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Curcumin-Mediated Inhibition of L-Tryptophanase activity: Purification and Characterization from *Escherichia coli*

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Abstract:

The study's objective was to use curcumin to limit the activity of the enzyme L-tryptophanase (Tnase).

Curcumin is known as diferuloylmethane ($C_{21}H_{20}O_6$) has minimal or no cytotoxicity and antioxidant qualities, curcumin is naturally occurring bioactive compound made from *Curcuma longa L. rhizomes*. According to the inhibition results higher curcumin levels of 250 µg/ml demonstrated 100% L-tryptophanase inhibitory activity, whereas lower curcumin concentration of 62.5 µg/ml demonstrated 95% inhibitory efficacy. The L-tryptophanase is considered a virulence-associated factor that play a role in the development and progression of infection. The enzyme was purified from *E. coli* isolated from a urinary tract infection via precipitation with 70% ammonium sulphate saturation and ion exchange chromatography, in addition to gel filtration chromatography using a Sephadex G-150 column. During the final purification stage, the crude enzyme's specific activity increased from 13.5 U/mg protein to 71.4 U/mg protein. According to the study's pure enzyme characterization, the impacts of pH and pH stability, along with temperature and temperature stability, were the results appeared 8, 8 and 37, 32, and 42 °C, respectively.

Keywords: *E. coli*, purification, inhibition, curcumin, and L-tryptophanase.
Note: The research is based on a doctoral thesis.

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Introduction

Tryptophanase (TnaA), a bacterial enzyme breaks down the amino acid tryptophan into metabolites that are crucial to bacterial survival, and physiology including indole, pyruvate, and ammonia. This enzyme, also known as tryptophan indole-lyase, is found in many bacterial species and uses α, β-elimination, and β-replacement reaction pathways to catalyze the breakdown of L-tryptophan (Boya *et al*., 2021). *Escherichia coli*, and several other harmful microbes such as *Vibrio cholerae* use the indole that Tnase generates as an intercellular signaling molecule. It is essential for quorum sensing, biofilm formation, and the control of genes related to multidrug efflux systems (Trastoy *et al*., 2019& Razzaq *et al*., 2025). The majority of research has used *Escherichia coli* as the main source of tryptophanase. Indole synthesis has been found in many other bacterial species and is now frequently used as a crucial characteristic for their identification, despite the fact that Tnase was previously believed to be exclusive to *E. coli* (Yuasa, 2023& Gao *et al*., 2025). Four identical subunits made up the tetrameric enzyme Tnase. Each subunit is composed of polypeptide chains that fold into a particular three-dimensional structure. TnaA protein's quaternary structure is composed of three subdomains: D1, D2, and D3. Whereas subdomains D1, and D3 together make a smaller structural domain, while D2 is a larger single domain. These domains connect with the other three TnaA subunits to create a tightly arranged tetrameric complex (Li& Young, 2015). This enzyme has been identified from a variety of bacterial species, including *E. coli*; *Bacillus alvei*; *Aeromonas alkalescens*; *Shigella alkalescens*; *Proteus vulgaris; Proteus morganii*, and *Symbiobacterium thermophilum* (Rety *et al*., 2015& Hufnagel *et al*., 2015). The most well studied type of Tnase is the enzyme derived from *E. coli*, this organism has tetramers enzyme which are composed of four identical subunits with molecular weights of about 52.8 kDa. Only in denaturing conditions or at pH values higher than 8.7 does the holo-enzyme split into monomers. Tnase's apo form, on the other hand, is less stable and dissociates into monomers at temperatures below 2°C, and dimers at temperatures below 25°C

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(Kogan *et al*., 2015). Thus, the goal of this study was to use a naturally occurring plant-derived compound with antioxidant qualities and little to no cytotoxicity to inhibit L-tryptophanase (Tnase) activity the cause of the urinary tract infection. In order to accomplish this goal, L-tryptophanase was isolated from *Escherichia coli* in Iraqi patients with urinary tract infections, and its inhibitory effect on L-tryptophanase activity was then characterized.

Material and Methods

Sample collection and Identification of *Escherichia coli* isolates: A total of 120 isolates of *Escherichia coli* were obtained from urine samples for Iraqi patients suffering from urinary tract infections of both sexes and all age groups. Specimens were obtained by Al-Shaheed AL-Sadr, Al-Karama Teaching Hospital, Alkadhmiya Teaching Hospital, and AL-Yarmouk Teaching Hospital in Baghdad during a time frame from February to May 2025. For the purpose of identifying *Escherichia coli*, they were promptly transported to the lab under aseptic conditions. To obtain pure, well-isolated colonies, the collection process involved streaking directly onto blood agar addition to MacConkey agar and incubating it for 24 hours at 37ºC in an aerobic environment (Abbas& Kahya, 2025). According to Bergy's Manual of Systematic Bacteriology, 2nd edition (Brenner *et al*., 2005), the isolates were identified using biochemical tests, cultural traits, and morphological features. Additionally, the Vitek-2 method (bioMérieux) was used in accordance with the manufacturer's instructions to confirm the classification of bacterial species according to the biochemical characteristics and typical culture of isolates.

Screening the ability of *E. coli* bacterial isolates for the production of L-tryptophanase: According to (Sasaki-Imamura *et al*., 2010)

(Qualitative Examination) in accordance with the manufacturer's guidelines, the young testing bacteria were inoculated into peptone water, which was then incubated for 24 hours at 37ºC. Gather the supernatant, then gently shake in ten drops of Kovacs' reagent. The development of a pink ring indicated a successful test. The ability of the bacteria to produce

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tryptophanase, which convert tryptophan to indole, pyruvic acid, along with ammonia is determined by this test.

(Quantitative Evaluation) using a spectrophotometer, the intensity of the red color caused by the hydrolysis of tryptophan was measured (usually at 520 nm). The amount of indole and, consequently, the activity of tryptophanase are directly correlated with color intensity.

Tryptophanase activity assay: *Escherichia coli* selected bacterial isolates were streaked onto nutrient agar medium, and the cells were kept at 37 °C for a whole day. Using a colorimetric technique (indole produced) that identifies the byproducts of the L-tryptophan degradation reaction, tryptophanase activity was assessed in vitro (Sasaki-Imamura *et al*., 2010). The following is how the assay was used: 150 microliters of the enzyme solution is added to 5 milliliters of peptone water. The same ingredients were used to prepare the control tube (blank), but 150 microliters of distilled water were added in place of the enzyme solution, then mixed using a vortex-mixing device and then incubated at 37°C for a whole day. next, centrifuge to collect the supernatant. Follow, adding Kovacs' reagent, color change (usually red or pink), signifies indole production, the evidence presence enzyme, and measure the intensity of red color using a spectrophotometer at 520 nanometers. The color intensity is proportional to the amount of indole production. Tryptophanase is an enzyme that breaks down tryptophan into ammonia, pyruvate, and indole. The activity of tryptophanase is often measured in terms of the amount of indole produced. The general reaction for tryptophanase activity is:

L-tryptophan + H_2O ⇌ indole + pyruvate + NH_3

The equation to measure tryptophanase activity usually involves quantifying the amount of indole produced, often through a colorimetric or spectrophotometric assay. One common method to quantify the enzyme activity is to use the following equation (Behbahani-Nejad *et al*., 1987):

$$\text{L- tryptophanase activity (U /ml)} = \frac{(\Delta OD \times \text{V reaction})}{(\epsilon \times l \times Sample\ volume)}$$

Where:

ΔOD: Change in absorbance (usually at 520 nm for indole)
V reaction: Volume of the reaction mixture in mL
ϵ: Molar extinction coefficient of indole at 520 nm (L mol $^{-1}$ cm $^{-1}$)
l: Path length of the cuvette (in cm)
Sample volume = Volume of the sample used in the reaction in mL

The Beer-Lambert rule, which states that the absorbance change is proportionate to the concentration of indole generated during the enzymatic process, is the foundation of this formula. The enzyme activity is commonly expressed in units (U), where 1 U is the quantity of enzyme that, under standard assay conditions, catalyzes the production of 1 µmol of indole per minute.

Purification of L-tryptophanase: Tryptophanase from *E. coli* was purified using the subsequent steps: ammonium sulfate precipitation, dialysis, ion-exchange chromatography, and gel filtration chromatography. 70% ammonium sulfate saturation was applied to the crude enzyme extract. Centrifugation at 6000 rpm for 20 minutes was used to gather the resultant precipitate. A dialysis membrane was used to dialyze the precipitate after it had been dissolved in the proper amount of phosphate buffer. A diethylaminoethyl cellulose (DEAE-C) ion-exchange column was subsequently filled with the dialyzed enzyme fraction. The fraction with the highest activity from the ion-exchange chromatography was gel filtered on a Sephadex G-150 column as a final stage of purification (Yoshida *et al*., 1974)

Characterization of L-tryptophanase:

Effect of pH on Tnase activity: Using acetate buffer (pH 5 and 6), phosphate buffer (pH 7 and 8), and Tris buffer (pH 9), the purified enzyme was added to a substrate solution (peptone water) that had been prepared at various pH values between 5 and 9. Following the measurement of enzyme activity, a plot of the correlation between pH and enzyme activity was created.

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Effect of pH on Tnase stability: The extracted enzyme was pre-incubated for 30 minutes at 37°C in buffers with different pH values (5–9). The tubes were chilled in an ice bath following incubation. After measuring the enzyme activity, the percentage of tryptophanase activity that remained was plotted against pH.

Effect of temperature on Tnase activity: L-tryptophanase activity was measured at 27, 32, 37, 42, and 47 °C, and a relationship between temperature and enzyme activity was displayed.

Effect of temperature on Tnase stability: After 30 minutes of pre-incubation at different temperatures (27, 32, 37, 42, and 47 °C), the purified enzyme was immediately cooled in an ice bath. The residual activity of tryptophanase (%) was then measured, and plotted as a function of temperature.

Determining the molecular weight of L-tryptophanase:

The molecular weight of L-tryptophanase was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli described by (Kielkopf *et al*., 2021). The purified L- tryptophanase was analyzed using SDS–PAGE with a 12% separating gel, and a 4% stacking gel. The enzyme samples were heated to 100°C for five minutes in sealed vials with β-mercaptoethanol, and 1% (w/v) SDS. In a Tris–HCl solution (pH 8.3), electrophoresis was performed for four hours at a steady voltage of 125 V. After electrophoresis, Coomassie Brilliant Blue R-250 staining was used to visualize the separated proteins.

Inhibition of L-tryptophanase action by curcumin in vitro:

Many bioactive compounds, such as curcumin, also known as diferuloylmethane (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), is the primary naturally occurring polyphenol present in the rhizome of *Curcuma longa* (turmeric) and other *Curcuma* species are plant-derived and can be isolated and purified for therapeutic use (Jiang *et al*., 2021& El-Saadony *et al.*, 2025). Curcumin ($C_{21}H_{20}O_6$) has a molecular weight of 368.39 g/mol and a purity ratio of 99.5% obtained from Avonchem (Cheshire, UK). The activity of L-tryptophanase was

inhibited by curcumin, following the initial determination of curcumin's minimum inhibitory concentrations (MICs) against *E. coli* using the established method (Gunes *et al*., 2016) with some modifications as follows: Working stock solutions of curcumin were prepared by dissolving 0.01 g of curcumin in 10 ml of ethanol. 500 µl of Mueller-Hinton Broth (MHB) was poured into each of ten sterile tubes that had been prepared. Next, the first tube was loaded with 500 µl of the curcumin solution. The solution was serially (twofold) diluted to obtain concentrations of 500, 250, 125, 62.5, 31.3, 15.7, 7.9, 4, 2, and 1 µg/ml. A standardized bacterial suspension equal to 0.5 McFarland was prepared by adjusting fresh cultures of the standard bacterial strains until the final concentration approximately equal to 1.5 x 10^8 CFU/ml. Then, 500 µl of the bacterial suspension was added to each tube, yielding final concentrations between 250 µg/ml to 0.5 µg/ml. A positive control containing the bacterial strains, and ethanol in a curcumin- free medium, and a negative control consisting of curcumin solution in a bacteria-free medium were included. For twenty-four hours, the tubes were incubated at 37 °C. Following incubation, the antibacterial activity of curcumin was evaluated by observing turbidity in each tube. The absence of turbidity indicated inhibition of bacterial growth. The lowest curcumin concentration at which there is no discernible turbidity is known as the minimum inhibitory concentration (MIC). Subsequently, using a concentration lower than the MIC to inhibit the action of L-tryptophanase. The purified enzyme was incubated with 500 µl of substrate solution (peptone water) and 500 µl of sub-MIC concentrations of curcumin. A positive control consisted of the enzyme, and ethanol in a curcumin- free medium, while a negative control contained the medium, and curcumin solution without the enzyme. Enzymatic activity was then measured under these conditions.

Results and discussion:

Identification of *Escherichia coli:* One hundred and twenty samples were taken from individuals who had urinary tract infection. From these isolates, 55 isolates of *Escherichia coli* were identified at a rate of 98-

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كلية التربية الاساسية – الجامعة المستنصرية
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99% based on biochemical tests, morphological characteristics on culture media, and confirmation using the VITEK 2 system. The isolates appeared as Gram-negative coccobacilli or bacilli, arranged singly or in pairs, and were presumed to be *E. coli* when examined under a light microscope. The spherical, tiny, and pink appearance of colonies on MacConkey agar demonstrated their ability to ferment lactose. Eosin Methylene Blue (EMB) agar appeared as a green metallic sheen with a dark center, while on blood agar, they appeared as opaque creamy colonies.

Table 1 shows the biochemical test for *E. coli* identification. The bacterial isolates were identified using the VITEK 2 system after preliminary confirmation. The majority of these isolates (99%) exhibited characteristics typical of *Escherichia coli*, as determined by the conventional GN (Gram- Negative) test. Similarly, Ines *et al*., (2009) reported a 90.1% success rate in identifying *E. coli* using the VITEK 2 technology.

Table 1: Biochemical examination for *Escherichia coli* isolates

Biochemical Examination	Result
Gram stain	-
Catalase	+
Oxidase	-
IMViC	++--
Urease production	-

+; positive result, -; negative result

Based on the appearance of a reddish ring at the interface of the culture medium containing tryptophan (peptone water) after incubation 37°C for a full day, subsequently addition of Kovac's reagent
(p- p-dimethylaminobenzaldehyde in hydrochloric acid), the intensity of the red color was measured spectrophotometrically at 520 nm. Accordingly, the isolate that produced the highest concentration of indole was selected for enzyme purification. Sasaki-Imamura *et a*l., (2010). found that *Escherichia coli* gene *TnaA*, which codes for tryptophanase

مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
P- ISSN: 3007-3650 E- ISSN: 2706-8536
Journal of the College of Basic Education Vol.32 (No.137) 2026,pp.1160-1178

and catalyzes the breakdown of L-tryptophan to generate indole, is a purported extracellular signaling molecule that affects the formation of biofilms in a variety of bacteria.

L-tryptophanase purification: Purification of L-tryptophanase was done in order to separate the target protein and get rid of undesired proteins. Ammonium sulphate precipitation; DEAE-cellulose; afterwards Sephadex G-150 column chromatography was used to purify an extracellular L-tryptophanase from *E. coli*. According to Table 2, the crude enzyme's specific activity was 13.5 U/mg protein with 2.7 U/ml enzyme activity. Revealed a 5.2-fold purification, a 51.8% total yield, and a rise in the specific activity of the purified enzyme, 71.4 U/mg.

Table 2: Purification steps for L-tryptophanase from *Escherichia coli* selected isolate

Fractions	*Volume (ml)*	*Enzyme activity (U/ml)*	*Protein concentration (mg/ml)*	*Specific activity (U/mg)*	*Total activity (U)*	*Purification (folds)*	*Yield (%)*
Crude enzyme	*75*	*2.7*	*0.2*	*13.5*	*202.5*	*1*	*100*
Ammonium sulphate precipitation 70%	*25*	*5*	*0.2*	*25*	*125*	*1.8*	*61.7*
Ion exchange on DEAE-cellulose	*24*	*4.5*	*0.1*	*45*	*108*	*3.3*	*53.3*
Gel filtration on Sephadex- G150	*21*	*5*	*0.07*	*71.4*	*105*	*5.2*	*51.8*

Characterization of L-tryptophanase:

Effect of pH on Tnase activity: The impact of pH on the activity of L-tryptophanase was evaluated across a pH range of 5 to 9. The activity of the enzyme rose progressively with rising pH values, reaching its maximum at pH 8. Beyond this point, a noticeable decline in activity was observed. Therefore, pH 8 was identified as the optimum pH for L-tryptophanase activity, at which the enzyme exhibited a specific activity

مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
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Journal of the College of Basic Education Vol.32 (No.137) 2026,pp.1160-1178

of 5 U/ml, as illustrated in Figure 1. When pH levels rose, the enzyme activity progressively increased until it reached the ideal level, after which the activity decreased. Most enzymes exhibit a bell-shaped activity profile, showing low activity under strongly alkaline conditions and rising from near zero in highly acidic environments until achieving a maximum at their optimal pH (Ahluwalia *et al*., 2022).

Effect of pH on Tnase stability: In order to assess the pH stability of the pure L-tryptophanase, it was incubated for 30 minutes at 37°C. The findings showed that the enzyme was stable over a wide pH range (5–9). Nonetheless, the highest residual activity (100%) was reported at pH 8, while maximum stability was noted at pH 7 and 8. After this, the activity decreased and, as Figure 2 illustrates, reached 85% at pH 9. These results show that L-tryptophanase can handle somewhat basic or alkaline environments. Since pH has an impact on the ionization state of the substrate as well as the side chains of amino acids within the enzyme, it is thought to be a crucial factor regulating the expression of enzymatic activity. Many enzymes can become irreversibly denatured and lose their activity when exposed to extremely acidic or excessively basic environments (Kotarkonda *et al*., 2023).

Effect of temperature on Tnase activity: One important factor influencing the rate of enzymatic processes is temperature. As shown in Figure 3 the temperature range in which L-tryptophanase was active was 27–47°C. Enzymatic activity peaked at 37°C at 5 U/ml, and then gradually decreased to 1.5 U/ml at 47°C. Temperature has a broad impact on biological systems, affecting both catalytic efficiency and protein stability. The enzyme may lose its three-dimensional conformation through structural destabilization or partial denaturation when temperatures rise or fall below its optimum. The reaction rate is significantly decreased as a result of this structural disturbance (Bartoo& Hussein, 2023).

مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
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Journal of the College of Basic Education Vol.32 (No.137) 2026,pp.1160-1178

Effect of temperature on Tnase stability:

The thermal stability of L-tryptophanase was assessed by incubating it at different temperatures (27–47°C) for 30 minutes. The enzymatic test was then used to determine the residual activity. The enzyme kept stable and maintained 100% of its activity between 32 and 37°C, as seen in Figure 4. However, residual activity gradually decreased as temperatures rose, reaching about 85% at 47°C.

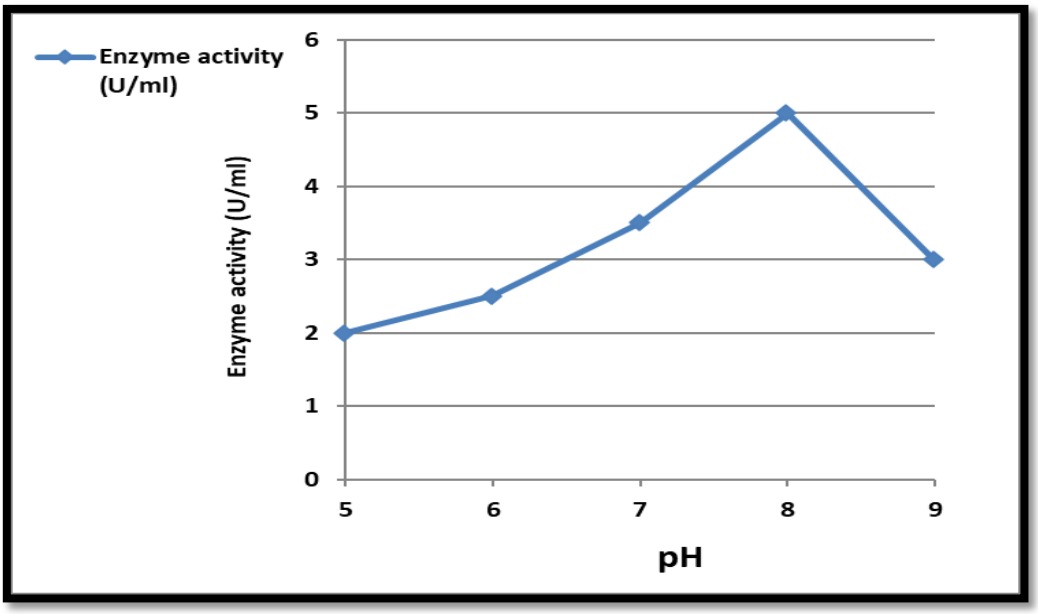

Figure (1): Impact of pH on activity of L-tryptophanase purified from *E. coli*

مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
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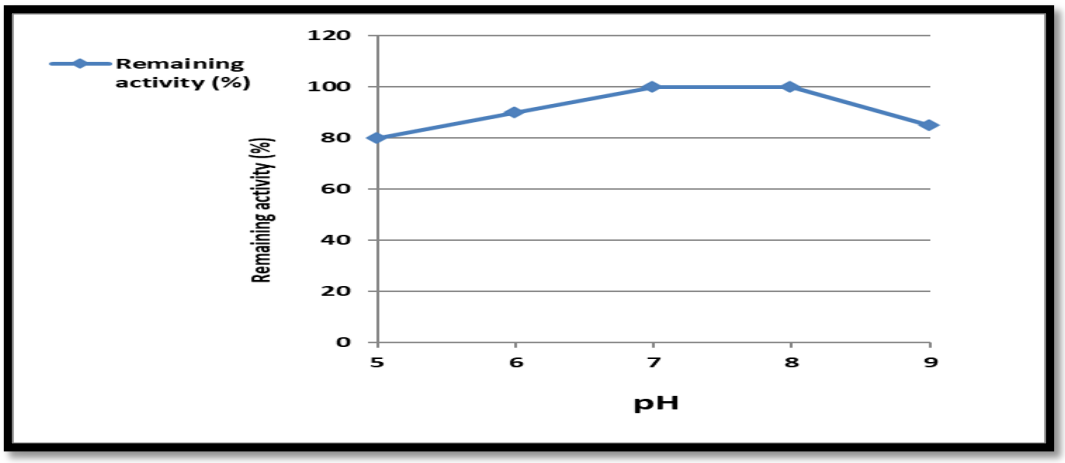

Figure (2): Influence of pH on stability of L-tryptophanase purified from *E. coli*

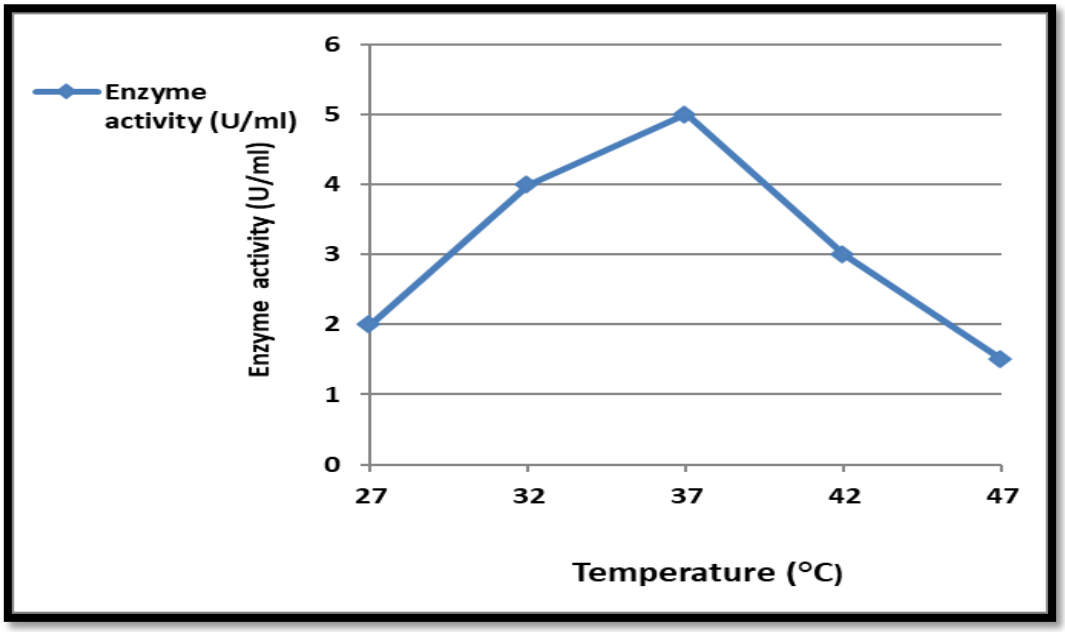

Figure (3): Temperature Impact on the activity of L-tryptophanase purified from *E. coli*

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كلية التربية الاساسية – الجامعة المستنصرية
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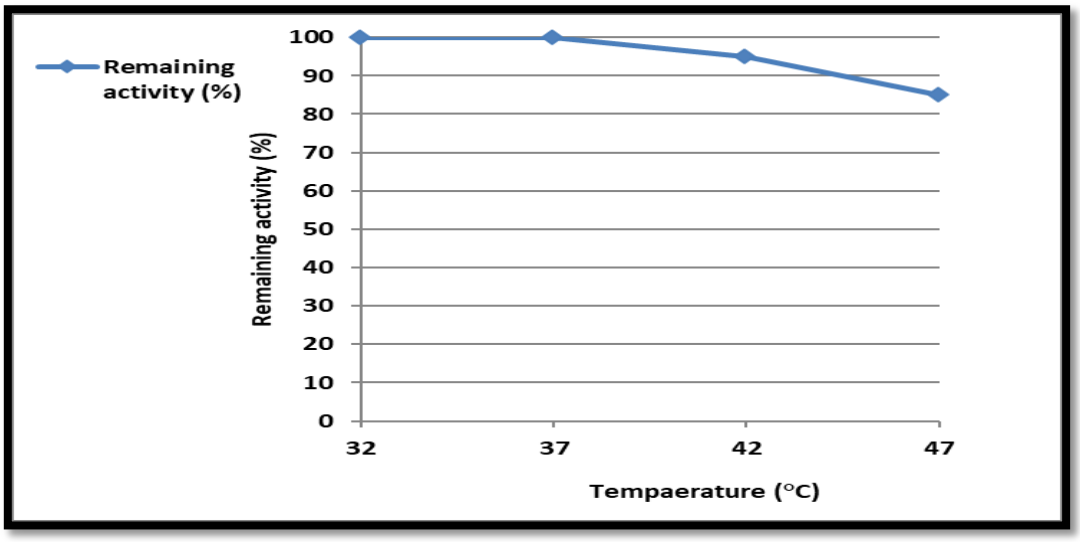

Figure (4): Temperature Influence on stability of L-tryptophanase purified from *E. coli*

Determination of molecular weight for Tnase: The molecular weight was estimated using SDS-PAGE based on the logarithmic relationship between molecular weight and Rf values, as illustrated in the corresponding Figure 5. The approximate molecular weight of the L-tryptophanase enzyme was 53 kDa.

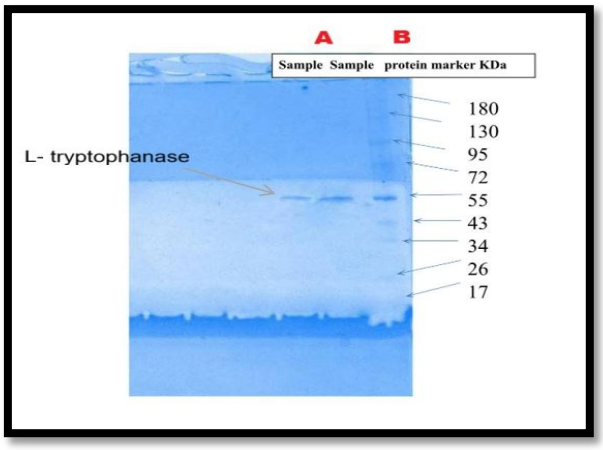

Figure (5): SDS-PAGE of the purified L- tryptophanase from *E. coli*, (**A**) L- tryptophanase after gel filtration (**B**) protein marker

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كلية التربية الاساسية – الجامعة المستنصرية
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Journal of the College of Basic Education Vol.32 (No.137) 2026,pp.1160-1178

L- tryptophanase a homo-tetrameric enzyme made up of four identical subunits. When analyzed by SDS-PAGE, the enzyme dissociates into its monomers, which typically appear as a band of approximately 53–55 kDa. Although the native tetrameric enzyme has an overall molecular weight of about 200–210 kDa, SDS-PAGE resolves it into the smaller monomeric units. For example, *E. coli* tryptophanase subunits appear around 52.8 kDa to 55 kDa on SDS-PAGE (Rety *et al*., 2015& Kogan *et al*., 2015). According to Suzuki *et al*., (1991), the molecular weights of Tnase enzyme are various according to the type of microorganisms, as follows: *E. coli* (55,000 Da), *Bacillus alvei* (52,000 Da), and *Providencia rettgeri* (55,000 Da). While the molecular weight of the Tnase extract from *Vibrio cholerae* single protein band of approximately 49 kDa. According to Nuidate *et al*. (2015), another study reported that the apparent monomeric molecular weight of Tnase extracted from *Bacillus alvei* was approximately 50,500 Da (Neil& DeMoss, 1972).

L- tryptophanase inhibition by curcumin: It is known that curcumin ($C_21H_{20}O_6$) has antibacterial activities against bacterial strains, including *Enterobacteriaceae*. *Escherichia coli*, one of the most significant members of this family, is found in the human gut and is responsible for septicemia, diarrhea, and lower urinary tract infections (Roy *et al*., 2024). The curcumin MIC value against *E. coli* in a prior investigation ranged from 93.8 µg/ml to 250 µg/ml (Tajbakhsh *et al*., 2008& Wang *et al*., 2009). Gune *et al.* (2016) indicate curcumin MIC values of 163 µg/ml against *E. coli*. MIC against *E. coli* in this study approximately 250 µg/ml, which is in line with previously reported levels. Therefore, it was determined that sub-MIC curcumin concentration or concentrations lower than the MIC values equivalent to 125 µg/ml in this study exhibited L-tryptophanase inhibitory activity 100 %. Consequently, the highest curcumin concentrations that inhibited the enzyme were 250 µg/ml, which demonstrated 100% inhibitory activity, while the lowest curcumin concentration that inhibited the enzyme was 62.5 µg/ml, which demonstrated a 95% inhibitory rate. This study concluded by demonstrating curcumin's antibacterial effect against harmful bacteria.

Curcumin was able to inhibit Tnase enzyme responsible for catalyzing the breakdown of tryptophan into ammonia, pyruvate, and indole. The literature indicates that indole, which is produced from tryptophan by tryptophanase, is crucial for a number of bacterial functions, such as the creation of persistent cells, biofilm formation, acid resistance, virulence, and antibiotic resistance (Ding *et al*., 2025). Furthermore, more research is necessary to determine curcumin's potential efficacy against other illnesses.

Table 3: Effect of MIC Curcumin Concentration on L-Tryptophanase Activity

Curcumin concentration (µg/ml)	Relation to MIC against *E. coli*	Effect on L-tryptophanase
250	MIC	100%
125	Sub-MIC (½ MIC)	100%
62.5	½ Sub-MIC	95%

Conclusions: In this study, the L-tryptophanase enzyme, which plays an important role in the pathogenicity of *E. coli* in urinary tract infections, was successfully purified through a series of steps including ammonium sulphate precipitation, ion-exchange chromatography, and gel filtration. The purified enzyme was then carefully characterized to determine its optimal pH, temperature conditions, stability, and molecular weight. The findings also showed that curcumin, a natural compound, was highly effective in inhibiting the activity of this enzyme. These results highlight the potential of curcumin as a promising natural inhibitor that could help reduce bacterial virulence.

Recommendations: Curcumin shows strong potential as a natural antibacterial agent and should be further tested in vivo. Its combination with antibiotics may help overcome resistant bacteria. More studies are needed to monitor antibiotic resistance in urinary infections and to better understand the mechanism of curcumin action.

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تثبيط نشاط أنزيم التربتوفانيز بواسطة الكركمين: تنقية وتوصيف الانزيم من الاشريكيه القولونية

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مستخلص البحث:
هدفت هذه الدراسة الى استخدام الكركمين للحد من نشاط انزيم التربتوفانيز. يعرف الكركمين بأسم Diferuloylmethane ($C_{21}H_{20}O_6$) ، وهو مركب حيوي طبيعي مستخلص من جذور نبات الكركم (*Curcuma longa L. rhizomes*) ، ويتميز بخصائص مضادة للأكسدة، و بسميه خلويه ضئيله أو معدومه. أظهرت نتائج التثبيط أن أعلى تركيز للكركمين (250 ميكروغرام/مل) حقق تثبيطًا كاملاً لإنزيم التربتوفاناز، بينما حقق أقل تركيز للكركمين (62.5 ميكروغرام/مل) فعالية تثبيطية بنسبة 95%. يُعتبر إنزيم التربتوفانازعاملًا مرتبطًا بضراوة البكتيريا، ويلعب دورًا في تطور العدوى وانتشارها. تم تنقية الانزيم من بكتريا الاشريكيه القولونية المعزولة من عدوى المسالك البولية عن طريق الترسيب باستخدام كبريتات الأمونيوم بنسبة تشبع 70% بالإضافة إلى كروماتوغرافيا التبادل الأيوني، وكروماتوغرافيا الترشيح الهلامي باستخدام عمود سيفادكس G-150 وخلال مرحلة التنقية النهائية، ازداد النشاط النوعي للإنزيم الخام من13.5 وحدة\ ملغم بروتين الى 71.4 وحدة\ ملغم بروتين ووفقًا لدراسة توصيف الإنزيم النقي، فقد كانت تأثيرات الاس الهيدروجيني وثباته عند الرقم الهيدروجيني، بالإضافة إلى درجة الحرارة وثباته عند درجة حرارة حيث اظهرت النتائج 8,8 (للأس الهيدروجيني)، و 32، 37، 42 درجة مئوية (لدرجة الحرارة)، على التوالي.

الكلمات المفتاحية : الإشريكية القولونية، التنقية، التثبيط، الكركمين، وإنزيم L-تريبتوفاناز.