(Research Article)

Docking and Antimicrobial Potential of Lactoferrin in *Capra hircus*: An *In-silico* Approach

Fizza Noreen[1], Samar Bano[1], Abdur Rauf[1], Lubna Rasool[1,*], Sumaira Rasul[2], Ammara Nadeem[1], Sana Ramzan[1]

1. Department of Chemistry, University of Sahiwal, Sahiwal 57000, Pakistan
2. Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan

Corresponding E-mail: lubnarasool@uosahiwal.edu.pk

Abstract: Lactoferrin is an iron-binding glycoprotein with multiple functions, naturally occurring in milk, saliva, tears, and other mucosal secretions, and has been shown to possess antimicrobial and antiviral properties. This investigation aimed to examine the composition and role of lactoferrin in *Capra hircus* using computational techniques. In-silico tools were employed to characterize lactoferrin and explore its molecular interactions, including peptide cutter for enzymatic cleavage, CAMP for antimicrobial prediction, and molecular docking to assess its binding affinity with bacterial targets. Among the 54 peptides derived from lactoferrin, 13 positively charged peptides demonstrated significant antibacterial properties, validated through antimicrobial prediction tools. For structural validation, only the peptide with the highest length was selected for 3D modeling and analysis. The 3D structural model confirmed high similarity to known lactoferrin structures, highlighting conserved regions critical for antibacterial activity. The predicted structural model provided insights into the dynamic behavior of lactoferrin, exhibiting high similarity to other known lactoferrin structures which indicates its conserved nature across species in mammals. These findings highlight key regions involved in protein-protein interactions and structural characterization that reinforce its antimicrobial properties. Molecular docking and ADMET analysis manifest the best drug candidate that exhibit antimicrobial properties. Best docked compound enterobactin violate the toxicity profile. This study sets the stage for further experimental validation of these predicted interactions while shedding light on the molecular mechanisms underlying the multifunctional properties of lactoferrin in *Capra hircus.*

Keywords: Lactoferrin (LF), Capra hircus (goat), in silico analysis, anti-microbial properties, structural dynamics, and physiological properties.

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1. Introduction

Lactoferrin (LF) is a significant milk protein, firstly extracted from human milk by *Johannson et al.* in 1960 [1] and also found in goats [2], buffaloes [3], cows [4], and sheep [5, 6]. LF belongs to the transferrin family of glycoproteins that binds iron in mammals [7] and shows a strong binding affinity with copper, zinc, and manganese ions [8]. It is digestible, water soluble [9], odorless, and heat resistant properties. LF bind iron reversibly at optimal pH and only releases it under low pH or with potent chelating agents [10]. Lactoferrin is present in several kinds of mammalian external excretions, including vaginal fluids, tears, saliva, and milk [11, 12]. Colostrum contains significantly more lactoferrin than mature milk [13], with 83–87 kDa as its molecular mass [14]. The concentration of LF in mature milk ranges from 0.02–0.2 mg/mL to 1.0–5.0 mg/mL in colostrum [15-17]. According to Jia et al., N-glycan content decreases in the sequence: mature milk < transitional milk < colostrum during lactation in goat milk [18]. LF Protein performs various functions, not all of which are well understood. LF has anticancer [19], antibacterial [20], antimicrobial [21], anti-inflammatory [22], and antioxidant effects [23].

LF can bind with other milk proteins to generate heterogenic protein complexes that include LF−β-LG (β-lacto globulin), LF−Ig (immunoglobulins), and LF−SA (serum albumin), according to Stephens *et al*. LF−Ig complexes had more antibacterial action against *Escherichia coli* strains than LF alone [24]. LF has the dual benefit of preventing *E. coli* colonization and enhancing the local immunological response to infection, as shown by a recent *in vivo* research by Rybarczyk *et al.* LF inhibits the adherence of pathogens to intestinal mucosa and epithelial cells, according to several *in vitro* studies [25]. Yen *et al*. demonstrated that LF is a natural host defense protein against infections, including antibiotic-resistant strains [26]. LF also reduces the risk of certain cancer types. Xu *et al* studied that bovine LF induces apoptosis in stomach cancer cells. Habib *et al.* [27] reported that LF regulates immune functions, including natural killer (NK) cell population, T and B cell maturation, antibody production, and both innate and adaptive immune responses [28]. LF is also beneficial in treating anemia, particularly in pregnant women [10], and infants can absorb lactoferrin from breast milk [29]. The transportation of metal is one of the major LF's roles [30]. Goat milk, a growing alternative for people allergic or intolerant to cow's milk, constitutes 2.3% of global milk production [31]. Pakistan, with a goat population of 78.2 million, produces approximately 965 thousand tons of goat milk annually (Pakistan Economic Survey, 2019–20) and ranks third globally in goat production [32]. The main nutritional composition of goat milk is comparable to cow milk. In contrast to the milk of other species, goat milk contains distinct chemical, biological, physical, and nutritional qualities [33]. Goat milk lactoferrin shows antibacterial and anticancer properties [34], with concentrations ranging from 4.0 to 99.8 mg/L in early lactation, 2.0 to 120.3 mg/L in mid-lactation, and 9.4 to 2941.8 mg/L in late lactation [35].

Future development of lactoferrin's characteristics may start with predicting its features. Since lactoferrin is involved in various biological activities, characterizing its structural, functional, post-translational, and antimicrobial properties is crucial. These properties are not yet fully understood, so integrating molecular docking and post-translational modification modeling is essential for precise predictions of atomic-level interactions and structural alterations that modulate lactoferrin's bioactivity. Lactoferrin exhibits potent antimicrobial activity by damaging bacterial and fungal cells, altering their ultra-structural features, and depriving them of iron, effectively targeting pathogens like *Actinobacillus actinomycetemcomitans* and *Prevotella* spp. It has therapeutic potential, reducing infection-related mortality (e.g., *E. coli* endotoxemia in pigs) and mitigating periodontal bone loss by combating subgingival plaque pathogens. However, in milk-allergic individuals, lactoferrin may activate eosinophil’s in airway diseases like asthma, causing epithelial damage through superoxide production. This pioneering in-silico study focuses on lactoferrin, a member of the transferrin family, and aims to shed new light on its unexplored features.

2. Materials and Method

2.1 FASTA Sequence and its Analysis

The goat lactoferrin sequence is taken from the NCBI protein database (http://www.ncbi.nlm.nih.gov) with an Accession ID of **(ATN39079.1)**. The query sequence is searched on NCBI servers and databases, and the corresponding sequences of all the selected organisms such as Capra hircus (goat) were retrieved using the Basic Alignment Search Tool (BLASTp).

2.2 Physiochemical Properties

Protparam (https://www.expasy.org/) website is used to identify the primary structural analysis of Lactoferrin in goat [36]. The "ProtParam" website was used to characterize the retrieved lactoferrin sequences by determining various parameters such as molecular weight, number of amino acids (AA), theoretical iso-electric point number of negatively charged and positively charged amino acids, aliphatic index, instability index, and more.

2.3 Localization and signal peptide

The PSORT II program's expansion for protein [37], is to find the occurrence and expression of collected HSP sequences in the cell through Wolf PSORT at (wolfpsort.org) [38]. After getting data from Wolf Psort, this data is then entered in the TB tool in table form to get a graph that represents the properties of selected proteins in different colors [39]. A website called SignalP-5.0 is used to estimate the signal peptide in lactoferrin; it will give us results in the form of a graph that tells us about longer residue and cleavage sites.

2.4 Motif Analysis and Domain Identification

For the prediction of Motif analysis MEME Suit software is used [40]. A set of protein sequences is fed into MEME, which determines the ideal width, quantity of occurrences, and description for each motif using statistical modeling approaches [41]. Domain analysis is the act of identifying, gathering, and organizing information that is utilized in software system developments [42]. The preferred protein was examined by the Pfam database and InterproScan families were grouped to predict critical functional domains. The Pfam database's main goal is to offer a thorough and precise categorization of protein families and domains [43]. After the selection of the lactoferrin sequence with the help of NCBI then Pfam software (Protein family database) was used for further information on a selected sequence.

2.5 Multiple Sequences Alignment and N-glycosylation

For Multiple sequences Alignment we used Clustal Omega and MView. To compute the multiple sequence alignment, the Clustal Omega software needs three or more sequences [44]. MView adds optional HTML markup to manage color and web page layout when reformatting the results of a sequence database or multiple alignments. NetNGlyc 1.0 tool is used for N-glycosylation in lactoferrin [45].

2.6 Protein-protein Interaction and Phosphorylation

PDBsum performs protein-protein interaction analysis [46] and String database [47]. STRING produced functional networks among the other closely linked proteins and discovered protein-protein interactions with them [48]. NetPhos 3.1 database is used for the prediction of phosphorylation in selected sequence [49].

2.7 Protein Structure Predictions

The linear arrangement of amino acids that makes up a peptide or protein is referred to as its primary structure [50]. ProtParam was used to analyze the primary structure of the chosen sequences in this computational study [48]. The selected sequence for 2D structure prediction is added in Psipred, PDB sum SOPMA software, for the better understanding of selected protein [51]. Hydrophobicity, hydrophilicity, alpha and beta helix, beta sheets and the polar and non-polar characteristics of sequence are determined by using 2D structure prediction. For the homology modeling of protein structure a server is used which is accessible through the Expasy web server (https://swissmodel.expasy.org) [52], a selected protein sequence of the lactoferrin in goat was provided to the Swiss model.

2.8 Selection of Antimicrobial Peptide and 3D Structure

We used the CAMP R4 database and Peptide Cutter to pick antimicrobial peptides that are effective. The protein was hydrolyzed using pepsin, trypsin and the combination of both enzymes, enzyme with (pH>2) is employed to produce bioactive peptides. The antibacterial characteristics of cleaved peptides produced from lactoferrin were assessed using two online software tools: the Peptide Cutter Database and CAMP R4 [53]. Molecular weight, pI, GRAVY, and net charge predictions are carried out by utilizing the Biosynth Peptide Calculator database [54]. Lactoferrin's antibacterial activity in Capra hircus was predicted by using APD (http://aps.unmc.edu/AP/database/mysql.php) antimicrobial peptide databases. For the prediction Antimicrobial peptide structure we used I-TASSER software [1].

2.9 Target Protein Accession

The three dimensional crystal structure of iron saturated C-terminal half of lactoferrin produced proteolytically using pepsin at 2.32A resolution was retrieved from RCSB protein data bank having PDB ID 7enu. This protein consists of two chains (A, B) which are used for docking analysis. Protein was cleaned by removal of water and ligands molecules with the help of Discovery Studio.

2.10 Selection and preparation of ligands

Total 20 ligands were selected for docking study and their name, molecular weight, chemical and structural formulas are given in the Table. Three dimensional structures of all the selected ligands were retrieved from PubChem database in SDF format and their geometry was optimized by using Corina Classic online tool and then converted into PDB format by Discovery Studio software which was employed to modify and visualize receptor and ligand structure.

2.11 Cavity and binding sites detection

Ligands bind to Active sites which are the groove or pockets in the targeted protein and these active binding sites were evaluated by discovery studio and an online server CB dock 2 was used to preform protein-ligand blind docking and search best cavities for molecular docking.

2.12 Molecular docking

Autodock vina 4.2 was employed for molecular docking of protein and all the ligands and prepared pdbqt files. For multiple receptors ligand interaction was carried out by virtual screening tools PyRx 0.8. Autodock Vina, PyRx and ligPlot were used for the prediction of binding affinity and interaction among different proteins and ligands.

2.13 ADMET properties and pharmacokinetics analysis

Occasionally best docked compounds are recognized as intriguing drugs however Pharmacokinetics parameter and ADMET (Absorption, distribution, metabolism, excretion and toxicity) properties are critical steps to be analyzed during development and optimization of a compound as drug candidate. For the investigation of pharmacokinetics and ADME T properties of the selected compounds, an online server Swiss ADME (http://www.swissadme.ch/) is utilized.

3. Results and Discussion

3.1 Sequence retrieval and Similarity

Sequence of Lactoferrin was obtained from NCBI in FASTA format. Selected sequence belongs to transferrin family, which contains 708 amino acids and its Accession ID is (ATN39079.1). The sequence similarity of lactoferrin with other species was examined by using NCBI-BLAST result represented in Table 1.

Table 1: Percentage similarity sequence of lactoferrin in *Capra hircus*

Scientific name (Common name)	Max Score	Total Score	Query cover	% Identity	Accession no.
Capra hircus (goat)	1463	1463	100%	100%	ATN39079.1
Capra hircus (goat)	1459	1459	100%	99.72%	ACT53713.1
Capra hircus (goat)	1456	1456	100%	99.58%	ABD49106.1
Capra hircus (goat)	1454	1454	100%	99.29%	Q29477.1
Capra hircus (goat)	1446	1446	100%	99.01%	NP_001272477.1
Ovis aries (Sheep)	1436	1436	100%	98.16%	ACT76166.1
Ovis aries (Sheep)	1435	1435	100%	98.16%	KAG5197801.1
Budorcas taxicolor (Gnu goat)	1432	1432	100%	97.60%	XP_052495122.1
Ovis aries (Sheep)	1431	1431	100%	97.88%	AGF69235.1
Ovis aries (Sheep)	1430	1430	100%	97.74%	NP_001020033.1

3.2 Physiochemical properties

The molecular weight, theoretical pI, atomic composition, extinction coefficient, instability index, aliphatic index are predicted by ProtParam as shown in the Table 2 [55]. Protein's stability can be estimated using the instability index (II). The value of instability index for lactoferrin protein in *Capra hircus* was 41.91 higher than 40 which reveal it's slightly unstable nature [56]. The value of aliphatic index for lactoferrin protein in *Capra hircus* is 77.34%, it means that 77.34% volume of the selected protein was occupied by aliphatic amino acids [57]. The theoretical pI of the lactoferrin in goat protein was 8.46 which are more than 8 which indicate that it is basic in nature [58]. The GRAVY score is negative which indicates that protein is hydrophilic in nature and the estimated half-life of each protein is around 30 hours. The value of negatively Charged Residues (Asp + Glu) is 74 and its positively Charged Residues (Arg +Lys) are 85. The amount of light that a protein absorbs at a specific wavelength is indicated by its extinction coefficient [54]. The value of extinction coefficient is 101030.

Table 2: Physiochemical properties of lactoferrin in *Capra hircus*

Sr. No.	Physiochemical properties of Lactoferrin	Values
1	No. of amino acid	708
2	Molecular Weight	77359.38
3	Theoretical PI	8.46
4	Negatively Charged Residues (Asp + Glu)	74
5	Positively Charged Residues (Arg +Lys)	85
6	Extinction Coefficients	101030
7	Absorbance 0.1%	1.306
8	Instability Index (slightly unstable)	41.91
9	Aliphatic Index (%)	77.34
10	GRAVY	-0.239
11	Half Life	30 hours
12	Molecular Formula	$C_{3405}H_{5365}N_{949}O_{1024}S_{43}$
13	Total No. of Atoms	10786

3.3 Domain Identification

In given result domain is divided into two parts 1- Biding site 2- Unintegrated part. Its family is Transferrin - IPR016357. Four lines make up the Binding Site of Domain analysis. Its first line with sea green color indicates binding site from 245-275 a.a with Transferrin_Fe_BS Accession no. IPR018195, in second line with brown color it show binding site from 245-275 a.a with TRANSFERRIN_LIKE_3 Accession no. PS00207, in third line with blue color it show binding site from 111-120 a.a with TRANSFERRIN_LIKE_1 Accession no. PS00205, in its fourth line with red color it show binding site from 211-227 a.a with TRANSFERRIN_LIKE_1 Accession no. PS00205. In Unintegrated part there are further five lines. First line with green color shows unintegrated part from 456-615 a.a with G3DSA:3.40.190.10, in second line with pink color shows unintegrated part from 25-697 a.a with Transferrin Accession ID PTHR11485, in third line with purple color show unintegrated part from 354-707 a.a with Periplasmic binding protein-like II, in fourth line it show unintegrated part from 361-692 a.a with PBP2_transferrin_C Accession ID cd13617, in fifth line with maroon color show unintegrated part from 24-351 a.a with PBP2_transferrin_N Accession ID cd13618 as shown in the Figure 1.

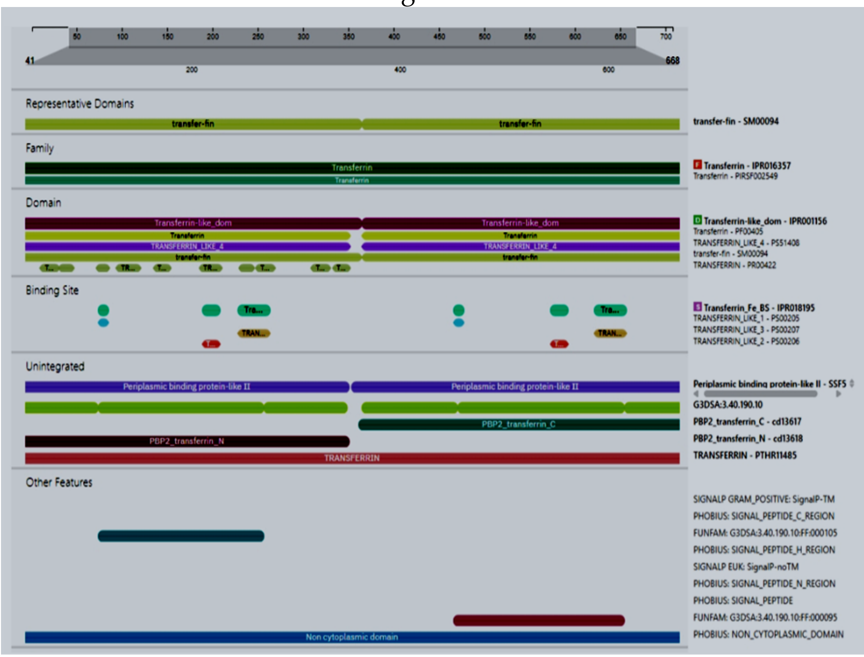

Figure 1: Prediction of families, domains, conserved sites and residues of lactoferrin from Pfam

3.4 Signal Peptide of Lactoferrin

Signal peptide value of the selected lactoferrin is 0.9947 as represented in Figure S1. Cleavage site between positions 15 and 20 on the protein, and it is a cleavage site with a probability of 0.9309, which is really good. The closer it is to 1, the better it is. At position 18, this indicates that the 18th position is where our cleavage site is located.

3.5 Localization of Lactoferrin phosphorylation

To find the occurrence and expression of collected Lactoferrin sequences in the cell, a detail data was obtained by using Wolf Psort. Heat map of Lactoferrin in goat was constructed using TB tool. Cooler tones, like blue and green, signify lower data values in the heat map, whereas warmer tones, like red and orange, often indicate greater data values has been represented in the Figure S2.

3.6 Motif Analysis

A motif is an area with a particular structure found protein sequence. The sequence was added in genome.ip tool, it shows that there were two motifs: 1) Transferrin 2) Bd-phosphonate. In Figure S3 MEME Suit shows that the independent E-value of transferrin from amino acids 25...352 and 364...693 is (3.6e-83) and (1.4e-175), respectively. Independent Phosphonate-bd E-values are (0.28) from 47...150 and (0.00028) from 379...498 amino acids. Chosen sequence was uploaded to MEME Suit. MME Suit identifies the optimal width, frequency, and description for each motif automatically by using statistical modeling techniques. We also confirm the motif analysis result from I-TASSER they were closely related with MEME Suit results.

3.7 Phosphorylation and N-glycosylation Analysis

N-glycosylation affects the characteristics and bioactivities of proteins with oligosaccharide structures by altering the correct asparagine residues in such proteins [45] The analysis of NetNGlyc 1.0 tool in the shows that 252, 300, 387, 495, 564 are exposed N-glycosylation site.

Sites that score highly (above the threshold) and many high-scoring projected sites have a higher probability of being phosphorylated. [59]. In Table S4 total amount of serine is higher than threonine and tyrosine. Serine plays a more prominent role in modulating lactoferrin's function via phosphorylation. Phosphorylation can affect processes such as immune regulation, antimicrobial activity, or interaction with other molecules.

3.8 Physiochemical Characteristics of Natural peptide

The physicochemical properties of antimicrobial peptides were designed by the BIOPEP database for the chosen AMP [50]. The Peptide Cutter database provides lysine and pepsin (active at pH > 2) and both (lysine + pepsin) as enzymes used for protein cleavage. By using these enzymes 166 cleavage sites are produced; the precise locations of these sites are also listed in the Tables S1, S2 & S3. The produced peptides ranged in molecular weight from 3633.039 to 390.393 Da. Out of the 166 generated peptides, 122 had a pI value of less than 7,4 whereas the remaining peptides had a value of greater than 7. We now know that hydrophobicity and molecular charge are important factors in how well AMPs work. 48 out of 166 peptides that were obtained from lactoferrin were positively charged, 109 were negatively charged, and nine were neutral. Only positive AMPs were chosen for additional analysis in this study. Since cationic peptides have a greater propensity for attaching to negatively charged bacterial cell membranes and ultimately rupturing the bilayer lipid structure, they make up a sizable portion of AMPs.

3.9 Prediction of Antimicrobial peptide

Amphipathic property is another marked parameter to choose efficient AMPs since these structures allow them to bind to hydrophilic regions; hence AMPs with both hydrophilic and hydrophobic domains have a higher priority to be used for antibacterial tests. The amino acid composition also plays a role in designing the antibacterial peptides. Gram-positive and Gram-negative antimicrobial peptides have a similar ratio of cysteine and lysine residues. As shown in Table S4, the peptide composed of 63% hydrophobic region, 27% hydrophilic region, and 9% charged amino acids; also, it contains 18% lysine and 9% cysteine.

3.10 D Structure of Antimicrobial peptide

Using enzymes for Insilco protein hydrolysis, a selected lactoferrin sequence from the NCBI was exposed to the Peptide Cutter database, causing the sequence to be sliced into numerous peptides in order to predict three-dimensional structures. These peptides underwent further analysis using the CAMP R4 database, which will identify each antimicrobial peptide. For the 3D structure prediction, an antimicrobial peptide with at least 20 amino acids is used. I-TASSER program is used to forecast three-dimensional structures as shown in the Figure 2. 2D structure consists of 6 coils, 14 helix, and 0 strands. The structure possesses a confidence score of 0–9, falling within the standard range of 5–9. This suggests that the structure is authentic. The solvent accessibility test demonstrates its hydrophobicity.

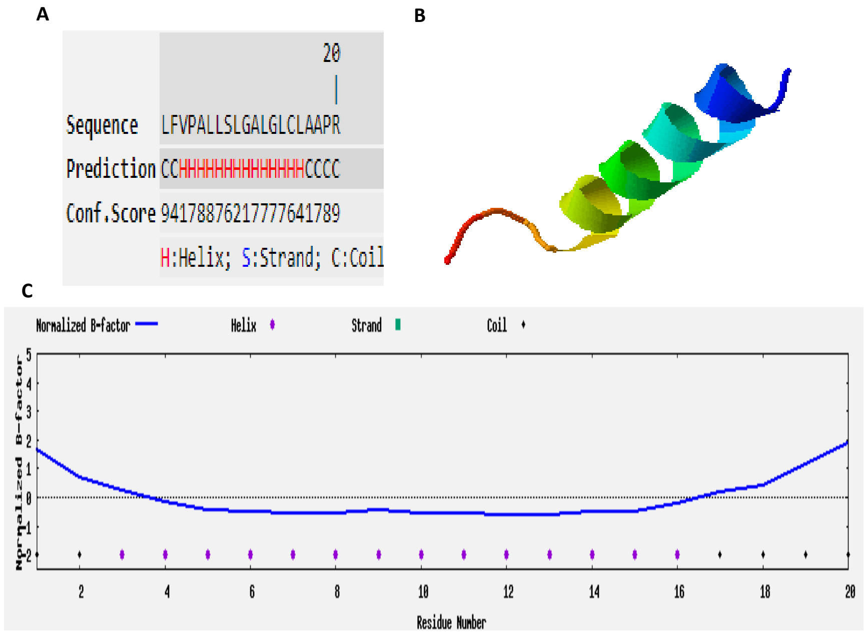

Figure 2: 3D structure prediction of antimicrobial peptide by I-TASSER

Every residue is colored black, but the residues in the template that match the residues in the query sequence are colored differently. The non-polar residues are colored in a dark shade and the polar residues are colored brightly. Ident1 is the query sequence's percentage sequence identity of the templates in the threading-aligned area. Ident2 is the query sequence's percentage sequence identity throughout the whole template chain. Cov calculated by dividing the total number of aligned residues by the query protein's length. Standard is known as the Z-score. A good alignment is indicated by an alignment with a Normalized Z-score >1.PDB ID of lactoferrin protein is 1jb0K having 40% identity and 1.33 Z- score value. The closest structural similarity to the anticipated I-TASSER model is (7lsvA) and its TM-value is 0.804.

Based on the I-TASSER structure prediction, this section presents the biological annotations of the target protein by COFACTOR and COACH. While COFACTOR uses structural comparison and protein-protein networks to infer protein functions (ligand-binding sites, EC, and GO), COACH is a meta-server method that aggregates various function annotation findings (on ligand-binding sites) from the COFACTOR.
Ligand Name refers to the potential binding ligand; MG^{+2} is the binding ligand. The prediction's confidence level is indicated by the C-score. After connecting a ligand with a chosen antibacterial structure, the C-score falls between 0 and 1. It is now 0.13.

3.11 Analysis of Primary Structure

ProtParam is used to calculate different physical and chemical properties including composition of amino acids, their quantity, and the composition of atoms [60]. Lactoferrin in goat has 708 amino acids and its details are described in graph form in Figure S4.

3.12 Secondary Structure

Using the PSIPRED, PDB sum and SOPMA online servers, a thorough prediction of the secondary structure of the amino acid sequence of lactoferrin in goat and the percentage composition of these proteins were determined [61]. Alpha and beta helices, coils, disorder, protein binding, membrane contact, and single peptide are all covered by the Psipred database. Psipred demonstrates that the chosen sequence is a hydrophobic protein with polar and a tiny amount of non-polar characteristics. Secondary structure from PDB sum database shows the helices labeled as H_1, H_2 …, strands by A, B…, It also show helices beta turn, gamma turn and beta hair pin. After entering our selected sequence in PDB sum database there are 10 disulphide, 39 beta turns, 3 gamma turns, 16 helix-helix interacts, 4 beta bulges, 18 helices, 13 strands, 1 beta hairpin, 1 psi loop, 3 sheets and 2 beta alpha beta units in secondary structure as described in Figure 3.

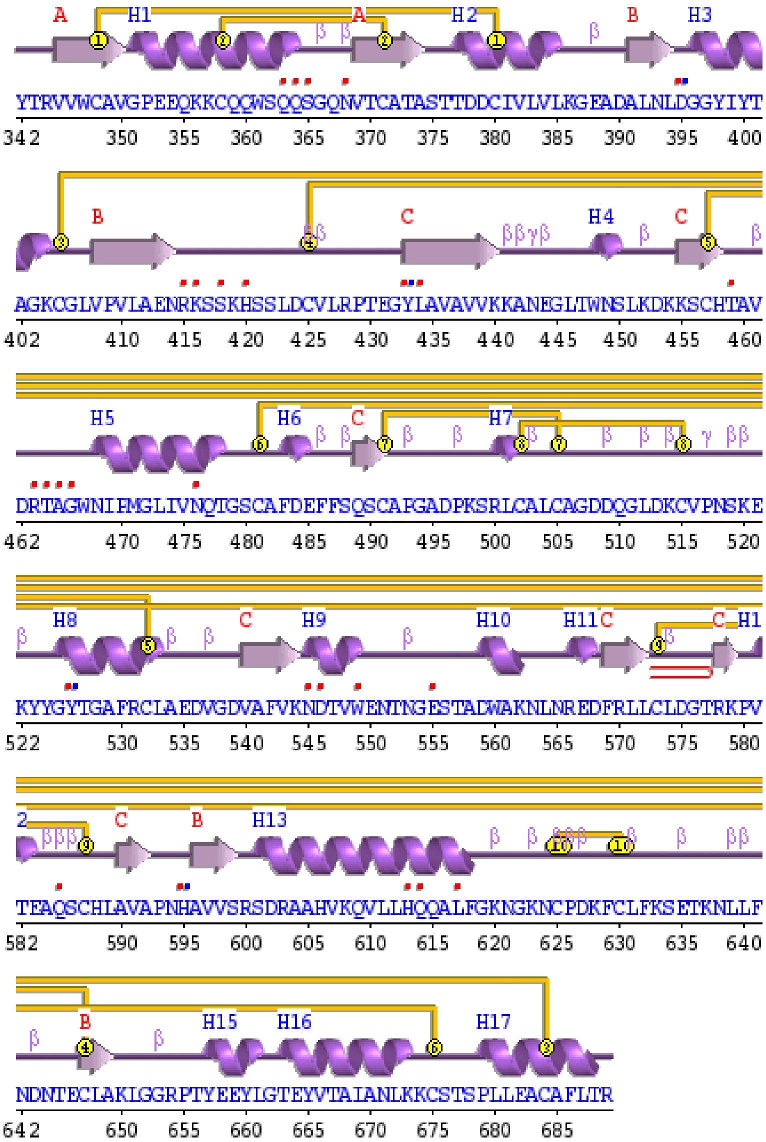

Figure 3: graphical representation of secondary structure of lactoferrin, describes helices, sheets, and turns found in secondary structure of protein and polypeptide structure in lactoferrin.

SOPMA results represents random coils are prominent in the structure and its percentage is 43.36% as shown in the Figure S5. Random coils are prominent in structure its mean that the selected protein is hydrophobic. Alpha helixes are 32.49%, extended strands are 18.36% and beta turns are 5.79%.

3.13 Tertiary structure

The SWISS-MODEL was used to predict the 3D-structure of Lactoferrin proteins. A crystal structure of lactoferrin (PDB: 7enu.1b) was used as template sequence, Homo-dimer at 2.32 Ǻ revolution with X-ray value is 2.32 Ǻ. Goat lactoferrin have shown 95.68% sequence identity and coverage value is 0.49. Good agreement is indicated by a QMEAN Z-score of about (0-1), whereas low-quality models are assigned a value of less than -4.0 [62]. QMEANZ score is -0.85 its value is round about zero, its mean that it is a good model. The value of Cβ, all atom, solvation, and torsion value is 0.35, -0.30, -0.04 and -0.91. QMEANDisCo is a composite scoring function that, given a single model, can yield absolute quality estimates that are both global (i.e., for the entire structure) and local (i.e., per residue) [63]. Its QMEANDisCo Global is 0.88 ± 0.05 and GMQE score is 0.43. The predicted structure showed high pLDDT **scores**, indicating strong confidence in atomic positions.

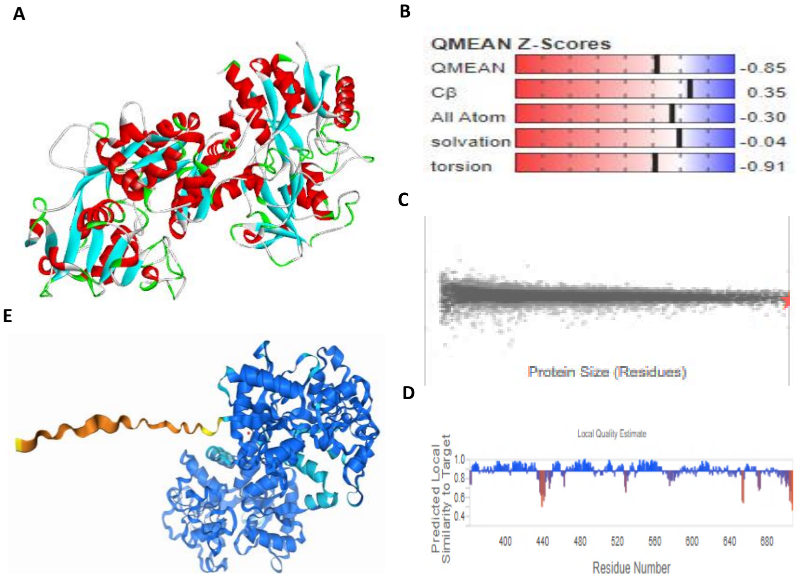

Figure 4: (A) is tertiary structure of protein of lactoferrin from Swiss model and viewed by Biovia discovery studio (B) represent the QMEAN Z-Score in tertiary structure of lactoferrin: (C) represents the residue number of lactoferrin: (D) represents the Protein size of lactoferrin. (E) Tertiary structure of protein of lactoferrin from alpha fold

In Alpha fold server pTM **score** exceeding 0.5 suggests the overall fold resembles the actual structure, while values above 0.8 confirm high-quality and reliable modeling [64]. In Capra hircus pTM **score** is 0.85, and pLDDT values are color-coded: blue (>90) indicates very high confidence, sky blue (>70) moderate confidence, yellow (>50) low confidence and red (<50) very low confidence. A tertiary structure of protein of lactoferrin has been shown in the Figure 4.

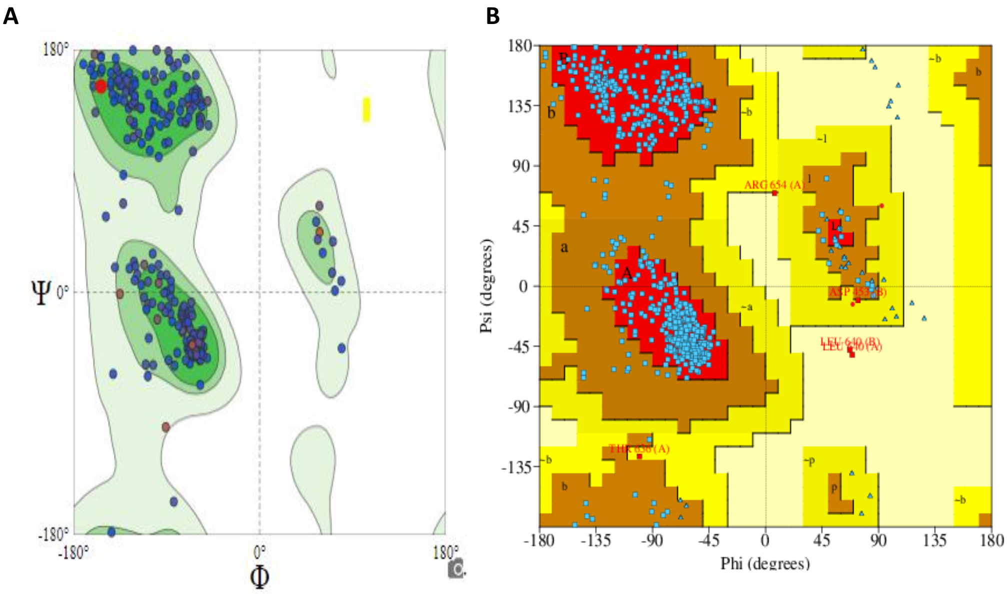

Figure 5: (A) represent ramachandran plot of lactoferrin by using PDB Sum and viewed by PROCHECK (B) represent ramachandran plot of lactoferrin by using Swiss model

Ramachandran plot (also referred to as a [φ, ψ] plot, a Rama plot, or a Ramachandran diagram is shown in the Figure 5. The peptide bond is kept planar by the partial double bond character, the ω angle at the peptide bond is typically 180° [65]. The favored region **of** Ramachandran plot analysis is 96.23%, MolProbity Score 1.44, Clash Score is 0.39, Ramachandran Outliers 0.00%, Rotamer Outliers 5.26%, Bad Bods 0 / 2686, Bad Angles 12 / 3649.

The initial study evaluated the model's quality using ERRAT [66]. For a high-quality model, the acceptable range is often greater than 50 [67]. The model's ERRAT score in this instance is 93.47% for *Capra hircus;* indicate that it is a high resolution structure as shown.

3.14 Multiple Sequences Alignment

The sequence from NCBI is aligned using the programs Clustal Omega and MView. Sequence motifs with possible antibacterial activity were found[68]. Conserved areas and functional sites of sequence were identified by combining the colors and percentages. Blue is highly conserved residue and its percentage identity is 90-100%, green is moderately conserved residue and its percentage is 89-60%, red is nonconserved residue and its percentage is less than 40%. Purple color show hydrophobic residue, orange color show polar residue and pink color show charged residue.

3.15 Protein-protein interaction

An summary of every 3D macromolecular structure deposited in the Protein Data Bank (PDB) can be found in the PDBSum database [69] and STRING database [70]. The quantity of disulfide bond contacts, salt bridges, hydrogen bonds, and non-bonded interactions between the atoms was all satisfactorily identified using PDBSum. The Figure S6 displays the quantity of bonding and non-bonding interactions. Results from PDBSum indicate how many salt bridges, hydrogen bonds, disulphide bond and non-bonded contacts. The STRING database has detected eleven detecting protein such as BPI, LOC102175113, LOC102174648, MPO, LTF, TFRC, ALB, LALBA, CSN2, LPO, PAEP which are involved in different hydrolyzing pathways. There are 11 nodes, 30 no. of edges and their average node degree is 5.45. Its p-value is 1.18e-06. Among them LTF has 81.8% similarity with PDB ID (1lfc and it shows a preferential cleavage at -Arg-Ser-Arg-Arg, Arg-Arg-Ser-Arg and of Z-Phe-Arg aminomethylcoumarin sites.

3.16 Molecular Docking

Docking was performed between selected macromolecule and the ligand by Autodock Vina and PyRx. Polar hydrogen and kollman charges was added before preparing pdbqt files and the grid box size was set keeping in view the specific angles and centers so that best binding affinity can be gained during molecular docking. Protein-ligand binding affinity indicates the stability of the specified ligand and receptor molecules which is an essential characteristic of effective drug. PyRx, a virtual screening tool for computational drug designing that perform multiple ligand docking against potential receptor at a single time was also used employed for molecular docking of selected protein and ligands. Enterobactin, a potent iron chelator can bind to iron more effectively than lactoferrin was docked with iron saturated C terminal half of lactoferrin receptor by using Autodock vina showed -9.4 Kcal/mol binding energy and by pyrx it showed -9.2Kcal/mol. Other effective ligand named Corynebactin, ferrichrome, Heparin which inhibit the activity of lactoferrin by sequestering iron and reduce its availability to lactoferrin showed -9.2 K/calmol, -8.9K/calmol and 7.3K/calmol binding energy respectively as shown in Table 3. Binding energy of all the compounds is elaborated by graph as shown in the Figure S7. The receptor and ligand molecules show different types of chemical interactions such as covalent bonds, hydrogen bonds, salt bridges, hydrophobic interactions and Van der Waal forces. These chemical interactions can be classified as polar (Hydrogen bond) and non-polar (Hydrophobic) interactions.

3.16.1 Interpretation of protein ligand complexes

In the field of drug discovery for the identification of a potential drug candidate receptor- ligands interaction study is essential. Interaction study helps to evaluate the active site of protein molecule and position of small ligand molecule and also predict their behavior in biological activities. Different bonding interaction of the complexed structure such as hydrogen bonds, hydrophobic Interactions, electrostatic bonds has been analyzed. Protein ligands interaction was analyzed by Discovery studio and ligplot. In the Figure 6 and Figure S8 3D structures of the protein ligand complex showed interaction of the ligand with protein and 2D Structures showed different chemical interaction with residual (amino acids) molecules of protein. Dark green amino acids such as Lys, ASp, Asn Glu, and Trp are conventional hydrogen bonds and the sky color Amino acids showed Van der Waals interactions. LigPlot Program generates 2D structures of protein ligand complexes and the output showed intermolecular interaction and their strengths including hydrogen and hydrophobic interactions. Some amino acid linked with the help of hydrogen bonds and some amino acid protein in the form of coils showed hydrophobic interaction.2D structure of pyerodine and ferrichrome is shown in the Figure S9 and caffeine and capric acid is shown in the Figure 7 describe different amino acid and their interaction.

Table 3: Binding energies of the selected ligands

Sr No	Name	Binding Energies by autodock vina (K/calmol)	Binding Energies by pyrx (K/calmol)
1	Enterobactin	-9.4	-9.2
2	Corynebactin	-9.1	-9.1
3	Ferrichrome	-8.9	-8.2
4	Lactoferrin	-8.0	-7.9
5	Heparin	-7.3	-7.3
6	Pyoverdine	-7.3	-7.3
7	Caffine	-7.1	-7.2
8	Aquachelin	-6.7	-6.9
9	Citric Acid	-6.8	-6.7
10	Linoleic Acid	-6.6	-5.3
11	Stearic Acid	-6.5	-6.5
12	Oleic Acid	-6.4	-6.3
13	Palmitic Acid	-6.2	-5.9
14	Capric Acid	-5.9	-5.5
15	Malic Acid	-5.7	-5.8
16	Lactic Acid	-4.6	-4.6
17	Phosphoric Acid	-4.3	-4.2
18	Monophosphate	-4.1	-4.2
19	Orthophosphate	-3.6	-3.8
20	Acetic Acid	-3.4	-3.5

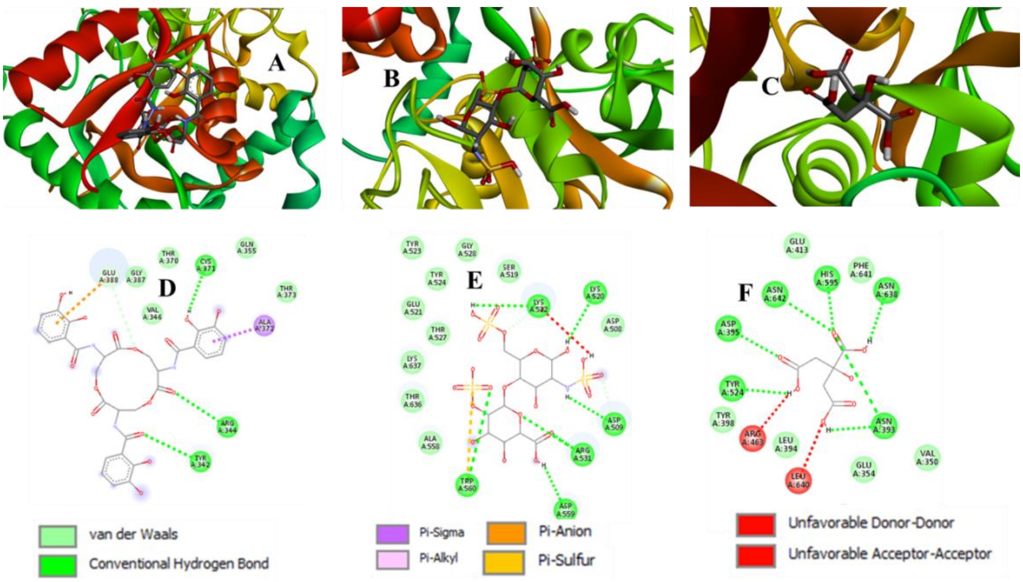

Figure 6: 3D docking models and 2D binding interaction of protein and the selected ligand as (A&G) enterobactin (B&H) heparin (C&I) citric acid (D&J) aquachelin (E&K) lactoferrin (F&L) corynebactin respectively

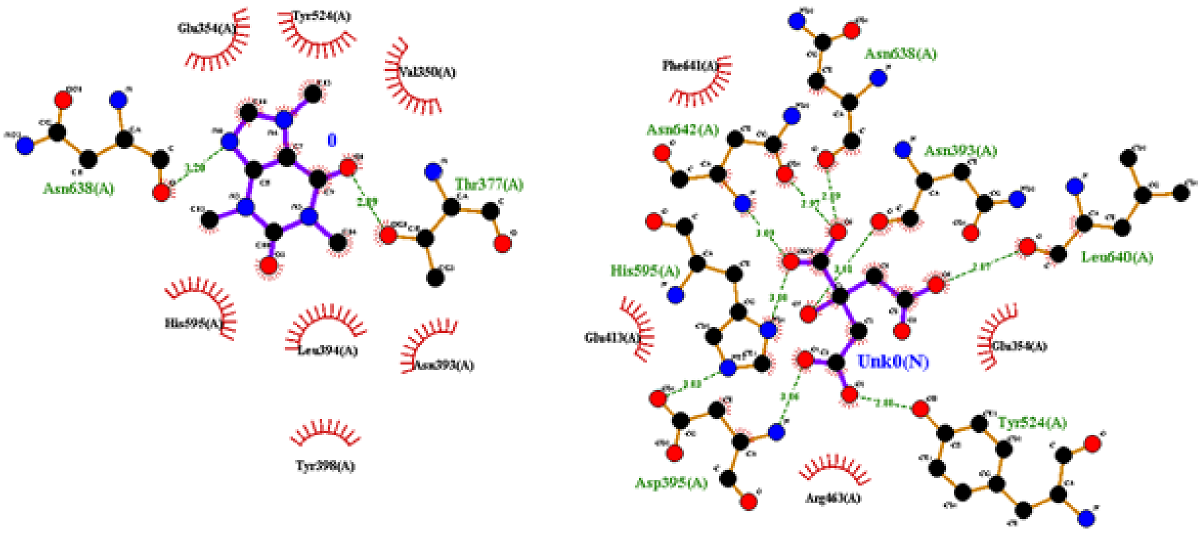

Figure 7: 2D protein-ligand interaction shown of caffeine and capric acid by ligPlot

3.16.2 ADMET Analysis

Occasionally best docked compounds are recognized as intriguing drugs however pharmacokinetics parameter and ADMET properties are the potential characteristics must be employed in drug designing and development that aids in identifying the harmful effect of substance on living organisms. Some physicochemical and pharmacokinetics properties were analyzed which include molecular weight, Consensus Log Po/w, GI absorption (gastrointestinal absorption), BBB permeant (Blood Brain Barrier), P-gp substrate (If p-gp+ is in blue it means it is approved by central nervous system by P glycoprotein and if P-gp- is in red it is not approved by central nervous system), skin permeation and Lipinski Rule violation as shown in the Table 4. The BOILED-Egg has been simplistic to construe and effective to translate to the molecular arrangement across a number of drug discovery environments. The white area denotes high likelihood of passive uptake by the gastrointestinal tract and the yellow color area (yolk) denotes high likelihood penetration by the brain.. All the compounds showed red dots that indicate all compounds are not approved by the central nervous system and called as non-substrate of p- gp. Palmitic acid and capric acid show high penetration in the brain as they lie in the yolk and stearic acid, oleic acid, acetic acid, lactic acid, caffeine and malic acid manifest high absorption in gastrointestinal tract. Bioavailability radar helps to rapidly evaluate the drug likeness of the molecule. In the bioavailability radar these physicochemical parameters must be in the optimal range for the validation of best drug candidate like (LIPO) lipophilicity, Size, Polarity (POLA), Insolubility (INSOLU), Insaturation (INSATU), and Flexibility (FLEX). Bioavailability radar and Boiled egg model of some compounds like Caffeine, Citric acid, Capric acid, Malic acid, Lactic acid and Acetic acid follow all the parameters and can be considered as a best drug as shown in the Figure 8 and Figure S10.

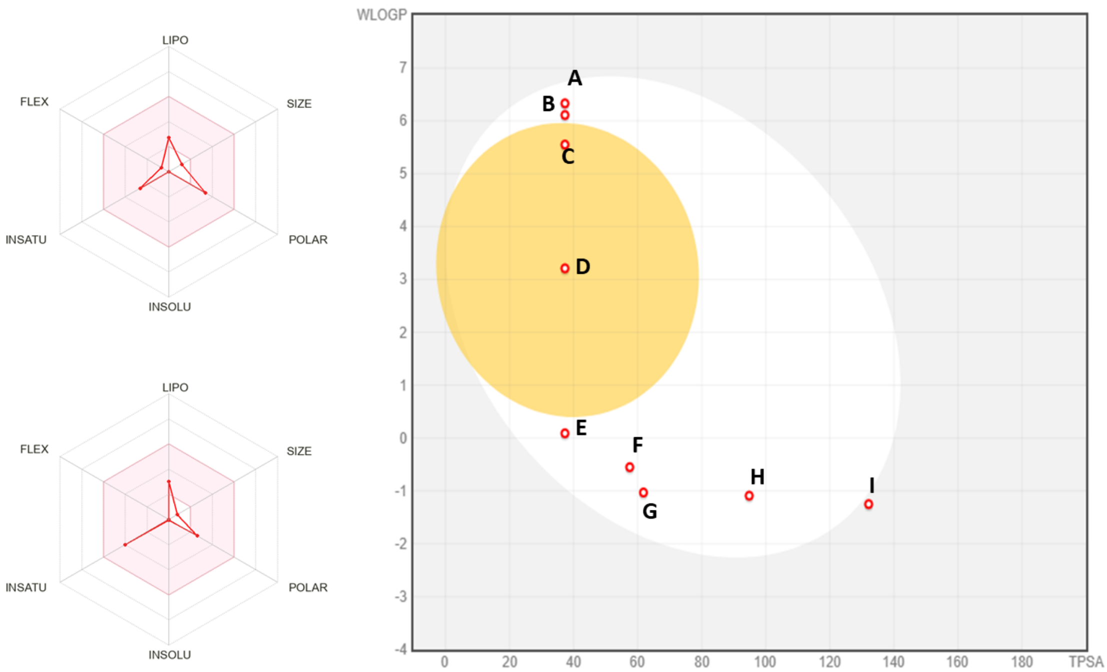

Figure 8: Bioavailability Radar and Boiled egg model of Stearic acid (A), oleic acid (B) , palmitic acid (C),capric acid (D), acetic acid (E), lactic acid (F), caffeine (G), malic acid (H), citric acid (I)

Table 4: ADMET and pharmacokinetics properties of the compound

Compounds	Molecular weight (g/mol)	GI Absorption	BBB Permeant	P-gp substrate	Consensus Log Po/w	Skin permeation	Lipinski Violation
Enterobactin	699.55	Low	No	Yes	0.15	-8.74 cm/s	3
Ferrichrome	740.52	Low	No	Yes	-44.42	-14.13cm/s	3
Heparin	595.48	Low	No	Yes	-5.13	-14.16cm/s	3
Caffine	194.19	High	No	No	0.08	-7.53cm/s	0
Citric Acid	192.12	Low	No	No	-1.51	-8.69cm/s	0
Palmitic Acid	256.42	High	Yes	No	5.20	-2.77cm/s	1
Oleic Acid	282.46	High	No	No	5.65	-2.60cm/s	1
Capric Acid	172. 26	High	Yes	No	3.00	-4.45cm/s	0
Malic Acid	134.09	High	No	No	-1.00	-8.01cm/s	0
Lactic Acid	90.08	High	No	No	-0.48	-7.36cm/s	0
Acetic Acid	60.05	High	No	No	-0.09	-6.82cm/s	0
Corynebactin	882.78	Low	No	Yes	-0.56	-10.52cm/s	3
Aquachelin	1065.13	Low	No	Yes	-2.64	-15.53cm/s	3
Lactoferrin	791.85	Low	No	Yes	-2.18	-14.37cm/s	3
Stearic Acid	284.48	High	No	No	5.93	-2.19cm/s	1

Discussion

Lactoferrin (LF) is a physiologically active cationic protein with a molecular weight of 80 kDa glycosylated protein with about 700 amino acids and strong interspecies resemblance [71]. It is present in exocrine secretions such as tears, salvia, bile, and neutrophil granules and belonging to the transferrin family, lactoferrin is one of the most important components of milk [72, 73]. LF is a vital protein in the immune system, playing a central role in protecting against infections and diseases [74]. The dairy industry is under pressure from two opposing forces: the escalating threat of diseases and extreme weather conditions [75]. The analysis consisted of several key tasks:

Physiochemical characterization: determining molecular weight, amino acid composition, theoretical pI, absorbance, and extinction coefficient, Functional region identification: recognizing motifs and domains to understand their roles, Structural analysis: examining secondary structure parameters and percentage similarity among organisms, Interaction mapping: exploring protein-protein interactions to understand molecular partnerships, 3D modeling: creating models of human and sheep LF, validated by Ramachandran plots to ensure structural accuracy. This integrated approach aimed to provide a thorough understanding of the LF protein's structure, function, and evolutionary history [76], instability index, aliphatic index, GRAVY, half-life and expression of LF in different organelles. The isoelectric point (pI) described the acidic and basic nature of the LF. Theoretical PI value of *Capra hircus* was 8.46 which indicate basicity in this protein. The value of negatively Charged Residues (Asp + Glu) is 74 and its positively Charged Residues (Arg +Lys) are 85. Aliphatic index for *Capra hircus* was 77.34% which is greater than 50% which indicates hydrophilic protein. Similar results were seen in literature with LF [77]. In silico protein localization prediction identifies the specific subcellular compartments where proteins reside, such as the nucleus, mitochondria, or plasma membrane. By pinpointing a protein's exact location, it is helpful to deduce its functional roles and responsibilities, predict potential interactions with other biomolecules, and understand its involvement in specific cellular processes [78]. Signal peptide not only exhibits a good connection between the predictions made using this bioinformatics technique and previously observed experimental data, but it also validates the validity of the utilized approach [79]. Signal peptide value of lactoferrin in *Capra hircus* is 0.9947, which is really good. The closer it is to 1, the better it is. Four lines make up the Binding Site of Domain analysis. Transferrin (IPR018195); TRANSFERRIN (PS00207); TRANSFERRIN (PS00205), and TRANSFERRIN (PS00205) are four Domains found in *Capra hircus*. Percentage similarity among selected organism protein sequence was analyzed by BLASTp while in other article, it was visualized by jalview [80]. N-glycosylation affects the characteristics and bioactivities of proteins with oligosaccharide structures by altering the correct asparagine residues in such proteins [45] In *Capra hircus* there are total 5 motifs. Motif analysis plays a crucial role in protein in silico studies, enabling researchers to identify conserved patterns and functional motifs within protein sequences [81]. Accurate modeling of secondary structural elements, including alpha helices, beta-sheets, and beta turns or random coils, is crucial for understanding protein structure. Moreover, reliable secondary structure prediction enables the identification of structural motifs and patterns, facilitating a deeper understanding of protein function, stability, and interactions [82]. In secondary structure of *Capra hircus*, Random coils are prominent in structure its mean that the selected protein is hydrophobic in nature. *Capra hircus* LF protein tertiary structures are generated using the Swiss model. Many factors, including GMQE, QSQE, sequence identity, coverage, range, sequence similarity, Qmean value, and Ramachandran's plot, are taken into account while choosing the final model [83]. A crystal structure of lactoferrin (PDB: 7enu.1b) was used as template sequence. Good agreement is indicated by a QMEAN Z-score of about (0-1) [62]. QMEANZ score is -0.85 its value is round about zero, its mean that it is a good model. String database indicates that *Capra hircus* LF create complexes through interactions with proteins. This protein interacted with several proteins and had a large number of functional partners, according to a protein–protein interaction network study. String database indicates that *Capra hircus* LF create complexes through interactions with proteins. To explain the relationship between LF proteins and other interacting proteins, a variety of characteristics were examined, including gene fusion, gene co-expression, co-occurrence, neighborhood, text mining, protein homology, and database [84]. Protein-protein interaction in *Capra hircus* among all results has 81.8% similarity with PDB ID (1lfc). In order to choose the right AMPs, the physicochemical characteristics of the synthesized peptides were examined utilizing internet resources including peptide cutter databases. To choose the correct antimicrobial peptides, an analysis was conducted on the general attributes of the produced peptides, such as molecular weight, charge, and PI values. The study utilized four CAMP database techniques, namely ANN, RF, and SVM, to evaluate the antibacterial efficacy of the peptides generated. Seven of the generated peptides satisfied the first set of requirements; however, since the AMPs' lysine and cysteine amino acids are advantageous, the peptide with an ID of 1 (as determined by trypsin) was chosen for additional examination. Trypsin was used to determine the ID of 1, and the peptide was found to be an AMP since its parameters had scores greater than 0.45 [53]. The chosen peptide's 3D structure has a confidence score between 0 and 9, which is within the usual range of 5 to 9. This implies the authenticity of the construction. Its hydrophobicity is demonstrated by the solvent accessibility test. Enterobactin shows highest binding affinity of about 9.4 Kcal/mol followed by cornyebactin, ferrichrome, lactoferin, heparin, Pyoverdine, caffine and so on. But toxicity analysis shows that the best docked compounds enterobactin do not fulfilled the given parameters of ADMET. Caffeine, capric acids, citric acid and malic acid, lactic acid and acetic acid fulfilled the given parameters and can be future analyzed as drug candidate.

4. Conclusion

The lactoferrin has shed light on the protein's many functions and possible medicinal uses as antimicrobial behavior. The prediction of the structural characteristics and antimicrobial properties of lactoferrin has been made through *in silico* method. The present research showed that lactoferrin is hydrophobic and thermostable, with a molecular weight of 83–87 kDa. It is a polar protein, but a part of it also shows a non-polar nature. Seven peptides out of 166 have antimicrobial characteristics. The sequence similarity of tertiary structure is 95.86%, QMEAN z-scores is -0.85; it is near zero its mean that it is a good model. This study provides virtuous understanding regarding functional aspect of goat lactoferrin. It also provides knowledge for future research in the field of peptide and analogs produced from lactoferrin. Additionally, there is a need to investigate the interactions between lactoferrin and conventional antibiotics as well as the creation of combination medicines to overcome resistance mechanisms.

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