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DNA Cleavage and Molecular Docking studies using Green Synthesized Annona muricata silver nanoparticles

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Abstract: Annona muricata has been attributed with numerous health benefits including antidiabetic, antimicrobial, etc. The aim of this study was to carry out DNA cleavage of pUC18 plasmid DNA and molecular docking against targeted protein. The DNA cleavage study was performed to know whether the isolated extract has the ability to cleave the DNA. Once the DNA was cleaved into fragments it may also lead to the death of the microbes containing the DNA which can also use as the antimicrobial agent in the drug formulation process. The molecular docking were performed to study the interaction between the molecule and the protein by which the drug interaction with the body can be known by In-Silico approach. For the studies, green synthesis of silver nanoparticles using leaf, fruit and seed of Annona muricata. Then with the sample produced the DNA cleavage studies were done with the agarose gel electrophoresis made using ethidium bromide to view the DNA under the UV light. The molecular docking was studied between alpha-d-glucopyranoside and 3VF6. The DNA cleavage studies revealed that the complete cleavage of the pUC18 plasmid DNA. In-silico Molecular docking was performed to analyse the interaction between the bioactive compound **alpha-d-glucopyranoside** ***present in Annona muricate and the targeted protein 3VF6.***

Keywords: Anonna muricata, molecular docking, DNA cleavage, silver nanoparticles, alpha-d-glucopyranoside, Anti-diabetic

1. INTRODUCTION

Anonna muricata is an evergreen tree, flowering and possessing broadleaf belongs to the Annonaceae family that bears sour and sweet flavoured fruit commonly known as soursop, gives the flavour of custard fruit they have fibrous texture, unique and pleas able aroma and are creamy and juicy it is the key tropical fruit contributes to the economic growth of the tropical countries like America, Australia, Africa and Malaysia. The fruit of Graviola holds many compounds to the highest of 114 volatile compounds they are in charge of the aroma, five hydrocarbons, forty-four esters, three acids, twenty-five terpenes, three lactones, ten alcohols, seven aromatic compounds, nine aldehyde and ketones and miscellaneous compounds. They possess the largest fruit among their family the juice and pulp of this fruit have been used in many food products like jam and jellies, syrups and nectars and ice-creams [1, 2]. Mono-THF acetogenins were present in the leaves and seeds of this tree, the compounds

responsible for the biosynthesis of the later compound [3]. *Anonna muricata* tree is a rich source of acetogenins [4] and they also contain minerals like potassium, calcium, sodium, copper, iron and magnesium, thus the constant intake of this fruit may provide good nutrients to the human body [5]. Different parts of this tree are in the treatment of many diseases and used as traditional medicine, the diseases cured were rheumatism [6], parasitic infestation [7][8], diabetes [8], inflammation [9] and hypertension [10]. The medical properties of this plant affect the treatment of many medical ailments, so this can be used as an effective source in the improvement of human health. Among the Annonaceae family species, the *Anonna muricata* has many potential properties, phytochemical constituents, many bioactive compounds that make them very special and has taken the attention of many scientific researchers. They are very much used in the field of pharmacy due to their pharmacological properties.

2. MATERIALS AND METHODS

Preparation of extract

The leaf, seed and fruit peel of Annonamuricatawas collected. The samples were washed in running tap water to remove the dirt,dehydrated to remove the moisture content,powdered, sieved to fine powder and kept separately for the experiments. The aqueous extract of the three samples were prepared by adding 50ml of distilled water in the three stored samples and kept in a magnetic stirrer for 24 hours, and then it is filtered using a Whatman filter. The pH and colour change was noted.

Synthesis of silver nanoparticles

The 100ml of each sample extract was mixed with 150ml of 1Mm silver nitrate solution (silver nitrate in water provides silver ions for the reaction), and then the mixture was incubated at 25°C in the dark (to avoid phytochemical activation of silver nitrate). The observation was silvery brown precipitation at the end of 20 minutes indicates the formation of silver nanoparticles. The formed product was washed well in double distilled water, dried and stored for further experiments[8]

DNA cleavage

Cleavage experiments were performed with mupid-ex agarose electrophoresis [12] connected with 50-200 V power supply visualized by UV trannsilluminator and photographed. Cleavage experiments of pUC18 plasmid DNA (300 ng) by complex 8 and 9 (1.0-7.0 μM) in a 5 mM Tris-HCl / 50 mM NaCl buffer at pH 7.2 were carried out and the reaction followed by Agarose gel electrophoresis. The samples were incubated for 1 h at 37°C. A loading buffer, containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol, was added and electrophoresis was carried out at 60 V for 1 h in a Tris-HCl buffer using a 1% agarose gel containing 1.0 mg/mL of ethidium bromide.

Molecular Docking

The Glide XP ligand docking protocol was employed to predict the scoring and binding interactions between the 3VF6 and the ligand alpha-d-glucopyranoside . The 3VF6 protein was taken from the RCSB protein data bank which is the protein present in the glucokinase regulates protein level in the blood. The alpha-d-glucopyranoside is a small molecule that present in the nanoparticle extract which was confirmed by the GC-MS result they have the ability to activate glucokinase. Then for optimization, the taken 3VF6 protein was run under protein prep wizard where the missing side chain and loops are added and the steric clashes between atoms are adjusted. The grid box was formed everywhere around the ligand 0H6 co-crystal in the 3VF6. The 0H6 ligand co-crystal was eliminated during the molecular docking process. XP glide was performed for the protein and the compound to know the glide score which determines the

interaction effect of the protein and the compound is chosen, that is lower the glide score good the binding effect greater negative value greater the interaction between the compounds. The Glide score, which distinguishes molecules based on interacting ability, was calculated for ligands. A more negative glide score indicates better fitting to the receptor active sites. The best ligand binding pose with the least Glide score or energy was chosen. The alpha-d-glucopyranoside binding site was polar which gave them the property of tight binding towards the inhibitors.

3. RESULTS AND DISCUSSION

DNA Cleavage

The cleavage activity was demonstrated by gel-electrophoresis experiments using pUC18 plasmid DNA in a medium TAE buffer. The first lane contains the plasmid DNA without any sample in it and gave the results with no cleavage in it. The second lane contains the silver nanoparticle synthesised from seed of Annona muricata (DSC1) and the pUC18 plasmid DNA which showed the partial cleavage of the DNA. The third and the fourth lane contain silver nanoparticle synthesised from fruit of Annona muricata (DSC2) with the plasmid DNA and silver nanoparticle synthesised from leaf of Annona muricata DSC3 with the plasmid DNA respectively. Both the lanes show complete digestion of the loaded along with the samples. The experiment shows that the all the nanoparticles synthesised from *Anonna muricata* has the ability to cleave DNA into fragments.

Figure 1: DNA Cleavage, Lane 1: Control (Plasmid DNA)**,** Lane 2: Plasmid DNA + DSC 1- Partially Cleaved the Plasmid DNA**,** Lane 3: Plasmid DNA + DSC 2- Completely Cleaved the Plasmid DNA**,** Lane 4: Plasmid DNA + DSC 3- Completely Cleaved the Plasmid DNA

Molecular Docking

The molecular docking for the alpha-d-glucopyranoside against 3VF6 gave the results that all alpha-d-glucopyranoside antagonists predicted were bind to the same hydrophobic pocket, previous studies indicated that ligands may occupy different sub cavities also. The

active site residues of alpha-d-glucopyranoside were found to be MET 235, TYR 214, ILE 211, MET 210, VAL 62, ARG 63, SER 64, THR 65, PRO 66, and GLU 67. Among the screened synthetic compounds shows good interactions with alpha-d-glucopyranoside having the least Glide score of -8.1 which has the least glide score. By using the help of the hydrogen bonds the specificity of the interacting molecules was known and also the 3D structure of the interacting macromolecules and protein. Glucokinase forms two hydrogen bond with alpha-d-glucopyranoside involving the residues ARG 63 and THR 65.

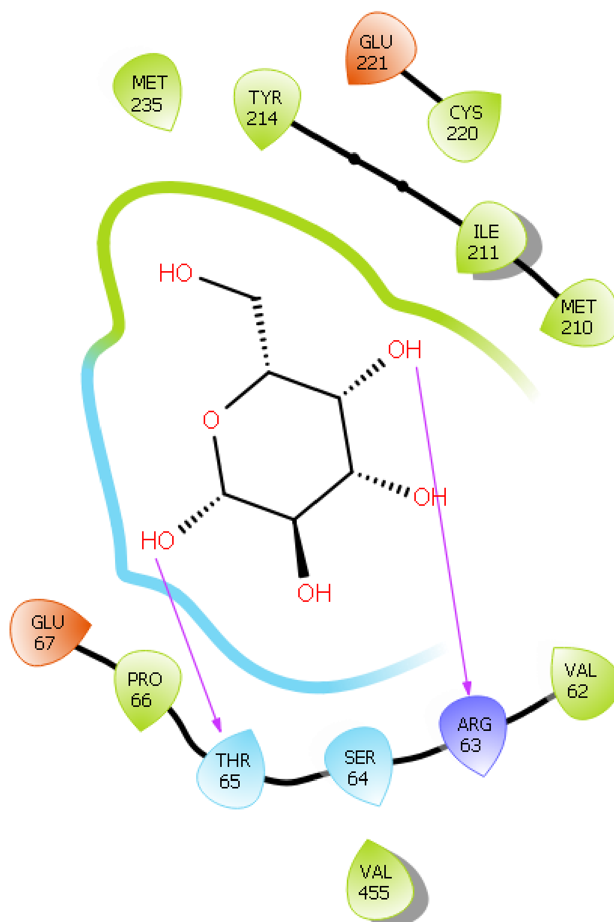

Figure 2: Molecular Docking of alpha-d-glucopyranoside against 3VF6

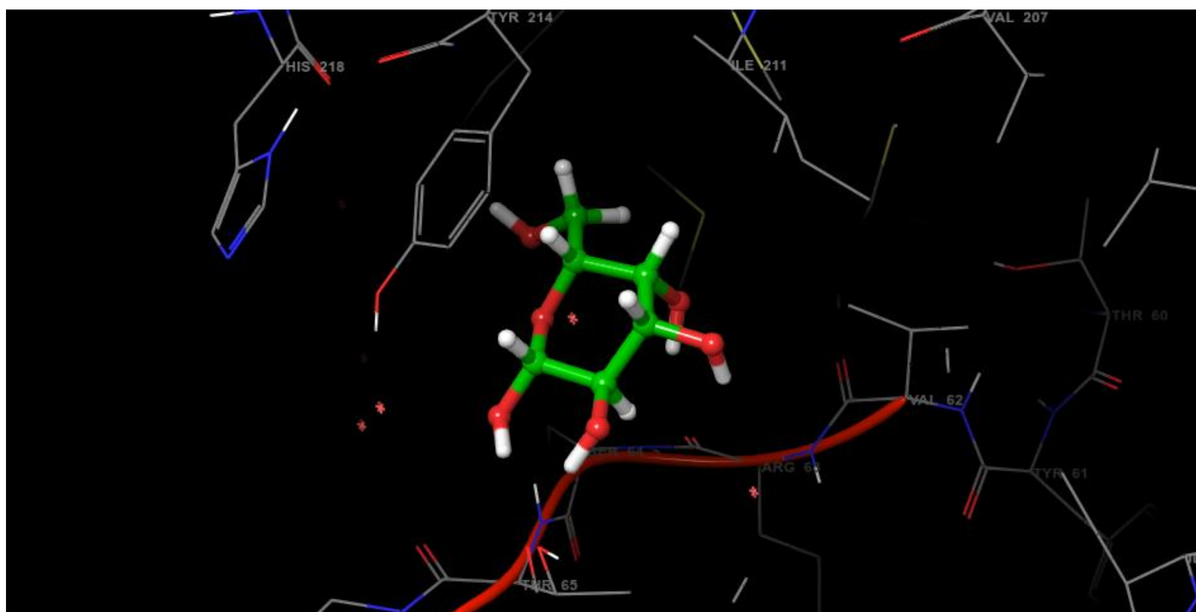

Figure 3: 3D model of molecular docking

4. CONCLUSION

The cleavage and molecular docking studies undertaken in the present work are in total agreement with the primary intercalative mode of binding, although the van der Waals and other types of interactions can also be argued. Bioactivity score also depicted the drug nature of these complexes. Hence, the present study has shown that these synthesized complexes can be used as template for future development through modification and derivatization to design more potent and selective drugs.

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