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Molecular Docking analysis of the antibacterial effect of pomegranate (Punica granatum) fruit molasses bioactive compounds.

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ABSTRACT

Pomegranate molasses is a functional element that has generated extensive scientific attention in the search for effective bioactive natural compounds. The in-silico effects of pomegranate molasses on foodborne pathogens were investigated using molecular docking analysis, using the crystal structures of E. coli topoisomerase II DNA gyrase B as a target. On the ATP-active pocket of E. coli DNA gyrase, the binding affinities and interactions of ellagic acid, gallic acid, punicalagin, and punicalin were examined. According to the findings, novobiocin had a docking score of -6.30 Kcal/mol, whereas the bioactive components of pomegranate molasses (punicalagin, punicalin, gallagic acid, and ellagic acid) adopted the best binding style with a score of -7.2 to -9.8 Kcal/mol. Our findings suggested that pomegranate molasses could provide powerful antibacterial agents and DNA gyrase inhibitors via unique structural properties that address antimicrobial resistance. They are being investigated further as a natural biocontrol agent. Future research to investigate the actual effects of pomegranate fruit molasses against E. coli using both plate inhibition assays and different food matrices is required.

1. INTRODUCTION

The potent antibacterial qualities and elevated concentration of physiologically active compounds in pomegranate are widely recognized (Lieu et al., 2023). Pomegranate has proven to have significant antibacterial activity against a range of pathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other harmful bacteria (Ibrahim et al., 2011; Belgacem et al., 2019; El-Beltagi et al., 2022; Das et al., 2021; Gadallah et al., 2022; Haghayeghi et al., 2013).

Pomegranate molasses's antioxidant capacity is attributed to its high concentrations of polyphenols, anthocyanins, and flavonoids (Akpinar-Bayizit et al., 2016; Ergin, 2020; Arafa, 2013). Pomegranate molasses's polyphenols, ellagic and gallagic acid, punicalin, and punicalagin have all been shown to have strong antibacterial and antifungal properties (Bikiaris et al., 2020). The number of bioactive compounds found in pomegranates, particularly polyphenols with antibacterial activity, including punicalin, gallagic acid, ellagic acid, and punicalagin, is thought to be responsible for the fruit's antibacterial properties (Ibrahim et al., 2011). Moreover, it has been suggested that the phyto-complex of pomegranate extracts' synergistic activity contributes to their antibacterial qualities (Pagliarulo et al., 2016).

The literature supports the use of pomegranate peel as a rich source of antibacterial components. Pomegranate peel is a significant source of polyphenols, particularly ellagitannins, which are responsible for its powerful antibacterial and antioxidant effects (Belgacem et al., 2019; El-Beltagi et al., 2022). Pomegranate peel has been studied as a natural antibacterial agent for different uses, including biodegradable packaging materials (Valdés et al., 2019; Emam-Djomeh et al., 2015). Furthermore, the antibacterial properties of pomegranate peel have been explored in food products, with studies confirming its effectiveness in reducing the development and survival of pathogenic bacteria in food matrices (Das et al., 2021; Gadallah et al., 2022; Haghayeghi et al., 2013).

Molecular docking is an important method in structure-based drug discovery since it predicts the binding mechanism and ranks small compounds for inhibitor discovery campaigns (Meng et al., 2011; Berenger et al., 2021). It has grown more essential in academic and corporate drug screening and discovery operations (Kamal and Chakrabarti, 2023). The method involves the automated docking of flexible ligand molecules into the active site of flexible protein targets (Sousa et al., 2013).

Pomegranate peel and its extract have been shown in numerous studies to have the potential to act as natural sources of antimicrobial agents due to their antibacterial properties. This is the first computational study that we are aware of that looks into the antibacterial properties of pomegranate molasses. The objective was to help enhance food technology, especially in the field of food preservation, and to create a reference for upcoming research.

2. MATERIAL AND METHODS

2.1. Protein preparation

The present study discovered that *E. Coli* DNA gyrase's ATP-active spaces are crucial proteins for foodborne

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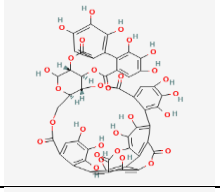
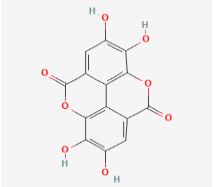
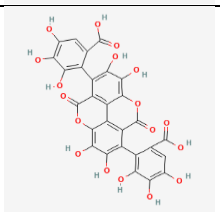
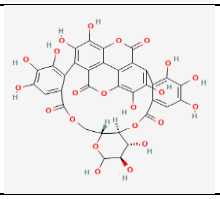
pathogen DNA gyrase and possible targets for inhibitors. The Protein Data Bank provided the crystal structures of *E. Coli* topoisomerase II DNA gyrase B complexed with Novobiocin that were used in docking research (PDB; www.pdb.org, PDB ID:1AJ6). Using PyRx academic, all protein structures were created for docking analysis (https://pyrx.sourceforge.io/). This method involved doing energy minimization, adding hydrogen and charges to the structure, and extracting solvents from PDB files.

2.2. Ligands

The structures of pomegranate molasses active molecules of Punicalagin, Punicalin, Ellagic acid, and Gallagic acid were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1).

Table 1: Ingredients with the highest activity in pomegranate molasses

No.	Ingredient	CAS Number	Formula	Structure
1	Punicalagin	65995-63-3	C48H28O30	
2	Ellagic acid	476-66-4	C14H6O8	
3	Gallagic acid	65995-i62-2	C28H14O18	
4	Punicalin	65995-64-4	C34H22O22	

Structures of pomegranate molasses (PM) probable core components were imported into Chem3D software version 12.0.2. (Cambridgesoft, USA). For energy minimization, assigning ligand atom kinds and components tested according to docking score affinity (Figure 1).

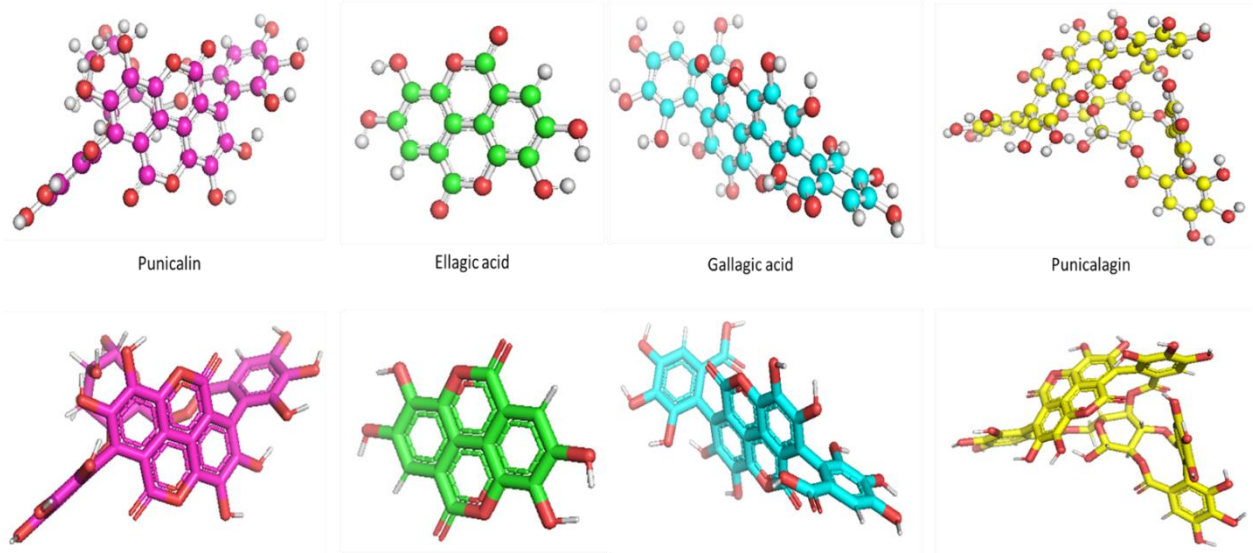


Figure 1: 3D Structures of pomegranate active compounds drawn by Chem3D software version 12.0.2 (Cambridgesoft, USA) Suite.

2.3. Molecular docking

Working cavities were built by anticipating druggable active spots and generating a grid box around them. The docking method was then carried out using the PyRx academic edition (https://pyrx.sourceforge.io/), a software based on the AutoDock Vina algorithm. Then, using visualization tools, the output files of the top binding affinity docking sites were assessed.

2.4. Analysis and visualization

A docking score of <−4.25 kcal/mol indicates ligand binding to the target site, <−5.0 kcal/mol1 indicates greater binding, and <−7.0 kcal/mol indicates a strong affinity between the two. The visualization was performed by PyMol open-source visualizer (http://www.pymol.org/pyMol).

3. RESULTS

In order to learn more about the process of binding between the novel bioactive chemical and the enzyme's active binding site, as well as possible interactions and the docking score, docking experiments were carried out in this work. Re-docking of the co-crystallized ligand novobiocin in the *E. coli* DNA gyrase B active site (PDB code: 1AJ6) resulted in an energy score of -6.4 kcal/mol. This ligand forms a single hydrogen bond with Asp73 to engage with *E. coli* DNA gyrase B kinase (Figure 2).

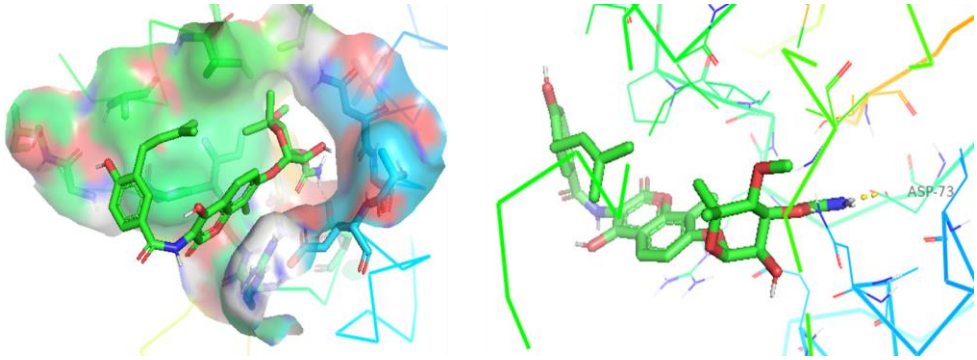


Figure 2: 3D binding modes of Novobiocin in the ATP active site of DNA gyrase (PDB ID: 1AJ6). Yellow dashed lines represent h-bonds. Analyzed by PyMol visualizer (http://www.pymol.org/pyMol).

Using the 3D protein structure, the four most active pomegranate molasses compounds were docked into the ATP-active region of *E. coli* DNA gyrase B. (PDB ID: 1AJ6) It was evident from the docking data that the compound Punicalagin, Punicalin, had the best binding style and had the highest *E. coli* DNA gyrase B inhibitory activity. Punicalin showed hydrogen bonding between VAL118 and HIS98's side chains and nitrogen. A new hydrogen bond with an affinity binding score of - 9.50 kcal/mol was found between the oxygen of the carboxamide group and ALA100 (Figure 3). Punicalagin has an energy score of - 9.80 kcal/mol and demonstrated hydrogen bonding with ARG76, VAL9, and GLY117 (Figure 4).

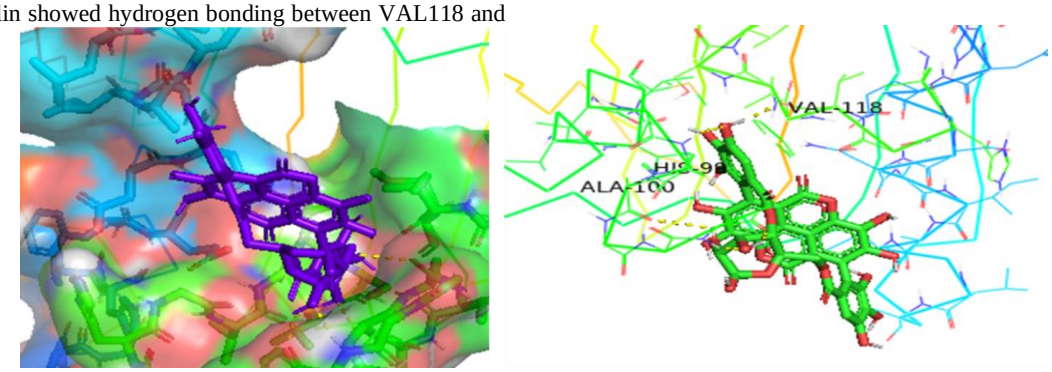


Figure 3: Punicalin's 3D binding modes in DNA gyrase's ATP active site (PDB ID: 1AJ6).Yellow dashed lines represent h-bonds. Analyzed by PyMol visualizer (http://www.pymol.org/pyMol).

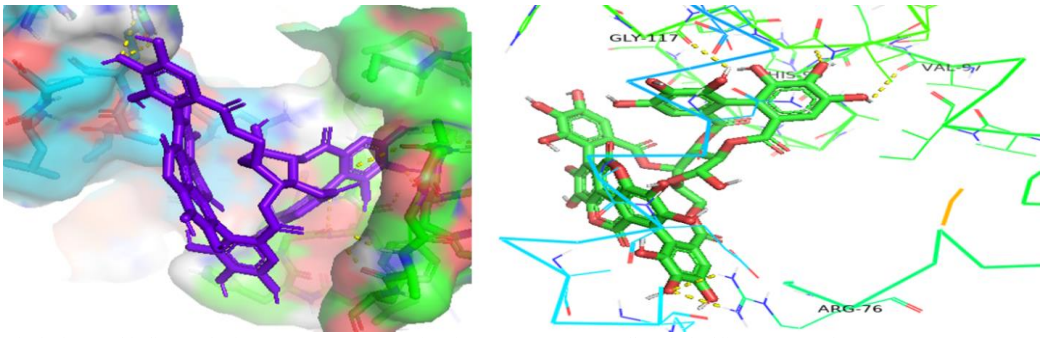


Figure 4: Punicalagin's 3D binding modes in DNA gyrase's ATP active site (PDB ID: 1AJ6). Yellow dashed lines represent h-bonds. Analyzed by PyMol visualizer (http://www.pymol.org/pyMol).

The other two pomegranate molasses compounds showed the same binding affinity with score – 7.2 kcal/mol for both Ellagic and Gallagic acid. Ellagic acid exhibited hydrogen bonds with ARG190 (Figure 5), while Gallagic acid exhibited hydrogen bonds with HIS116, HIS99 and VAL97 (Figure 6).

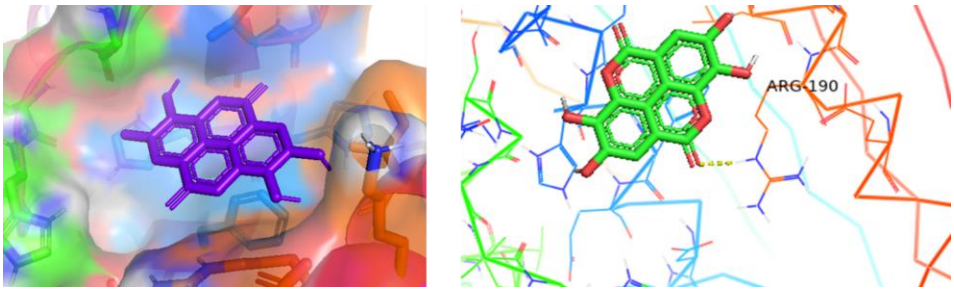


Figure 5: Ellagic acid's 3D binding modes in DNA gyrase's ATP active site (PDB ID: 1AJ6). Yellow dashed lines represent h-bonds. Analyzed by PyMol visualizer (http://www.pymol.org/pyMol).

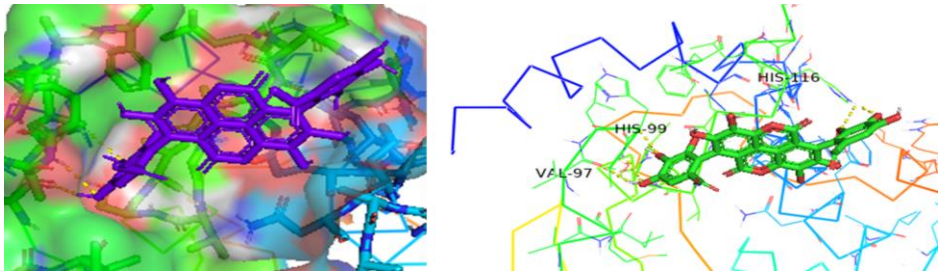


Figure 6: Gallagic acid's 3D binding modes in DNA gyrase's ATP active site (PDB ID: 1AJ6). Yellow dashed lines represent h-bonds. Analyzed by PyMol visualizer (http://www.pymol.org/pyMol).

4. DISCUSSION

Pomegranate molasses, a concentrated form of pomegranate juice, contains various bioactive compounds such as punicalagin, punicalin, and ellagic acid, which contribute to its potential health benefits. Punicalagin, an important polyphenolic compound in pomegranate peels, is reported to have strong antioxidant effects (Sun et al., 2017). Additionally, punicalin and other compounds extracted from pomegranates have been shown to kill harmful bacteria such as Escherichia coli and Staphylococcus aureus (Ibrahim et al., 2011; Opara et al., 2008; Bikiaris et al., 2020; Karwasra et al., 2019). In addition, pomegranate molasses has been found to have significant antibacterial activity against *Salmonella typhimurium* and increase the microbial safety of foods (Ergin, 2020).

The presence of these bioactive compounds in pomegranate molasses contributes to its potential as an antimicrobial agent. Molecular docking is a computational technique used to predict the binding affinity and orientation of a small molecule ligand in the active site of a target protein. In pomegranate molasses, molecular docking studies have investigated the possible interactions of the bioactive compounds present in pomegranate with the target protein of foodborne pathogens. For example, ellagic acid (EA) was found to form hydrogen bonds and aromatic interactions within the ATP binding site of the VEGFR-2 kinase, indicating its potential antitumor, antimetastatic, and antiangiogenic activities (Ceci et al., 2018). Similarly, molecular docking studies confirmed the binding of tacrolimus (TAC) to the CYP3A4 protein and provided insights into how the phytoconstituents of pomegranate interact with the CYP isoenzyme (Karwasra et al., 2023). In addition, docking parameters were investigated and selected before conducting docking studies of pomegranate-derived angiotensin-converting enzyme (ACE), which highlighted the potential of pomegranates to treat hypertension (Ali et al., 2023).

In addition, molecular docking analysis of pomegranate fruit powder showed its inhibitory effect against pancreatic lipase and amylase, with quercetin, a compound in pomegranate, binding to the amylase enzyme similar to acarbose (Dewi et al., 2020). In addition, punicalagin, a polyphenol in pomegranate, was analyzed using molecular docking to predict its interaction with proteins, demonstrating its potential to optimize assays to find targets for the treatment of fungal infections (Neto, 2023). Moreover, molecular docking studies have been used to evaluate the potential of pomegranate compounds to inhibit SARS-CoV-2 infection. Certain biologically active compounds, such as gallic acid, quercetin, naringin, and capsaicin, were investigated for their inhibition of SARS-CoV-2 infection (Liskova et al., 2021). Furthermore, molecular docking of pomegranate seed compounds with the enzyme cyclooxygenase demonstrated their potential as anti-inflammatory agents (Iqbal, 2023). These results highlight the diverse applications of molecular docking in exploring the therapeutic potential of pomegranate compounds against various diseases and infections.

5. CONCLUSIONS

In summary, the literature supports the potential of pomegranate peels and byproducts as a rich source of antimicrobial components, particularly due to their polyphenol content and their application in various fields, including food science, medicine, and materials science. To our knowledge, this is the first computational study to utilize molecular docking for the antibacterial effects of pomegranate molasses. Molecular docking has proven to be an effective method for studying ligand-receptor interactions, classifying small compounds, and predicting binding modalities. In comparison to the binding manner of

novobiocin, docking experiments were used to predict the binding mode of the most active molecules to the active binding site of E. coli DNA gyrase B. It is evident from the docking data analysis that the bioactive ingredients in pomegranate molasses interact meaningfully with the chosen bacterial protein target. These findings demonstrate pomegranate molasses' potential as a natural biocontrol agent with a range of health advantages.

Declaration

Ethical approval.

The study was conducted after the research proposal was approved by the Care and Use Committee Research Ethics, Faculty of Veterinary Medicine, Benha University (BUFVTM, 15/10/23), Egypt.

Conflicts of Interest:

The authors declare no conflict of interest.

Authorship contribution statement:

Hanaa S. Bekier: Formal analysis, Data curation, Writing - review and editing. Ahmed Hamad: Methodology, conceptualization, Formal analysis, Data curation, visualization, Writing - review and editing. Nesreen Z. Eleiwa: Supervision, roles/Writing. Reham A. Amin: Writing - review and editing, Supervision. All authors have read and agree to the published version of the manuscript.

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Data Availability Statement: Data is available on request.

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