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Bioinformatic studies of the effect of *Curcumin glucuronide* in the plants of the *Curcuma longa* species on DNA gyrase inhibition as antimicrobial agent

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ABSTRACT

DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. DNA gyrase has long been known as an attractive target for antibacterial drugs. Curcumin is a polyphenol, found in the spice turmeric, that has promising anticancer and antimicrobial properties. The aim of this research is the bioinformatical study of DNA gyrase inhibition by a Curcumin derivative. In order to investigate the mode of interaction of the compound with DNA gyrase active site, the chemical structure of Curcumin glucuronide wase designed using ChemDraw program, then transferred into Hyperchem software for energy minimization. Docking study was performed by AutoDock 4.2 program and the resulting docking poses were analyzed in AutoDockTools, DS Visualizer 3.5 and Ligplot software. Curcumin glucuronide was able to occupy the active site of the enzyme. In fact, this compound indicated favorable interactions with the key amino acid residues at active site of DNA gyrase. Docking results for this compound are in accordance with those of cocrystallized ligand. The Asn46, Glu50, Ala47, Val71, Val43 of DNA gyrase were the sites for hydrogen bonding interactions with this compound. Finally, in respect to high effectiveness and docking results, we can conclude that the Curcumin glucuronide may be regarded as antimicrobial agent.

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Introduction

Escherichia coli is classified as a rod-shaped, Gram-negative bacterium in the family Enterobacteriaceae. The bacterium mainly inhabits the lower intestinal tract of warm-blooded animals, including humans, and is often discharged into the environment through faeces or wastewater effluent(Jang et al., 2017) .Escherichia coli remains one of the most frequent causes of several common bacterial infections in humans and animals. *E. coli* is the prominent cause of enteritis, urinary tract infection, septicaemia and other clinical infections, such as neonatal meningitis. E. coli is also prominently associated with diarrhoea in pet and farm animals (Allocati et al., 2013). Particular strains belonging to Escherichia coli have been identified as a potential risk factor for colorectal cancer (CRC) (Wassenaar, 2018). DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA (Reece and Maxwell, 1991). DNA topoisomerases are enzymes that control the topology of DNA in all cells. There are two types, I and II, classified according to whether they make transient single- or double-stranded breaks in DNA. Their reactions generally involve the passage of a single-

or double-strand segment of DNA through this transient break, stabilized by DNA-protein covalent bonds. All topoisomerases can relax DNA, but DNA gyrase, present in all bacteria, can also introduce supercoils into DNA. Because of their essentiality in all cells and the fact that their reactions proceed via DNA breaks, topoisomerases have become important drug targets; the bacterial enzymes are key targets for antibacterial agents (Bush et al., 2015). DNA gyrase is comprised of two distinct subunits, GyrA and GyrB (molecular mass ≈96 kDa and 88 kDa, respectively) and is arranged as an A_2B_2 tetramer. GyrA contains the active site tyrosine used in DNA cleavage and ligation, and GyrB contains the binding site for ATP(Vélez and Osheroff, 2004). DNA gyrase is an essential enzyme in bacteria, and its inhibition results in the disruption of DNA synthesis and, subsequently, cell death (Eakin et al., 2021).

Curcumin (1, 7 - bis - (4 – hydroxyl – 3 methoxyphenyl) – hepta -1,6-diene-3,5-dione) (Kotha and Luthria, 2019) is a substance obtained from the root of the turmeric plant, which has the feature of being a yellow or orange pigment. It is also the main component of curry powder commonly used in Asian cuisine (Unlu et al., 2016). Curcumin is one of the most important components of the curcuminoids famil. it is also called as diferuloylmethane, which can be isolated from the rhizome of Curcuma longa L. It was first discovered in 1815, though its chemical structure was

identified in 1973 by Roughley and Whiting with a melting point ranging from 176 °C to 177 °C (Mbese et al., 2019). Extensive research over the past half century has shown that curcumin (diferuloylmethane), a component of the golden spice turmeric (Curcuma longa), can modulate multiple cells signaling pathways.Extensive clinical trials over the past quarter century have addressed the pharmacokinetics, safety, and efficacy of this nutraceutical against numerous diseases in humans. Some promising effects have been observed in patients with various pro-inflammatory diseases including cancer, cardiovascular disease, arthritis, uveitis, ulcerative proctitis, Crohn's disease, ulcerative colitis, irritable bowel disease, tropical pancreatitis, peptic ulcer, gastric ulcer, idiopathic orbital inflammatory pseudotumor, oral lichen planus, gastric inflammation, vitiligo, psoriasis, acute coronary syndrome, atherosclerosis, diabetes, diabetic nephropathy, diabetic microangiopathy, lupus nephritis, renal conditions, acquired immunodeficiency syndrome, β-thalassemia, biliary dyskinesia, Dejerine-Sottas disease, cholecystitis, and chronic bacterial prostatitis. Curcumin has also shown protection against hepatic conditions, chronic arsenic exposure, and alcohol intoxication (Gupta et al., 2013). An increasing amount of evidence suggests that curcumin may represent an effective agent in the treatment

of several skin conditions (Vollono et al., 2019). In vivo and in vitro studies have uncovered many important bioactivities of curcumin, such as antioxidant activity, inducing cell apoptosis, inhibiting cell proliferation, anti-cell adhesion and motility, anti-angiogenesis and anti-microbe properties (Fan et al., 2013). In spite of all these benefits, the therapeutic application of curcumin in clinical medicine and its bioavailability are still limited due to its poor absorption and rapid metabolism. Structural modification of curcumin through the synthesis of curcumin-based derivatives is a potential approach to overcome the above limitations. Curcumin derivatives can overcome the disadvantages of curcumin while enhancing the overall efficacy and hindering drug resistance (Mbese et al., 2019).

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure (Morris and Lim-Wilby, 2008).

Materials and Methods

Protein and Ligand Structure Preparation:

The crystal structure of DNA gyrase from Escherichia coli (1kzn) was chosen as the protein model for the present study. Co-crystallized ligands (CBN1), and water molecules of crystallization were removed from the complex using Discovery StudioVisualizer. All

missing hydrogens were added and after determining the Kolman united atom charges, non-polar hydrogens were merged to their corresponding carbons using Autodock tools. The structural detailes of the compound subjected to molecular docking simulation is provided in Figure (1). structure of compound was built using ChemDraw program, then were transferred into Hyperchem 8.0 software and energy minimized. This optimized structure was used as input of the AutoDock tools. Then the partial charges of atoms were calculated using the Gasteiger-Marsili procedure implemented in the AutoDock tools package. Non-polar hydrogens of the compound were merged and then rotatable bonds were assigned.

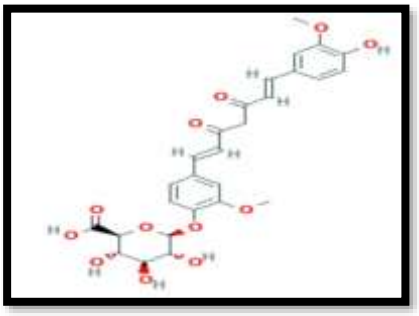

Fig. 1. Structural details of the studied compound.

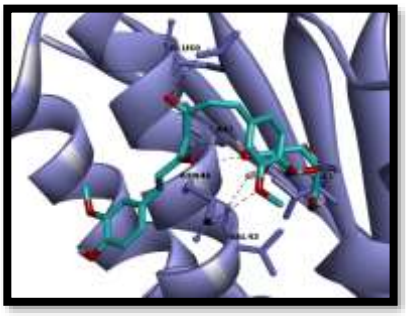

Fig. 2. Docking results of curcumin glucuronide in the active site of DNA gyrase. This figure was prepared using the Accelrys discovery studio visualizer program.

Docking Procedure:

The AutoGrid program performs precalculations for the docking of a ligand to a set of grids that describe the effect that the protein has on point charges. The effect of these forces on the ligand is then analyzed by the

AutoDock program. Using Autogrid as a part of the Auto dock package, desolvation parameters and electrostatic interactions were assigned to each protein atom. The grid points were set as 40 × 40 × 40 with the spacing valued at 0.375 to the catalytic site of the DNA gyrase. The Lamarckian Genetic Algorithm (LGA) approach was selected as the search algorithm for the global optimum binding position search among the three different search algorithms offered by AutoDock 4.2. The resulting docking poses were analyzed in AutoDockTools, DS Visualizer 3.5 and Ligplot softwares (Asadzadeh et al., 2015).

Results and Discussion

After docking various ligand interactions with the protein can be observed and analyzed. The binding mode of the study compound against DNA gyrase was investigated by performing molecular docking smulations. Possible interactions assessed using the Discovery StudioVisualizer program.the Asn46, Ala47, Glu50, Val71, Val43 residues of DNA gyrase were the sites for hydrogen bonding interactions with studied compound. this molecule makes no pi interactions in active site (Figure2). DNA gyrase from Escherichia coli that was obtained from the RCSB Protein Data Bank(1kzn) was transferred into Discovery StudioVisualizer. At the active site of the enzyme Asn46, Asp73, Arg136 residues were the sites for hydrogen bonding and it showed one Pi-cationic interaction with Arg136. DNA

gyrase is a very selective and validated target for the development of novel antibiotics that target DNA replication (Korrapati et al., 2021). The docking results show that *Curcumin glucuronide* can bind to the active site of DNA gyrase and inhibit it. Co-crystal molecule reveals that hydrogen bonding with Asn46 residue is the same for Curcumin glucuronide and Co-crystal molecule.

The docking results for these compoundare in accordance with the docking results reportedby others in terms of the amino residues involved in interaction with the inhibitor molecule. Clorobiocin, inhibitor of E. coli DNA gyrase binding to the amino acid residues N46, D73, I78, P79, I90, R136, T165 were highly conserved in the selected group of pathogens (Korrapati et al., 2021) [17]. reported that this binding complex involves 3 hydrogen Bonds with residues Arg136, Asp73 and Asn46 and one Pi-cationic interaction with Arg76 (Mohamed A. Ismail et al., 2013). Clorobiocin is a based coumarin antibiotics, which prohibits the cell division of bacteria by inhibition of the DNA gyrase enzyme. There is an article in 2021, The innovative arylthioureas were docked to the active site of DNA gyrase enzyme using Autodock4 to comprehend their possible intermolecular interactions with the receptor. The residues Asp73, Asn46, and Arg136 are vital in making hydrogen bonds and are very important for the biological activity. some compounds also

displayed a strong hydrogen bond with Asn46. Docked compounds also stabilize the DNA gyrase via hydrophobic interactions with Ala47, Glu50, Val71, Asp73, Arg76, Gly77, Ile78, Pro79, Met91, Val43, Thr165, and Val167 (Khidre and Radini, 2021).

In a study on benzimidazole derivatives as antibacterial compounds for inhibition of DNA gyrase (subunit b-PDB ID: 1kzn) Glu50 and Ans46 residue were the site for hydrogen binding interactions (Gullapelli et al., 2017). The docking study on the DNA gyrase topoisomerase II (1 kzn) enzyme of E. coli bacteria was performed exclusively on heterocycles including triazoles.The synthesized compounds exhibited potent antibacterial activities against the tested bacterial strains. The alkyne CH of 5 was found to be involved in p-alkyl interaction with ALA47 and VAL43. The middle phenoxy ring of alkyne 5 showed p-anion interaction with GLU50 and p-alkyl interaction with ILE78.The carbonyl oxygen of the semicarbazone moiety showed conventional H-bond interaction with ARG136. The amide group of the semicarbazone moiety showed conventional H-bond interaction with GLU50 and GLY77. The benzene ring of triazole showed p-alkyl interaction with ALA47 (Kumar et al.,2019). in Docking Studies of Substituted Acetylphenoxymethyl - triazolyl - N - phenylacetamides the most significant steric interactions were observed through 2-(4-((4 acetylphenoxy) methyl) with Val71, Glu50,

Ala47, Val43 Val167, Met166, Thr165, Asp74, Asp73, residues (Pramod et al., 2019). In 2018, docking studies were performed for the synthetic compounds. The hydrophobic interactions of best conformer with DNAG receptor VAL43, VAL71, VAL93, VAL118, VAL120, VAL167, ILE90, ILE78, ILE59 (Hanan et al., 2018).

Also 4-anilinoquinazoline derivatives were studied for their antimicrobial activities against Gram-positive and Gram-negative bacteria All compounds showed good results especially compound with Glu50, Arg 76 residues in place of hydrogen bonds

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