have the ability to protect the skin from the adverse effects of ultraviolet radiation (19).

The human skin is equipped with a complex system of safeguarding antioxidants. These include protective enzyme-based antioxidants such as catalase and superoxide dismutase, alongside non-enzymatic antioxidants like glutathione, bilirubin, ferritin, and coenzyme Q10. Exposure to UV radiation triggers the oxidation of biomolecules within the human skin, leading to a depletion of endogenous antioxidants (20). Therefore, the application of antioxidants and enzyme inhibitors in cosmeceuticals may be highly effective candidates for the protection and treatment of skin photoaging. Commonly used antioxidants in topical formulations are vitamins E, and C, lipoic acid, and ubiquinone (CoQ10). Antioxidants can mitigate photoaging by suppressing the generation of reactive oxygen species caused by photooxidative stress and enhancing the epidermal defense mechanism against UV damage (21).

Berberine is an isoquinoline alkaloid with a tetracyclic skeleton. It is the ammonium salt of benzylisoquinoline and is present in the barks, rhizomes, stems, and roots of plants such as Berberis vulgaris, Berberis thunbergii, Berberis aristata, Hydrastis canadensis, Xanthorhiza simplicissima, Mahonia aquifolium, and Phellodendron amurense. Berberine is considered an active ingredient in these plants and serves both edible and medicinal functions (22). Several methods have been applied for the extraction of berberine from different plants, including maceration, percolation or Soxhlet extraction, solvent extraction, and green extraction approach. In another study, scientists synthesized special magnetic nanoparticles for the extraction of berberine (23-27).

Berberine exhibits various pharmacological properties, including anticancer, antiarrhythmic, ischemic, cardioprotective, antidepressant, antidiabetic, and anti-asthmatic effects. It also serves as a potential new therapy for neurodegenerative disorders such as Parkinson's disease (28-33).

In a comprehensive study conducted by Shekarabi, SPH et al. (34), the antioxidant and antibacterial properties of berberine fruit were investigated, along with its potential to enhance immune function. The study involved the examination of dietary Berberine fruit extract in the serum of one hundred and fifty fish specimens. Their findings strongly support the notion that dietary berberine fruit extract, particularly at a dosage of 750 mg/kg, represents a promising candidate for the regulation of immune responses and bolstering antioxidant defense systems (34).

El-Zahar, KM et al. (35) demonstrated the high antioxidant activities of phenolic and flavonoid components derived from the ethanolic extract of barberry leaf and roots in-vitro. In a subsequent in-vivo study by Gholampour, et al. (36), evidence emerged supporting the administration of barberry hydro-alcoholic extract at 160 mg/kg/day for ten days as an effective measure against cisplatin-induced nephro- and hepato-toxicity. This treatment exhibited an increase in exogenous enzyme activation and a concomitant decrease in malodialdehyde levels compared to cisplatin-induced toxicity. They concluded that Barberry extract,

notably its active component berberine, exhibits significant potential in mitigating cisplatin-induced oxidative stress within hepatic and kidney tissues (36).

Ashraf, H and Zare, S. (37) have reported on the antidiabetic potential of the aqueous extract derived from Berberis integerrima Bge root. Their research findings underscore the preventive capabilities of berberine extract against hepatic impairment and oxidative stress instigated by streptozotocin-induced diabetes mellitus in rats. These results substantiate the utility of Berberis integerrima Bge root extracts as a promising agent for managing diabetes and its associated complications (37).

Yadawa, et al. (38) explored the biological effectiveness of Berberine in rat models induced with aging through D-galactose treatment. Their investigation revealed that D-galactose administration elevated pro-oxidants, including malondialdehyde, plasma membrane redox system, and advanced oxidation protein products in the plasma. This led to a decrease in antioxidant levels such as reduced glutathione (GSH), plasma thiols and sialic acid. However, when berberine was administered alongside D-galactose in rat models, it successfully reinstated both pro-oxidants and antioxidant levels in erythrocytes (38).

Recent studies have identified moisturizing properties of berberine hydrochloride when incorporated into cellulose-derived and biodegradable hydrogel films. These films were analyzed for their water uptake profile and skin permeation properties (39). Kim, SM et al. (40) provided evidence that berberine reduces the expression of MMP-1 genes and increases type I procollagen expression in human dermal fibroblasts. The cells were treated with berberine for 72 hours prior to a one-hour period of exposure to fluorescent sun lamps with an emission spectrum of 275–380 nm and 310–315 nm. Their findings suggest that the reduction of MMP-1 and induction of type I procollagen may be mediated by the antioxidant activity of berberine in human dermal fibroblasts (40). While the mentioned study investigated the impact of Berberine on MMP-1 expression levels, there has been limited scientific inquiry into the inhibitory action of berberine chloride on MMPs. MMPs constitute a family of enzymes critical in remodeling the extracellular matrix (ECM) within tissues, including skin. The ECM is a complex network of proteins and carbohydrates that provides structural support to cells and influences various cellular processes. In the context of skin health, MMPs are involved in maintaining skin structure, wound healing, tissue repair, and turnover. They function by breaking down ECM components, such as collagen and elastin, responsible for skin's elasticity and strength. This breakdown facilitates the removal of damaged tissue and supports tissue regeneration during healing. In skin health, MMPs contribute to balancing ECM synethesis and degradation, which is crucial for maintaining skin elasticity, texture, and overall function. However, their dysregulation can lead to skin disorders such as premature aging and inflammatory conditions. Exposure to UV light also activates the MMPs (41).

Consequently, we aimed to evaluate berberine's potential as an active ingredient in cosmeceuticals. We conducted primary tests on enzyme inhibition and the kinetics of elastase and collagenase, which are relevant in skin health and rejuvenation. we assessed Berberine antioxidant capacity using three methods: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, and we also formulated a cream containing 1.5% berberine. We then evaluated the cream's SPF, pH, and heat stability to determine its relevance in the skin anti-aging segment of the cosmetic industry. However, further in vitro and in vivo investigations are necessary to fully substantiate this potential.

2. EXPERIMENTAL SECTION

2.1. Materials

The Berberine chloride (purity >95%), ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)], DPPH [2,2-Diphenyl-1-picrylhydrazyl], and all other reagents were purchased from sigma-Adrich.

2.2. Elastase (Type IV, EC 3.4.21.36) Inhibitory Activity

The inhibitory potential of berberine was measured using a modified spectrophotometric method based on Kraunsoe et al. (42) and Deniz et al. (43). A mixture of 10 µL of berberine at various concentrations (50, 100, and 200 µg/mL), 10 µL of elastase (from porcine pancreas) at a concentration of 0.5 mU/mL, and 180 µL of 0.1 M tris buffer (pH 8.0) was pre-incubated for 20 minutes at 25°C. To the reaction mixture, 10 µL of N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide substrate (1 mM) was added, followed by a 10-minute incubation at 25 °C. The amount of product (p-nitroaniline) produced from the substrate was recorded at 410 nm using an ELISA microplate reader. Each experiment was performed in triplicate and enzyme inhibitory activity was calculated using the equation below:

$$\text{Enzyme inhibitory activity (\%)} = (\Delta A/\text{min})_{\text{control}} - (\Delta A/\text{min})_{\text{sample}} / (\Delta A/\text{min})_{\text{control}} \times 100 \quad \text{(Eq. 1)}$$

The IC_{50} value was obtained by plotting a graph between concentration and percentage inhibition at a substrate concentration of 1 µmol/mL using GraphPad Prism software version 9.5.1.

2.3. Collagenase (Type I, EC 3.4.24.3) Inhibitory Activity

The collagenase inhibitory activity of berberine was measured by spectrophotometer according to the modified method of Kozachok et al. (44). A reaction mixture was prepared by dissolving different concentrations of berberine (25, 50, and 100 µg/mL) in 700 µL of 50 mM tricine buffer (pH 7.5) and

incubated with 100 µL of collagenase enzyme solution for 20 min at 37 °C. Subsequently, 200 µL of N-(3-[2-furyl]-acryloyl)-Leu-Gly-Pro-Ala (FALGPA) was added as the substrate. Optical densities were measured in 5-minute intervals for 20 minutes at 345 nm. Each experiment was performed in triplicate and enzyme inhibitory activity was calculated according to the equation below:

Enzyme inhibitory activity (%)= (ΔA/min) control - (ΔA/min) sample / (ΔA/min) control ×100 (Eq. 2)

The IC_{50} value was obtained by plotting a graph between concentration and percentage inhibition at a substrate concentration of 1 µmol/mL using GraphPad Prism software version 9.5.1.

The inhibitory effectiveness of berberine chloride against collagenase and elastase was studied by observing its action against different substrate concentrations. The enzymatic reaction took place at a temperature of 25 °C for a duration of 20 minutes. The most effective doses of berberine were determined based on the results of the inhibitory activity test. The data obtained from the Lineweave-Burk plot analysis, calculated using Michaelis-Menten kinetics, were fitted to a sigmoidal dose-response equation with a variable slope through linear regression.

2.4. Antioxidant Assays

The antioxidant activity of berberine hydrochloride was quantified using three methods; DPPH, ABTS, and FRAP assays. A detailed description of the antioxidant assays can be reviewed in our previous study. IC_{50} values were calculated using GraphPad Prism software version 9.5.1. (45).

2.5. Cream Formulation

The oil-in-water (O/W) creams used in this study were prepared based on the solubility of the different components of the formulation:

Water 67%, Glycerin 2.3%, Tetrasodium EDTA 0.2%, Xanthan Gum 0.5%, Mineral Oil 15%, Polysorbate 60+Cetearyl Alcohol (1:1) 4%, Cetyl Alcohol 2%, Stearic Acid 1%, Triglyceride 7%, Phenoxyethanol 1%.

The prepared cream was divided into three batches. The first batch served as the negative control and did not contain berberine, while the second batch contained 1.5% berberine and the third batch contained 1.5% vitamin C as the positive control.

Table 1: Cream constituents with percentage content.

Aqueous Phase (70%) Constituents	
Water	67%
Glycerin	2.3%
Tetrasodium EDTA	0.2%
Xanthan Gum	0.5%
Oily Phase (30%)	
Mineral Oil	15%
Polysorbate 60 + Cetearyl Alcohol (1:1)	4%
Cetyl Alcohol	2%
Stearic Acid	1%
Triglyceride	7%
Phenoxyethanol	1%

Table 2: Antiaging lotions' oil-in-water cream formulas.

Formulations	**Contents**
F1	Base cream without active compound (negative control)
F2	Base cream + 1.5% Berberine
F3	Base cream + 1.5% Vitamin C (positive control)

2.6. In-vitro SPF Determination

The cream's SPF was assessed using the UV absorbance technique. A 0.1% hydro-alcoholic solution of berberine was prepared, and its absorbance was measured within the 290–320 nm range. The in-vitro photoprotection level was calculated using the Mansur equation, which was employed to establish the SPF values of the formulations. The equation is as follows:

$$SPF = CF \sum_{290}^{320} EE(\lambda) .I(\lambda). Abs(\lambda) \quad \text{(Eq. 3)}$$

Where CF=10 (Correction Factor), EE(λ)= Erythemogenic Effect of radiation at wavelength λ, I (λ) = Intensity of solar light at wavelength λ, and Abs (λ) = Absorbance of wavelength λ by a solution of the preparation (46).

2.7. pH Determination

The pH of the lotions was measured using a pH meter (EDGE pH meter) at 25°C.

2.8. Stability Studies

To assess the formulation's stability, heat stability study were carried out as per ICH guidelines. Cream-

filled bottles were placed in a humidity chamber maintained at temperatures of 8°C ± 0.1°C, 25°C ± 1°C, 40°C ± 1, and 65 ± 5°C in 20-30% humidity for 3 months. At the end of the studies, the samples were analyzed for their physical properties.

2.9. Statistical Analysis

The results were expressed as mean value ± standard error mean (SEM) of triplicate experiments. The analysis of the data was performed using GraphPad Prism software version 9.5.1.

3. RESULTS AND DISCUSSION

3.1. Inhibitory Effect of Berberine on Elastase and Collagenase

The IC_{50} values of berberine for elastase and collagenase were estimated to be 47.54 and 22.16 µg/mL respectively, indicating that berberine exhibited stronger inhibition of collagenase compared to elastase. Several natural compounds were identified with MMP inhibitory activities. For instance, Curcuma mangga Val. Contains bioactive compounds such as curcumin, known for its anti-aging properties. The IC_{50} values for elastase inhibitory activities by C. mangga extracts from different solvent types were reported to range from 26.34 to 532.38 µg/mL (47). The inhibition of elastase by phytochemicals, such as phenolic compounds, is known to enhance capillary wall integrity, protect components of the extracellular matrix, and initiate the process of tissue reconstruction (48).

In our study to identify the inhibitory kinetics of elastase by berberine, Lineweaver−Burk plots were employed. Different substrate concentrations (1-8 mM) were incubated with elastase along with berberine (at concentrations of 50, 100, and 200 mM). An uninhibited enzyme reaction (control) was carried out in the absence of berberine. The results are illustrated in Figure 1a. As depicted in Figure 1a, the reciprocal graph reveals that the slope corresponds to Km/Vmax, while the intercept on the 1/v –axis is augmented by a factor of (1+ci/ki). Consequently, increasing the concentration of the inhibitor results in a set of parallel lines, wherein the intercept on the y-axis progressively rises in response to the increasing inhibitor concentration, suggesting that berberine functions as an uncompetitive inhibitor of elastase. Both Vmax and Km values were reduced from 1.821 to 0.744 mU/min and 0.351 to 0.191 mM, respectively.

Figure 1b illustrates the kinetics of collagenase enzyme inhibition by berberine. Lineweaver-Burk plots of the enzymatic reaction catalyzed by collagenase were generated in the absence or presence of berberine at different concentrations (25, 50, and 100 µg/mL). The reverse of substrate concentrations plotted on the X-axis versus 1/V (mU/min)-1 values (obtained from the Lineweaver-Burk analysis) plotted on the Y-axis. Results indicated that berberine acts as an uncompetitive inhibitor of collagenase, as evidenced by the reduction in both Vmax and Km values, from 1.222 to 0.516 mU/min for Vmax and 0.33 to 0.125 mM for Km, with increasing berberine concentrations.

Elastase and collagenase were both found to be un-competitively inhibited by berberine against their respective substrates. In uncompetitive inhibition, the inhibitor binds to the enzyme that is already associated with the substrate, making substrate concentration an important factor for inhibition. In the current study, both Vmax and Km decreased with increasing inhibitor concentration. Km is considered as the substrate concentration at which the rate of enzymatic reaction reaches half of Vmax. The lower Km values were observed in the presence of different concentrations of berberine, which suggested that the presence of berberine as an uncompetitive inhibitor causes the pretense affinity of the enzyme for the substrate to increase, meaning that Km decreases (49).

Uncompetitive inhibitors bind to the enzyme–substrate complex, but they do so at a site other than the enzyme's active site. Since the substrate is already bound to the enzyme, this binding by the inhibitor decreases both the Km and Vmax. Berberine exhibited significant inhibitory activity against the studied MMPs, which might be attributed to the formation of hydrogen bonds between berberine and the site of the enzyme-substrate complex or other critical sites, disrupting the essential catalytic configuration (50).

Table 3 presents the data on the inhibitory effect of berberine on enzymatic activity and the kinetics of enzyme inhibition. IC_{50} is the concentration of an inhibitor that produces half-maximum inhibitory effect against the enzyme. The inhibition constant K_i, referred to as the inhibitor concentration required to produce half of maximum inhibition, indicates the potency of the inhibitor; the lower the value of K_i, the more potent the inhibitor. K_i is a constant value for an inhibitor and enzyme pairing, whereas IC_{50} is a relative value that varies based on the substrate concentration employed in the assay. As listed in Table 3, berberine exhibited lower K_i values, demonstrating its stronger affinity for binding to elastase and collagenase (50).

Inhibiting MMPs presents a promising strategy for combating and preventing skin photoaging. Notably, numerous studies have identified plant secondary metabolites, such as polyphenols, as effective inhibitors of MMPs (51). For instance, Madhan et al. (52) demonstrated that polyphenols, namely catechin and epigallocatechin gallate (EGCG) from green tea, possess potential inhibitory activity against collagenase, with rates of inhibition reaching 70 and 88 % respectively. Their kinetic study on the inhibition of collagenase by catechin and EGCG, using the hydrolysis of 2-furanacryloyl-l-leucyl-glycyl-l-prolyl-l-alanine as a substrate, revealed that both catechin and EGCG exhibited competitive type of inhibition against collagenase. They concluded that EGCG's heightened inhibitory effect, in contrast to catechin, is a result of EGCGs superior ability to form hydrogen bonds and engage in hydrophobic interactions with collagenase (52). As a result,

phytochemicals hold potential applications in both pharmaceuticals and cosmeceuticals for skin care. MMP inhibitors can be formulated within cosmeceutically compatible formulations or pharmaceutically approved carriers such as creams, lotions, or ointments. When used in cosmeceuticals, this innovative MMP inhibitor composition can be applied to the skin to proactively diminish wrinkles, pigmentation irregularities, loss of skin elasticity, and other manifestations linked to aging or sun-induced damage (53).

Table 3: Inhibitory Effect of Berberine on Elastase and Collagenase; IC_{50} values and values of inhibition constant from kinetic studies.

	Berberine IC_{50} value (µg/mL)	**Berberine K_i value**
Elastase[1]	47.54	0.003733
Collagenase[1]	22.16	0.003557

[1] IC_{50} values for both enzyme inhibitions were calculated at substrate concentration of 1 µg/mL.

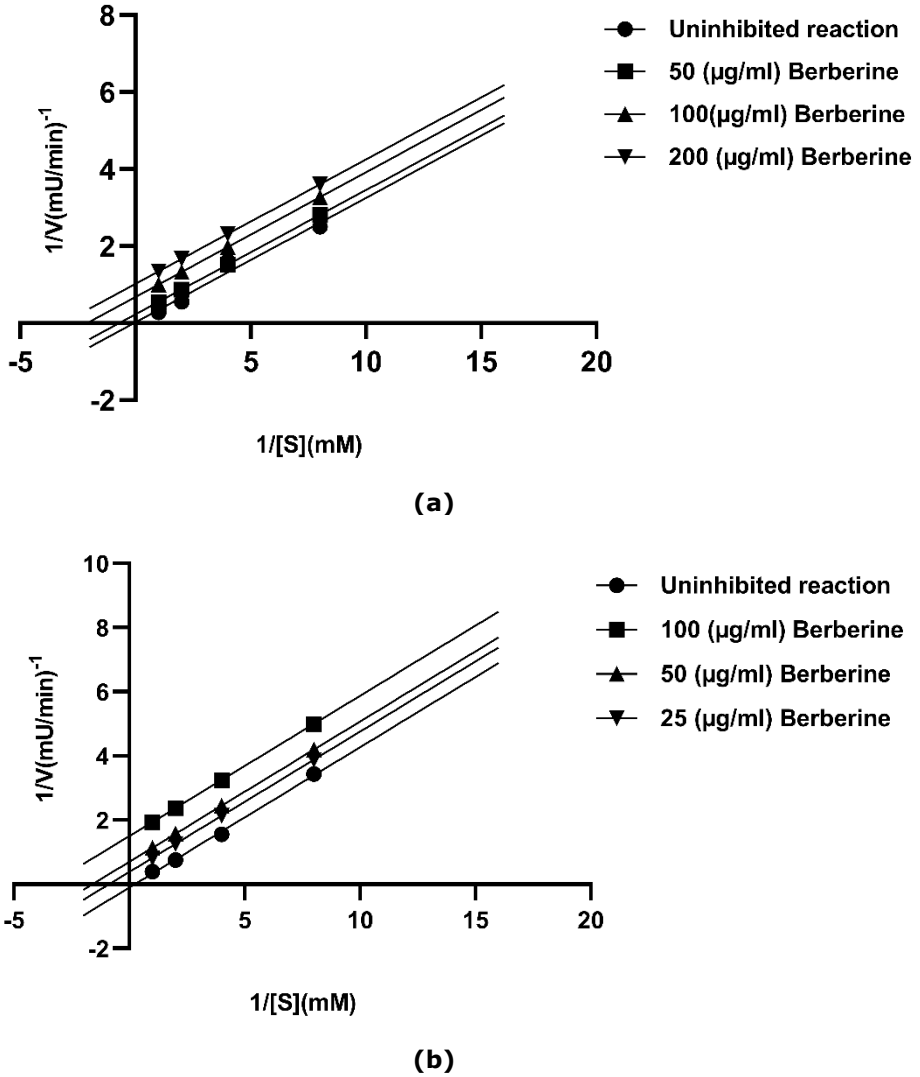

Figure 1: Effect of berberine on (a) elastase and (b) collagenase enzyme kinetics.

One of the primary limitations is the limited solubility of berberine in aqueous solutions, which can affect its bioavailability and make it challenging to achieve desired concentrations in formulations. To address this, we dissolved berberine in ethanol, given its solubility in ethanol, DMSO, and DMF, and used this solution for various experiments in the study. Additionally, using high concentrations of berberine for inhibition studies may interfere with assay components and skew results. To minimize this potential interference, we selected a concentration range of 50-200 µg/mL Despite these challenges, the study provides valuable insights, but the findings should be interpreted with these limitations in mind.

3.2. Antioxidant Capacity

Prolonged and repeated exposure to UV can lead to the accumulation of a substantial number of active oxygen products within skin tissues. The mechanisms underlying photoaging induced by UV radiation primarily involve the generation of ROS and DNA damage, ultimately resulting in cellular damage. A key factor in this process is the imbalance between ROS production and their neutralization by the body's natural antioxidant systems, leading to oxidative stress. ROS can promote the peroxidation of the lipid components in cell membranes and alter the structure and function of various enzymatic systems (54).

The results from the current study indicate that berberine possesses significant potential in neutralizing free radicals and demonstrates remarkable reduction capabilities when compared to the control substance, ascorbic acid. Our findings, as determined by the FRAP assay, indicate that 210.387 mg of berberine is equivalent to 1 mM of ascorbic acid in reducing power. A comprehensive investigation into berberine's antioxidant capacity was conducted by Aoxue Luo and Yijun Fan (55) employing six distinct methods. Their research revealed that berberine displayed scavenging abilities against radicals, particularly with regards to ABTS, hydroxyl, and DPPH radicals. Overall, berberine's impressive antioxidant prowess can be attributed to the existence of electron-donating groups within the molecular structure of this alkaloid compound (56).

Table 4: Antioxidant activity of berberine using FRAP, DPPH and ABTS radical scavenging assays.

Antioxidant Assays	Berberine	Ascorbic acid (AA)
DPPH Radical Scavenging Activity IC_{50} Values (µg/mL)	66.81	55.1
ABTS Radical Scavenging Activity IC_{50} Values (µg/mL)	180.5	125.7
FRAP	210.387 mg/L Berberine/176 mg/L AA	

3.3. Determination of SPF, pH and Stability of Berberine in Formulated Cream

In daily life, the skin's exposure to sunlight stands out as the primary factor that accelerates photoaging. This condition can activate the extrinsic mechanism responsible for ROS synthesis throughout skin cells. Excessive ROS production, in turn, leads to the appearance of wrinkles and a reduction in skin elasticity. Sunscreens were developed with the aim of averting these effects by either dispersing or absorbing the UV light (57).

In this study, the SPF value of the berberine in formulated creams was evaluated using UV spectrophotometry and the Mansur mathematical equation. Figure 2 shows the SPFs of the formulated creams. It was demonstrated that the SPF of the berberine-containing cream (F2) was significantly higher than that of the base cream, comparable to the cream containing 1.5% ascorbic acid (F1). These results indicate that berberine can effectively function as a highly efficient anti-solar agent against harmful UV radiation.

Brinda, S et al. (58) evidenced the effectiveness of aqueous extract of three types of naturally occurring herbs separately and in combinations, in the formulation of sunscreen creams aiming to avoid the addition of synthetic active ingredients due to their adverse effects. They estimated the SPF value of creams using the spectrophotometric method and Mansur equation. They found the highest SPF value for the creams containing integrated herbal ingredients (58).

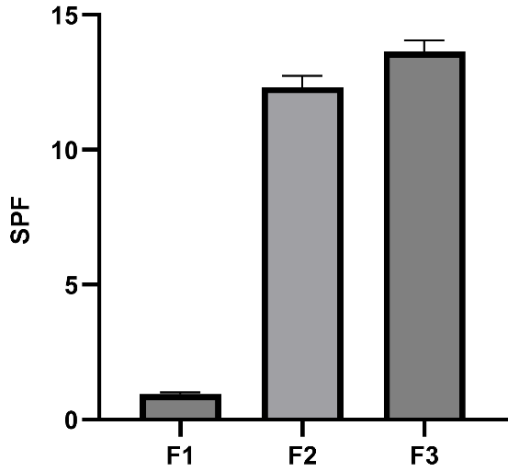

Figure 2: Determination of the Sun Protection Factor (SPF) of Oil-in-Water Creams containing berberine (F2) and Ascorbic Acid (F3) and negative control without active ingredient (F1). Values are the mean ± standard deviation (n=3).

The pH values of formulated creams are presented in Table 2. The pH of F3, compared to F2, was lower; however, the changes in the pH values during three-time intervals were not significantly different at P<0.01. All samples exhibited a pH level close to skin's natural pH.

The stability of formulations was determined at three different temperatures: 8°C ± 0.1°C, 25°C ± 1°C, 40°C ± 1, and 65 ± 5°C relative humidity for a duration of 3 months. All of the formulations demonstrated strong stability when exposed to different temperatures for three months.

The pH level of sunscreen is a critical factor that affects both its effectiveness in providing sun protection and heat stability of the cream. It should be compatible with the skin's natural pH, which is typically around 5.5 (59). The pH of our formulations was found to be 5.6 and remained stable for a duration of 24 days.

Table 5: pH values of formulated creams.

Formulations	0 Day	14 days	28 days
F1[1a]	6.72 ± 0.1	6.75 ± 0.08	6.77 ± 0.05
F2[1b]	5.62 ± 0.08	5.62 ± 0.2	5.6 ± 0.3
F3[1c]	5.39 ± 0.1	5.4 ± 0.1	5.4 ± 0.15

[1]The change in the pH values during three time intervals was not significantly different at p<0.01. [a] Negative control without active ingredient (F1), [b] berberine (F2) and [c] Ascorbic Acid (F3).

4. CONCLUSION

Collagen and elastin are crucial structural proteins that maintain skin integrity and elasticity. A decrease in their levels is closely associated with the appearance of aging signs, such as wrinkles and sagging skin.

In recent years, Cosmeceuticals-cosmetic products with bioactive compounds- have gained significant attention for their potential to combat skin aging. These products often contain active antioxidant and anti-aging compounds derived from various plant sources, offering natural and effective solutions for skincare.

Our research highlights Berberine, as a potent antioxidant and an effective agent in inhibiting collagenase and elastase activity. In our studies, a formulated cream containing Berberine demonstrated promising results, showing reasonable sun protection factor (SPF), skin-suitable pH values, and excellent heat stability. These findings suggest that Berberine is a promising candidate for use as an active ingredient in cosmeceuticals, offering a natural approach to enhance skin health and reduce the visible signs of aging.

5. CONFLICT OF INTEREST

The authors declare no competing financial interest.

6. ACKNOWLEDGMENTS

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