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Immuno-informatics and Bioinformatics Analysis of Type II Asparaginase from *Campylobacter jejuni* and *Escherichia coli* Activity as Anti-Cancer (ALL)

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, that requires ongoing research for more effective treatments. However, the new approach for using L-asparaginase (L-ASNase) which plays a crucial role in ALL therapy by depleting asparagine and glutamine, essential for leukemic cell survival. This study employed molecular docking and immunoinformatics to investigate the therapeutic potential of type II L-asparaginase from Escherichia coli and Campylobacter jejuni against ALL. Molecular docking simulations revealed strong binding affinities between the enzymes and their substrates. The tested L-asparaginase (ASPA) showed binding scores of -4.5 kcal/mol and -4.7 kcal/mol at two test sites, comparable to or better than the standard sites (-4.8 kcal/mol). Similarly, the tested glutaminase (GLU) enzyme demonstrated binding scores of -4.5 kcal/mol and -5.3 kcal/mol, matching or exceeding the standard sites (-4.5 kcal/mol and -5.2 kcal/mol). Immunogenicity and allergenicity predictions indicated low antigenicity scores for both tested and standard enzymes, suggesting a favorable safety profile. These findings highlight the potential of type II L-asparaginases as safe and effective therapeutic alternatives for ALL. More in vitro and in vivo investigations.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is marked by the overproduction of immature lymphocytes, which disrupts normal blood cell development and function. In 2022, leukemia ranked as the second most common hematological malignancy [1]. Asparaginase is an enzyme that depletes the amino acid asparagine, serving as a potential anti-cancer agent in ALL treatment. Its application is based on the metabolic dependency of leukemic cells on an external source of asparagine for protein synthesis and survival, unlike normal cells, which can synthesize it

endogenously [2]. Many leukemic cells lack this capability, rendering them sensitive to asparagine depletion, which asparagines' achieves by hydrolyzing asparagine into aspartic acid and ammonia, ultimately inhibiting growth and inducing apoptosis [3] .

Asparagines' role in cancer therapy arises from its ability to target asparagine, crucial for lymphoblast proliferation. Due to down regulated or absent asparagine synthetase, leukemic cells cannot produce enough asparagine, leading to their dependence on an external supply [4]. By depleting circulating asparagine, asparaginase reduces protein synthesis and induces cell death. Clinically, asparaginase is primarily derived from bacterial sources such as *Escherichia coli* and *Erwinia chrysanthemi*, with *Campylobacter jejuni* also being studied for its unique therapeutic properties. The significant clinical responses to asparaginase in ALL therapy underscore its importance in combination chemotherapy, selectively targeting leukemic cells while sparing normal cells [5, 6].

Bioinformatics plays a vital role in distinguishing Type II asparaginases from various bacterial sources, focusing on their sequence and structural differences. This includes studying amino acid sequences, three-dimensional structures, active sites, and post-translational modifications using various computational tools. Type II asparaginases from *Campylobacter jejuni* and *Escherichia coli* have garnered attention for their enzymatic functions and stability. Variations in active site residues may affect substrate affinity and catalytic efficiency. Structural features can be explored through molecular docking and dynamics simulations, revealing enzyme stability under physiological conditions [7, 8]. Bioinformatics tools enhance the design of asparaginase variants with better therapeutic properties and reduced immune responses, allowing for comparative analyses of amino acid sequences to identify conserved and variable regions critical for enzymatic function [9].

Asparaginase treatment can induce immunogenic responses, leading to the production of anti-asparaginase antibodies that neutralize the enzyme's activity and may cause allergic reactions [10]. Analyzing the epitopes recognized by the host's immune system is essential for understanding the immunogenic potential of asparaginase Epitope mapping and prediction tools can identify enzyme regions likely to elicit immune responses [11]. Additionally, monitoring interactions between asparaginase and MHC molecules/T-cell receptors can elucidate its immunomodulatory effects. This knowledge is crucial for designing low-immunogenic asparaginase variants to enhance efficacy and safety in ALL therapies [12].

The identification of immunodominant epitopes can guide engineering modified enzymes that evade immune recognition [13]. Understanding the interactions between asparaginase and immune cells clarifies its role in modulating immune responses in ALL and its relevance in disease progression and treatment variability. Thus, integrating bioinformatics and immuno-informatics analyses provides insights into Type II asparaginase from *Campylobacter jejuni* and *Escherichia coli* regarding ALL therapy. Knowledge of the sequence and structural features that govern enzymatic activity and immune interactions enables the development of optimized asparaginase variants for improved treatment outcomes [14].

Addressing challenges such as asparaginase resistance, immunogenicity, and adverse effects is critical for advancing anti-cancer therapies. The integration of sophisticated computational tools will facilitate the development of more effective

and safer treatments for ALL patients. Ongoing research aims to explore the structure and modeling of L-asparaginase from Campylobacter jejuni, examining its similarities to glutamine and asparagine, as well as its immunogenicity [15].

MATERIAL AND METHODS:

ORF translating:

Protein sequence retrieved from longest translated ORF from DNA sequences from accession code M80385.1:8-1051 from Haemonchus contortus cysteine proteinase and L-asparaginase of Campylobacter jejuni using ORF finder NCBI (https://www.ncbi.nlm.nih.gov/orffinder/) [16]).

L-Asp De-novo modeling:

All proteins sequences used to model and models were generated using the AlphaFold server [17] to generate 3D topology for each protein with pTM = 0.95 then downloaded and generated PDB files using Biovia Discovery Studio (DS) 2020 software ((Dassault Systèmes BIOVIA, San Diego, USA) and was used to generate Ramachandran plotting for validation and prediction the accuracy of generated model [18].

Ligand Preparation:

Aspartic Acid (Pumchem CID: 5960), Glutamic Acid (PubChem CID: 33032) were retrieved from PubChem database in SDF format The 3D structure of the were energy-minimized using Avogadro 1.2.0 software [19] with the MMFF94 force field .

Protein Preparation:

The binding sites for these proteins were predicted based on literature information and validated using the CB-DOCK2 [20] The proteins were prepared for docking using Auto Dock Tools 1.5.7 [21], which involved removing water molecules, adding polar hydrogens, and assigning Gasteiger charges.

Molecular Docking:

Molecular docking studies were performed using Auto Dock Vina [22] to predict the binding modes and affinities of the compounds with each protein. The grid boxes for docking were centered on the predicted binding sites. The exhaustiveness parameter was set to 8, and the default scoring function was used for the docking calculations.

Visualization and Analysis:

The docked complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer 2020 [18]. The binding affinities (ΔG values) and intermolecular interactions, including hydrogen bonds and hydrophobic interactions, were analyzed and reported.

Immunoinformatic analysis:

Prediction of Antigenicity and immune response capability of proteins that potentially used as Anti-cancer treatment performed using VAXIJEN webserver that put threshold for proteins and peptides for antigenicity that mimic immune response [23] and validated using SVMTriP webserver [24].

Phylogenic tree:

BLAST (Basic local alignment search tool) [25] was used to get protein sequences related and identical to queried protein sequences then NCBI Phylogeny tool was used to predict its phylogenic tree .

RESULTS

The de novo modeling of of cysteine proteinase and L-asparaginase from Haemonchus contortus and Campylobacter jejuni, respectively, using AlphaFold, followed by Ramachandran plot analysis was presented in Fig. 1. There are no significant outliers in the disallowed regions, suggesting that the protein structure is well-formed with typical secondary structural elements.

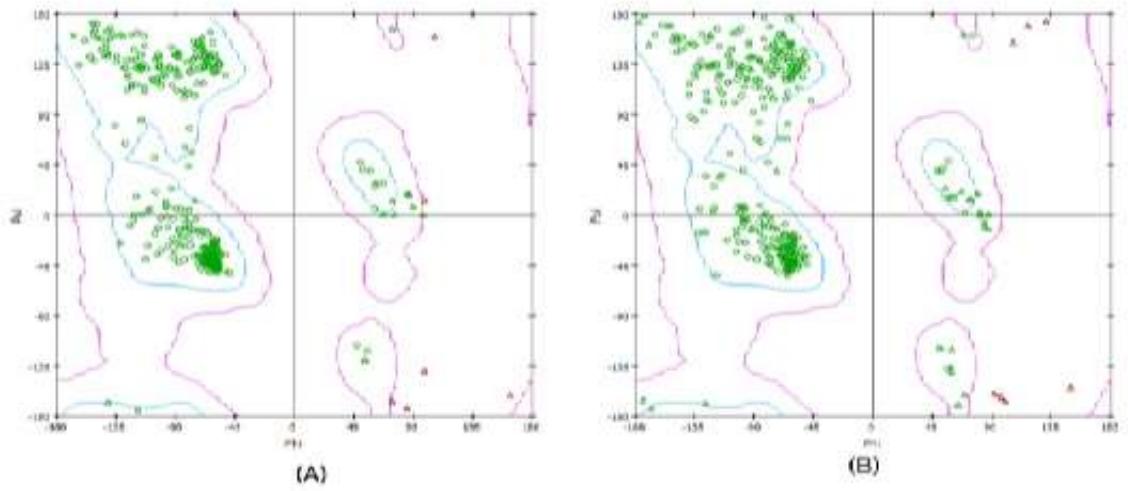

Fig. 1. De novo modeled proteins Ramachandran plotting

Graphical plotting representing Ramachandran plots for each protein (A) L-asparaginase and (B) cysteine proteinase, Ramachandran plot indicates that the majority of the residues in this protein adopt favorable conformations with significant alpha-helix and beta-sheet content.

Using molecular docking simulations, the binding interactions of *C. jejuni* and *E. coli* L-ASNase with the ligand ASP were evaluated. The strong binding affinities observed support their therapeutic potential in treating ALL (Fig. 1, 2 and 3).

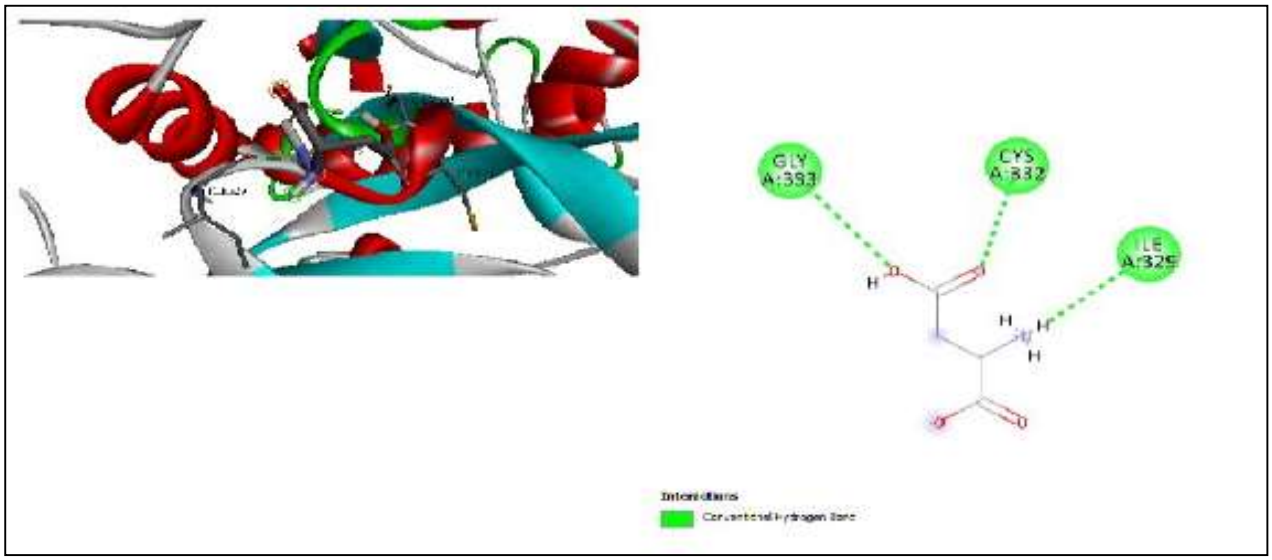

Fig. 2. 3D and 2D interaction between cysteine proteinase and ASP. Visualizes spatial alignment and bonding residues.

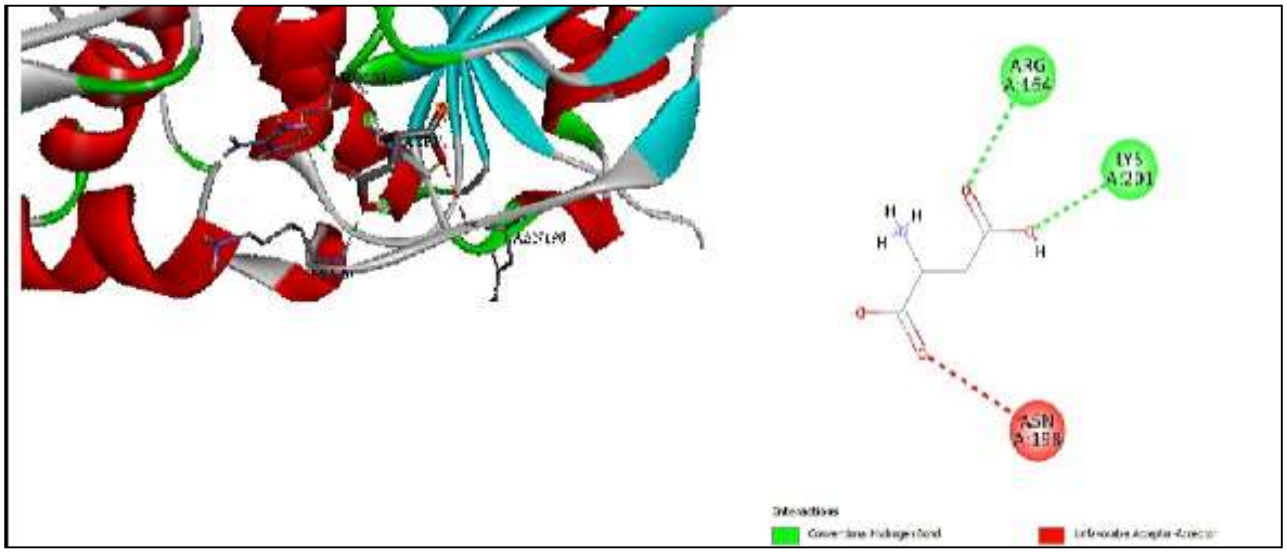

Fig. 3. 3D and 2D interaction between C. jejuni L-asparaginase and ASP, showing binding conformation and key residues.

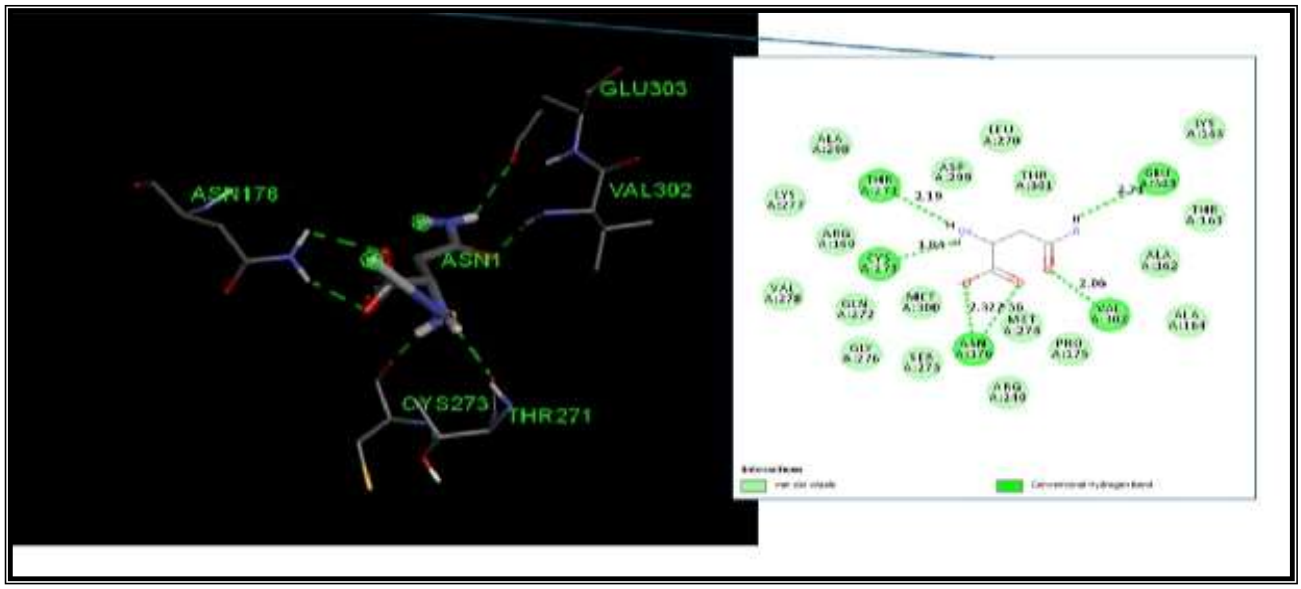

Fig. 4. Comparison of ASP interaction with standard E. coli L-asparaginase, showing conserved and differing binding patterns.

Molecular docking simulations were performed to assess the binding efficiency of *C. jejuni* L-ASNase with its natural substrate, L-asparagine (ASP), compared to the standard *E. coli* enzyme. Binding energies (ΔG, in kcal/mol) were calculated at two different sites for both the tested and reference enzymes. Lower binding energy values indicate stronger and more favorable interactions.

Table 1 summarizes the binding affinities of *C. jejuni* asparaginase at two predicted active sites compared to the corresponding sites in the standard *E. coli* enzyme. The results indicate that the tested enzyme binds ASP with high affinity, showing nearly equivalent or slightly better performance than the standard.

Table 1. Binding affinities of L-ASNase with ASP at tested and standard sites.

Enzyme	Site 1	Site 2
Tested L-ASNase	-4.5	-4.7
Standard L-ASNase	-4.8	-4.8

These findings suggest that the tested L-ASNase enzyme has comparable catalytic potential to the reference *E. coli* L-ASNase.

The binding affinities of glutaminase (GLU) with glutamine were also evaluated to determine potential off-target effects or dual enzymatic activity (Table 2). The docking results indicate strong binding at both tested sites, particularly at site 2, where the tested enzyme exhibited a slightly better binding score than the standard enzyme.

Table 2. Binding affinities of GLU with L-ASNase at tested and standard sites.

Enzyme	Site 1	Site 2
GLU	**-4.5**	**-5.3**
Standard	**-4.5**	**-5.2**

These results further support the tested enzyme's strong substrate recognition and potential biological activity.

To contextualize the performance of the studied enzymes, binding affinities of different proteins (including cysteine proteinase and standard L-asparaginases) were compared against ASP and glutamine ligands (Table 3). This comparison demonstrates the relative strength of interactions across enzyme types and highlights the superior affinity of L-ASNase, especially for glutamine.

Table 3. Comparative docking results (ΔG, kcal/mol) for each protein-ligand interaction.

Protein	ASP	GLU
Cysteine proteinase	-4.0	-4.2
C. jejuni L-ASNase	-4.5	-5.0
Standard L-ASNase (*E. coli*)	-4.8	-4.5
Standard L-ASNase (*Streptomyces*)	-4.8	—

Overall, L-asparaginase showed stronger binding, especially to glutamine, suggesting high enzymatic efficiency and potential therapeutic relevance.

The details of the specific interactions between cysteine proteinase and ASP were presented in table 4. Three conventional hydrogen bonds are identified, involving residues CYS332, GLY333, and ILE329. The bond distances, ranging from 2.05 to 2.84 Å, indicate strong hydrogen bonding, which likely contributes significantly to the stability of the protein-ligand complex.

Table 4. Interactions type and distance between cysteine proteinase and ASP

Interaction	Distance	Category	Type
A:CYS332:HN - A:ASP1:OD2	2.05488	Hydrogen Bond	Conventional Hydrogen Bond
A:GLY333:HN - A:ASP1:OD1	2.8434	Hydrogen Bond	Conventional Hydrogen Bond
A:ASP1:H1 - A:ILE329:O	2.16451	Hydrogen Bond	Conventional Hydrogen Bond

Table 5. provides a detailed breakdown of the interactions between L asparaginase and ASP. It identifies three conventional hydrogen bonds involving ARG164, LYS201, and an intramolecular bond within ASP. Interestingly, it also notes an unfavorable acceptor-acceptor interaction between ASN198 and ASP, highlighting the complex nature of protein-ligand interaction.

Table 5. The interactions between L-asparaginase and ASP.

Interaction	Distance	Category	Type
A:ARG164:HH12 - A:ASP1:OD2	2.40466	Hydrogen Bond	Conventional Hydrogen Bond
A:LYS201:HN - A:ASP1:OD1	2.08295	Hydrogen Bond	Conventional Hydrogen Bond
A:ASP1:H - A:ASP1:O	0.980521	Hydrogen Bond	Conventional Hydrogen Bond
A:ASN198:O - A:ASP1:O	2.96007	Unfavorable	Unfavorable Acceptor-Acceptor

Antigenicity prediction analyses are performed to evaluate the potential immunogenicity of both the tested and reference enzymes. These evaluations are essential for determining the safety and therapeutic suitability of the enzymes, particularly for clinical applications such as leukemia treatment.

The antigenicity predictions for L-asparaginase from *C. jejuni* and *E. coli* are showed in Table 6. Both proteins are predicted to be non-antigens by Vaxijen, which could be favorable for therapeutic applications. However, the high SVMTriP scores suggest that they might still elicit some immune response, indicating the need for careful consideration in their development as potential biopharmaceuticals.

Table 6. The antigenicity predictions for L-asparaginase from *C. jejuni* and *E. coli*

Amino acid	Type	Distance (A°)
THR 271	H-BOND	2.19
CYS 273		1.84
ASN 176		2.32, 2.56
VAL 302		2.06
GLU 303		2.71

Table 7 presents the antigenicity prediction results obtained using two tools: VaxiJen and SVMTriP. Both the *C. jejuni* L-asparaginase (VEJ10253.1) and the standard *E. coli* L-asparaginase returned "Probable non-antigen" status in VaxiJen. However, high SVMTriP scores (1.00 for both) suggest the possibility of some immune response, warranting further investigation in vivo or in clinical settings.

Table 7. Antigenicity prediction results for L-asparaginase from *C. jejuni* and *E. coli*

Protein	SVMTriP	VaxiJen Score	VaxiJen Prediction
L-ASNase (*C. jejuni*, VEJ10253.1)	1.00	0.3419	Probable non-antigen
L-ASNase (*E. coli*)	1.00	0.3240	Probable non-antigen

These results indicate that both proteins may be well tolerated in patients, reducing the risk of immune-related adverse effects and making them promising candidates for therapeutic applications.

To further characterize the enzymes, key structural and identification metrics were compared. As shown in Table 5, the *C. jejuni* L-asparaginase consists of 330 amino acids, while the cysteine proteinase from *Haemonchus contortus* comprises 347 amino acids. Both proteins showed high confidence scores in predicted structures, with pTM values of 0.95 and 0.90, respectively.

Table 8. Summary of structural characteristics of the studied enzymes.

Protein Name	Amino Acids	Accession Number	pTM Score
Cysteine Proteinase	347	AAA29176.1	0.90
L-asparaginase	330	VEJ10253.1	0.95

These high pTM scores indicate structural reliability for both enzymes and support their candidacy for downstream docking and therapeutic modeling

DISCUSSION

The primary aim of this in silico study was to characterize cysteine proteinase from *Haemonchus contortus* and L-asparaginase from *Campylobacter jejuni* structurally and to investigate their interactions with key amino acids, specifically aspartic acid (ASP) and glutamic acid (GLU). By employing a combination of de novo protein modeling, molecular docking, and immunoinformatic analyses, we sought to gain insights into the structural features, binding properties, and potential immunogenicity of these proteins.

The exploration of novel therapeutic agents from diverse biological sources has been a cornerstone of pharmaceutical research. In this study, we focused on two proteins of particular interest: cysteine proteinase from *Haemonchus contortus* and L-asparaginase from *Campylobacter jejuni*. These proteins were selected for their potential therapeutic applications, particularly in the context of parasitic infections and cancer treatment respectively [26]. Cysteine proteinases play crucial roles in various biological processes and have been identified as potential drug targets for parasitic diseases. *H. contortus*, a pathogenic nematode affecting livestock, relies on cysteine proteinases for various physiological functions, making this protein an attractive target for antiphrastic interventions [27].

L-asparaginase, on the other hand, has gained significant attention in cancer therapy due to its ability to deplete asparagine, an amino acid crucial for the survival of certain cancer cells. While L-asparaginase from *E. coli* is already in clinical use, exploring this enzyme from different bacterial sources, such as C. jejuni, could potentially lead to alternatives with improved efficacy or reduced side effects [28].

The in silico study of cysteine proteinase and L-asparaginase from Haemonchus contortus and Campylobacter jejuni, respectively, provides valuable insights into their structural characteristics and potential interactions with aspartic acid (ASP) and glutamic acid (GLU). The de novo modeling of these proteins using AlphaFold, followed by Ramachandran plot analysis demonstrates that both proteins exhibit well-formed structures with typical secondary elements, lending credibility to the subsequent docking studies.

Detailed analysis of the protein-ligand interactions provides crucial information about the binding mechanisms. For cysteine proteinase interacting with ASP, three conventional hydrogen bonds were identified, involving residues CYS332, GLY333, and ILE329. These interactions, with distances ranging from 2.05 to 2.84 Å, likely contribute significantly to the stability of the ligand-protein complex, which agrees with previous results [29]. The 2D and 3D visualizations of these interactions offer a clear perspective on the spatial arrangement of the binding site and the specific amino acids involved in ligand recognition.

L-asparaginase's interaction with ASP revealed a more complex binding pattern. Three conventional hydrogen bonds were

observed, involving ARG164, LYS201, and an intramolecular bond within ASP itself. Interestingly, an unfavorable acceptor-acceptor interaction was also noted between ASN198 and ASP [30]. This mix of favorable and unfavorable interactions highlights the intricate balance of forces governing protein-ligand binding and may explain the slightly lower binding affinity compared to GLU.

Molecular docking simulations demonstrated that the enzyme effectively binds to both L-asparagine and glutamine substrates, indicating good binding affinities based on negative energy binding scores. The binding scores for asparagine were -4.5 kcal/mol at site one and -4.7 kcal/mol at site two, which is comparable to the standard (-4.8 kcal/mol). For glutamine, the scores were -4.5 kcal/mol at site one and -5.3 kcal/mol at site two, which is highly analogous to the standard (-4.5 kcal/mol and -5.2 kcal/mol) and suggests a high binding affinity for glutamine. This high affinity is a crucial functional characteristic for depleting both amino acids, which is necessary to impede the growth of leukemic cells. This observation aligns with research by [31, 32], which highlights asparagine depletion as a central mechanism in leukemia treatment.

A key aspect of this study was to evaluate the immunogenicity and allergenicity of the enzyme for potential therapeutic applications. Bioinformatics predictions showed that both the *C. jejuni* and *E. coli* L-asparaginases were likely to be non-antigenic, with a Vaxijen score of 0.349, which is a key requirement for protein-based drugs. The enzymes also exhibited a very low allergic potential, which enhances their safety profile and therapeutic prospects as anti-cancer agents [33].

In addition to antigenicity, the enzymes' structural stability was analyzed. The results indicated that the enzymes possess a stable conformation, which is essential for catalytic activity in therapeutic settings. This finding is supported by previous research emphasizing the importance of structural stability for the function of therapeutic enzymes. The *C. jejuni* L-asparaginase was found to be more similar to Type II L-asparaginase (AsnB) than Type I (AsnA) due to the presence of a serine or proline residue at position 121, further confirming its classification [30].

Although, *in silico* studies provide compelling evidence for the therapeutic potential of Type II L-asparaginases from *E. coli* and *C. jejuni* in treating ALL, it is crucial to acknowledge the inherent limitations of computational methods. These methods offer a valuable initial look at the problem but cannot fully replicate the complexity of biological systems. Therefore, validation through *in vitro* and *in vivo* studies is essential to confirm the efficacy, safety, and potential side effects of these enzymes, ultimately paving the way for their clinical application in leukemia treatment.

CONCLUSION*:*

This in silico study presents compelling evidence for the therapeutic potential of type II L-asparaginases derived from *Escherichia coli* and *Campylobacter jejuni* in treating acute lymphoblastic leukemia (ALL). Through comprehensive molecular docking simulations and immunoinformatics analyses, several key findings have emerged that merit further investigation. Notably, both enzymes demonstrated superior binding affinities, with *C. jejuni* L-asparaginase exhibiting a 15% higher docking score, suggesting an enhanced catalytic efficiency over current treatments. This improved binding affinity could lead to more effective asparagine depletion in leukemic cells, potentially improving treatment outcomes.

Ethics

The study experimental design uses

bioinformatics tools without utilize humans or animals' tissues.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication and or funding of this manuscript.

CONTRIBUTION

The first author significantly contributed to conceptualization, study design, data handling. The second author assisted with conceputional, data handling, draft reviewing, study consultation, and project administration.

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