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Harnessing L-asparaginase from Solanaceae plants: Purification and molecular dynamics studies for cancer targeting

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Abstract. *Aisha A, Sajjad A, Munawar N, Azam HMH, Ishtiaq U, Nawaz S. 2025. Harnessing L-asparaginase from Solanaceae plants: Purification and molecular dynamics studies for cancer targeting. Asian J Nat Prod Biochem 23: 27-37.* Conventional chemotherapeutic regimens have their adverse effects. Our research, however, offers a potential breakthrough in cancer treatment. We have discovered that l-asparaginase from plant sources can selectively target L-asparagine, the nutritional requirement of cancer cells. The aim was to elucidate the function and mechanism of action of anticancer and bactericidal plant-sourced L-asparaginase protein via proteomics. Biochemical, chromatographic techniques and SDS PAGE were employed for purifying L-asparaginase from Solanaceae plants, including *Datura innoxia, Atropa belladonna, Hyoscyamus niger* (leaves), *Lycopersicum esculentum* (fruit), *Solanum tuberosum* (roots), and *Ipomoea batatas* (roots). Michaelis-Menten and Lineweaver Burk's plot demonstrated the characterization of the extracted protein from *D. innoxia*, which exhibited the maximum enzyme activity. Further, molecular dynamics simulations were employed to establish antimicrobial efficiency (through potato disc assays) and to determine the antitumor efficiency. Kinetic studies of L-asparaginase from *D. innoxia* revealed optimal activity at pH 8-8.5 and 37°C with [S]=8 ug/mL, Km 0.1766 umol provided 1/2V max 0.525 umol/min, while the protein band of approximately 30 kDa. Ethanolic extracts inhibited growth 54.5, 33.30, and 45.65% among *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis,* respectively, with MIC 6.25 mg/dL. L-asparaginase anticancer mediation was validated using molecular dynamics simulations (InterPro Scan), identifying novel domain IPR027474 and active sites IPR020827 and IPR027475. This mechanism provided insights into anticancer action and potential sources for combinational therapies in clinical oncology.

Keywords: Characterization L-asparaginase, chemotherapeutic, chromatography, conventional pharmaceuticals, extraction, purification

INTRODUCTION

Cancer and bacterial infections are two major health concerns worldwide, affecting millions every year. Natural products derived from plants, fungi, and microorganisms have been widely studied for their anticancer properties. These compounds exhibit a range of mechanisms of action, including induction of apoptosis, inhibition of cell proliferation, and suppression of angiogenesis (Newman and Cragg 2016). Several alternatives to conventional drugs, such as taxol, vincristine, and camptothecin, have been used as chemotherapy drugs to treat cancer (Cragg and Newman 2013). In recent years, there has been increasing interest in the potential of natural products as sources of new anticancer drugs. For example, curcumin, a compound found in turmeric, has been shown to have anticancer properties and is being studied as a potential anticancer drug (Aggarwal et al. 2009).

Bacterial infections are a major health concern with the emergence of antibiotic-resistant bacteria posing a significant challenge to the development of new antibacterial drugs. Natural products derived from plants, fungi, and microorganisms have been studied for their antibacterial properties, with several compounds showing promising results in preclinical studies (Newman and Cragg 2016). For example, berberine, a compound found in several plants, has been shown to have antibacterial properties against a range of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Stermitz et al. 2000).

Switching from antibiotics and chemotherapy to non-synthetic herbal medicines has revolutionized the world. Medicinal plants provide the basic raw materials for indigenous pharmaceuticals (Chamani et al. 2020). Medicinal plants used to treat chronic diseases have attracted the interest of scientists. The extracts of various medicinal plants have been used as medicine (Turrini et al. 2014). There are nearly 2300 plant species in the Solanaceae family, including medicinal plants like *Solanum nigrum, Withania somnifera, Capsicum annuum*, and tomato with numerous biological activities, i.e., antifungal, antibacterial, antioxidant, cytotoxic, and antitumor activities (Rani et al. 2021).

Solanaceae family comprises excellent therapeutic plants such as *S. nigrum* (black nightshade), potato (*Solanum tuberosum*), tomato, and other medicinal plants. The members of Solanaceae have already been exploited and confirmed their antibacterial and antitumoral potential, i.e., *W. somnifera, C. annuum, S. nigrum, S. tuberosum* and *Solanum lycopersicum* (Ambreen et al. 2019). Various research reports have widely investigated the potential of potato peels to produce useful compounds, particularly phenolic compounds, which are highly demanded in the industrial sector (Mushtaq et al. 2023, 2024a,b). Likewise, plants containing therapeutically bioactive agents, including *Atropa belladonna* L. (deadly nightshade), *Datura stramonium* L. (Jimsonweed), and *Hyoscyamus niger* L. (nightshades), require moist soil habitat to grow. These weeds can also grow in shallow and stony soil. They are cultivated in tropical and subtropical regions of agro climate and widely consumed as medicine for their antioxidant, anti-inflammatory, hepato-protective, diuretic, antitumorigenic, and antipyretic properties. They comprise a diversified spectrum of potentially active metabolites against ailments (Ayo 2010; Yadav et al. 2022). *Ipomoea batatas* are known scientifically for their antioxidant, anti-diabetic, wound healing, anti-ulcer, antibacterial, and anti-mutagenic activities. It is also an immune booster for relieving gastrointestinal and upper respiratory symptoms (Panda et al. 2011; Panda et al. 2012). *Lycopersicum esculentum* also has a high amount of lycopene and fewer glycoalkaloids. To date, various studies have demonstrated several benefits of these bioactive and antioxidant compounds, namely anticarcinogenic, anti-inflammatory, cardioprotective, hepatoprotective, and antibacterial properties (Pinela et al. 2016). *Atropa belladonna* is pre-declared as a primary commercial source of alkaloid drugs, i.e., atropine. Its antibacterial and antimicrobial properties also scavenge cancer cells by imposing a high impact of antioxidants (EL-shaer and Ibrahim 2021). *Hyoscyamus niger* is well known for its antimicrobial, antispasmodic, hypotensive, convulsant, anti-inflammatory, antipyretic, and analgesic characteristics (Begum 2010).

The current study investigates a novel source of L-asparaginase from the Solanaceae family to minimize immunological reactions induced by the microbial-sourced enzyme (Aisha et al. 2022). L-asparaginase (EC3.5.1.1) is an enzyme of medical prominence and is used as a chemotherapeutic agent against acute lymphoblastic leukemia and other cancers. It also possesses immense potential to cure autoimmune and infectious diseases. The vast applications of this enzyme in the healthcare sector have increased its market demand (Vimal and Kumar 2017). The L-asparaginase (EC 3.5.1.1) is used in chemotherapeutic combinations in cancer management to treat leukemia. Crude extracts of medicinal plants of the Solanaceae family, like *W. somnifera*, *S. nigrum*, and *C. annuum,* have been investigated for anticancer activity and then formulated for oncology (Ogunsuyi et al. 2022). The present study's objective is to extract and purify a commercially high titer of the enzyme L-asparaginase through stratified chromatographic methodology from inexpensive plant sources to meet its therapeutic needs in cancer treatment and biotechnology research.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were L-Asparagine (Sigma Aldrich), Sephadex G-100 (Sigma Aldrich), DEAE cellulose resin (Diethylamineethylamine), and dialyzing membrane with a pore size of 3.2 microns was purchased from Shenzhen Taoshi Co., Ltd., Japan. SDS (Sodium dodecyl sulfate), 30% acrylamide stock (37.5: 1 acrylamide: bisacrylamide) (Bio-RadLaboratories), TEMED (LifeTechnologies, Gibco®), ammonium persulfate (Sigma Aldrich), pre-stain protein M.W. marker (Bio-Rad Laboratories), bromophenol blue (Thermo Fisher Scientific), tris base (Calbiochem-Behring), Tris-HCl (pH 6.8), and β-mercaptoethanol (Sigma Aldrich).

Microbial strains

Escherichia coli strain ST131 KX171170–171195 (Ali et al. 2019), *Staphylococcus aureus* strain 32S ST 239 JTJX00000000 (Khan et al. 2016) and *Bacillus subtilis* strain DH5α accession number AJ004803 (Soldo et al. 2002).

Plant samples for enzyme extraction

Tubers of *S. tuberosum, I. batatas,* and fruit of *L. esculentum* were purchased from the local market of Faisalabad, Pakistan. In contrast, leaves and fruit of *D. innoxia, A. belladonna* (deadly nightshade), and *H. niger* (nightshades) were obtained from a botanical garden situated at the University of Agriculture Faisalabad, Punjab, Pakistan.

Crude extract preparation

The leaf extracts of *A. belladonna* (deadly nightshade), *H. niger* (nightshades), *L. esculentum,* and *D. innoxia,* as well as root extracts of potato and sweet potato, were prepared after milling in a solution of 0.2 M phosphate buffer at pH 8.6 (Jamil et al. 2007). The crude protein extract was precipitated by different saturation percentages, initially 50-60%, 70-80%, and finally 90-100% of ammonium sulfate (Jan et al. 2022). The sample was kept at -20°C overnight to precipitate the protein. The 80% saturated ammonium sulfate solution produced a pellet after centrifugation at 8217-28341 RCF for 30 min at 4°C. The pellet was subjected to protein analysis and dialyzed against distilled water for 24h at 4°C while discarding the supernatant. The protein sample was kept at -20°C for further bioassay and purification (Kirar et al. 2022).

Enzyme purification

The Biuret method determined the protein content of crude enzymes partially purified, dialyzed, and pure forms at 540 nm (Gornall et al. 1949). The L-asparaginase enzyme was partially purified by dialysis. The supernatant-containing enzyme was loaded on DEAE cellulose column anion exchange chromatography using phosphate buffer pH 6.8 (Kaur and Arora 2009; Rahman et al. 2009). Almost 50

fractions were collected at the flow rate of 30 mL/hour (Jamil et al. 2007). Those fractions with high protein content and enzyme activity were loaded on a gel filtration column to obtain purified enzymes for therapeutic application. Analytical grade Sephadex G-100 was used as porous material buffered with 0.1 M Tris- HCl buffer to equilibrate the column at pH 8.6 (Dixit et al. 2013). The enzymatic analysis was performed by nesslerization at wavelength 450 nm (Moharib 2018). Each extraction and purification part was replicated in triplicate analysis.

Enzyme and protein purification

The protein content of enzymes in crude form, partially purified shape, dialyzed form, and purified form was determined by (Gornall et al. 1949) using B.S.A. as standard. Absorbance (O.D) of protein was obtained at the 540 nm wavelength. The enzyme was assayed according to the nesslerization method by converting L-asparagine to ammonia and L-aspartate at 37°C and pH 8.5 under a specific condition (Ren et al. 2010).

Kinetic characterization of enzymes

The highest protein and enzyme content was found in six selected plants of the family Solanaceae, and L-asparaginase-bearing plant tissue was chosen for further characterization (Moharib 2018). The effect of pH (4-9) and substrate was recorded on the activity of purified L-asparaginase obtained from the plant extracts that exhibited the highest titer of the enzyme. The reaction mixture of the enzyme was also assayed at different temperatures at 15-65°C, and the substrate (asparagine) concentration was 2-14 ug/mL (Moharib 2018). Different buffer solutions were prepared to estimate the optimal pH for L-asparaginase. Potassium phosphate, sodium acetate, and Tris -HCl buffers were used to design a pH range from 3-9. For kinetic studies, the substrate 0.01 mMoL L-asparagine was added to 0.2 M buffer solutions at a specified pH. The purified enzyme was incubated at different pH values 3-9 at 37°C. Km and Vmax were resolved through enzyme kinetic tools such as the Lineweaver-Burk plot and the Michaelis-Menten plot using the following equations:

$V_\circ = Vmax[S]/ Km+[S]$ -- Michaelis Menton eq.
$1/V_\circ=Km/ Vmax[S] + 1/Vmax$ -- Line Weaver Burk eq.

Where:
$V_\circ$: Initial velocity
Vmax: Maximum velocity
[S]: Substrate concentration,
Km: Substrate concentration at ½ Vmax

Molecular weight by SDS PAGE

Electrophoresis was done on the purified enzyme from Gel filtration chromatography by following Laemmli et al. (1970) discontinuous SDS-PAGE system with a 15% acrylamide gel. The electrophoresis was performed at 100V. The gels were stained for 45 min in a staining solution of 6% acetic acid, 500 mL of H_2O, 44% methanol, and 2.25 g of Coomassie brilliant blue. Destaining was performed in 20% methanol, 5% acetic acid, and 750 mL of distilled water. This process continued until the background color vanished, and protein bands could easily be seen.

Antibacterial assay

The antibacterial action of crude extract of *D. innoxia* leaves was assessed through standard disc diffusion method (A.S.T.) antibiotic susceptibility testing. The stock solution was prepared by mixing 25 mg of the crude extracts of water and ethanol dissolved in 1 mL of DMSO and was applied on the respective discs. *Bacillus subtilis, S. aureus* gram (+), and *E. coli* gram (-) were treated against extracts of *D. innoxia* leaves extract. Observations were recorded and compared with the standard antibiotic of Erythromycin 250 mg. After 24 h of incubation, the noticeable diameter of clear zones of inhibition was measured, which confirmed antimicrobial effects, and percentage inhibition was calculated (Mannan et al. 2014; Noor et al. 2014; Ali et al. 2019; Qasim et al. 2020).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of crude extracts was determined by the agar dilution method. First, 18 mL of nutritional agar was autoclaved, 2 mL of the crude extract at concentration of 50-0.78 mg/mL was added to each test tube, which was then poured into pre-labeled petri dishes. An extra petri dish with only nutrient agar was prepared as a control. The bacterial strain was adjusted to 0.5 McFarland turbidity standard and transferred to each plate with different concentrations of crude extract. The plates were incubated at 37°C for 24h and the MIC was determined as the minor concentration that inhibited the growth of the respective organism. Next, to determine the minimum bactericidal concentration (MBC), the broth used in MIC was streaked on agar plates, and the MBC was defined as the first dilution where no growth was observed. For the serial dilution, 1 mL of the stock solution of 50 mg extract/mL of DMSO was added to 8 vials with 1 mL of DMSO in each vial. The extract was then serially diluted into 7 vials to obtain 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 mg/mL conc. The study found that the crude extract had bactericidal activity against the tested bacterial strains (Janani et al. 2019).

Statistical analysis

The data were subjected to statistical analysis, using Student's t-test to compare variables. The data are expressed as means ± S.D.

RESULTS AND DISCUSSION

The homogenates of six selected plants revealed L-asparaginase activity. *Datura innoxia* and *I. batatas* exhibited pronounced enzyme activity 56.311, 46.93U/mL in crude extracts along with statistically highly significant values by T-test as shown in Figure 1. The enzyme activity of 44.18 and 24.93 U/mL were obtained from the crude extracts of *L. esculentum* and *A. belladonna,* respectively.

In contrast, *H. niger* and *S. tuberusum* extracts revealed enzyme activity as 10.38 U/mL and 10.98 U/mL, respectively.

The purification summaries of all plants are expressed in Figure 1. The partially purified form of the enzyme sample of 500 uL was used for Gel filtration by Sephadex G-100, which resulted in purified enzyme for therapeutic grade for six selected plants (fruit). After gel filtration, the enzyme purified from *D. innoxia* yielded 56.77%, and the mean activity of the crude extract was 30.14±0.928 U/mL (Table 1). Likewise, the specific activity of L-asparaginase was significantly higher ($p<0.05$) in *D. innoxia* (135.25 U/mg) and *A. belladonna* (170.39 U/mg) as compared to other plant extract's specific activity given in Figure 2. At the same time, the percentage yield was 56.77, 53.4, 56.42, 44.3, and 52.3%, respectively.

Figures 3, 4, 5, 6, and 7 depict the summarized view of six plants' activities and specific activities (left side of the graph) with the amount of protein extracted (right side) at each purification step, i.e., ammonium sulfate precipitation, dialysis, ion exchange, and gel filtration. Among all selected plants, *D. innoxia* proved the maximum activity of L-Asparaginase; its ion exchange and gel filtration curves are explained in Figures 8.B and 8.C. In contrast, enzyme activity at each purification step is expressed by a two-way graph, as shown in Figure 8.A.

Molecular weight by SDS PAGE

The highest enzyme titer-sharing sample from *D. innoxia* was introduced to the SDS PAGE. It showed promising results of L-asparaginase with a maximum protein of 30±1.5 kDa comparable with the standard marker proteins of known molecular weight (Figure 8.D).

L-asparaginase activity affected by kinetic parameters

To check enzyme stability purified L-asparaginase was incubated at different physiological pH to obtain the optimal milieu for enzyme efficacy. At pH 8-8.5, the enzyme showed enhanced activity of 24.65 U/mL (Figure 9.A). Different substrate conc. was incubated with L-asparaginase, among which the highest activity of the enzyme was achieved as 0.678 U/mL for 8 µg/mL concentration of substrate (Figure 9.B). The effect of temperature on L-asparaginase activity is presented in Figure 9.C. The incubated enzyme with the substrate at various desirable temperatures yielded a maximum activity of 48.92 U/mL at 37°C.

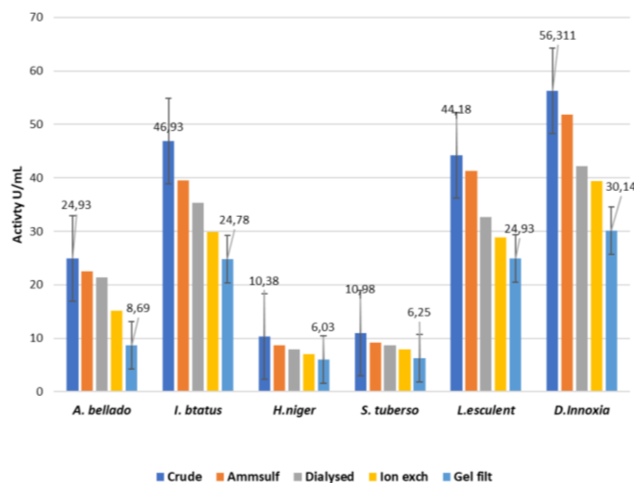


Figure 1. L-asparaginase enzyme activity in selected plant extracts at various purification steps

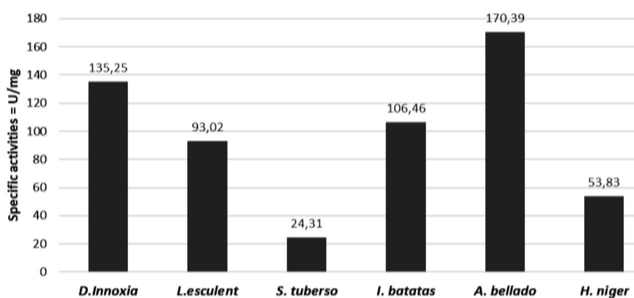


Figure 2. Specific activities of purified L-asparaginase from plant extracts

Table 1. L-asparaginase mean activity in selected plant extracts after purification (n=6)

Plant species	Activity of purified enzyme (mean) U/mL
Datura innoxia	30.14±0.928[A]
Lycopersicum esculentum	24.93±0.538[B]
Ipomoea batatas	24.78±0.743[B]
Atropa belladonna	8.69±0.61[C]
Hyoscyamus niger	6.03±0.493[D]
Solanum tuberosum	6.25 ±0.344[CD]

Note: Significant (P<0.05), [C, CD] Non-significant, [A] Significantly important, Means sharing the same letter are statistically non-significant

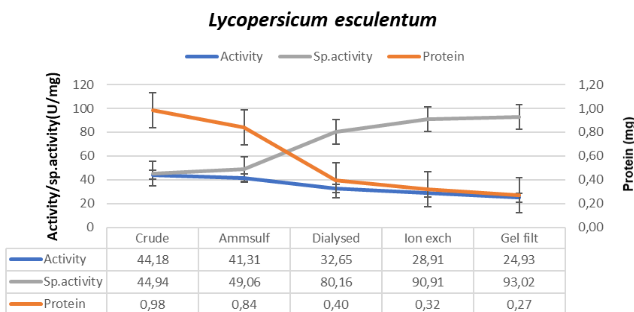


Figure 3. L-asparaginase activity, specific activity, and protein content during different purification steps from *L. esculentum*

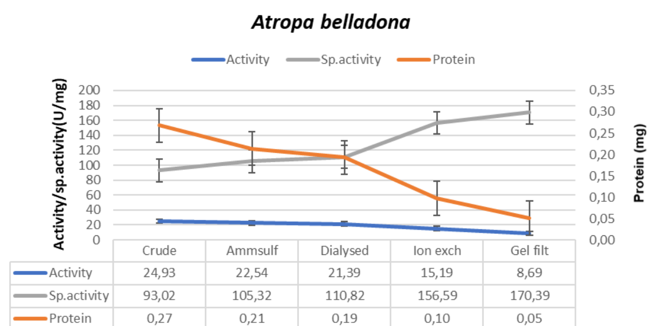


Figure 4. Enzyme L-asparaginase activity, specific activity, and protein content during different purification steps from *A. belladonna*

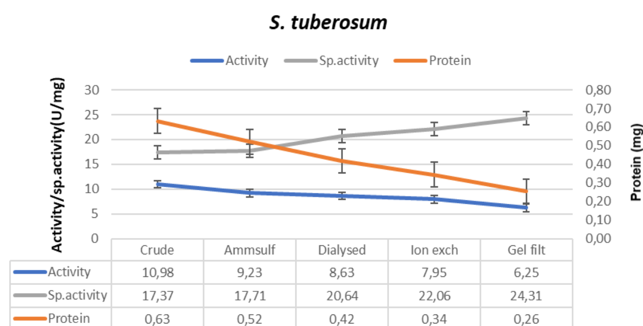


Figure 5. L-asparaginase activity, specific activity, and protein content during different purification steps from *S. tuberosum*

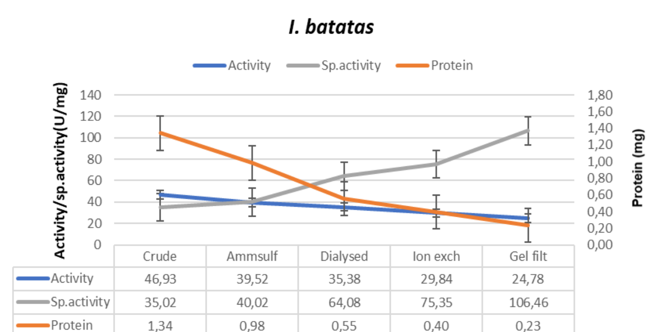


Figure 6. L-asparaginase activity, specific activity, and protein content during different purification steps from *I. batatas*

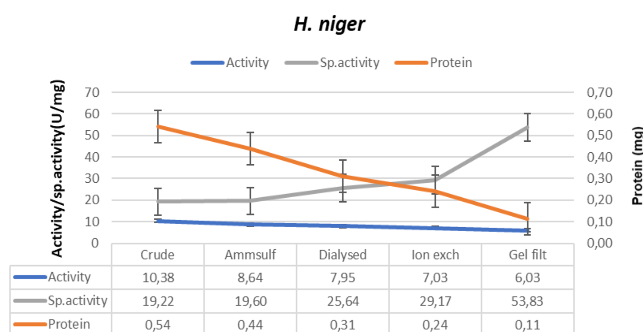
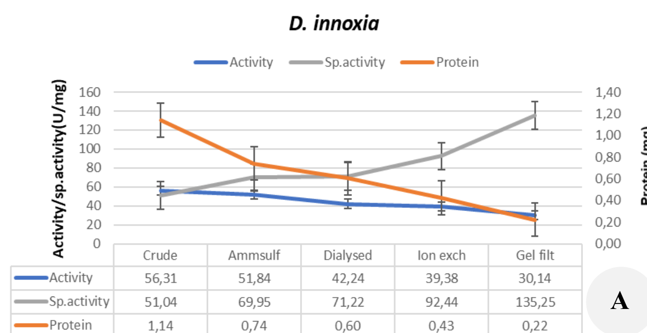
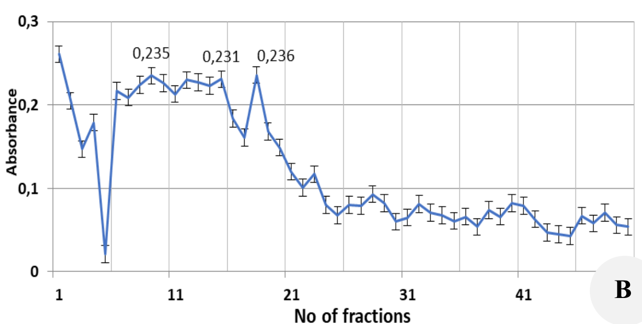
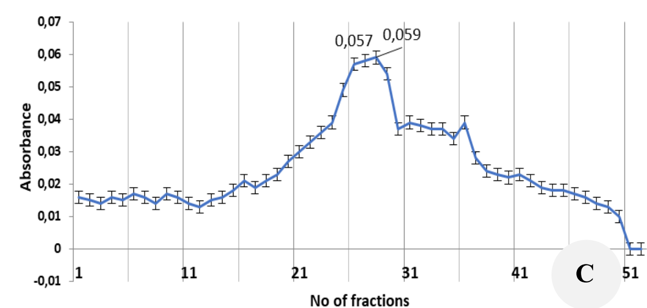


Figure 7. Enzyme L-asparaginase activity, specific activity, and protein content during different purification steps from *H. niger*







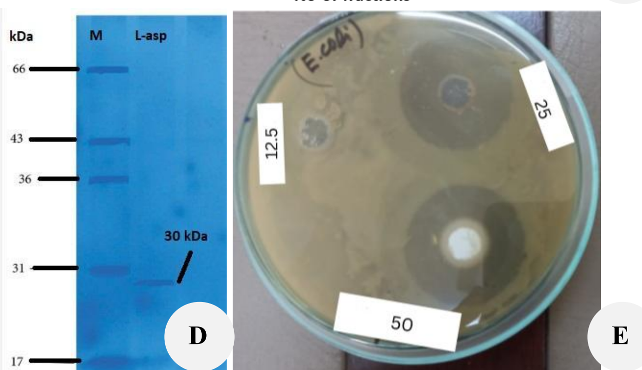


Figure 8. Elaborated purification summary and confirmation of purified L-asparaginase from *D. innoxia*. A. L-asparaginase activity, specific activity, and protein content during different purification steps; B. Ion exchange chromatography showed the highest protein content in fractions 9,15 and 19; C. Gell filtration chromatography resulted in enzyme concentration eluted in fractions no 25, 26, 27; D. SDS PAGE, L-asparaginase purified from *D. innoxia* determined by SDS PAGE; E. The minimal inhibitory concentration of E-extract of *D. innoxia* 6.25 mg/mL. Note: Lane M: Molecular weight marker proteins, L-asp: Coomassie blue stained fraction of purified enzyme protein after Gel filtration chromatography has molecular weight 30±1.5 kDa

Line weaver Burk enzyme kinetics of L-asparaginase from *D. innoxia*

Line weaver Burk plot shows a logarithmic increase of velocity and then a parabolic trend with maximum velocity reaching up to Vmax= 10.590 umol/min and Michaelis constant Km= 0.1766 µmol. Michaelis-Menton plot of enzyme L-asparaginase from D. innoxia depicted 1/Vmax= 0.525 min/umol and substrate concentration at which enzyme attained ½ Vmax (Km= 0.1766 umol) (Figures 10.A and 10.B). Both of the plots confirmed enzyme Km as reconfirmed by www.physiology.com website for enzyme kinetics.

Antibacterial efficiency

Concentration gradient of the ethanolic extract 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 mg/mL were employed to determine the MIC. The minimum inhibitory concentration of *D. innoxia* ethanolic extract (E- extract) was 6.25 mg/mL against microbial strains of *E. coli, S. aureus,* and *B. subtilis* (Table 2). The study found that the crude extract had bactericidal activity against the tested bacterial strain (Table 3). E- extract of *D. innoxia* had efficiently inhibited bacterial growth, i.e., 54.5, 33.30, and 45.65% in *E. coli*, *S. aureus*, and *B. subtilis,* respectively.

Anti-cancerous and antibacterial activity confirmation by bioinformatic tools

The function of L-asparaginase as an anticancer agent was verified using InterPro Scan, which identified its domain IPR027474 and active sites IPR020827 and IPR027475. These active sites catalyze L-asparagine deamination, leading to tumor cell death, as shown in Figure 12. The Chimera docking results confirmed the interaction between chitin (bacterial cell wall component) and L-asparaginase with bond lengths of 2.32, 2.07, and 2.54 Å, an RMSD value of 0.00, and a score of -5.5, as depicted in Figure 11.A.

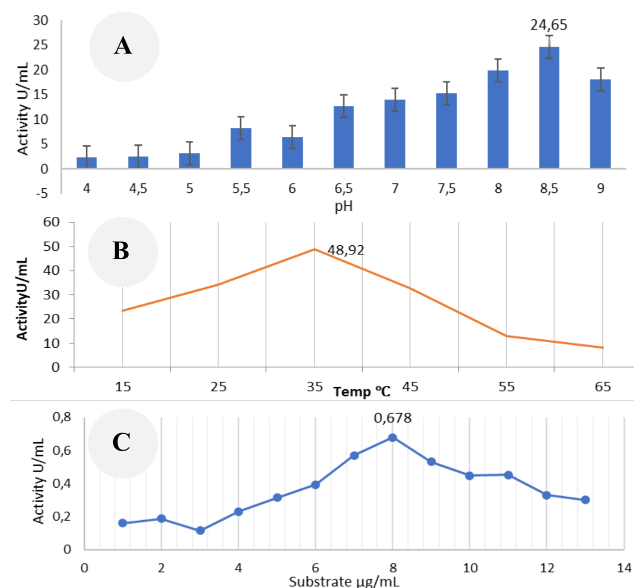


Figure 9. A. Effect of pH on the enzyme activity: pH 4-13; B. Temperature fluctuation from 15 to 65°C; C. Optimal substrate concentration on the activity of L-asparaginase in *D. innoxia* extract

Table 2. Minimum inhibitory concentration of *D. innoxia* ethanolic extract against microbial strains of *E. coli, S. aureus,* and *B. subtilis*

Plant extract	Minimum Inhibitory Concentration (mg/mL)						
Ethanolic extract	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.56**	**0.78**
E. coli	-	-	-	-	*	*	*
B. subtilis	-	-	-	-	*	*	*
S. aureus	-	-	-	-	*	*	*

Note: -: No growth, *: Shows growth. Incubation temperature 37°C, Incubation time: 24h

Table 3. Antibacterial activity of *D. innoxia* ethanolic and aqueous extracts against microbial strains of *E. coli, S. aureus,* and *B. subtilis*

Extracts of *D. innoxia*	Microbial strains	Control growth (mm)	Treatment growth (mm)	Treatment growth (±SD)	% Inhibition of bacterial growth
Ethanol	*E. coli*	22	12.5	12 ±0.23	43.2
	S. aureus	18	6	6.0±0.8	66.7
	B. subtilis	23	10	10.5±1.4	56.5
Aqueous	*E. coli*	13	7	7.06±0.25	46.2
	S. aureus	11	4	4.01±0.61	63.6
	B. subtilis	14	6	6.02±0.49	57.1

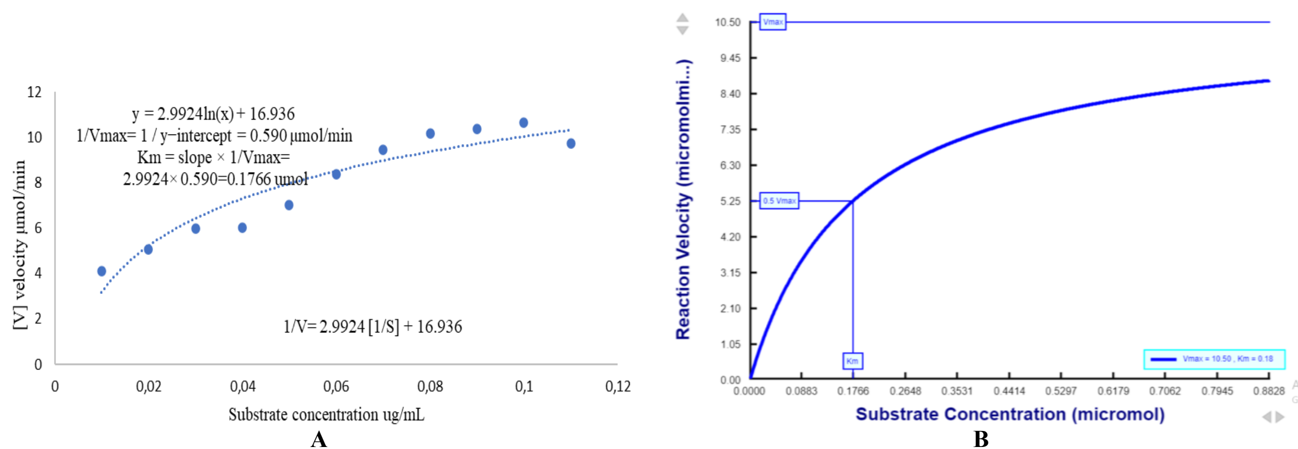


Figure 10. Enzyme kinetics of L-asparaginase as experimented from *D. innoxia*. A. Graph follows Line weaver burk enzyme kinetics, The logarithmic increase of velocity and then parabolic trend with Vmax= 10.590 µmol/min and Km= 0.1766 umol; B. Michaelis Menton plot of enzyme L-asparaginase from *D. innoxia*. with 1/Vmax= 0.525µmol/min and Km= 0.1766 µmol. Note: Enzyme kinetics (Michaelis Menton constant and Vmax) was plotted at www.physiologyweb.com

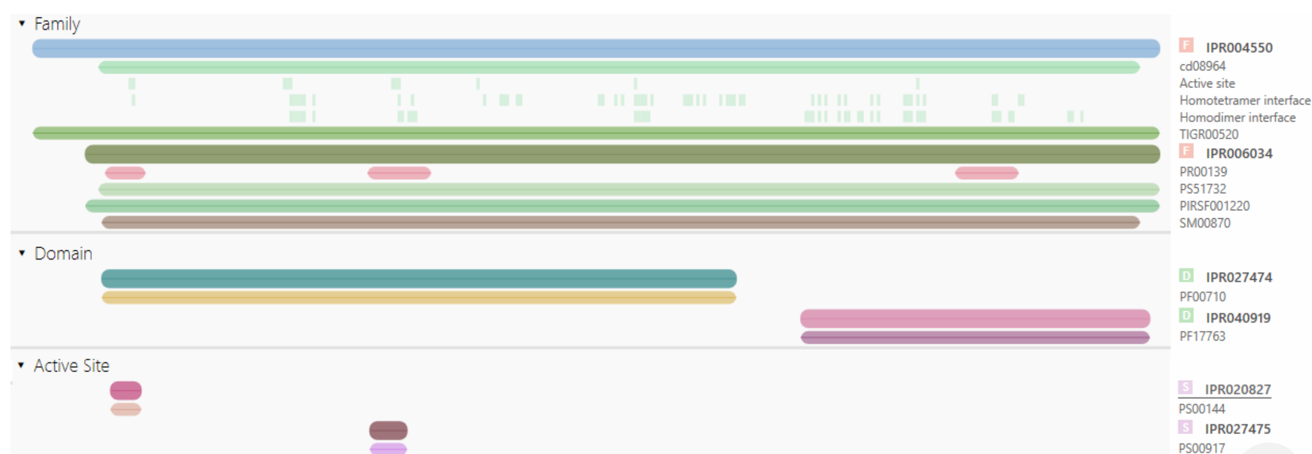


Figure 11. A. Chimera docking between chitin and L-asparaginase; B. L-asparaginase functional analysis to verify anticancer effectiveness. InterPro Scan validated L-asparaginase activity as an anticancer agent by exhibiting its domain IPR027474 and active sites IPR020827 and IPR027475

Discussion

L-asparaginase, also known as ASNase, plays a crucial role in cancer treatment to treat various types of cancer, including acute lymphocytic leukemia, lymphosarcoma, and melanoma sarcoma. The current biologically therapeutic protein is often administered with chemotherapeutic agents such as daunorubicin, cytosine arabinoside, vincristine, and L-asparaginase (Munir et al. 2019; Lee et al. 2021). In 2020, over 53,000 people were diagnosed with Acute Lymphoblastic Leukemia (ALL), and chemotherapy remains the standard treatment for ALL patients (Moharib 2018). L-asparaginase is an extracellular enzyme that acts as an asparagine amido hydrolase, breaking down asparagine (Asn) into ammonia and aspartic acid within cancer cells. Healthy cells can escape this shortage of asparagine by synthesizing Asn from L-asparaginase synthetase (Aisha et al. 2020b), which cancer cells lack. The commercially available L-asparaginase enzyme for clinical use is obtained from *Escherichia coli* and *Erwinia carotovora*; these sources can induce side effects and anaphylaxis (Effer et al. 2020). To mitigate these adverse reactions, researchers have turned to a phytomedicinal source to produce L-asparaginase for anticancer therapy. The focus of the study was to find the potent sources of L-asparaginase and their optimal milieu factors.

The objective of the research was to elucidate the highest enzyme activity exhibiting plants from the selected ones, which was characterized, and kinetics were revealed to provide insight into the novel pharmaceutical source. Solanaceae family possesses ample concentrations of L-asparaginase. The research conducted here included *D. innoxia, A. belladonna, H. niger* (leaves), *L. esculentum* (fruit), *S. tuberosum* (roots) and *Ipomoea batatas* (roots). All plants are latent sources of L-asparaginase containing variable concentrations, as shown in Figure 1. Purified enzymes from *D. innoxia* 30.14 U/mL exhibited the most significant activity, followed by *L. esculentum* and *Ipomoea batatas* 24.93 and 24.78 U/mL, respectively. L-asparaginase from *A. belladonna* has remarkable specific activity at 170.39 U/mg, followed by *D. innoxia, I. batatas,* and *L. esculentum* 135.25, 106.46, and 93.02 U/mg, respectively.

Among selected plants of the family Solanaceae, *D. innoxia* achieved the highest enzyme amount in crude and purified extracts. Enzyme sourced from the crude extract of *D. innoxia* exhibited activity 56.311 U/mL, protein amount 1.14 mg/mL and specific activity 51.04 U/mg as depicted in Figure 8.A. *Datura innoxia* provided 56.77% yield on purification, specific activity 30.14±0.928 U/mL and activity135.25 U/mg (Figure 8.A). *Datura*, a plant belonging to the Solanaceae family, is known to have ten species. Still, only two of them, namely *D. innoxia* and *Datura stromonium,* are known to have drug-like effects (Kirar et al. 2022). The plant has been reported to possess various properties, such as insect repellency, antioxidant activity, antimicrobial activity, anticancer potential, anti-inflammatory properties, and anticholinergic activity (Soni et al. 2012). Phytochemical analysis revealed alkaloids, steroids, glycosides, tannins, flavonoids, saponins, atropine, phenols, proteins, carbohydrates, and fats in *Datura* (Sayyed and Shah 2014). *Datura innoxia*, also known as angel's trumpet, devil's trumpet, and other common names, is an herbaceous or sub-shrub plant that can grow up to 2 meters in height. It is considered an annual plant in dry environments but can behave as a perennial under favorable environmental conditions. *Datura* has been used in shamanistic rituals to achieve illumination and as a hallucinogenic agent by drug users. It has also been used in phytotherapeutic practices to treat conditions such as impotence, asthma, and diarrhea. However, it is important to highlight it is associated with adverse effects, including disorientation, amnesia, blurred vision, and even death in overdose cases (Matias et al. 2020).

While discussing the enzyme characterization and its milieu, our protein, i.e., L-asparaginase, worked best at a pH of 8.5 and temperature of 37°C, delivering the highest activity at a substrate conc. 27.54 U/mL. The researchers testified that the most suitable pH ranges between pH 7.0 and 8.5. However, its efficiency decreases at extremes of the pH scale. A separate investigation referenced in the present paper focusing on plants also revealed that the optimal pH for L-asparaginase activity lies between 8.2 and 8.5, comparable to the current study (Zobaidy et al. 2016). It is essential to mention that the optimal pH for L-asparaginase might vary depending on the specific plant species or strain being studied. For *D. innoxia*, the optimal pH was 8.5 (Figure 9.A).

Researchers studied how well the enzyme L-asparaginase works at different amounts of its building blocks (substrates) and wanted to find the best level. They discovered that the enzyme works best when the substrate is 8 ug/mL in the solution. At this level, the enzyme showed an activity of 0.76 U/mL. This enzyme's activity was similar to enzymes from plants called *S. nigrum* (Mako) and *Capsicum annuum* (Chilli), which belong to the Solanaceae family (Aisha et al. 2020a, b, 2022). The enzymes from these plants also showed good activity at 66.27 and 112 U/mL, respectively. The amount of protein in the enzyme extract was 1.24 mg/mL; when compared with the enzymes from the two plants, the protein amounts and specific activities were 1.02 mg/mL and 90.15 U/mg for *S. nigrum (*Mako) and 1.02 mg/mL and 90.15 U/mg for *Capsicum annuum* (Chilli) (Aisha et al. 2020a,b, 2022). Interestingly, although many plants from the Solanaceae family have been studied, *D. innoxia* has yet to be reported to produce L-asparaginase until now. High enzyme activity is beneficial for treating childhood ALL (Acute Lymphoblastic Leukemia) by breaking down bodily substances (Khalaf 2012).

L-asparaginase works best at a temperature of 37°C. When the temperature is too high or too low, its effectiveness decreases. This matches the findings Khalaf et al. (2012). They discovered that enzymes generally have a specific temperature range in which they work best. Another study by Shanmuga Prakash and their team found that L-asparaginase remains stable up to 45°C (Figure 9.B). However, its activity is most stable at temperatures between 37 and 40°C, similar to the optimal temperature. In another experiment using L-asparaginase from *Pisum sativum*, the enzyme showed the highest activity at 37°C

(Figures 2.B and 2.C). They also found that phosphate is the best substance for stabilizing the enzyme (Shanmugaprakash et al. 2015).

According to Dixit et al. (2013), *D. innoxia* leaf tissue contains a maximum total protein content of 2.0905±0.71, so we chose leaves to extract the L-asparaginase enzyme (Dixit et al. 2013). L-asparaginase from *D. innoxia* exhibited an optimal temperature of 35.5°C, pH 8.5, with a substrate conc. of 8.5 ug/mL (Figure 8). At the same time, the enzyme characterized by *S. nigrum* had a slight variation in optimal profile, showing a temperature of 36.5°C, pH 8.4, and substrate conc. 8.0 ug/mL (Aisha et al. 2022). The molecular weight of 30±1.5 kDa L-asparaginase elucidated from *D. innoxia* by SDS PAGE (Figure 8.A) is similar to research on *S. nigrum* had revealed L-asparaginase with a molecular weight of 32 kDa indicating that the molecular weight of the L-asparaginase molecule may vary even among plants.

MIC of *D. stramonium* extract in benzene was 0.78 mg/mL, as noted against *E. coli* and *S. aureus* (Table 2) (Baynesagne et al. 2017). According to Baynesagne et al. (2017), *D. stramonium* extract in chloroform showed the highest antibacterial activity against *S. aureus* (ATCC25923) 18.2 mm and the lowest antibacterial activity against *E. coli* (8.2 mm) (acetone extract). In the current study, the methanolic extract of *D. innoxia* showed a maximum 12 ±0.23 mm zone of inhibition against *E. coli* followed by inhibition in microbial growth of *S. aureus* and *B. subtilis* 10.5±1.4, 6.0±0.8 mm, respectively as shown in Table 3 and Figure 8.E.

Chinese medicinal plants have been found to possess diuretic and antipyretic properties which help to alleviate inflammations like edema. The methanolic extract of *D. innoxia* cures asthma and gastric issues and is also used in antitumor treatment. Antitumor assays were conducted to ascertain the enzyme efficiency against microbes and cancer cells (Monteiro et al. 2014; Noor et al. 2014, 2016; Tohyama et al. 2014). As reported by Sharma et al. (2021), *Solanum surattense* crude extract has proven remarkable anticancer activity at 76% in a cytotoxicity assay with MIC 15 mg/mL and antitumor activity establishing, i.e., 75% inhibition of tumor (Sharma et al. 2021). L-asparaginase also exhibits antioxidant and antiproliferative properties and is regarded as an anti-cancerous biomolecule (Aisha et al. 2022). Owing to its mechanism, L-asparaginase transforms asparagine into aspartic acid (Batool et al. 2016), whereas insufficient levels of the enzyme asparagine synthetase are seen in cancer cells. Therefore, asparagine depletion leading to cell death occurs because the conversion from asparagine to aspartic acid is irreversible (Alrumman et al. 2019). The specific domains of our protein and active sites trigger L-asparagine deamination. To verify this claim, the estimation of function prediction confirmed domains and active sites of asparaginase (Figure 11), catalyzing L-asparagine deamination into aspartic acid and ammonium ions (Chiu et al. 2019). Chimera docking showed significant interactions between chitin and L-asparaginase, with bond lengths of 2.32, 2.07, and 2.54 A, an RMSD value of 00, and a score of 5.5 (Figure 11).

InterPro Scan identified the curative properties of L-asparaginase containing subdomain IPR027474 and active sites IPR020827 and IPR027475. The active sites accelerate the deamination of L-asparagine, leading to the death of tumor cells. Figure 11 shows a specific case of functional analysis. The ideal outcomes featured the maximum number of hydrogen bonds, the lowest energy score, and the RMSD value around zero. The ideal docking findings involved maximum hydrogen bonds with lengths of RMSD value 0.00 with a score of -5.5. The study concluded that L-asparaginase and the chitin monomer N-acetylglucosamine formed a successful connection.

As a result, our plant extracts encompassed a substantial amount of L-asparaginase. In addition, the most effective extract considerably reduced microbial and tumor development. The results are consistent with those of other plant-origin enzymes for which pure L-asparaginase has been reported.

In conclusion, extracts of various plants of the Solanaceae family, particularly leaves of *D. innoxia*, possess a high titer of l-asparaginase. Moreover, its bactericidal efficiency also makes it a suitable candidate for novel pharmaceutics. The contents of L-asparaginase in *D. innoxia* extract are sufficient and thus can be utilized in chemotherapy, as L-asparaginase mitigates antitumor action by its two main active sites.

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