

Investigation of Bioactivities of Morel Mushrooms (*Morchella* Spp.) and Their Application in Facial Mask Formulation

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ABSTRACT

Mushrooms have historically been a significant natural resource due to their nutritional and medicinal properties. Today, the bioactive compounds in mushrooms are attracting considerable interest, particularly in the food, nutrition, and cosmetics sectors. Approximately 14,000 mushroom species have been identified, of which only 2,000 are suitable for human consumption and 650 are reported to have medicinal properties. Recent studies on the biological activities of mushrooms demonstrate that these organisms possess remarkable properties not only as nutrients but also from a pharmacological and cosmetic perspective. The aim of this study was to purify the protease enzyme from *Morchella* spp. mushrooms collected from Muğla region by Three-Phase Partitioning (TPP) method and to evaluate the potential of the obtained pure enzyme in cosmetic mask formulations. In this context, morel mushrooms exhibit antioxidant, anti-inflammatory, moisturizing, and anti-aging effects thanks to their rich content of polysaccharides, β -glucans, phenolics, flavonoids, amino acids, vitamins, and minerals. The biological activities of *Morchella* extracts, such as scavenging free radicals, inhibiting lipid peroxidation, reducing UV-induced oxidative stress, supporting epidermal barrier integrity, and accelerating wound healing, have been reported in the literature. Extracts with antimicrobial activity can also serve as natural preservatives in cosmetic formulations. Furthermore, due to their moisture-retaining properties, they are effective in masks and tonics, protecting and strengthening the skin against oxidative stress. In this study, the protein content of naturally grown morel mushrooms from the Muğla region was determined using the Three-Phase Partitioning (TPP) method, and protease enzyme activity was investigated. Cosmetic face mask formulations were developed with the obtained proteins. The yield of the protein purified from morel mushrooms using the TPP method was calculated as 40.26% in the middle phase. As a result of the applications, the masks were observed to increase skin moisture, radiance, and vitality. In conclusion, the morel mushroom stands out as a high-value-added natural raw material in the cosmetic industry due to its rich biochemical components and versatile biological activities. This study not only highlights the potential of *Morchella* species in functional cosmetic products but also proposes a model that can contribute to the local economy through the valorization of regional natural resources.

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1. Introduction

Mushrooms have a long history and a profound biological and economic impact on humanity [1]. Wild mushrooms have been consumed with great pleasure since ancient times for their taste and pleasant aroma [2]. Many of them have been used in folk medicine for thousands of years [3]. In ancient Egyptian and Chinese cultures, mushrooms were favored for better health and longevity [4]. In traditional Chinese medicine, mushrooms have been used for various therapeutic effects [5].

Of the approximately 14,000 known mushroom species, only 2,000 are considered safe for human consumption, and about 650 of these possess medicinal properties [6, 7]. Over the last two decades, knowledge about the bioactive composition and nutritional value of mushrooms has grown steadily [4–8].

Various studies have revealed the potential use of mushrooms and the bioactive nutraceuticals isolated from them as antitumor, antiviral, anti-inflammatory, antioxidant, antifungal, antidiabetic, antibacterial, cardioprotective, and hepatoprotective agents [1, 3, 9]. Consequently, understanding the health benefits and mechanisms of action of mushrooms has garnered significant interest in the fields of food, nutrition, and cosmetics [4, 10].

Morel mushrooms (*Morchella* species) are a genus of mushrooms that are both highly valued for their gastronomic qualities and rich in biologically active compounds [11]. In recent years, they have begun to attract attention in the biotechnology and, especially, the cosmetics sector [12]. The main reason for this is their antioxidant, moisturizing, and anti-aging effects, which are attributed to their content of polysaccharides, phenolic compounds, flavonoids, amino acids, vitamins, and minerals [13–15].

Morchella extracts contain high levels of polyphenols, flavonoids, and ascorbic acid (Vitamin C) [13, 15]. These compounds neutralize free radicals, reducing oxidative stress in the skin [16]. They also inhibit collagen breakdown, delaying the formation of wrinkles [17]. Therefore, they can be evaluated for use in anti-aging serums, creams, and masks [18].

The β -glucans and other polysaccharides in the mushroom increase the skin's moisture-retention capacity [1–19]. They support the epidermal barrier, enhancing the skin's resistance to dryness and external factors [20]. Due to these properties, they can be used in moisturizing creams and products for sensitive skin [21].

Morchella extracts have shown anti-inflammatory effects in various studies [13–22]. They can be used in soothing products for skin redness, irritation, and inflammation [20].

They offer potential benefits, especially for acne-prone and atopic skin [21].

The phenolic compounds they contain provide protection against UV-induced oxidative damage [23, 24]. For this reason, they have the potential to be used in after-sun care products or as supporting ingredients in sunscreen formulations [25].

Some research indicates that *Morchella* extracts have antimicrobial activity against bacteria and fungi [13, 15]. In this regard, they can serve as natural preservatives, offering supplementary benefits in cosmetic formulations [26].

Polysaccharides and amino acids strengthen hair strands and maintain moisture balance [1–15]. Their antioxidant structure protects the scalp from free radical damage [20]. For these reasons, they can be used in anti-hair loss tonics, shampoos, and hair masks [27].

Morel mushrooms are edible fungi rich in phenolics, amino acid, sterols, fatty acids, and especially polysaccharides (including β -glucans), with antioxidant and anti-inflammatory biological activities that have been extensively studied [1, 3]. Recent reviews on *Morchella* polysaccharides highlight their capacity to scavenge free radicals, inhibit lipid peroxidation, and modulate oxidative stress pathways [15, 28]. These effects directly align with cosmetic goals such as reducing skin aging, improving barrier integrity, and managing irritation [4].

Polysaccharides/ β -glucans are the primary mediators of antioxidant activity in *Morchella* [15, 28]. Both in vitro and in vivo studies report their ability to reduce ROS, scavenge free radicals (in DPPH, ABTS, and CUPRAC tests), and regulate signaling pathways (e.g., NF- κ B) [1–19]. β -glucans may also contribute to skin moisture retention and repair through their humectant properties that support the epidermal barrier [21]. For phenolic compounds and sterols, a correlation has been reported between phenolic levels and antioxidant capacity in *Morchella* species [13–28]. These compounds may offer support against oxidative damage associated with photoaging [23].

Modified polysaccharides and extracellular vesicles from *Morchella* species have shown anti-inflammatory activity in both in vitro and in vivo models. These compounds dose-dependently suppressed the expression of inflammatory markers such as TNF- α , IL-6, iNOS, and COX-2 in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells, and additionally inhibited the phosphorylation of the NF- κ B and p38 MAPK signaling pathways [29]. They have a potential soothing effect on redness and reactivity and can be used in products for sensitive, acne-prone, or compromised skin.

The polysaccharides of *Morchella* species, particularly after the deproteination process, exhibit significant free radical

scavenging activity. Studies have reported that deproteinized polysaccharides show a moderate antioxidant effect in DPPH, ABTS, and CUPRAC assays [30]. These compounds offer potential benefits against signs of aging such as collagen degradation, fine lines, and skin dullness caused by oxidative stress. These properties of *Morchella* polysaccharides can be evaluated to provide an anti-aging effect in cosmetic formulations.

The polysaccharides of *Morchella* species, especially β -glucans, offer potential benefits in skincare. These compounds have been reported to exhibit effects such as moisture retention (humectant properties), strengthening the epidermal barrier, soothing irritation, and accelerating wound healing. Specifically, studies on the structural properties and biological activities of *Morchella esculenta* polysaccharides reveal the potential of these compounds to support skin health [15].

The extracts and polysaccharides of *Morchella* species, which are rich in phenolic compounds, have the potential to reduce the accumulation of UV-induced reactive oxygen species (ROS). The antioxidant activities of these compounds can provide anti-aging effects by reducing oxidative damage in the skin. The antioxidant, anti-inflammatory, and immunomodulatory properties of *Morchella esculenta* polysaccharides, in particular, support the use of these compounds as potential auxiliary active ingredients in skincare products [30, 31].

The polysaccharides of *Morchella* species possess biological properties that offer significant benefits in hair care products [29, 30]. The antioxidant and anti-inflammatory profiles of these compounds can improve scalp comfort by protecting it from oxidative stress [32]. Additionally, their film-forming and moisture-retaining (humectant) properties can enhance their effectiveness in shampoos, masks, and tonics [21].

The existing literature suggests that *Morchella* polysaccharides are a promising natural product for moisturizing, restorative, soothing, anti-aging, and brightening cosmetic products, based on their antioxidant and anti-inflammatory biology. In this research, we aimed to determine the protein content of the *Morchella* species using the three-phase partitioning method, investigate its protease enzyme activity, and develop and apply face mask formulations.

2. Materials and Methods

2.1. Plant Material Collection

Morel mushrooms were collected from the rural areas of Muğla during April-May 2024. The collected mushrooms were stored at -18°C in a cold room until they were used for experimental procedures.

2.2. Homogenate Preparation

The mushrooms stored in the cold room were weighed to 5 g using a precision balance. They were then mechanically crushed using a phosphate buffer. They were placed in 25 ml of 0.1 M phosphate buffer (pH 7.0) and left to mix with a magnetic stirrer for half an hour at room temperature. After this process, they were subjected to several freeze-thaw

cycles at -18°C . The resulting homogenate was filtered through filter paper. The homogenate was then centrifuged at 6000 xg for half an hour, the pellet portion was discarded, and the supernatant was used in subsequent stages of the work.

2.3. Purification of Protease Enzyme from Morel Mushroom by Three-Phase Partitioning (TPP) Method

In the TPP system, the mutual interaction between t-butanol and $(\text{NH}_4)_2\text{SO}_4$ was utilized to effectively concentrate the enzymes at the interface. The optimal ratio of t-butanol and amount of $(\text{NH}_4)_2\text{SO}_4$ were determined. To 10 mL of homogenate, 4 g of $(\text{NH}_4)_2\text{SO}_4$ was added, and t-butanol was added at a 1:1 ratio. The reaction was stirred with a magnetic stirrer for 80 min, and to separate the phases, the mixture was centrifuged at 9000 rpm for 20 min. After the process, the resulting phases were separated into top, middle, and bottom phases and subjected to dialysis [33].

The preparation of the homogenate from morel mushrooms and the determination of the protein content were obtained by following the organizational chart below (Figure 1).



Figure 1 Preparation of a homogenate from morel mushrooms (*Morchella* spp.) (stepwise workflow from left to right) and quantification of protein content using the Bradford method

2.4. Protein Determination by Coomassie Brilliant Blue (Bradford) Method – Chemical Principle

This method was developed by utilizing the fact that Coomassie brilliant blue G-250 dye produces different intensities of blue color in protein solutions of varying concentrations. The dye was observed to have a tendency to bind to basic amino acids like arginine and some aromatic amino acids like tyrosine and tryptophan. Coomassie brilliant blue G-250 binds to proteins in a phosphoric acid medium, and the resulting complex shows maximum absorbance at 595 nm. The sensitivity of the method is between 1-100 mg [34].

2.5. Determination of Protease Enzyme Activity

Proteolytic activity was determined by the casein digestion method in the presence of 1% casein. For proteolytic activity, 0.5 ml of enzyme solution was added to 1 ml of casein solution, mixed thoroughly, and incubated for at 40 °C during 20 minutes. Subsequently, 3 ml of 5% TCA was added and left to stand for 1 hour. It was then was centrifuged at 10000xg for 5 minutes, and the resulting supernatant was filtered [35].

2.6. Development of Mask Formulation with Enzyme and Homogenate

The mask formulation was optimized based on viscosity, homogeneity, spreadability, and skin compatibility parameters. Enzyme/homogenate concentration was standardized at 2% (w/v) to ensure stability and activity in the cosmetic matrix. Facial masks are cosmetic formulations designed for topical application over a specified duration to provide multiple benefits, such as moisturizing the skin, exfoliating dead cells, preventing the formation of pigmentation, and mitigating skin aging. The mask was formulated at a concentration of 100% (w/v). The constituents in the mask formulation is presented in Table 1.

Table 1 Mask Formulation

Material	Quantity
Distilled water	%95
Homogenate, Enzyme, Extract	%2
Ethyl Alcohol	%3

3. Results and Discussion

The preparation of the homogenate from morel mushrooms and the determination of the protein content were obtained by following the organizational chart below (Figure 1).

In our literature search for this study, it was found that morel mushrooms have a high protein content. We used the three-phase partitioning method to purify the protease enzyme from morel mushrooms, as it is more efficient, less costly, and faster for industrial use. The protease enzyme activity and protein amount purified from morel mushrooms showed good yield with the three-phase partitioning method. The middle phase yield of the morel mushroom was calculated as 40.26%. The purification results are given in Table 2. The purification yield obtained in this study is comparable to TPP-based purification efficiencies reported in other fungal protease studies [36].

Table 2 Purification results from the protease enzyme homogenate obtained by the Three-Phase Partitioning Method from Morel Mushrooms

Samples	Homogenate	Middle Phase
Activity (EU/ μ g)	0.571	0.249
Total Activity(U)	57.1	24.9
Total Protein (μ g)	67.375	27.125
Spesific Activity (U/ μ g)	0.85	0.92
Purification Factor	1	1.082
Yield (%)	100	40.26

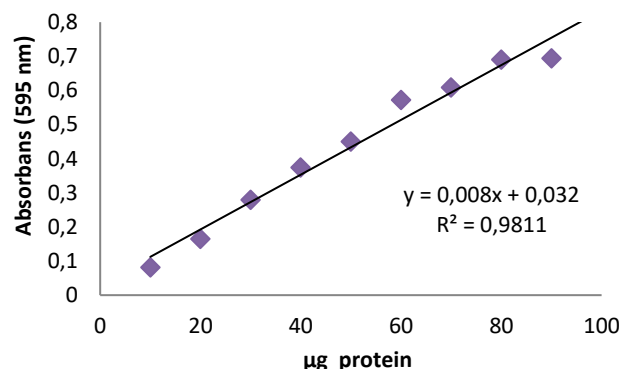


Figure 2 In the quantitative protein determination, the Coomassie brilliant blue method was used. A standard curve was first prepared using BSA. This curve was used for the determination of proteolytic activity in the homogenates, purified enzyme solutions, and for calculating the amount of protein.

Mask formulations were developed using the proteins obtained from the middle phase, and characterization studies were performed on these products. As a result of applying the developed masks, a significant increase in skin moisture, radiance, freshness, and vitality was observed. After regular and long-term use, the skin was found to have a healthier and more vibrant appearance. The increase in skin moisture observed in our formulations is consistent with previous findings showing that β -glucans enhance water retention and support barrier function [37].

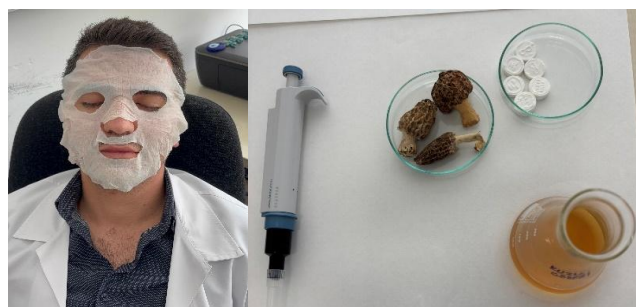


Figure 3 Photographs product testing study

4. Conclusion

Throughout human history, people have utilized natural products to improve their quality of life and prevent diseases, with early studies often conducted through alchemy and experimental methods. These investigations, particularly those focused on longevity and youth, were limited by the knowledge of the time but popularized the use of natural products.

Today, with technological and scientific advancements, research on the bioactive properties of natural products has become systematic and scientifically grounded. Consumers are increasingly seeking and preferring cosmetic products with scientifically proven natural ingredients. This trend supports the use of natural cosmetics in personal care and addresses the body's needs at a micro-level, while also shaping research and development activities.

Studies have reported that *Morchella* extracts possess the capacity to scavenge free radicals, inhibit lipid peroxidation, and modulate the signaling pathways of oxidative stress (e.g., NF- κ B, MAPK) [30]. These activities provide a strong scientific basis for using *Morchella* in cosmetic products to

reduce oxidative damage associated with photoaging, strengthen the epidermal barrier, maintain skin moisture, and soothe inflammation. Additionally, the humectant properties of *Morchella* polysaccharides increase the skin's water-retention capacity, support barrier functions, and accelerate the wound healing process [21].

In this study, some bioactive properties of the morel mushroom (*Morchella* spp.) naturally grown in the Muğla region were examined, studies on the purification of the protease enzyme were conducted, and skin mask design and characterization were performed using extracts obtained from the mushroom. The protein amount that passed from the homogenate to the middle phase was calculated to have a total activity of 24.9 U, total protein of 27.125 (µg), a purification factor of 1.082, and a yield of 40.26%.

The aim of the research was to reveal the industrial potential of the morel mushroom and increase its recognition as a natural product with socioeconomic value for the local community. Furthermore, skin mask trials with anti-aging and moisturizing properties were carried out with mushroom extracts, establishing a foundation for the development of high added value cosmetic products from natural ingredients.

Author Contributions

ND: Conceptualization, Investigation, Methodology, Data Curation, Formal Analysis, Visualization, Writing-Original Draft, Writing – Review & Editing

YDv: Data Curation, Formal Analysis, Writing-Original Draft

YD: Formal Analysis, Writing – Review & Editing, Supervision

Conflict of Interest

The authors have no conflicts of interest to declare.

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