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The Biological Activity of the Protease Enzyme Extracted from Basidiomycete *Inocutis tammaricis*: Antibacterial, antioxidant, and antitumor roles of naturally produced protease enzyme by *Inocutis tammaricis*

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ABSTRACT

The basidiomycete *Inocutis tamaricis* is a medicinal fungus belonging to the family Hymenochaetaceae. This study evaluates the biological activities of a protease enzyme extracted from I. tamaricis, focusing on its antimicrobial, antioxidant, and antitumor potential. Antimicrobial activity was assessed using the agar well diffusion method against several pathogens at concentrations of 25, 50, and 100 µg/ml. The results demonstrated that the enzyme has exclusive inhibitory activity against gram-positive bacteria, specifically Enterococcus faecalis and Staphylococcus aureus, but shows no activity against gram-negative bacteria or yeasts. Notably, E. faecalis showed greater sensitivity to the enzyme than S. aureus at all tested concentrations. Antioxidant capacity was evaluated via the DPPH radical scavenging assay. The enzyme showed a dose-dependent response, achieving maximum effectiveness (74.88%) at 200 µg/ml, with activity decreasing proportionally at lower concentrations. Furthermore, antitumor assessments were conducted on the breast cancer cell line (MCF-7) and a normal human dermal fibroblast line (HdFn). The findings revealed that the concentration of 400 µg/ml resulted in the lowest cell viability for both lines (40.5% for MCF-7 and 75.7% for HdFn). The calculated IC50 values were 118.6 µg/ml for the cancerous MCF-7 cells and 781.7 µg/ml for the normal HdFn cells. These results indicate that the protease enzyme from I. tamaricis possesses significant antioxidant properties and selective antitumor activity with low toxicity to normal cells, highlighting its potential for pharmaceutical applications.

Keywords: **protease** enzyme, antimicrobial, antioxidant, anticancer

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الفعالية البيولوجية لأنزيم البروتياز المستخلص من الفطر البازيدي الكبير*Inocutis* tammaricis

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الملخص

تناولت هذه الدراسة تقييم الفعاليات البيولوجية لإنزيم "البروتياز" المستخلص من الفطر الطبي*Inocutis tamaricis* ، وهو فطر بازيدي ينتمي لعائلة *Hymenochaetaceae*. أُجري البحث في كلية العلوم بجامعة تكريت، وشمل اختبارات الفعالية المضادة للميكروبات، ومضادات الأكسدة، والنشاط المضاد للأورام.

أظهرت نتائج اختبار المضادات الميكروبية، باستخدام طريقة الانتشار في الحفر بتركيزات (25، 50، 100 ميكروغرام/مل)، كفاءة حصرية للإنزيم في استهداف البكتيريا الموجبة لصبغة غرام، مع عدم تسجيل أي تأثير تجاه الخمائر أو البكتيريا السالبة لصبغة غرام. وقد تفوق الإنزيم في تثبيط بكتيريا *Enterococcus faecalis* مقارنة بـ *Staphylococcus aureus* عند جميع التركيزات، مع غياب الفروق المعنوية بين العزلات التابعة لنفس النوع البكتيري.

وفيما يخص النشاط المضاد للأكسدة المقاس بطريقة(DPPH) ، أظهر الإنزيم قدرة تثبيطية تصاعدية مرتبطة بالتركيز؛ حيث سجلت أعلى نسبة تثبيط (74.88%) عند تركيز 200 ميكروغرام/مل، بينما انخفضت النسبة تدريجياً مع تقليل التركيز لتصل إلى (35.37%) عند تركيز 12.5 ميكروغرام/مل.

أما اختبار الفعالية المضادة للأورام، فقد استهدف خط خلايا سرطان الثدي (MCF-7) والخط الخلوي الطبيعي .(HdFn) أظهرت النتائج علاقة عكسية بين التركيز وحيوية الخلايا؛ إذ انخفضت حيوية خلايا (MCF-7) من 85.8% عند تركيز 25 ميكروغرام/مل إلى 40.5% عند تركيز 400 ميكروغرام/مل. وبالمقارنة، كانت الخلايا الطبيعية (HdFn) أكثر مقاومة للإنزيم. وأكدت قيم التركيز المثبط النصفي (IC50) هذه الانتقائية، حيث بلغت 118.6 ميكروغرام/مل لـ (MCF-7) مقابل 781.7 ميكروغرام/مل لـ(HdFn) ، مما يشير إلى إمكانية استخدام الإنزيم كعامل علاجي واعد ضد السرطان مع سمية منخفضة للخلايا الطبيعية .

INTRODUCTION

The basidiomycete *Inocutis tamaricis* is a medicinal fungus belonging to the division Basidiomycota, class Basidiomycetes, order Hymenochaetales, and family Hymenochaetaceae. The species grows on live and dead tamarisk trees (Tamarix spp.) and is distributed in Southern Europe, North Africa, and South Asia. Although *Inocutis* spp. are among the main causes of white rot and ulcers, these fungi have shown many interesting medicinal activities, such as potential anti-inflammatory and antimicrobial properties that could be beneficial in treating various health conditions. [(1).] For instance, in addition to the sedative and pain-relieving properties of Inocutis rheades, the species *Inocutis levis* has been discovered to have immunostimulatory, antitumor, and hypoglycemic effects. However, techniques for the artificial cultivation of these fungi for large-scale production have rarely been developed, despite their potential as a promising alternative for the effective production of valuable metabolites. [(2)].

Finally, researchers are investigating issues related to cancer and antibiotic resistance in a resource that allows for the use of available factors [(3)]. Compare it with a variety of factors, including biological factors that may be based on a different type of blood sugar, and, in this case, use the original juice. An inscription on the various biological and

diagnostic activities that exist here. Investigations into the disease of this metabolite. Extracting a severe form of it has a fetal stage in cancer and hemorrhoids, and it is labeled as an antioxidant. There are different types of ways [4].

Protease production is the final step in this process. This book is the latest guide to enzyme production, which is very easy and convenient for mycelium to be tested against harmful bacteria. Fungi are also a safe source of enzymes because most proteases produced by them are extracellular, making isolation and purification simpler. Fungi produce around 60% of the enzymes used in industries such as food, medicine, and therapy. [5].

The study aims to investigate the biological activity of the purified protease enzyme from I. tammaricis, focusing on antimicrobial activity. Antioxidant activity and anticancer activity

MATERIALS AND METHODS

Protease enzyme

The purified protease enzyme was obtained from basidiomycete *I. Tamaricis are stored at -19°C in the laboratories of the College of Science / Department of Biology / Tikrit University until* use. [6].

The microorganisms used in the experiment

The study samples were obtained from Burns and UTI samples from the Emiz Research Laboratory in Baghdad. The samples were already diagnosed by the lab staff and confirmed by the Vitek system, which included the following types shown in Table 1. Six bacterial and Candida isolates were obtained, and three replicates per isolate were used at three concentrations (25%, 50%, and 100%).

The I. I. *I.tammaricis* were obtained from Duhok City and Al-Alaam in Tikrit.

Table 1: The used isolates in the antimicrobial activity

Isolates	Stain
Candida albicans	Gram-positive
Enterococcus faecalis	Gram-positive
Escherichia coli	Gram-negative
Klebsiella pneumonia	Gram-negative
Pseudomonas aeruginosa	Gram-negative
Staphylococcus aureus	Gram-positive

The solutions and dyes used in the experiments:

Solution of 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$):

One hundred ninety-seven grams of DPPH powder were dissolved in fifty milliliters of pure methanol to produce the 0.1 millimolar solution. To prevent photodegradation, the bottle was wrapped in aluminum foil and refrigerated until needed. [7].

Streptomycin solution

In accordance with the manufacturer's instructions, the stock solution was prepared at a concentration of 200,000 µg/mL by dissolving the components in 5 mL of deionized distilled water. Subsequently, it was preserved at -18°C until required. [8].

Benzyl penicillin solution

The stock solution of benzyl penicillin at 200,000 IU/mL was prepared by dissolving the contents of the vial in 5 mL of sterile, ion-free distilled water. The solution was subsequently preserved at -18°C until required. [8].

Phosphate-Buffered Saline (PBS)

The solution was prepared by dissolving 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 0.2 g of sodium hydrogen phosphate (Na_2HPO_4), and 1.15 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of deionized water. Stir the mixture vigorously and adjust the pH to 7.2. Then, sterilize the solution for 15 minutes at a temperature of 121°C and 1 atm in an autoclave, and then the solution is left to cool before being stored at 4°C until use[9].

Sodium Bicarbonate Solution

The solution was prepared by dissolving 2.2 g of sodium bicarbonate (NaH_2CO_3) in 1000 mL of deionized water. Then, sterilize in an autoclave at 121°C and 1 atm for 15 minutes and subsequently preserve at 4°C until needed. ([8].

Ethylenediaminetetraacetic acid (EDTA) solution

The solution was prepared by dissolving 1 g of EDTA in 100 mL of PBS, autoclaving for 10 minutes, and storing at 4°C until use. [8].

Trypsin Solution

A Millipore filter (0.22 µm) was used to sterilize 1 g of trypsin powder dissolved in 100 ml of PBS. Until it was needed, the solution was stored at -20°C [8].

Trypsin-EDTA Solution

According to [8]The solution was prepared by dissolving 20 mL of trypsin solution, 10 mL of EDTA solution, and 370 mL of PBS. After mixing, the mixture was stored at 4°C until needed.

Thiazolyl Blue Tetrazolium Bromide (MTT) dye

A phosphate-buffered saline solution was used to dissolve the MTT dye powder, resulting in a final concentration of 5 mg/mL. After thoroughly mixing the dye solution with a magnetic stirrer, it was sterilized using a Millipore filter (0.22 µm) and stored at -20°C until needed. [10].

Trypan Blue Stain

After autoclaving, dissolve 1 gram of dye in 100 milliliters of PBS, and then filter the mixture through Whatman No. 1 filter paper for sterilization. After the dye cooled, it was stored at 4°C until required. According to [11]The use of the dye allows for the evaluation of cell viability and the number of live cells in the samples.

Antimicrobial Activity

This test was conducted using the technique of agar well diffusion [12] First, the bacterial isolates were cultured in nutrient broth for 18-24 hours at 37°C. Then, 0.1 ml of each bacterial suspension was transferred to nutrient agar and left for 24 hours at 37°C. Finally, a single colony was added to a test tube containing 5 mL of regular saline to make the bacterial suspension. plates were incubated for 10 minutes after adding a portion of the bacterial suspension to Mueller-Hinton agar, and the concentration was adjusted to 1.5 × 10^8 bacterial cells/ml according to McFarland standards. Using a sterile cork borer, 5 mm wells were bored further. Using a micropipette, approximately 0.1 ml of the concentrated enzyme solution was added to each well, and the plates were incubated at 37°C for 1 day. Following the inhibitory zone width measurements. As a control, sterile distilled water was put in place of the enzyme solution. Each isolate was tested three times in the experiment.[13].

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to evaluate the protease's antioxidant activity. 0.5 mL of protease was added to test tubes at 12.5, 25, 50, and 100 µg/mL. Then, each concentration received 3 mL of a methanol-DMSO mixture and 0.3 mL of DPPH solution. The tubes were then incubated at 37°C for an additional hour. Using an ELISA at 517 nm, the samples' free radical-scavenging ability against stable DPPH radicals was measured with a spectrophotometer. According to [14]DPPH free radical scavenging is indicated by a color change from dark purple to pale yellow. Three milliliters of a methanol-DMSO mixture and three-quarters of a milliliter of DPPH solution made up the negative control treatment. At different concentrations (12.5, 25, 50, and 100 µg/ml), the enzyme's antioxidant activity was contrasted with that of ascorbic acid, which served as a positive control. A decrease in absorbance indicates that the substance is more effective in removing free radicals. The following equation was used to determine the percentage of free radical inhibition:

Scavenging effect (%) ={(A0-A1)/A0*100}

A0: represents the control absorbance, while A1: represents the absorbance of the samples and ascorbic acid; three replicates for each concentration were conducted, after which the average for each was taken.

Anticancer activity

Development and Preparation of Cancer Cell and Normal Cell Lines:

The study used two cell lines: the human breast cancer cell line MCF-7 and the primary dermal neonatal fibroblast cell line (HdFn), obtained from the Biotechnology Research Center—Al-Nahrain University—Baghdad (Table 2). The cell lines were transferred to the tissue culture laboratory, and a cell culture was established for each line by propagating and maintaining them according to the method of [11].

Table 2: The Cell lines used in the current study and their sources

cell line	Sources
MCF-7 cancer cell line	Breast cancer
HdFn cell line	Primary Dermal Fibroblast Normal; Human, Neonatal

Propagation and Maintenance of Cell Lines

The cell lines have been warmed in a water bath at 37°C. Then the cells were placed in 25 cm² Falcon plastic flasks containing RPMI-1640 culture medium and 10% bovine serum. The dishes were then incubated at 37°C and 5% carbon dioxide for 24 hours, and the cells formed a near-complete layer (approximately 70–80%) in the culture dishes. Secondary subcultures of the two cell lines were then performed. The cells were transferred to a growth cabinet, and the incubated medium was removed by washing the cells in the dish with 2–3 ml of PBS. This process was repeated twice, each time for 10 minutes. After the washing process was completed, 2 ml of trypsin solution was added to the dish to detach the cells, and the dish was incubated for approximately 3 minutes at 37°C. Next, the dishes were removed from the incubator and gently tapped to ensure that the cells were detached from the bottom of the culture dish. At this point, enzyme activity was inactivated by adding fresh RBMI 1640 medium containing 10% bovine serum. The cells were collected in centrifuge tubes and centrifuged at 2,000 rpm at room temperature to precipitate the cells and remove trypsin and growth media. The supernatant was eliminated, and the cells were resuspended in fresh growth media supplemented with 10% bovine serum. Finally, the cell suspension was distributed into new containers and incubated at 37°C and 5% CO_2 for 24 hours. To count cells, a specified volume of the cell suspension was taken, an equal volume of trypan blue dye was added, and the cells were placed on a counting slide (blood cell counter). Using a light microscope, dead cells were counted when they stained blue, while live cells remained colorless and transparent. According to the following equation, the number of cells per unit volume (1 µl) was calculated.

$C = n \times d \times 10^4$

C = the number of living cells in 1 ml, n = the number of cells in the slide, d = the dilution factor in which the cells were suspended, 10^4 = Slide dimensions.

MTT Cytotoxicity Assay

Following the protocol provided by [15]The cytotoxicity was evaluated. To achieve a total volume of 200 µl of complete culture media in each well, a 96-well flat plate was supplemented with a cancer cell suspension ranging from 1×10^6 to 1×10^4 cells/ml. Following this, sterile parafilm was applied to the plates, and they were gently mixed before incubation at 37°C with 5% CO2 for 1 day. Then, the medium was removed, and 200 µl of purified protease enzyme was added to the wells. The protease enzyme concentration was varied and measured in milligrams per milliliter; three replicates were used for each concentration. A control group was included, consisting of serum-free medium. The next step was to incubate the plates for 4 hours at 37°C with 5% CO_2. Each well was treated with 10 mL of MTT solution and incubated according to the parameters described previously. 100 µL dimethyl sulfoxide (DMSO) was added to each well for 5 minutes after the media was carefully removed. The ELISA method was used to measure absorbance at 575 nm. Using the following equation, we statistically analyzed the optical density readings to find the rate of cell growth inhibition (cytotoxicity percentage):

Percentage of live cells = (optical density of the control - optical density of the sample) / (optical density of the control) * 100

Statistical analysis

Data were analyzed using Minitab software version 17, and an ANOVA test was conducted. The means were compared using Duncan's Multiple Range Test at a $P \leq 0.05$ significance threshold.

RESULTS AND DISCUSSION

The Antimicrobial Activity:

The findings presented in Table 3 and Figure 1 illustrate the enzyme's efficacy against the

examined isolates at three concentrations (25, 50, and 100 µg/ml). The enzyme demonstrated exclusive efficiency in targeting gram-positive bacteria, but showed no effectiveness against yeasts or gram-negative bacteria. The table indicates that the enzyme demonstrated greater inhibitory activity against *E. faecalis* than against S. aureus across all concentrations tested, with notable differences observed between the two. In contrast, no significant differences were observed among isolates of the same bacterial species.

Table 3: The inhibitory activity(mm) of protease enzyme

25 µg /ml	50 µg /ml	100 µg /ml	25 µg /ml	50 µg /ml	100µg /ml
E. feacalis(1)	E. feacalis(2)	E. feacalis(3)	S. aureus (1)	S. aureus (2)	S. aureus (3)
18.33 a	19.67 a	21.00 a	12.67 b	13.67 b	13.00 b

The similar letters are referring to not significantly different
The different letters are referring to significantly different

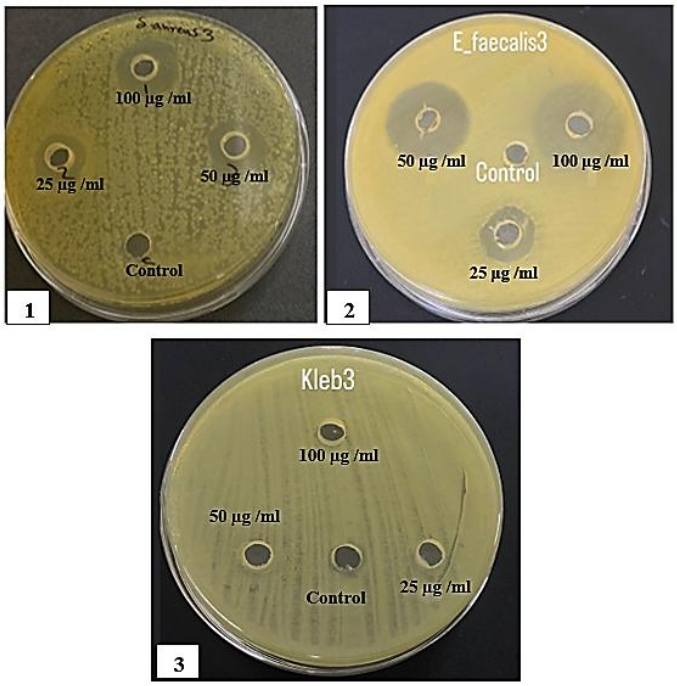

Fig. 1: Efficacy of the antibacterial protease enzyme against (1): *S.aureus*, (2): *E.feacalis*, (3*): K. pneumoniae*

The results of this study were in agreement with (16), which showed that the enzyme was effective against *Salmonella typhi; however,* it had no inhibitory effect against *Listeria monocytogenes* or *Pseudomonas aeruginosa*. The reason Gram-negative bacteria are less susceptible may be their outer membrane, which confers strong hydrophilicity and serves as a strong permeability barrier. [5].

Antibacterial agents can easily penetrate the bacterial cell wall and cytoplasmic membrane, resulting in cytoplasmic leakage and coagulation.[13]. The results aligned with those of [17], who investigated the antibacterial properties of Ergosta-5,7,22-triene-3β,14α-diol (22Z) proteins isolated from Ganoderma lucidum at 100 mg/ml against Methicillin-Resistant S. aureus (MRSA) and Streptococcus pyogenes, and found that the same concentration produced an inhibition zone of 10.3 ± 0.3 mm. On the other hand, gram-negative bacterial isolates (e.g., E. coli, P. aeruginosa, and K. pneumoniae) were not included in the same investigation.

In addition, a study by[18] also supported this finding, recorded that only two isolates of the five pathogenic bacteria tested— *S.aureus* and

Micrococcus sp showed an inhibition zone on protease-enriched culture media after 24 hours, measuring 9 mm for *Micrococcus* sp. and 12 mm for *S. aureus,* while no antibacterial activity against gram-negative bacteria, such as *E. Coli*, *S.typhi*, and *P. aeruginosa*. In contrast to[19], whose findings revealed that the peptides extracted from t *G. lucidum* had antibacterial action against gram-negative (*E. coli* and *S. typhi*), but no antibacterial activity against gram-positive bacteria, such as *S. aureus*, this antibacterial activity, due to the protease enzyme, was responsible for breaking down the proteins in bacteria by cleaving several peptide bonds between amino acids, which is why an inhibitory zone occurred[19, 20]. Regarding the enzyme's ability to inhibit gram-positive bacteria, this may be explained by the cell wall construction, especially the peptidoglycan nature. Proteins linked to peptidoglycan, or even those found in the cell wall itself, can be broken down by proteolytic enzymes, leading to a weaker cell wall and greater vulnerability to antibacterial and environmental effects. Its ineffectiveness against Gram-negative bacteria might be due to the lipopolysaccharide-based outer membrane, which may be resistant to or inhibit proteolytic enzymes. This is consistent with several studies indicating that Gram-negative bacteria are a major cause of disease and death in humans and animals, and that they are also among the most susceptible to antibiotic resistance. [21].

The Antioxidant Activity

The data shown in Table 4 and Figure 2 demonstrate significant disparities across the various concentrations of protease and ascorbic acid. However, no notable changes were detected between the enzyme and the control group. The table indicates that the protease enzyme had the maximum antioxidant activity at 200 µg/ml, with a value of 74.88 ± 3.1. The rate diminished markedly with concentrations of 100, 50, 25, and 12.5 µg/ml, yielding rates of 67.55, 51.96, 43.90, and 35.37, respectively.

The findings indicated significant differences among the various ascorbic acid concentrations (see Table 4 and Figure 2). The 200 µg/ml concentration achieved the highest value of 77.70, demonstrating a significant difference from the other treatments, with a standard deviation of 1.6. The 100 µg/ml concentration showed a notable difference from the other concentrations, yielding a value of 71.45 and a standard deviation of 6.1. At the same time, the concentration of 12.5 µg/ml yielded a value of 40.39, accompanied by a standard deviation of 2.6. The concentrations of 50 and 25 µg/ml showed the greatest variation, with values of 66.0 and 55.86, respectively.

Table 4: Antioxidant activity at different concentrations of protease enzyme and ascorbic acid

Conc (µg/ml)	Ascorbic acid		Protease	
	Mean	SD	Mean	SD
200	77.70067 a	1.678334	74.8843 A	3.131415
100	71.45067 B	6.135 462	67.5540 B	1.100019
50	66.04933 c	2.411 951	51.9677 c	1.5045
25	55.86433 D	1.801796	43.9043 D	1.290243
12.5	40.39367 e	2.697388	35.3783 E	7.069249

The similar letters are referring to not significantly different
The different letters are referring to significantly different

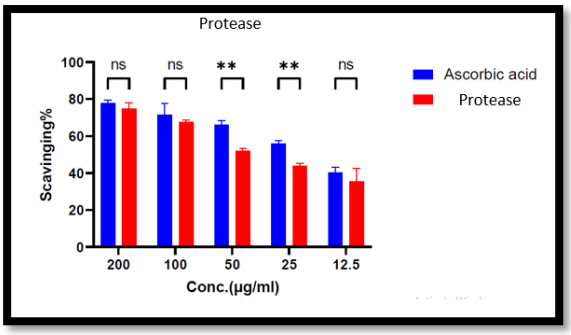

Fig. 2: The antioxidant activity of protease enzyme and ascorbic acid

The results were consistent with the findings of the study by [22], which isolated the protease from Aspergillus niger and demonstrated its antioxidant activity using the DPPH method. The study samples showed the highest antioxidant activity at 62.37%, while the control sample recorded 20%. In line with (23), the study also found that extracts of the two species, Blakeslea trispora and Rhizomucor pusillus, inhibited free radical activity at rates

ranging from 33.4% to 52.4%. Several mechanisms explain proteases' antioxidant activity. DPPH radicals are stable free radicals that exhibit maximum absorption at a wavelength of 517 nm in ethanol. They have been widely used to assess the antioxidant potential of natural compounds that act as free radical scavengers or hydrogen donors. DPPH free radicals were eliminated, and their absorbance decreased upon contact with a proton-donating material, such as an antioxidant [24]. Proteases can also break down damaged or oxidized proteins that accumulate as a result of oxidative stress, preventing their toxic accumulation and thereby maintaining protein homeostasis under these conditions. This proteolytic activity is essential in protecting cells from oxidative damage that could impair cellular functions [25].

Anticancer Activity and Cytotoxicity

The results presented in Table 5 demonstrate the viability of MCF-7 cells and the HdFn cells following treatment with several concentrations of protease enzyme (25, 50, 100, 200, 400 µg/ml). The findings indicate that the maximum viability rates were 85.8% and 95.4% at 25 µg/ml for MCF-7 and HdFn line, respectively. The concentration of 400 µg/ml resulted in the lowest viability for the MCF-7 and HdFn cell lines, at 40.5% and 75.7%, respectively. Furthermore, the IC50 values were 118.6 and 781.7 µg/ml for the MCF-7 and HdFn lines, respectively, as illustrated in Figure 3.

Table 5: Anticancer activity and cytotoxicity of protease enzyme

1	HdFn		MCF-7	
Conc.	Mean	SD	Mean	SD
400	75.772 c	2.184578	40.586 e	1.280027
200	87.346 b	1.562853	51.775 d	2.135402
100	92.631 a	0.582331	64.005 c	2.168135
50	94.637 a	1.037389	76.543 b	2.224263
25	95.409 a	0.481826	85.841 a	1.674551

The similar letters are referring to not significantly different
The different letters are referring to significantly different

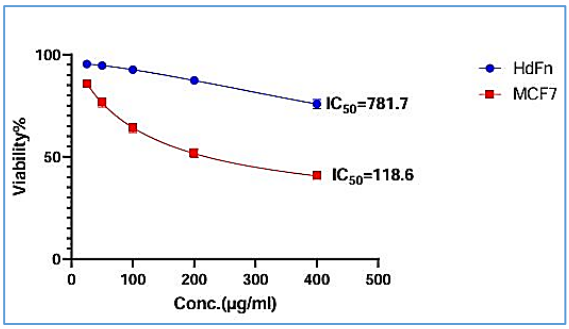

Fig. 3: IC50 of the protease enzyme for the MCF-7 and HdFn cell line

The finding agreed with the results obtained by[26]In which they identified the efficacy of the purified protease enzyme from the fungus Cordyceps militaris against cancer cells such as MCF-7, 5637, and A-549 cells, at a concentration of 15 µM, the viability of MCF-7 cells decreased to 33.41 ± 3.81%, and the IC50 value was 9.3 µM. In comparison, the viability of the 5637 cell line was approximately 39.06 ± 15.60% with an IC50 value of 8.1 µM. The enzyme showed lower effectiveness against A-549 cells (64.68 ± 13.23%). The results of the current study were in agreement with the findings reported by [27], which showed that the serine protease isolated from the fungus Lignosus rhinocerus exhibited strong selective toxicity against MCF7 cells, with an IC50 value of 3.0 µg/ml.

Numerous studies have identified multiple mechanisms that elucidate the anti-cancer properties of protease enzymes. A study suggests that protease enzymes from various catalytic classes possess tumor-suppressing properties by obstructing cancer progression stages, including the inhibition of angiogenesis essential for tumor growth and dissemination, as well as modulating inflammatory responses induced by cancer cells [27, 28] demonstrated that the F5 protein fraction extracted from the cold water solution of *Lignosus rhinocerus* mycelium contained serine protease enzymes. The study indicated its role in the apoptosis of MCF7 breast cancer cells and in cell growth inhibition. It was explained that this occurs through interactions between the extrinsic and intrinsic apoptosis pathways, with upregulation of

caspase activity (caspase-8 and caspase-9, the main enzymes in the apoptosis process), as well as a significant decrease in Bcl-2 (a protein that protects cells from apoptosis). At the molecular level, the same study showed increased levels of Bax and BID, which stimulate apoptosis, accompanied by significant actin reorganization, indicating structural changes in the cell. At the gene level, F5 consists of three predicted non-synonymous single-nucleotide polymorphisms (T > C) and an alternative 5'-splicing site.

CONCLUSION

The enzyme exhibited activity against gram-positive bacteria, with greater inhibitory efficacy against E. faecalis isolates than against S. aureus isolates. It exhibited no efficacy against Gram-negative bacteria or yeasts. The enzyme showed peak antioxidant activity at 200 µg/ml, yielding a value of 74.88 ± 3.1. The enzyme exhibited anticancer activity, resulting in 40.5% and 75.7% viability in cell lines at a dose of 400 µg/ml, respectively.

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Author contributions: The authors contributed equally to the study.

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