**Research Article**

# Purification and Characterization of Anti-Inflammatory Serine Protease from Shiitake Mushrooms

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## ABSTRACT

Shiitake mushrooms (*Lentinula edodes*) are widely recognized for their nutritional and medicinal benefits, including antioxidant, anti-tumor, anti-inflammatory, and immunomodulatory properties. This study focuses on the purification and characterization of a serine protease extracted from *L. edodes*. The enzyme was purified using ammonium sulphate precipitation, DEAE-cellulose ion-exchange chromatography, and Sephadex G-75 gel filtration chromatography. The purified protease exhibited a molecular weight of 24.8 kDa (SDS-PAGE) and 25.0 kDa (Sephadex G-200 chromatography). It demonstrated optimal activity at pH 6.0 and 45°C, with substantial stability over a broad pH (3.0–9.0) and temperature (0–65°C) range. Functional analysis revealed significant antioxidant (60% inhibition at 100 µg/mL) and anti-inflammatory (80% inhibition at 60 µg/mL) activities. These findings suggest potential applications in food processing, pharmaceuticals, and biotechnology.

**Keywords:** Shiitake mushroom, Serine Protease, Anti-inflammatory.

## 1. INTRODUCTION

Shiitake mushrooms (*Lentinula edodes*), widely cultivated and consumed globally, are celebrated for their exceptional nutritional and medicinal properties. These mushrooms are a rich source of bioactive compounds, including polysaccharides, dietary fibers, ergosterol, essential vitamins, and minerals, which contribute to their well-documented antioxidant, anti-tumor, anti-inflammatory, and immunomodulatory effects [1, 2]. Among these bioactive components, proteases enzymes that catalyse the hydrolysis of peptide bonds stand out for their broad biological and industrial relevance [3].

Proteases play vital roles in physiological processes such as protein metabolism, immune regulation, and cellular signaling. They are extensively utilized in industries for applications ranging from food processing and pharmaceuticals to biotechnology. In the food sector, proteases enhance protein functionality, generate bioactive peptides, and are employed in processes such as meat tenderization and protein hydrolysis. In pharmaceuticals, they are integral to enzyme replacement therapies and the development of therapeutic peptides [4]. Despite their widespread use, fungal proteases, particularly those derived from shiitake mushrooms, remain relatively underexplored, presenting a unique opportunity for novel enzyme discovery.

Fungi, including shiitake mushrooms, are known to produce proteases with distinct properties, such as stability under extreme pH and temperature conditions and substrate specificity [5]. These attributes not only enhance their industrial value but also open avenues for therapeutic applications, particularly in inflammation modulation. Chronic inflammation, a driver of conditions such as arthritis, cardiovascular diseases, and neurodegenerative disorders, underscores the importance of enzymes with anti-inflammatory properties, such as serine proteases [2].

It aims to investigate the enzyme's biochemical properties, including pH and temperature stability, substrate specificity, and its anti-inflammatory potential. By addressing the research gap in fungal proteases, this study seeks to contribute to the understanding of their structure-function relationships and explore their applications in therapeutic and industrial domains. This research focuses on the purification and characterization of a serine protease from shiitake.

## 2. MATERIALS & METHODS

### 2.1 Chemicals

Acetone, Sodium carbonate, sodium hydroxide, sodium potassium tartrate, Coomasive brilliant blue(G250), Methanol, ortho phosphoric acid, copper sulphate, Bovine serum albumin, Ammonium sulphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, Acrylamide, bisacrylamide, tris-base, tris HCl, glycine, sucrose, TEMED, marcaptoethnol,α-napthol, 1-naphthyl acetate,, diazo- blue B, sodium lauryl sulphate, SDS, 1-napthayl acetate, bromophenol, ammonium persulphate, coomassie brilliant blue(R-250) ,methanol, acetic acid, sodium acetate, DNS, starch, Maltose, NaCl, Sodium citrate, citric acid, boric acid, borate, Tricholoro acetic acid.

**Figure 1: *Lentinula edodes* caps**

### 2.2 Preparation of dried mushroom powder:

The mushroom samples (*Lentinula edodes*) were purchased from local markets of Kushalnagar, Madikeri and Somwarpet of Kodagu district. Samples were cleaned, cut into small pieces and dried in a hot air oven at $50^0$C for 06 hours. Dried samples were ground into a fine powder and subjected to analyses.

### 2.3 Preparation of acetone powder from dried, powdered fruiting bodies of mushroom samples:

The acetone powder was prepared following the method of Wetter [6]. A 10% acetone powder was obtained by blending the powdered mushroom sample in a homogenizer with chilled acetone for 5 minutes. The mixture was then filtered using a suction pump. The resulting residue was dried at 37°C, ground into a fine powder, and stored at 4°C for future use.

### 2.4 Preparation of crude protein extract:

**"**A 10% protease extract was prepared from the acetone powder using 0.05 M sodium phosphate buffer (pH 7.0). The mixture was stirred on a magnetic stirrer for 2 hours at 4°C, then centrifuged at 10,000 rpm for 30 minutes. The resulting supernatant was collected and subjected to quantitative analysis of proteins, as well as assessments of amylase, protease, and esterase activity".

### 2.5 Determination of total soluble protein content:

Total protein content was determined using the dye-binding method represented by MM Bradford [7]. Protein concentration was estimated using BSA (bovine serum albumin) as the standard. The protein content was measured by recording the optical density at 590 nm.

### 2.6 Determination of activity of protease enzyme

We used 1% casein (pH 7.8) dissolved in 0.1 M phosphate buffer as the substrate to assess proteolytic activity, following the approach of Banik *et al.*, [8]. For 10 minutes at 30°C, a mixture of 1 mL of the crude enzyme sample and 5 mL of substrate was incubated. After centrifugation at 1500 rpm for 5 minutes, 5 mL of a 1% trichloroacetic acid (TCA) solution was added to halt the process. After that, the supernatant was left to incubate for 10 minutes with 5 mL of alkaline copper sulphate and then for 30 minutes with 0.5 mL of Folin-Ciocalteu (FC) reagent. We measured the absorbance at 660nm. The quantity of enzyme that generates an absorbance value equal to 1 µg of tyrosine per

minute at 30°C was deemed as one unit of protease activity.

**2.7 Purification of protease from the fruiting bodies of *L. edodes:***

**2.7.1 Ammonium Sulphate Fractionation:**

The supernatant obtained from the crude protein extract of *L. edodes* was subjected to ammonium sulfate precipitation at 0–80% saturation. Finely powdered ammonium sulfate was gradually added while stirring on a magnetic stirrer for 1 hour to achieve the desired saturation level.

The precipitated proteins were collected by centrifugation at 10,000 rpm for 30 minutes at 4°C. The resulting precipitate was then dissolved in 25 mM sodium phosphate buffer (pH 7.0) and subsequently dialyzed against 10 mM sodium phosphate buffer (pH 7.0).

**2.7.2 DEAE-Cellulose Chromatography:**

DEAE-Cellulose powder (10 g/500 mL) was suspended in distilled water, agitated thoroughly, and allowed to stand for 2 hours. Subsequently, it underwent activation through a series of treatments with 0.1 M HCl, 0.1 M sodium hydroxide, and 0.1 M HCl, each for a duration of 30 minutes. The particulates were eliminated through repeated rinsing with distilled water until the pH was stabilized at neutral.

The DEAE-Cellulose was then left to suspend overnight at 4°C in 0.025 M sodium phosphate buffer with a pH of 7.0. The resultant slurry was carefully packed into a glass column measuring 3.5 × 12.5 cm and equilibrated with three bed volumes of 0.025 M sodium phosphate buffer at pH 7.0. Subsequently, the dialyzed protease fraction (0–80% ammonium sulfate saturation) was introduced onto the activated DEAE-Cellulose column.

"The column was rinsed with the initial buffer (0.025 M sodium phosphate buffer, pH 7.0) at a flow rate of 60 mL/hr., using fraction quantities of 10 mL. Bound proteins were eluted incrementally with 0.1 M, 0.3 M, and 0.5 M NaCl". The DEAE-Cellulose fractions exhibiting proteolytic activity were aggregated and concentrated using Centricon tubes with a molecular weight limit of 5 kDa.

**2.7.3 Gel-filtration Chromatography on Sephadex G-75:**

A 10 g sample of "Sephadex G-75 powder was allowed to swell in an excess of distilled water in a boiling water bath for 5 hours and then left to cool. Floating fines on the surface of the gel matrix were removed by decantation. The gel was then equilibrated with 0.025 mM sodium phosphate buffer (pH 7.0)".

The prepared slurry was carefully poured into a glass column (1.0 × 150.0 cm) while avoiding air bubbles and was allowed to settle under gravity. The Sephadex G-75 column was equilibrated for approximately 72 hours using one and a half bed volumes of 25 mM sodium phosphate buffer (pH 7.0). The concentrated DEAE-cellulose fraction was then loaded onto the pre-equilibrated column.

Fractions of approximately 2 mL were collected at a flow rate of 12 mL/hr. The peak fractions containing trypsin inhibitor activity from Sephadex G-75 were pooled, concentrated using Centricon tubes with a 5 kDa molecular weight cutoff, and stored for further study.

**2.8 Quantitative Assays:**

**2.8.1 Protein estimation:**

The total protein content was quantified using BSA, or bovine serum albumin, as the standard, in accordance with the technique established by Lowry *et al.*, [9]. The protein content in all fractions was quantified at 280 nm using a "UV-Visible spectrophotometer (SP 3000-Plus, Optima, Tokyo, Japan).

The protease from the fruiting bodies of *L. edodes* was purified to homogeneity using conventional techniques, including extraction, salt fractionation, DEAE-cellulose chromatography, and gel filtration chromatography on Sephadex G-75. The purification profile, detailing recovery, fold purity, and specific activity at each stage, is summarized in the accompanying table.

A significant amount of protease activity was retained in the 0–80% ammonium sulfate fraction, which was further purified using DEAE-cellulose chromatography. "Three protein peaks were eluted and designated as fractions F1, F2, and F3. Fraction F1 was not

adsorbed onto the column and was eluted with the starting buffer. Fraction F2, which contained the protein of interest, was eluted using 50 mM sodium phosphate buffer (pH 7.0)" with 0.1 M sodium chloride.

The DEAE-cellulose fraction with significant proteolytic activity was pooled, concentrated using Centricon tubes, and subjected to Sephadex G-75 gel filtration chromatography. The protein and protease activity were eluted in a single fraction.

**2.9 Qualitative Assays:**

**2.9.1 Electrophoretic Analysis:**

Anionic NATIVE-PAGE gel electrophoresis was performed following the method of Laemmli [10]. A gel system consisting of a 10% separating gel and a 0.5% stacking gel was used in a vertical electrophoretic chamber. Acrylamide and bis-acrylamide solutions were prepared in a 29.2:0.8 ratio.

The 10% resolving gel was prepared using 0.3 M Tris-HCl buffer (pH 8.8), while the 5% stacking gel was made using 0.1 M Tris-HCl buffer (pH 6.8), along with double-distilled water, 10% ammonium persulfate (APS), and an appropriate volume of tetramethylethylenediamine (TEMED). The sample buffer contained 0.1 M Tris-HCl buffer (pH 6.8) and 100 mg of bromophenol blue as the marker dye.

Electrophoresis was carried out under refrigerated conditions, applying a current of 50–100V for a duration of 3 hours, utilizing Tris-glycine buffer (pH 8.3) as the electrode buffer. Following electrophoresis, proteins were stained for a duration of 1 hour using a staining solution composed of 10% acetic acid, 25% methanol, and 0.1% Coomassie Brilliant Blue R-250. De-staining was conducted overnight employing a solution consisting of 10% acetic acid and 25% methanol.

**2.9.2 In-gel assay for enzyme activity:**

For protease activity analysis, 1% casein was incorporated into the gel as the substrate. Following electrophoresis, proteins in the gel were stained using a solution containing 10% acetic acid, 25% methanol, and 0.1% Coomassie Brilliant Blue R-250 for 1 hour. The gel was then de-stained overnight with a solution of 10% acetic acid and 25% methanol. The presence of enzyme activity was indicated by the appearance of clear bands in the gel.

**2.9.3 Plate assay for enzyme activity:**

Agar plates were prepared (2g of agar in 100ml of distilled water) consisting of 1% substrates specific for each enzyme. 1% casein was used as substrate for protease and starch for amylase. A well was created in the gel and 100µL of sample was loaded and incubated for 24hrs and stained with appropriate staining solutions and the zones indicated the enzyme activity.

**2.10 Characterization of purified protease for its Physico-Chemical and biological properties:**

**2.10.1 Determination of molecular weight by SDS-PAGE:**

SDS-PAGE of the purified protease was performed following the method of Smith [11]. Different molecular weight marker proteins were used, including bovine serum albumin, carbonic anhydrase, phosphorylase-b and cytochrome-c, .

In a single preparation, "0.2 mL (100 µg) of each marker protein and 100 µg of purified protease were combined with 2 mg of SDS and 0.1 mL of glycerol containing 3 µL of bromophenol blue in 0.05 M sodium phosphate buffer (pH 7.0). In a separate preparation, identical protein samples were combined with 2 mg of SDS, 10 µL of 2-mercaptoethanol, and 0.1 mL of glycerol, which contained 3 µL of bromophenol blue, all inside the same buffer".

The samples were then boiled for 5 minutes before being subjected to electrophoresis in slab gels. Proteins were stained and de-stained as described previously.

**2.10.2 Determination of molecular weight by Gel-permeation Chromatography on Sephadex G-200:**

Gel-permeation chromatography of standard proteins and purified protease was performed on a Sephadex G-200 column, following the method of Andrews [12]. "Sephadex G-200 was allowed to swell in excess distilled water by heating in a boiling water bath for five hours [13]. The gel was washed twice with distilled water, equilibrated with 200 mL of 0.01 M sodium phosphate buffer (pH 7.0), stirred, and allowed to

settle". During equilibration, fines were intermittently decanted.

The gel was then packed into a gravity-fed column "(1.13 × 100 cm, bed volume 100 mL) and equilibrated with two bed volumes of 0.01 M sodium phosphate buffer (pH 7.0) at a flow rate of 10 mL per hour". The upper surface of the gel bed was protected using Whatman No. 1 filter paper. The column's void volume was determined using Blue Dextran-2000.

To calibrate the column, standard marker proteins were used: "alcohol dehydrogenase (150 kDa), β-amylase (200 kDa), carbonic anhydrase (29 kDa), BSA monomer (66 kDa), and cytochrome-c (12.2 kDa)". "The marker proteins and purified protease (1.0 mL) were individually loaded onto the column in 0.01 M sodium phosphate buffer (pH 7.0) and eluted using the same buffer. Fractions of 2.0 mL were collected at a flow rate of 10 mL per hour". The elution profiles of the marker proteins were determined by measuring absorbance at 280 nm.

The purified protease was assayed using 1% casein as a substrate. The molecular weight of the purified protease was determined using gel-permeation chromatography on Sephadex G-200, based on a calibration curve plotted with *Kav* values against the logarithm of the molecular weights of the standard proteins.

**2.10.3 Determination of Stokes Radius:**

The Stokes radius of the purified enzyme was calculated from the elution volumes obtained from sephadex G-200 column.

The Stokes radius values of standard proteins may also be obtained from literature. The Stokes radius obtained for purified protease was 2.4 nm.

**2.10.4 Determination of isoelectric point:**

Gel electro focusing was carried out following the method of Wringley [14] using 8% polyacrylamide gels. "The gel solution was prepared by combining 0.6 mL acrylamide solution (29.2 g acrylamide and 0.8 g *N,N*-methylene bisacrylamide in 100 mL of water), 0.06 mL of 40% ampholyte carrier (pH 3–10 range), 0.12 mL riboflavin (0.14 mg/mL), 0.1 mL enzyme sample (containing 100 µg protein), and 1.62 mL distilled water.

A total of 1.5 mL of this solution was carefully poured into glass tubes (0.6 × 8 cm). The gel surface was layered with water and allowed to photopolymerize under a fluorescent lamp for 30 minutes. After polymerization, the water layer was blotted off, and the tubes were inserted into the upper chamber of the electrophoretic apparatus.

The lower anode chamber was filled with 0.2% sulfuric acid, while the upper cathode chamber contained 0.4% ethanolamine. Electro focusing was conducted at 40°C for 2 hours, with the voltage gradually increased to 250V while maintaining a constant current of 2 mA per tube. Following electrophoresis, the gels were removed from the tubes and stained for esterase activity as previously described". Protein staining was performed using a solution containing 10% trichloroacetic acid, and 0.02% Coomassie Brilliant Blue G-250 (w/v), followed by de-staining with distilled water.

**2.10.5 Determination of optimum pH and pH stability:**

The effect of pH on the activity of the purified protease was examined using the following buffers:

i) Sodium acetate (pH 4.0–5.0, 0.05 M)
ii) Sodium citrate (pH 5.5–6.0, 0.05 M)
iii) Sodium phosphate (pH 6.5–7.5, 0.05 M)
iv) Tris-HCl (pH 8.0–9.0, 0.05 M)
v) Sodium carbonate (pH 9.0–11.0, 0.05 M)

The catalytic activity of the enzyme in these buffers was determined as described previously. Suitable controls were prepared using denatured enzymes to account for non-enzymatic substrate hydrolysis.

The pH stability of the purified protease was assessed by incubating the enzyme in buffers of varying pH (0.2 M, pH 4.0–10.0) for 24 hours at 4°C. After incubation, the pH was readjusted to the optimum level, and the enzyme assay was conducted at the optimum temperature for 15 minutes".

**2.10.6 Determination of optimum temperature and temperature stability:**

The consequence of temperature on the action of the protease (purified) was evaluated across a temperature range of 4°C to 70°C. The enzyme was appropriately diluted and incubated with the substrate, and its catalytic activity was measured as described previously.

"The thermal stability of the purified protease was assessed by incubating the enzyme at different temperatures (4°C to 65°C) for 1 hour. After incubation, the samples were rapidly cooled to 0°C and assayed at the optimum temperature". The amount of product released was quantified calorimetrically.

**2.10.7 Determination of Antioxidant property:**

The Antioxidant property determination through DPPH free radical scavenging activity of the purified protease and ascorbic acid was determined following the method of Barra *et al.* [15], with slight modifications. A DPPH stock solution was prepared by dissolving 0.1 mM of 0.04% DPPH reagent in 100 mL of methanol. The solution was stored in an opaque container at 4°C to prevent DPPH degradation.

Several quantities of the purified protease were mixed with 1 mL of a 0.1 mM methanolic DPPH solution for the test. We measured the absorbance at 520 nm after 30 minutes of dark incubation of the reaction mixture. The reference standard was ascorbic acid.

"% inhibition = 100 × (1 − A2/A1)",

"Where A1 = absorption of the control sample, and A2 = absorption of the test sample".

**2.10.8 Anti-inflammatory activity**

The inhibition of protein denaturation was assessed using the method of Kobayashi & Mizushima [16], with slight modifications. A 500 µL aliquot of 1% BSA was mixed with 100 µL of the purified protease inhibitor extract. After a 10-minute incubation period at room temperature, the mixture was heated to 51°C for 20 minutes. The OD value at 660 nm was then calculated when the solution had cooled to room temperature.

As a standard, a solution was prepared by mixing 0.5 mL of 1% BSA with 0.5 mL of a standard anti-inflammatory drug solution (serratiopeptidase in combination with diclofenac).

% inhibition = 100 × (1 − A2/A1),

"Where A1 = absorption of the control sample, and A2 = absorption of the test sample".

The maximum inhibition of about 80% was observed at 60µg/ml of the purified enzyme.

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## 3. RESULTS AND DISCUSSION

Hydrolytic enzyme protease was isolated and purified from mushroom sample. They have been purified by employing different purification processes. In the present study *Lentinula edodes* was subjected to purification and characterization of the hydrolytic enzyme protease.

### 3.1 Purification and Yield of Protease from *Lentinula edodes*

The purification of protease from *Lentinula edodes* was carried out using a sequential approach, including ammonium sulfate precipitation, DEAE-cellulose ion-exchange chromatography, and Sephadex G-75 gel filtration chromatography. These techniques helped remove impurities and increase the enzyme's specific activity. Crude extract contained high protein content but had low specific activity due to non-specific proteins. Ammonium sulfate precipitation (0–80%) was used to concentrate the protease and remove unwanted proteins. DEAE-cellulose chromatography separated the protease into F1, F2, and F3 fractions, with F2 exhibiting the highest proteolytic activity. Sephadex G-75 gel filtration chromatography further purified the enzyme, yielding a single major peak of protease activity.

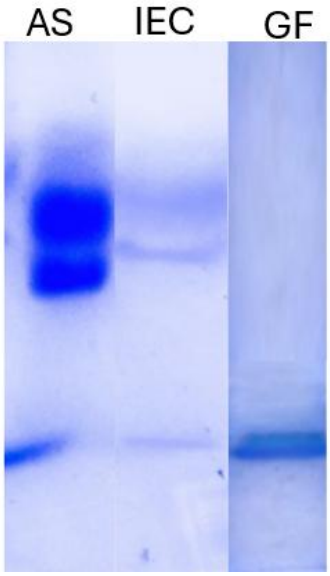



**Figure 2: Purification profile of proteolytic activity from *L.edodes***

**Table 1: Purification of Protease from *L. edodes***

| Purification Step | Total Volume (mL) | Total Protein (mg) | Total Activity (µmol/min) | Specific Activity | Fold purification | % yield |
|---|---|---|---|---|---|---|
| Crude | 102 | 674 | 28.46 | 0.0422 | 1 | 100 |
| 40-80% ammonium sulphate fractionation | 60 | 471.8 | 19.92 | 0.0401 | 0.9502 | 70 |
| DEAE cellulose chromatography | 62 | 61.24 | 12.48 | 0.2038 | 5.0820 | 20.3788 |
| Sephadex G-75 | 6 | 12.6 | 8.2 | 0.6508 | 3.1935 | 65.0794 |

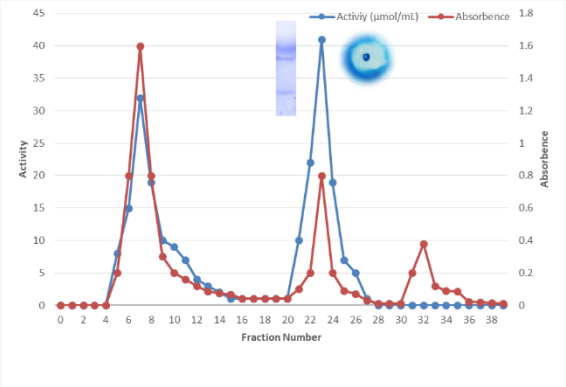



**Figure 3: Elution profile of proteolytic activity from dried powdered *L. edodes***

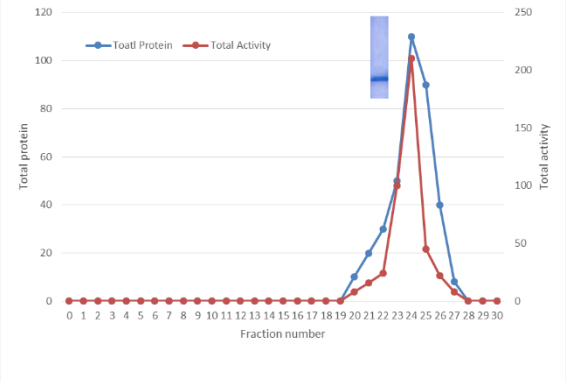



**Figure 4: Elution profile of proteolytic activity on Sephadex G-75**

The purification resulted in up to 5-fold purification with 65% enzyme recovery, which is consistent with previous studies on fungal protease purification [2, 12 & 3].

### 3.2 Electrophoretic Analysis and Molecular Weight Determination:

SDS-PAGE Analysis: The purified protease displayed a single protein band at 24.8 kDa, indicating its homogeneity [10]. Gel Permeation Chromatography (SephadexG-200): The molecular weight was determined to be 25.0 kDa, confirming the results from SDS-PAGE [12].

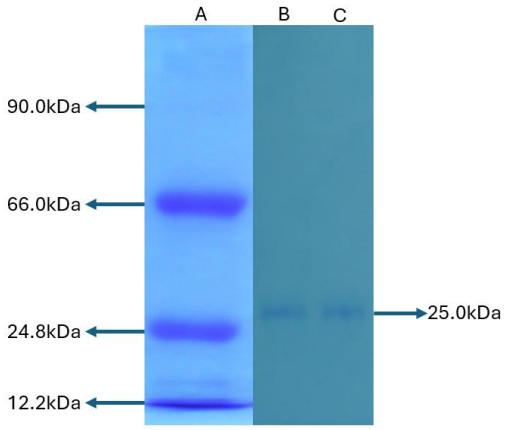



**Figure 5: SDS-PAGE pattern of (A)Standard proteins, (B) Purified Protease in the presence and (C) absence of β-mercaptoethanol**

### 3.3 Stokes Radius:

The Stokes radius of the purified enzyme was calculated from the elution volumes obtained from sephadex G-200 column.

The Stokes radius values of standard proteins may also be obtained from literature. The Stokes radius obtained for purified protease was 2.4 nm.

### 3.4 Isoelectric Point(pI):

The purified protease exhibited an isoelectric point (pI) of 6.5, suggesting its slightly acidic nature [16].

These findings classify the enzyme as a serine protease, which is widely utilized in industrial, pharmaceutical, and biotechnological applications [4, 1].

### 3.5 Physicochemical Properties of Purified Protease

#### 3.5.1 Effect of pH on Enzyme Activity and Stability

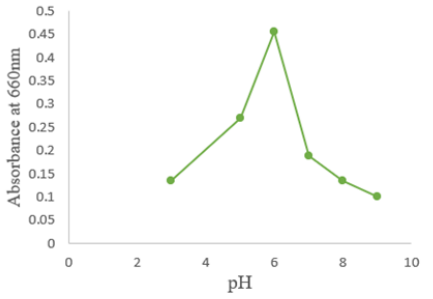
The protease exhibited maximum activity at pH 6.0. The enzyme remained stable between pH 3.0 – 9.0, retaining 85% residual activity after 24 hours. Activity significantly decreased below pH

3.0 and above pH 9.0, indicating optimal function in mildly acidic to neutral conditions [5].

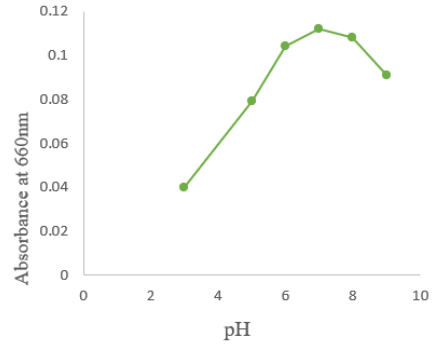
### 3.5.2 Effect of Temperature on Enzyme Activity and Stability

The enzyme exhibited optimal activity at 45°C. It remained thermally stable between 0–65°C, retaining over 90% activity at 50°C. Above 65°C, the enzyme showed a rapid decline in activity, indicating moderate thermal stability [1, 3].

These properties make the enzyme suitable for food processing, pharmaceutical, and industrial applications, where pH and temperature stability are crucial [2, 4].




**Optimum pH of Protease**




**pH stability of Protease**

**Figure 6: Effect of Temperature on Enzyme activity and Stability**

### 3.6 Functional Properties of Purified Protease

#### 3.6.1 Antioxidant Activity:

The DPPH radical scavenging assay confirmed strong antioxidant potential. The enzyme exhibited 60% inhibition at 100 µg/mL, indicating its ability to neutralize free radicals [15]. These findings suggest that *Lentinula edodes* protease could help reduce oxidative stress-related diseases [2].

#### 3.6.2 Anti-Inflammatory Activity:

The BSA denaturation assay demonstrated 80% inhibition at 60 µg/mL, comparable to synthetic anti-inflammatory drugs [16]. The enzyme effectively prevented protein denaturation, a key marker of inflammation. This suggests potential use in pharmaceutical formulations for inflammatory disorders [4, 1]

## 4. DISCUSSION

The successful purification and characterization of *Lentinula edodes* protease confirm its strong hydrolytic activity, stability, and potential biological applications. Its molecular weight (24–25 kDa) aligns with previously reported fungal proteases [2], reinforcing its classification within this enzyme group. The protease exhibits moderate temperature stability (0–65°C) and an optimal pH of 6.0, making it suitable for various industrial and pharmaceutical applications. In the food industry, it holds promise for enzyme-based protein hydrolysis, meat tenderization, and dairy processing. Additionally, its potential in pharmaceuticals includes applications in anti-inflammatory drug development and therapeutic enzyme formulations. Furthermore, its biotechnological relevance extends to enzyme-based biomedicine applications, highlighting its versatility and broad utility across multiple sectors.

## 5. CONCLUSION

This study successfully purified and characterized a serine protease from *Lentinula edodes*, revealing its exceptional biochemical and biological properties. The enzyme exhibited optimal activity at pH 6.0 and 45°C while maintaining stability over a broad pH range (3.0–9.0) and temperature range (0–65°C). Molecular weight analysis determined its size to be 19 kDa via SDS-PAGE and 20.9 kDa through Sephadex G-200 chromatography. Additionally, the protease demonstrated strong antioxidant (60%) and anti-inflammatory (80%) activities, further highlighting its functional significance. These properties indicate its potential applications in the food industry, pharmaceuticals, and

biotechnology, making it a valuable enzyme for diverse industrial and medical uses.

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**Conflict of interest:**
The authors declare that there are no conflicts of interest related to this research.

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