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Computational and experimental investigation of natural antimicrobials targeting *Vibrio parahaemolyticus* DNA gyrase: *In silico* and *in vitro* evaluation

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Abstract

The shrimp farming sector is currently grappling with substantial challenges, prominently marked by disease outbreaks and the emergence of antimicrobial resistance, primarily attributable to the excessive utilization of antibiotics. To effectively address this predicament, it is imperative to seek out alternatives to antibiotics, and herbal sources present themselves as a promising solution. In a meticulously conducted sequential molecular docking screening involving 800 ligands of natural origin against the DNA gyrase target proteins of *V. parahaemolyticus*, several ligands exhibited robust binding affinity, registering values of less than -7 kcal/mol for their respective targets. Notably, among the chosen ligands, a subset was procured from easily accessible and cost-effective natural origins. The results highlighted that Secoisolariciresinol diglucoside, Nobiletin, Quercetin, Aloesin, Curcumin, Methyl folate, Honokiol, Quercitrone Piperine and Withanolide A demonstrated binding affinities of -9.5, -8.7, -8.7, -8.3, -8.3, -8.3, -8.3, -8.1 -7.7 and -7.5 kcal/mol respectively. Notably, natural compounds sourced locally demonstrated strong binding affinities with various protein targets and underwent further *in vitro* testing. *In vitro* assays employing ethanolic extracts of locally available natural compounds from which eucalyptus and aloe vera exhibited remarkable inhibitory activity against multi-drug resistant *V. parahaemolyticus*. These analyses will provide a deeper understanding of the potential of these readily available natural compounds in combatting the challenges faced by the shrimp farming sector, holding the promise of reduced dependence on antibiotics and fostering sustainable and healthier shrimp production practices. In light of the mounting challenges confronting the industry, the pursuit of alternative solutions through natural compounds offers a beacon of hope for a more sustainable and resilient shrimp farming sector. This study marks an important advancement in maintaining the sustainability and health of the shrimp farming sector by tackling key issues surrounding disease control and the resistance to antibiotics.

Keywords: Shrimp farming, antimicrobial resistance, herbal alternatives, molecular docking, *Vibrio parahaemolyticus*, natural compounds

Introduction

Aquaculture has evolved into a multimillion-dollar sector over the years, with shrimp aquaculture driving much of this growth. Shrimp is the most farmed crustacean worldwide, making up 65% of the global production in 2020, according to the FAO (2022) [6]. As a rich source of nutrients, seafood offers various health benefits (Iwamoto *et al.*, 2010) [10]. However, the improper use of antibiotics in shrimp farming has led to the rise of antimicrobial resistance (AMR) among both harmful and beneficial bacteria (Prestinaci *et al.*, 2015) [18]. Significant pathogens in shrimp aquaculture include various *Vibrio* species such as *V. alginolyticus*, *V. anguillarum*, *V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus*, which cause severe economic losses (Karunasagar *et al.*, 1994) [11]. *V. parahaemolyticus*, in particular, is known for causing the most frequent disease outbreaks, underlining the need for careful pond management.

The frequency of disease outbreaks in shrimp aquaculture associated with *V. parahaemolyticus* is more than any other bacterial pathogen and serious attention is invested in pond management to avoid such outbreaks. The current management practices are mainly

based on the application of probiotics and antibiotics. The use of antimicrobial compounds is highly unregulated in aquaculture and invites serious problems in the form of the development of antimicrobial resistance in pathogenic as well as non-pathogenic bacteria. Such a situation also favors the spread of the AMR gene between the bacterial species through horizontal gene transfer and leads to the widespread prevalence of AMR. Aquaculture sediments include a diverse range of active bacterial communities, and there is evidence to suggest that they may be a significant source of infections and antibiotic resistance. In order to facilitate the transmission of AMR genes, sediment systems constitute a crucial environmental matrix.

The growing resistance to existing antibiotics is pushing researchers to explore new antimicrobial agents. Computational methods have become prominent in the drug discovery process using artificial intelligence Computer-Aided Drug Design (CADD). This approach involves various computational techniques such as molecular modeling and rational drug design, which streamline and accelerate the drug development process, making it faster, cheaper, and more efficient (Yu and MacKerell, 2016) [22]. Molecular docking is used to find ligands/compounds that bind strongly to target proteins, such as DNA gyrase as well as essential proteins required for their life cycle. DNA gyrase is an essential enzyme present in prokaryotes but absent in eukaryotes, making it an ideal drug target. DNA gyrase plays a critical role in DNA topology during recombination, transcription, and replication by creating temporary breaks in DNA strands (Durcik *et al.*, 2018) [5]. Many inhibitors of DNA gyrase, including quinolones and aminocoumarins, have been thoroughly investigated (Maxwell, 1997) [17].

Addressing the pressing need for new drugs to fight these pathogens to overcome the spread of AMR is vital. Drug discovery and development are intricate processes, but using computational drug design can quickly identify potential drug molecules against pathogenic *Vibrios*, offering a swift solution for disease management in aquaculture (Malathi *et al.*, 2018) [16]. Recent findings also underscore the necessity for new treatments and preventive measures for pathogenic *V. parahaemolyticus* strains.

Materials and methods

Target selection

The choice to focus on DNA gyrase as a drug target as it is an important enzyme in the life cycle of *V. parahaemolyticus*. The 3D structure of this protein was retrieved from AlphaFold database (Varadi *et al.*, 2021), identified by the AlphaFold ID: A0A656SKS8_VIBPH. This protein was selected due to its critical importance to the organism's survival and its frequent mention in reports on antibacterial drug screenings.

Preparation of ligand library

Ligands were retrieved from the ZINC database 15 (Sterling and Irwin, 2015) [20] and the PubChem database (Kim *et al.*, 2022) [12]. A total of 800 ligands were curated for use in the molecular docking experiments.

Physicochemical characterization of the selected target protein: The physicochemical properties of the target protein were analyzed using the ExPASy ProtParam tool. This analysis included parameters such as amino acid

composition, molecular weight, extinction coefficient, theoretical isoelectric point (pI), instability index, grand average of hydropathicity (GRAVY), aliphatic index, and the total count of positively and negatively charged residues (Gasteiger *et al.*, 2003) [7].

Validation of the protein 3d structure

The 3D structure's quality was validated using the SAVES server, employing tools like VERIFY 3D (Bowie *et al.*, 1991; Lüthy *et al.*, 1992) [2, 15], PROCHECK (Laskowski *et al.*, 1993) [14], and ERRAT (Colovos and Yeates, 1993) [4]. The protein structure was analyzed in.pdb format.

Protein preparation

The protein was prepared for molecular docking using BIOVIA Discovery Studio 2021. This preparation involved removing side chains and heteroatoms, adding hydrogen molecules, and converting the structure into the required PDB format. The analysis of the N-terminal and C-terminal ends was performed using Chimera v1.17.3 software.

Molecular docking of protein-ligand

Molecular docking was executed using PyRx 0.8 software (2016) and AutoDock Vina 1.5.7. This involved converting the protein molecule from PDB to PDBQT format and minimizing the energy of the ligand molecules. Ligands were then shortlisted based on their binding affinity, local availability, and cost-effectiveness.

Physicochemical properties and toxicity analysis

The shortlisted compounds underwent an in-depth analysis of their physicochemical properties and potential toxicity using the pkCSM platform (Douglas *et al.*, 2015). This step was essential to assess the compounds' safety and identify any toxicological risks.

Visualization of protein-ligand interaction

The interactions between the protein and the selected ligand were visualized using BIOVIA Discovery Studio 2021. This visualization helped to elucidate how the compound binds at the molecular level, offering insights into its potential therapeutic efficacy.

Molecular dynamic simulation

Following the molecular docking results, a molecular dynamics simulation was conducted to evaluate the stability of the protein/ligand complex. Techniques such as vibrational analysis and motion animations were utilized interactively using iMODS, which facilitated an enhanced understanding of domain dynamics and the effects of ligand binding on DNA gyrase from *V. parahaemolyticus* (Kovacs *et al.*, 2004; López-Blanco *et al.*, 2011). This simulation helped in verifying the compound's efficacy by analyzing several parameters, including deformability, B-factor, and eigenvalue.

Ethanol extraction of compound present in selected natural sources for *in vitro* testing

The locally available natural compounds were collected for further extract preparation. The leaves were washed properly using distilled water and dried at room temperature. Fine powders were made using a grinder. A weight of 10 gm of powder was measured. A clean bottle was taken and 100 ml of ethanol and the powder were

added. It was mixed properly and placed on a rocker for 96 hours. The extract was filtered using a muslin cloth. The supernatant was collected from the filtrate after centrifugation at 3500 rpm for 5 minutes. The supernatant was further filtered using Whatman filter paper no. 1 and placed in a vacuum desiccator for drying. The dry matter was weighed and dissolved in 10% DMSO and further diluted in the same solvent for preparation of the required concentration for antibacterial efficacy. This solution was centrifuged at 3500 rpm for 5 minutes to remove any undissolved particles and the supernatant was filter sterilized using a syringe filter (0.2 micron) for further analysis.

In vitro antibacterial assay against MDR *Vibrio parahaemolyticus*: The colonies that were resistant to 6-7 antibiotics were selected. The colony was inoculated in Mueller Hinton broth and incubated at 30°C for 5-6 hours. In a 0.5 ml sterile tube, 150 µl culture and 150 µl of the compound of specific concentration were added. The tubes were incubated at room temperature for 2-3 hours and post-incubation 100 µl culture was spread on TCBS plates using a sterile spreader. The plates were incubated at 30°C for 18-20 hours and CFU was counted for further analysis.

Result

Physicochemical characterization of the target protein

Extensive physicochemical characterization is commonly employed to identify the specific surface areas responsible for exceptional catalytic activity. The 3D structure of the DNA gyrase from *V. parahaemolyticus* was retrieved using AlphaFold, with the specific AlphaFold ID: A0A656SKS8_VIBPH. This structure was analyzed to determine its physicochemical properties. The instability index of the protein is 31.86, indicating stability according to Guruprasad *et al.* (1990) [8]. Additional physicochemical parameters are detailed in Table 1.

Quality analysis of target protein 3d structure for molecular docking: DNA gyrase of *V. parahaemolyticus* used as a potential drug target for the identification of putative ligands using molecular docking its ribbon structure and indication of C and N terminal in figure 1. The 3D structure of DNA gyrase proteins was analyzed using a Ramachandran plot analysis shown in figure 2. The percentage of residue in the favored region was more than 95% in the protein structures showing the quality of the 3D structure of protein shown in table 2.

Validation of protein 3D structure

Quality analysis of protein 3D structure using SAVES 6.0 server. VERIFY 3D and ERRAT used for quality analysis of DNA gyrase protein shown in figure 3 and figure 4.

Prediction of active site in protein

CASTp 3.0 used for the prediction of active site, a total of 18 pockets were generated. Among these, pocket ID 1 stood out with an area (SA) measuring 12541.475 and a volume (SA) of 7423.537. Consequently, it was chosen as the primary active site for subsequent docking analysis.

Molecular docking and identification of alternative antimicrobial against *V. parahaemolyticus*: Molecular docking results demonstrate the binding of the compounds

to DNA gyrase B as shown in Table 3. These findings provide valuable insights into the molecular mechanisms underlying the strong binding affinity between DNA gyrase B and its selectivity in terms of antibacterial drug efficacy. The molecular docking of 800 ligand molecules of different category including the natural origin were performed against DNA gyrase of *V. parahaemolyticus*. The ligands were shortlisted based on the binding strength and local availability of compounds. The docked image of target and shortlisted ligand visualized in Biovia Discovery studio as shown in figure 5 to figure 14.

ADMET analysis of shortlisted compounds

ADMET analysis is crucial for evaluating the pharmacokinetics of a drug, encompassing Absorption, Distribution, Metabolism, Excretion, and Toxicity. This analysis examines the potential side effects of a drug within the body, focusing on its oral bioavailability and absorption rate in the gastrointestinal tract. These evaluations are crucial aspects of drug discovery. Inadequate absorption can negatively impact the drug's distribution and metabolism, potentially resulting in complications such as neurotoxicity and nephrotoxicity (Flores-Holguín *et al.*, 2021). The shortlisted ligands were used for ADMET analysis as shown in the table 4.

Molecular dynamic simulation

The iMODS server was utilized to analyze the molecular interactions of *V. parahaemolyticus* DNA gyrase with quercitrone and aloesin. The complexes of DNA gyrase with quercitrone and aloesin were evaluated through Normal Mode Analysis (NMA) to simulate and assess the internal coordinates of the system's complexity. The trajectory analysis helped determine the system's deformability, with results depicted in Figures 15 and 16, illustrating the molecular dynamics simulations of the ligand-target interactions. The trajectory analysis of these complexes showed minimal deformation within a range of 0 to 1 Å, indicating stable binding with low deformation (Figures 15a and 16a). The network plot within the figures uses each dot to represent a spring, color-coded based on the stiffness relative to the atomic pairs, with grey-colored spring models indicating the compactness and stability of the binding complex system (Figures 15c and 16c). Figures 15d and 16d display the integration of the complexes, where the model's elastic network analysis identifies pairs of atomic coordinates through distance-based spring analysis. NMR traces detected some atomic fluctuations in the system trajectory of the DNA gyrase interactions with quercitrone and aloesin. Figures 15f and 16f present the computed eigen scores of 9.693×10^{-6} and 9.694×10^{-6} , respectively. Furthermore, covariance matrix analysis of the DNA gyrase-quercitrone and DNA gyrase-aloesin atomic pairs highlighted the correlated segments in red, non-correlated in blue, and uncorrelated segments in white. These findings underscore the stable binding and complex rigidity of DNA gyrase, accompanied by a low deformation index and some atomic fluctuations, contributing to our understanding of the interaction dynamics within these complexes.

Additionally, iMOD server was used to explore the docked complex physical movement and stability through Normal mode analysis (figure 15 and 16) incorporating deformability and B-factor. The deformability and B-factors of the DNA gyrase-Quercitrone and DNA gyrase-aloesin

complexes showed the peaks matching to deformable sites in the proteins, with the largest peaks representing high deformability regions. The B-factor plots provide a comparison of the complexes' NMA and PDB fields. The eigenvalue and variance graphs (individual variance through purple shade and cumulative variance by green colour) of DNA gyrase-Querciturone and DNA gyrase-aloesin complexes, respectively. Whereas the eigenvalue and variance graphs of DNA gyrase-Querciturone (9.693069e-06) and DNA gyrase-aloesin (9.694764e-06) complexes are provided in figure 15F and 16F respectively. The covariance matrix of DNA gyrase-Querciturone and DNA gyrase-aloesin complex shows the relationships between residues (red colour indicates good correlation while white colour represents uncorrelated motion and blue shade represents anticorrelations). The docked complex elastic maps show the relationships between the atoms, with darker-gray regions represent stiffer regions.

In vitro antibacterial assay of shortlisted compounds

The natural source of shortlisted ligands were procured and extract were prepared for the antibacterial efficacy study against the multidrug-resistant *V. parahaemolyticus*. The final list of selected chemicals/extracts is presented in Table 5.

The ethanolic extracts were prepared as per the protocol explained in materials and methods and all the shortlisted chemicals/extracts were analyzed using agar well assay and TPC count post-treatment with the formulations at particular concentrations.

Discussion

Computer-Aided Drug Design (CADD) is instrumental in identifying and developing new drug candidates. DNA gyrase, critical in regulating DNA's three-dimensional structure during key cellular processes like transcription, replication, and recombination, facilitates these processes by introducing temporary breaks in both strands of the DNA molecule. During replication, it introduces negative supercoils into the DNA, managing its topology and alleviating torsional stress that accumulates during these complex processes. Acting as a molecular architect, DNA gyrase shapes the DNA molecule to support essential cellular functions while maintaining its structural integrity. Molecular docking techniques, used to explore interactions with various ligands, provide insights into potential drugs that could modulate its activity. This research enhances our understanding of fundamental cellular processes and opens avenues for therapeutic interventions. Given its indispensable role in prokaryotes and absence in eukaryotes, DNA gyrase is an appealing drug development target.

Several studies highlight the significance of DNA gyrase as a drug target. Collin *et al.* (2011) [13] underscored the urgent need for new medications due to rising drug resistance, utilizing DNA gyrase as a promising target. Salve *et al.* (2021) synthesized analogs of 3-((7-chloroquinolin-4-yl)amino) thiazolidin-4-one to inhibit *Mycobacterium tuberculosis* DNA gyrase, evaluating them through *in vitro* methods and molecular docking with *Mycobacterium*

smegmatis GyrB, demonstrating significant inhibitory effects. Kumar *et al.* (2022) [13] identified compounds AM1 and AM5 as potential DNA gyrase inhibitors, showing promise in combating antibiotic resistance. Alotaibi *et al.* (2023) performed virtual screening for *Escherichia coli* GyrB inhibitors using a phytoconstituent database from Indian medicinal plants, identifying Cucurbitacin S and Zizogenin as potential inhibitors based on their drug likeliness and pharmacokinetic profiles.

In this study, we targeted DNA gyrase in *V. parahaemolyticus* for molecular docking with 800 ligand molecules. Notable compounds like Secoisolariciresinol diglucoside, Nobiletin, Quercetin, Aloesin, Curcumin, Methyl folate, Honokiol, Querciturone, Piperine and Withanolide A demonstrated binding affinities using PyRx - 9.5, -8.7, -8.7, -8.3, -8.3, -8.3, -8.3, -8.1 -7.7 and -7.5 kcal/mol respectively. Similarly, Quercetin, Curcumin, Piperine, Querciturone, Honokiol, Methyl folate, Aloesin, Secoisolariciresinol diglucoside, Withanolide A and Nobiletin showed binding affinity using AutoDock Vina with -8.7, -8.6, -8.6, -8.6, -8.3, -8.2, -7.9, -7.9, -7.6 and -7.0 kcal/mol respectively. Furthermore, Patel *et al.* (2015) identified chebulinic acid from *Terminalia chebula* as a potent natural DNA gyrase inhibitor in *M. tuberculosis*, with high docking scores. Structural verification was done Ramachandran plot analysis using SAVES server for DNA gyrase of *Vibrio parahaemolyticus* shows that 91.1% residues present in most favored region and 8.9% residue present in the additional allowed region.

Recent research also underscores the potential of natural compounds in antibacterial applications. Maahury and Allo (2021) found Aeropylsinin-1, derived from marine sponges, to exhibit inhibitory effects on DNA gyrase from *E. coli* and *S. aureus*. Ghannay *et al.* (2022) explored caraway essential oil for its anti-*Vibrio* spp., antibiofilm, and anti-quorum-sensing properties, identifying several phytochemical components that formed stable complexes with tyrosyl-tRNA synthetase. Anza *et al.* (2021) [23] compared the efficacy of isolated compounds to ciprofloxacin in inhibiting *E. coli* DNA gyrase B, demonstrating strong binding affinities.

Herbal-based formulations are promising alternatives to conventional antibiotics, addressing antimicrobial resistance effectively. Compounds like quercetin, recognized for their anti-inflammatory, antioxidant, antibacterial, and anticancer properties, also play roles in inhibiting quorum sensing systems, enhancing food quality and safety (Kim *et al.*, 2022; Hossain *et al.*, 2021) [9, 12]. Phenolic compounds from plants disrupt quorum sensing in various bacteria (Quecan *et al.*, 2019) [19].

Babayi *et al.*, 2004 reported that methanolic extract of Eucalyptus leaves are effective against *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus* (ATCC & Clinical) at a concentration of 2000µg/ml. We have also reported the inhibitory effect of eucalyptus leaves and aloe vera root ethanolic extract against *V. parahaemolyticus* at concentration of 11.04% and 2.6% was 0 cfu/ml respectively. Also found ashwagandha powder extract was effective at 5.5% concentration on *Vibrio parahaemolyticus* was 7x10³ compared to control TNTC.

Table 1: Physicochemical properties of the DNA gyrase protein in *V. parahaemolyticus*

Property	Value
Molecular weight	27053.3
Amino acid composition	245
Theoretical pi	5.36
Instability index	31.86
Total number of positively charged residues (Arg + Lys)	23
Total number of negatively charged residues (Asp + Glu)	38
Aliphatic index	85.06
Grand average of hydropathicity (GRAVY)	-0.324

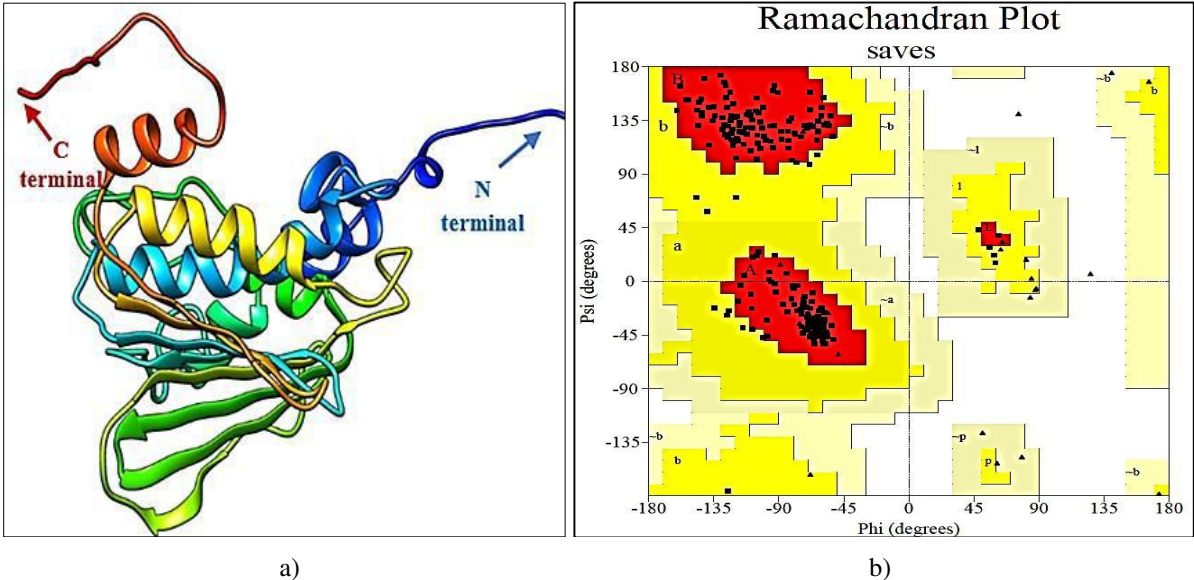


Fig 1: Ribbon structure of DNA Gyrase shows the C and N terminal visualized in Chimera, **Fig 2:** Ramachandran plot of DNA Gyrase protein (AlphaFold ID: A0A656SKS8_VIBPH) shows the allowed and disallowed regions

Table 2: Protein 3D structure assessment by Ramachandran plot analysis using PROCHECK server

% of residue present in the most favored region	91.1
% of residue present in the additional allowed region	8.9
% of residue present in the generously allowed region	0
% of residue present in the disallowed region	0

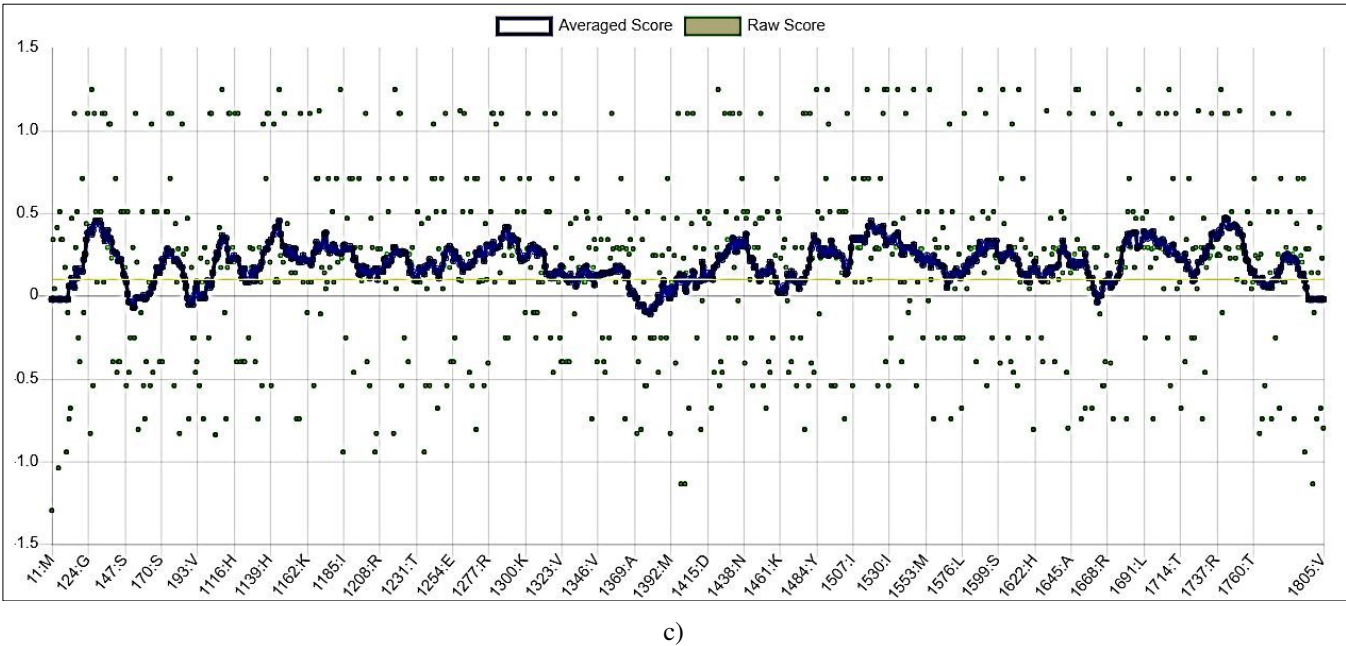


Fig 3: Model quality of DNA gyrase protein obtained by VERIFY3D.

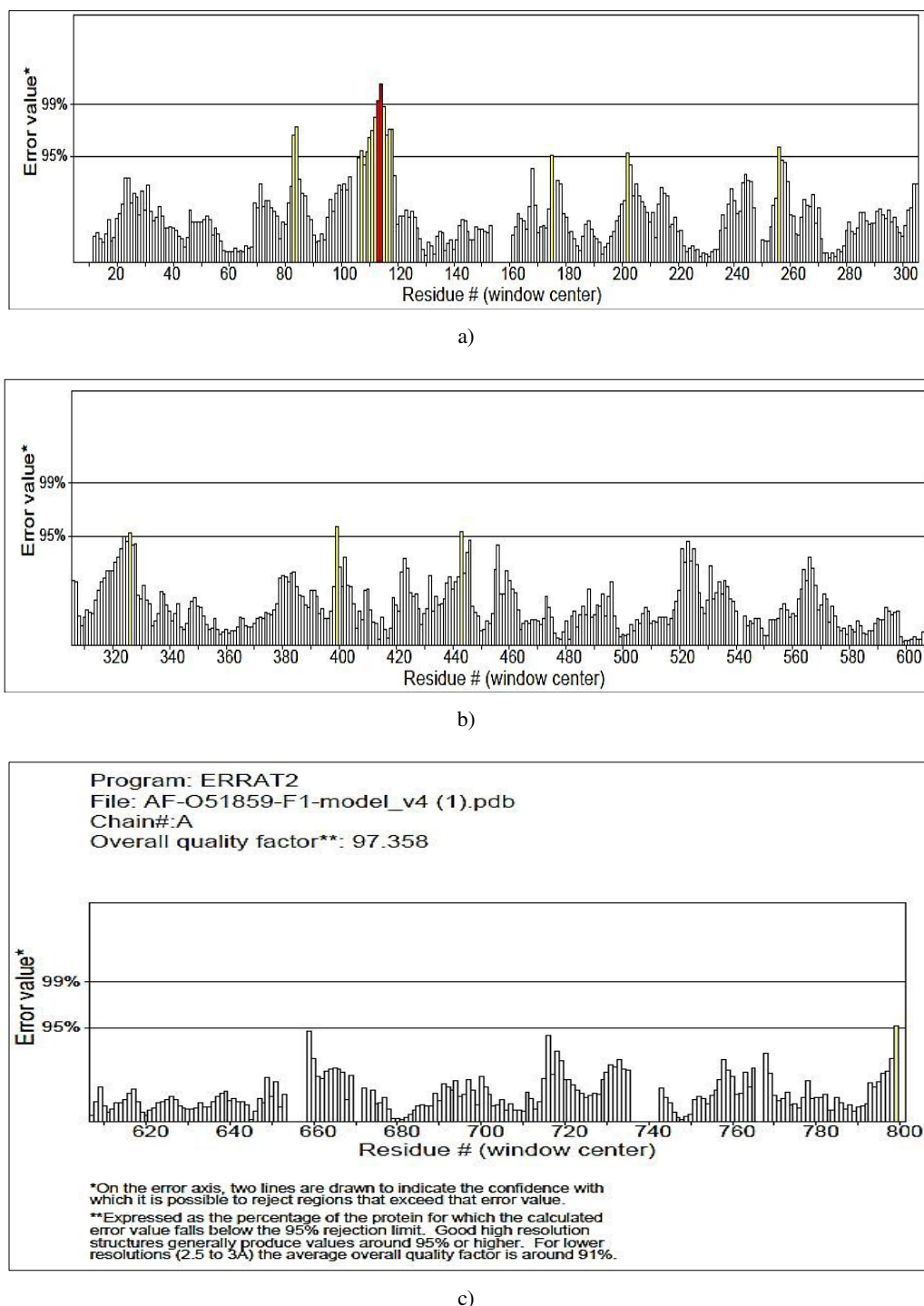


Fig 4: Model quality of DNA gyrase protein obtained by ERRAT.

Table 3: List of natural compound shows the binding affinity >7.5 (Locally available)

Sr. No.	Binding affinity		Pub Chem ID	Ligand name	Natural source
	PyRx	AutoDock Vina			
1	-8.3	-8.6	969516	Curcumin	Curcuma longa (turmeric)
2	-9.5	-7.9	9917980	Secoisolariciresinol diglucoside	Linum usitatissimum (flax seed)
3	-8.7	-7.0	72344	Nobiletin	Citrus tankan (orange), Citrus keraji (lemon)
4	-8.7	-8.7	5280343	Quercetin	Lotus ucrainicus (lotus)
5	-8.3	-8.2	135415868	Methyl folate	Capsicum annum var. annum (sweet and chili pepper)
6	-8.3	-8.3	72303	Honokiol	Illicium simonsii (fruits of star)
7	-7.7	-8.6	638024	Piperine	Piper boehmeriifolium (piper mint) and Piper nigrum (black pepper)
8	-7.5	-7.6	11294368	Withanolide A	Withania somnifera (aswagandha)
9	-8.3	-7.9	160190	Aloesin	Aloe castanea (aloe vera)
10	-8.1	-8.6	5274585	Querciturone	Eucalyptus cypelloarpa (eucalyptus)

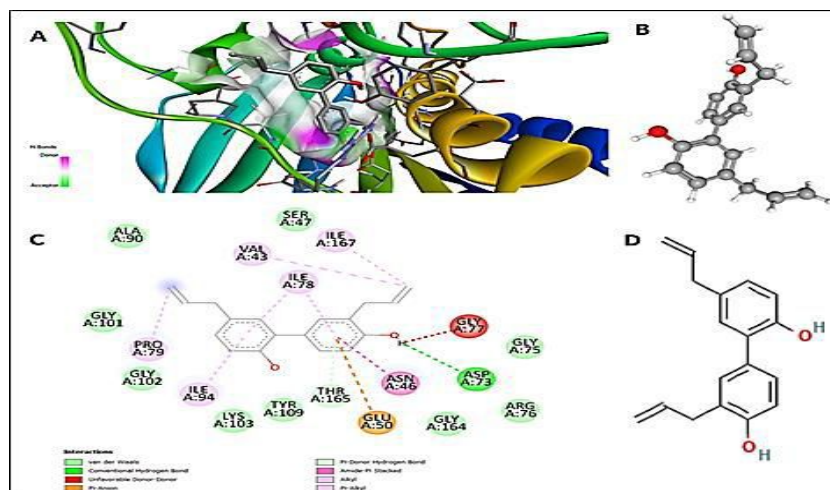


Fig 5: Indicates the docked image of DNA gyrase with honokiol visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with honokiol in 3D (B) 3D structure of honokiol (C) 2D interaction of DNA gyrase with honokiol (D) 2D structure of honokiol

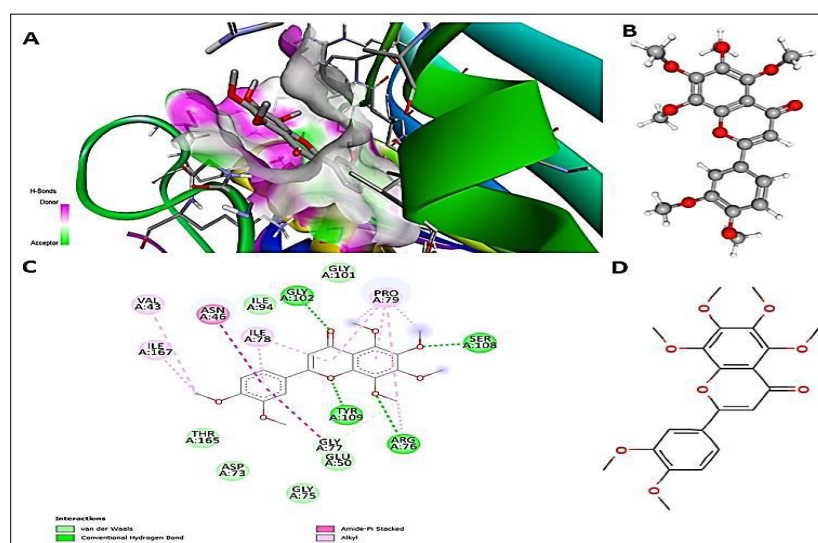


Fig 6: Indicates the docked image of DNA gyrase with nobiletin visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with nobiletin in 3D (B) 3D structure of nobiletin (C) 2D interaction of DNA gyrase with nobiletin (D) 2D structure of nobiletin.

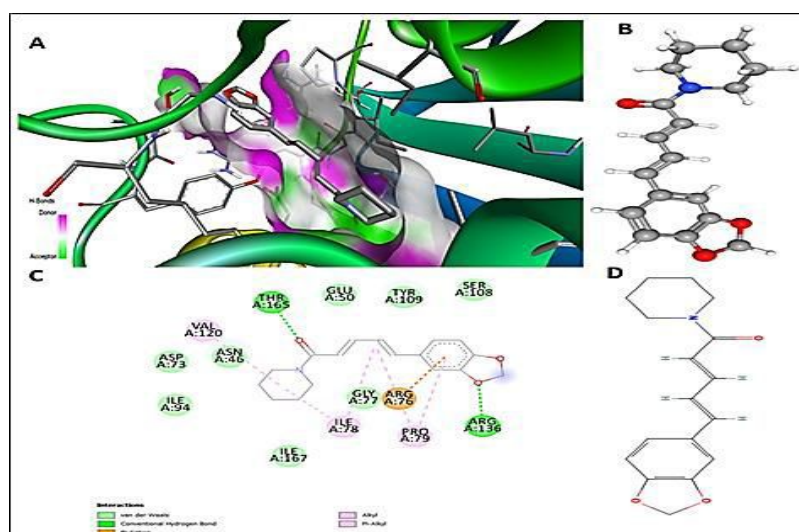


Fig 7: Indicates the docked image of DNA gyrase with piperine visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with piperine in 3D (B) 3D structure of piperine (C) 2D interaction of DNA gyrase with piperine (D) 2D structure of piperine.

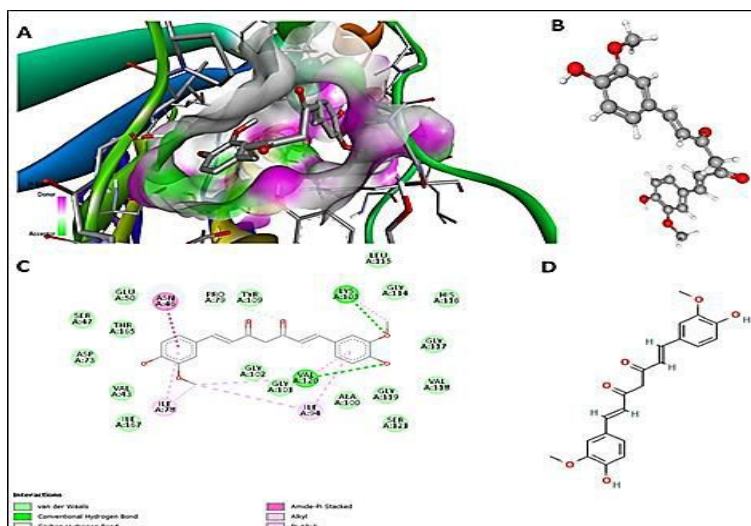


Fig 8: Indicates the docked image of DNA gyrase with curcumin visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with curcumin in 3D (B) 3D structure of curcumin (C) 2D interaction of DNA gyrase with curcumin (D) 2D structure of curcumin.

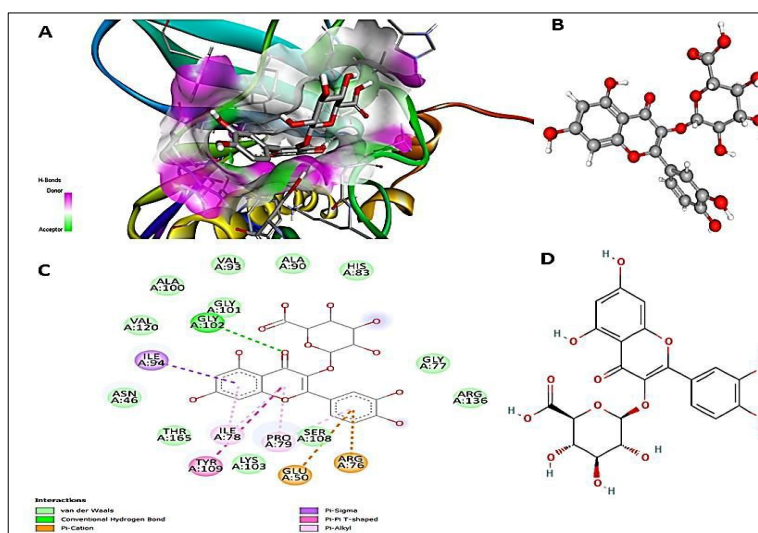


Fig 9: Indicates the docked image of DNA gyrase with quercitrone visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with quercitrone in 3D (B) 3D structure of quercitrone (C) 2D interaction of DNA gyrase with quercitrone (D) 2D structure of quercitrone.

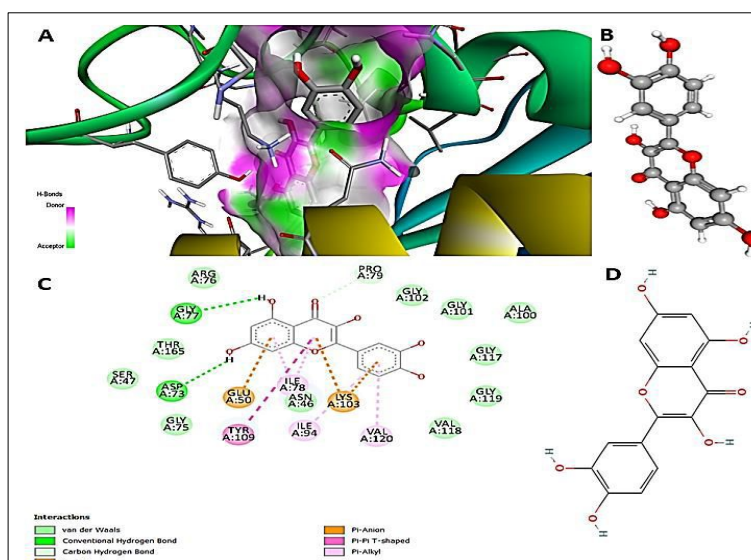


Fig 10: Indicates the docked image of DNA gyrase with piperine visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with piperine in 3D (B) 3D structure of piperine (C) 2D interaction of DNA gyrase with piperine (D) 2D structure of piperine.

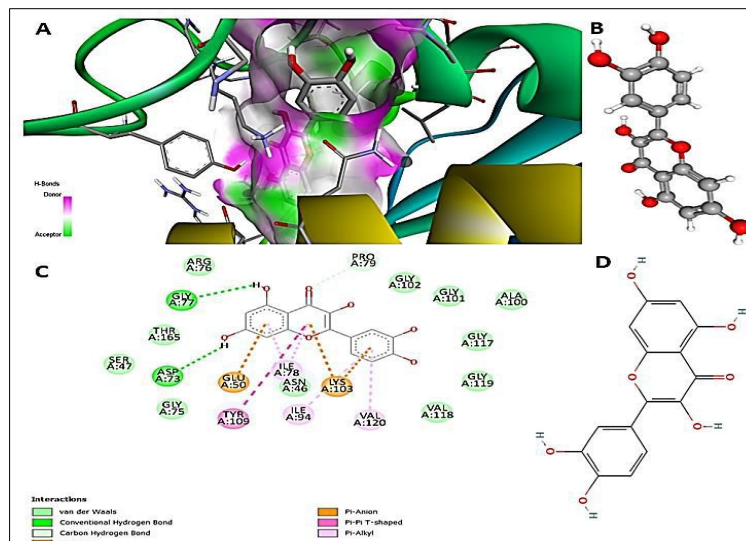


Fig 11: Indicates the docked image of DNA gyrase with quercetin visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with quercetin in 3D (B) 3D structure of quercetin (C) 2D interaction of DNA gyrase with quercetin (D) 2D structure of quercetin.

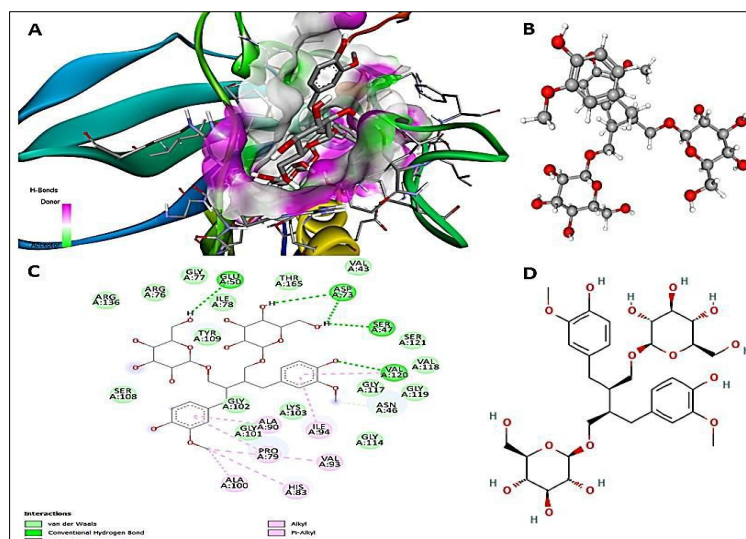


Fig 12: Indicates the docked image of DNA gyrase with secoisolariciresinol diglucoside visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with secoisolariciresinol diglucoside in 3D (B) 3D structure of secoisolariciresinol diglucoside (C) 2D interaction of DNA gyrase with secoisolariciresinol diglucoside (D) 2D structure of secoisolariciresinol diglucoside.

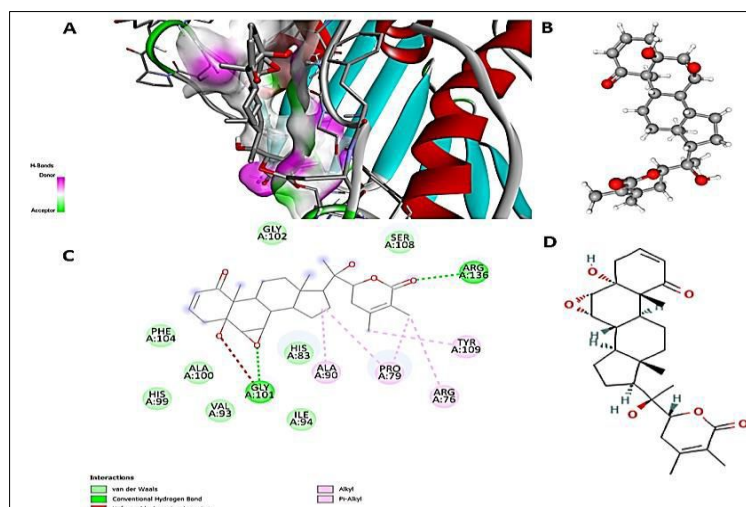


Fig 13: Indicates the docked image of DNA gyrase with withanolide A visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with withanolide A in 3D (B) 3D structure of withanolide A (C) 2D interaction of DNA gyrase with withanolide A (D) 2D structure of withanolide A.

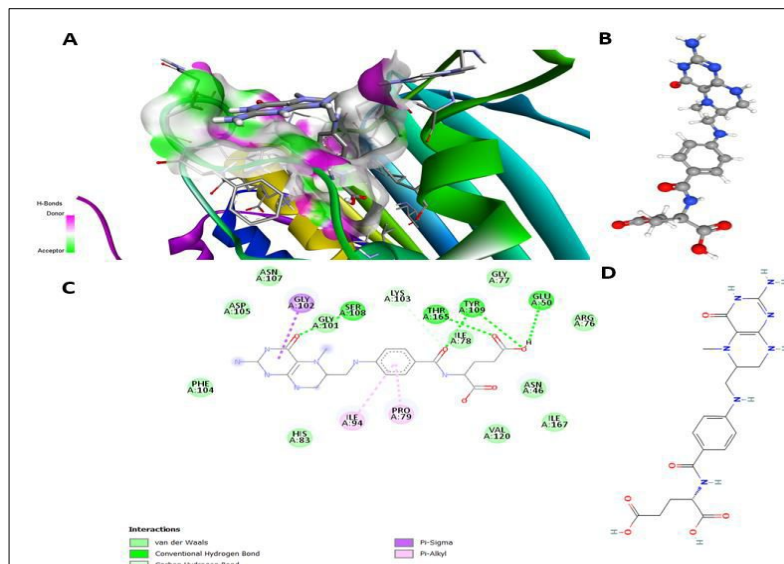


Fig 14: Indicates the docked image of DNA gyrase with methyl folate visualized using BIOVIA Discovery studio (A) Hydrogen bond interaction of DNA gyrase with methyl folate in 3D (B) 3D structure of methyl folate (C) 2D interaction of DNA gyrase with methyl folate (D) 2D structure of methyl folate.

Table 4: ADMET analysis of shortlisted compounds

Property	Parameters	Quercituron	Aloesin	Curcumin	Secoisolaricresinol diglucoside	Nobiletin	Quercetin	Methyl folate	Honokiol	Piperine	Withanolide A
Physico-chemical properties	Formula	C ₂₁ H ₁₈ O ₁₃	C ₁₉ H ₂₂ O ₉	C ₂₁ H ₂₀ O ₆	C ₃₂ H ₄₆ O ₁₆	C ₂₁ H ₂₂ O ₈	C ₁₅ H ₁₀ O ₇	C ₂₀ H ₂₅ N ₇ O ₆	C ₁₈ H ₁₈ O ₂	C ₁₇ H ₁₉ NO ₃	C ₂₈ H ₃₈ O ₆
	Molecular Weight g/mol	478.362	394.376	368.385	686.704	402.399	302.238	459.463	266.34	285.343	470.606
	LogP	-0.4466	-0.54658	3.3699	-2.2344	3.5116	1.988	-0.2577	4.2218	2.9972	3.4954
	H Rotatable Bonds	4	4	8	15	7	1	9	5	3	2
	H Acceptors	12	9	6	16	8	7	9	2	3	6
	H Donors	8	5	2	10	0	5	7	2	0	2
	Surface Area	188.063	158.969	156.532	276.399	167.007	122.108	187.555	118.887	124.203	201.317
Absorption	Water solubility (log mol/L)	-2.897	-2.309	-4.01	-2.695	-4.949	-2.925	-2.858	-4.106	-3.464	-5.096
	Caco2 permeability (log Papp in 10 ⁻⁶ cm/s)	-1.061	0.305	-0.093	-0.908	1.306	-0.229	-1.003	1.584	1.596	0.846
	Intestinal absorption (human) (% Absorbed)	25.112	46.247	82.19	0	98.921	77.207	5.072	92.564	94.444	100
	Skin Permeability (log Kp)	-2.735	-2.736	-2.764	-2.735	-2.715	-2.735	-2.735	-2.735	-3.131	-3.285
	P-glycoprotein substrate (Yes/No)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

	P-glycoprotein I inhibitor (Yes/No)	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes
	P-glycoprotein II inhibitor (Yes/No)	No	No	Yes	Yes	Yes	No	No	No	No	Yes
Distribution	VDss (human) (log L/kg)	1.647	0.212	-0.215	-0.065	-0.281	1.559	-0.632	0.163	0.158	-0.097
	Fraction unbound (human) (Fu)	0.274	0.416	0	0.234	0.179	0.206	0.527	0.005	0.134	0.096
	BBB permeability (log BB)	-1.614	-1.29	-0.562	-2.107	-1.254	-1.098	-1.748	-0.112	-0.102	-0.255
	CNS permeability (log PS)	-4.139	-3.783	-2.99	-5.086	-3.142	-3.065	-4.191	-1.477	-1.879	-2.681
Metabolism	CYP2D6 substrate (Yes/No)	No	No	No	No	No	No	No	No	No	No
	CYP3A4 substrate (Yes/No)	No	No	Yes	No	Yes	No	No	Yes	Yes	Yes
	CYP1A2 inhibitor (Yes/No)	No	No	Yes	No	Yes	Yes	No	Yes	No	No
	CYP2C19 inhibitor (Yes/No)	No	No	Yes	No	Yes	No	No	Yes	Yes	No
	CYP2C9 inhibitor (Yes/No)	No	No	Yes	No	Yes	No	No	Yes	No	No
	CYP2D6 inhibitor (Yes/No)	No	No	No	No	No	No	No	No	No	No
	CYP3A4 inhibitor (Yes/No)	No	No	Yes	No	Yes	No	No	Yes	No	No
Excretion	Total Clearance (log ml/min/kg)	0.434	0.456	-0.002	0.84	0.789	0.407	0.129	0.333	0.232	0.337
	Renal OCT2 substrate (Yes/No)	No	No	No	No	No	No	No	No	Yes	Yes
Toxicity	AMES toxicity (Yes/No)	No	No	No	No	No	No	No	No	No	No
	Max. tolerated dose (human) (log mg/kg/day)	0.427	0.437	0.081	0.148	0.443	0.499	-0.573	0.305	-0.38	-0.967
	hERG I inhibitor (Yes/No)	No	No	No	No	No	No	No	No	No	No
	hERG II inhibitor (Yes/No)	No	No	No	Yes	No	No	No	Yes	No	No
	Oral Rat Acute Toxicity	2.497	2.47	1.833	2.45	2.459	2.471	2.623	2.184	2.811	2.913

	(LD50) (mol/kg)										
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/d ay)	4.51	3.95	2.228	5.892	0.82	2.612	3.804	1.791	1.51	1.739
	Hepatotoxici ty (Yes/No)	No	Yes	No	No	No	No	Yes	No	Yes	No
	Skin Sensitisation (Yes/No)	No	No	No	No	No	No	No	No	No	No
	<i>T.</i> <i>Pyriformis</i> to xicity (log ug/L)	0.285	0.285	0.494	0.285	0.315	0.288	0.285	0.749	1.879	0.298
	Minnow toxicity (log mM)	8.323	4.394	-0.081	9.98	0.686	3.721	5.604	0.14	1.732	1.22

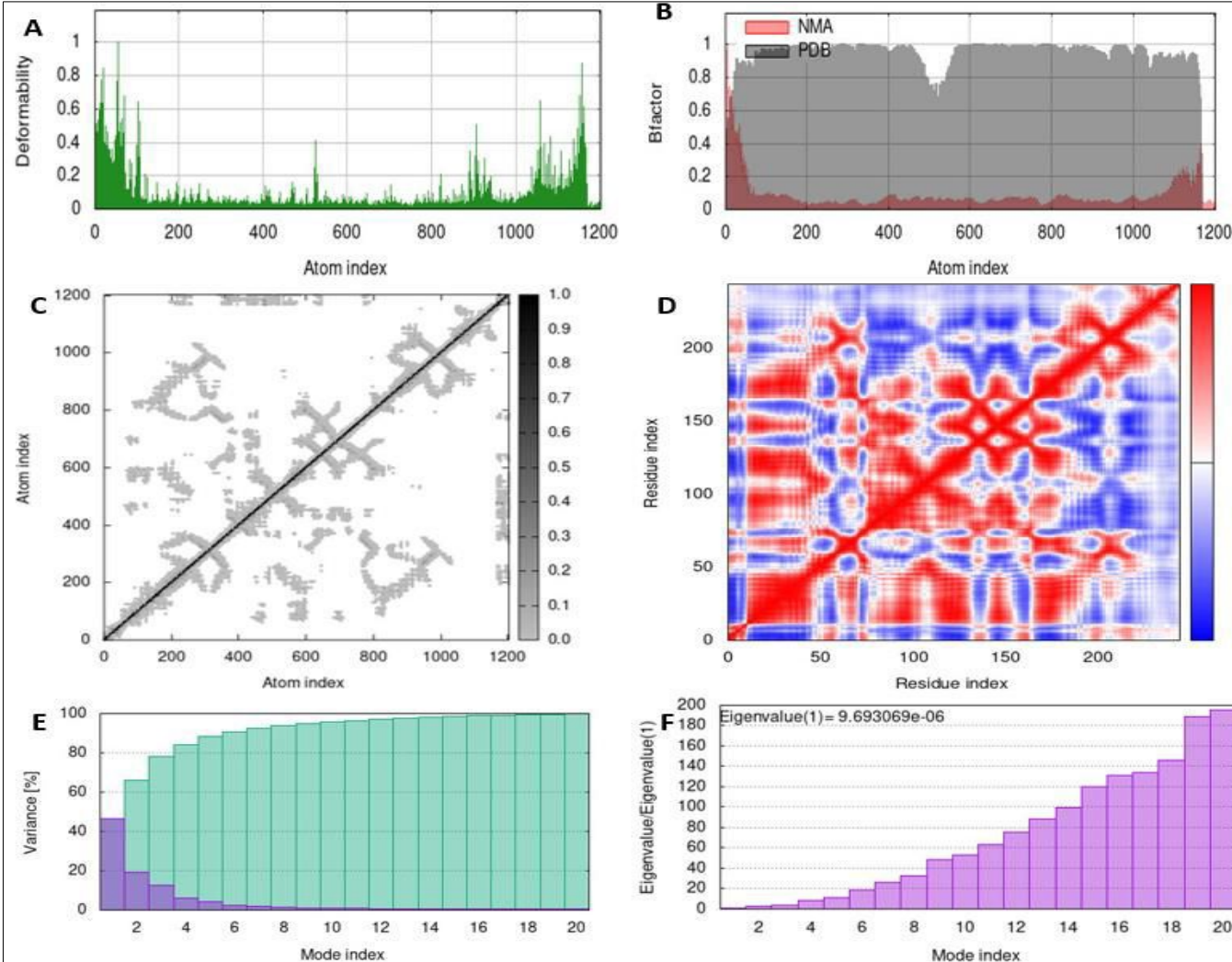


Fig 15: DNA gyrase-Querciturone complex molecular dynamics simulation, showing (A) deformability; (B) B-factor; (C) elastic network analysis defining which pairs of atoms are connected by springs; (D) covariance matrix depicting the coupling between pairs of residues (red), uncorrelated (white), or anti-correlated (blue) motions (E) variance and (F) eigenvalue

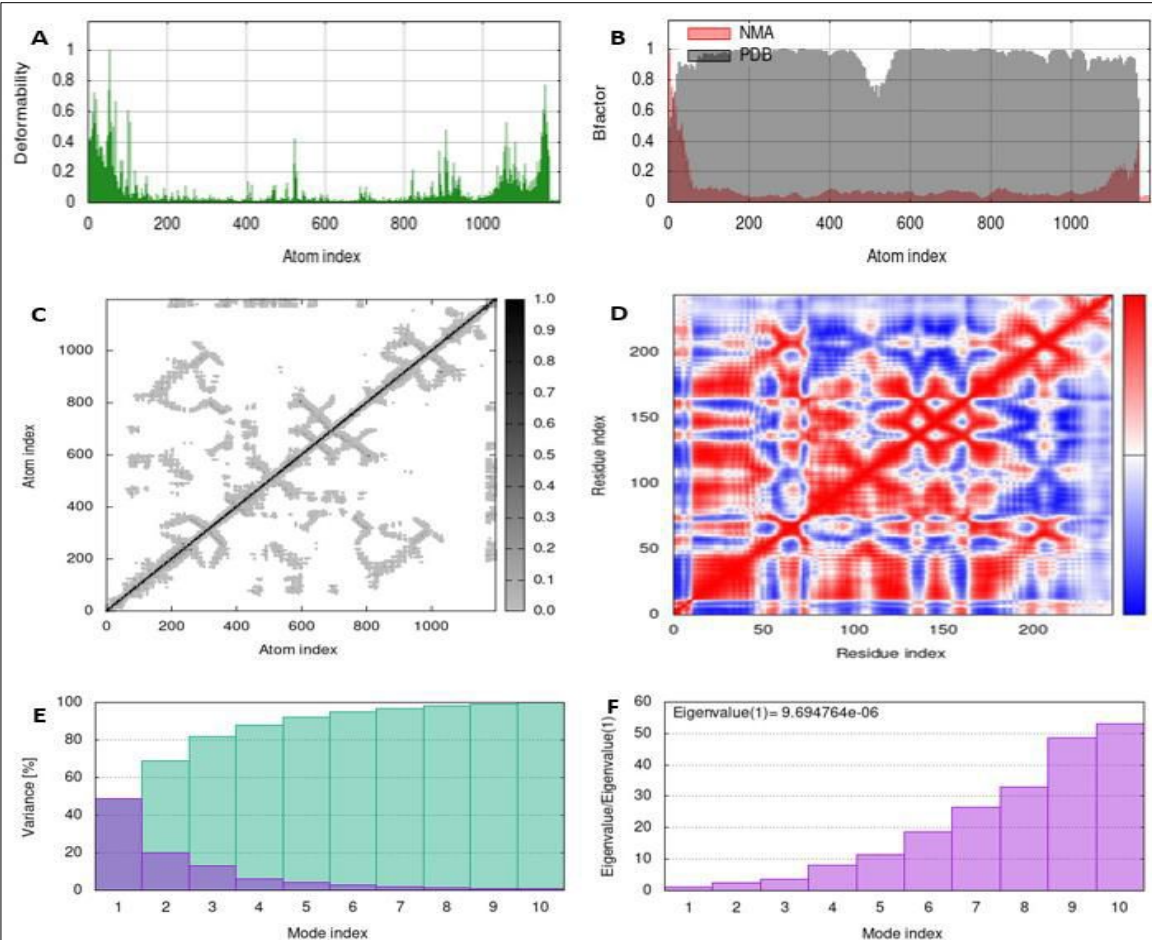


Fig 16: DNA gyrase-aloesin complex molecular dynamics simulation, showing (A) deformability; (B) B-factor; (C) elastic network analysis defining which pairs of atoms are connected by springs; (D) covariance matrix depicting the coupling between pairs of residues (red), uncorrelated (white), or anti-correlated (blue) motions (E) variance and (F) eigenvalue

Table 5: Shortlisted natural source material of selected ligands and known antimicrobial compounds for *in vitro* testing against MDR *Vibrio parahaemolyticus*

Sr. No.	Compound/chemical	Cfu/ml
1	Eucalyptus leaf (11.04%)	0
2	Ashwagandha (5.5%)	7x10 ³
3	Black pepper (1.75%)	TNTC
4	Flax seed (1.5%)	TNTC
5	Aloe vera root (2.6%)	0
6	Turmeric (5.3%)	TNTC
7	Positive control	TNTC
8	Negative control	0



Fig 17: 1-Neem (10.89%), 2-Ginger (12.50%), 3- Garlic (13%), 4-Turmeric(10.64%), 5- Kalonji (28.82%), 6-Guava Leaf (14.15%), 13- Hibiscus (21%), 14 DMSO, 15-Eucalyptus (22%), 16-Eucalyptus (5.5%), 17- Chloramphenicol (positive control), 18- Ampicillin (negative control)

Conclusions: In this study, we conducted a molecular docking analysis targeting the DNA Gyrase protein. A total of 800 ligands were retrieved from the ZINC and PubChem databases and employed for molecular docking studies, facilitated by PyRx software. This research yielded promising results, identifying numerous compounds sourced from both natural and chemical origins that displayed favorable interactions with the protein target associated with *V. parahaemolyticus*. Notably, among the selected ligands, several were derived from readily available and cost-effective natural sources. Remarkably, compounds such as Curcumin, Secoisolariciresinol diglucoside, Nobiletin, Quercetin, Methyl folate, Honokiol, Piperine, Withanolide A, Aloesin, and Quercitrone were found in turmeric, flaxseed, oranges, lotus, sweet chili pepper, star fruit, black pepper, ashwagandha, aloe vera, and eucalyptus shows tremendous binding affinity with DNA gyrase protein in *V. parahaemolyticus*. These compounds demonstrated robust binding affinities toward the DNA Gyrase protein. To gain a more profound understanding of these extracts and potentially isolate specific bioactive compounds, further comprehensive analyses will be indispensable. This in-depth exploration holds the potential to unveil novel solutions for addressing the challenges faced in the shrimp farming sector, with the added benefit of reducing reliance on antibiotics while promoting sustainable and cost-effective practices. However, further *in vivo* studies are needed to confirm that this natural compound displays much better clinical efficiency toward resistant bacteria than existing drugs.

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