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Tetrasiklin Antibiyotikleri ve Bromelain Enzimi Arasındaki Etkileşimlerin Kenetleme Araçları Kullanılarak İncelenmesi

Gülgün AYLAZ [1*]

Öne Çıkanlar:
- Bromelain
- Moleküler kenetleme
- Antibiyotikler

Anahtar Kelimeler:
- Bromelain,
- Moleküler kenetleme,
- Demeklosiklin
- Minosiklin
- Tetrasiklin

ÖZET:

Ananas sapından ekstrakte edilen bromelain, farklı amaçlar için kullanılan kompleks bir enzimdir. Bromelain takviyeleri genellikle sindirimi kolaylaştırmak, dolaşım sistemini iyileştirmek ve ağrı kesici özelliğinden dolayı artrit semptomlarını hafifletmek için kullanılır. Ancak antibiyotik kullanımı veya kanama riski olan bazı durumlarda bromelain kullanımı veya doğrudan ananas tüketimi sınırlandırılmalıdır. Bu amaçla antibiyotik bromelain etkileşiminin hangi mekanizma ile gerçekleştiğini göstermek amacıyla bu çalışma yapılmıştır. İlk olarak UCSF Chimera görselleştirme programında bromelain molekülü ve demeklosiklin, minosiklin ve tetrasiklin antibiyotikleri hazırlanmıştır. Etkileşimler, Auto Dock Molecular Modeling Toolkit moleküler modelleme programında görüntülenmiştir. Bu etkileşimlerin serbest bağlanma enerjileri de Auto Dock'ta hesaplanmıştır. Moleküler modelleme sonuçlarına göre, bromelain ve demeklosiklin, minosiklin, tetrasiklin antibiyotikleri, hidrojen bağları ve hidrofobik etkileşimler ile etkileşime girmiştir. Bromelain ve antibiyotikler arasındaki bu etkileşimler, serbest bağlanma enerjisi hesaplamalarına dayalı olarak enerjisel olarak uygun bulunmuştur.

Investigation of Interactions Between Tetracycline Antibiotics and Bromelain Enzyme Using Docking Tools

Highlights:
- Bromelain
- Molecular docking
- Antibiotics

Keywords:
- Bromelain,
- Molecular docking,
- Demeclocycline
- Minocycline
- Tetracycline

ABSTRACT:

Bromelain, extracted from the stem of the pineapple, is a complex enzyme used for different purposes. Bromelain supplements are often used to facilitate digestion, improve the circulatory system and relieve arthritis symptoms due to its pain relief. However, in some cases where there is a risk of antibiotic use or bleeding, the use of bromelain or direct consumption of pineapple should be limited. For this purpose, this study was carried out to show the mechanism by which the antibiotic bromelain interaction occurs. Firstly, the bromelain molecule and demeclocycline, minocycline, and tetracycline antibiotics were prepared in the UCSF Chimera visualizing program. The interactions were monitored in the Auto Dock Molecular Modelling Toolkit molecular modeling program. The free binding energies of these interactions were also calculated in Auto Dock. According the molecular modelling results, bromelain and demeclocycline, minocycline, tetracycline antibiotics were interact with hydrogen bonds and hydrophobic interactions. These interactions between bromelain and antibiotics were energetically favorable based on free binding energy calculations.

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INTRODUCTION

The discovery of penicillin is accepted as the beginning of the antibiotic era. Penicillin group antibiotics are used in the treatment of quite different types of infections, have a bactericidal effect, have low toxicity, and can easily penetrate tissues. Tetracycline is an antibiotic produced by Streptomyces rimosus bacteria (Petkovic et al., 2006). Tetracyclines is the general name given to a group of antibiotics, and tetracycline is one of these antibiotics. Tetracycline antibiotics are a group of broad-spectrum antibiotics that are widely used for the treatment of various bacterial infections. They work by inhibiting bacterial protein synthesis through binding to the bacterial ribosome, thus preventing the attachment of transfer RNA (tRNA) molecules to the ribosome-messenger RNA (mRNA) complex. This mechanism of action makes tetracyclines effective against a wide range of bacteria (LaPlante et al., 2022). Tetracyclines have a broad spectrum of activity against Gram-positive and Gram-negative bacteria, as well as against certain atypical pathogens (Leichtweis et al., 2022). Bacterial resistance to tetracyclines can arise through various mechanisms, including efflux pumps that expel the antibiotic from the cell, ribosomal protection proteins, and enzymatic inactivation (Hu et al., 2023; Hao Li et al., 2022). Tetracyclines are used to treat a wide range of infections, including respiratory tract infections, skin and soft tissue infections, urinary tract infections and some transmitted infections. Tetracyclines have a common basic structure consisting of four rings. They are classified into different generations based on their development and modifications over time (Angelette et al., 2023). Since the molecular structure differences of the antibiotics in this group and their interactions with the proteins are different, their antibacterial activities are also different (Li et al., 2019). Minocycline hydrochloride, or Minocycline, is a tetracycline antibiotic class. It is often used in the treatment of acne. Minocycline is the longest-acting type of tetracycline. Demeclocycline is another antibiotic from the tetracycline class (Singh et al., 2022). Demeclocycline produced by Streptomyces aureofaciens is frequently used in the treatment of acne and bronchitis. It binds to bacterial RNA and inhibits translation, thus preventing the development of bacteria. Therefore, its effect is biostatic (Allahverdiyeva et al., 2021; Wang et al., 2022).

Bromelain is an enzyme with protein breakdown feature obtained from the root, stem, and fruit parts of pineapple. The main component of the bromelain enzyme, also called pineapple extract or extract, is the sulfhydryl proteolytic fraction (Jancic & Gorgieva, 2022). That is, it is a small part of enzymes that help break down protein. In addition to being used primarily as a digestive support, bromelain is frequently preferred in the treatment of edema, relieving inflammation, accelerating the healing process, after skin burns, in the treatment of colon cancer, ulcerative colitis, inflammatory bowel disease, constipation and allergic asthma (Kumar et al., 2022; Pereira et al., 2023). A daily average of 500-800 milligrams is recommended, and although bromelain is generally well tolerated, there are some situations where it is not safe to use this enzyme. Bromelain promotes wound healing as it acts on cellular depolymerization and vascular permeability. Particularly, care should be taken when using bromelain supplements, as they can increase the risk of bleeding in situations such as accidents or surgery (Gupta et al., 2022; Sharma et al., 2022). Similarly, due to their effects on antibiotic absorption, caution should be exercised when using antibiotics and bromelain together. Bromelain has been observed to increase the absorption of antibiotics, whether administered orally, by subcutaneous or intramuscular injection (Chisci & Fredianelli, 2022). In short, it can increase the effectiveness of antibiotics and cause higher serum and tissue levels, as well as cause undesired side effects. There are many studies showing that bromelain alone has antimicrobial and antifungal properties. The use of bromelain with antibiotics has been found to have a synergistic effect and it has been found to be more

effective in chronic bronchitis, staph infection, thrombophilia, perirectal and rectal ulcers, sinus infections and bladder infections compared to the use of antibiotics alone Kumar et al., 2020; Maher et al., 2021). There are studies showing that bromelain affects the absorption mechanism of antibiotics in the body. However, the interactions between bromelain and antibiotics can be complex and may vary depending on the specific antibiotic and the formulation of bromelain used. It's important to note that interactions between dietary supplements, enzymes like bromelain, and pharmaceutical drugs can have different effects based on individual differences, dosages, and other factors (Maher et al., 2021). It may increase the absorption dose of amoxicillin or tetracycline antibiotics used or the side effects of antibiotics (Mameli et al., 2021; Maheshwari et al., 2023).

Thanks to computational molecular modeling programs, they provide information about the interaction sides of the molecules, the interaction profile, and the conformation of the molecules. Thanks to these molecular modeling tools, researchers see molecular binding patterns without the need for long, laborious, and costly experimental studies (Aylaz & Andaç, 2022). In molecular docking studies, the interaction between the stable form receptor molecule and the flexible ligand is monitored. There are many computational modelling programs developed for these purposes (Elton et al., 2019). In this study, molecular docking calculations and conformation of molecules were investigated with AutoDockTools. AutoDock is a widely used molecular docking software that plays a crucial role in pharmaceutical research for drug discovery and design. It allows scientists to predict and analyze the interactions between a ligand and a protein target, which is essential for understanding drug binding and optimizing drug candidates (Yong Yang, 2010; Wang et al., 2004). While using this tool, variables such as different interaction lattice coordinates of protein and ligand molecules and scoring parameters can be determined (Li et al., 2019; Sabe et al., 2021; Solis-Vasquez et al., 2022). For these purposes, the molecular interactions of bromelain and tetracycline group antibiotics were examined with AutoDockTools, and the interaction profile was investigated by calculating their free binding energies.

MATERIALS AND METHODS

Operating System and Software

UCSF Chimera Biocomputing software developed by UCSF Biocomputing, Visualization, and Informatics Resource (RBVI) (Pettersen et al., 2004; Ji et al., 2023), University of California, San Francisco, CA, USA was used in this study. AutoDockTools, a molecular modeling simulation program developed by the Scripps Research Institute, was operated as GUI (graphical user interface) (Morris et al., 2009). In addition, AutoGrid was also used as a grid calculation module developed by the Scripps Research Institute. Chimera-v1.15-win64 and AutoDockTools-v4.2.6 versions were used under a Windows 10 Home Single Language, Lenovo Ideapad 110-15ISK 80UD workstation with an Intel® Core (TM) i5-6200U CPU @ 2.30GHz processor, 4 GB RAM and 240 GB solid state drive running a Windows operating system. The molecules were prepared in Chimera using Protein Data Bank ID (PDB ID) from The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) and PubChem Compound Identifier (CID) number.

Molecular Docking Preparation

The antibiotic and bromelain molecules' PDB files were prepared and minimalized in Chimera. The clear structure of bromelain was directly obtained using the PDB ID 1W0Q. Bromelain was prepared for molecular modeling via Chimera with this specific ID, as using the clear state of the molecule allows for more realistic modeling of interactions that occur in biological systems. And PubChem CID numbers were 54675776, 54680690, and 54675783 for tetracycline, demeclocycline (dimethylchlortetracycline) and minocycline, respectively. Antibiotics and bromelain molecules were

prepared in Chimera for molecular modeling with the Molecular Modeling Toolkit. The antibiotic and bromelain molecules' PDB files were prepared and minimalized in Chimera. The PDB ID was 1W0Q for bromelain (Sorokin et al., 2023; Pankovaa et al., 2022). And PubChem CID numbers were 54675776 (Liang et al., 2023), 54680690 (Bharadwaj et al., 2020), and 54675783 (Juhi et al., 2023) for tetracycline, demeclocycline (dimethylchlortetracycline) and minocycline, respectively. Antibiotics and bromelain molecules were prepared for molecular modeling in Chimera with the Molecular Modeling Toolkit using the specified special codes. Non-standardized ligands in the bromelain structure were not removed to observe their interaction with antibiotics. However, other solvent, water and unwanted ligands were removed from the molecules (Olshannikova et al., 2022). During this dock preparation, hydrogen addition and removal steps were performed with the AddH and AddCharge commands (Kılıç et al., 2021). In addition, the Gasteiger charge, in which the energies are calculated according to the stationary and mobile atoms in the molecules, was applied. While analyzing which bonds in proteins and ligands are rotatable with AutoDockTools, the size of the molecules interacting in a cage was determined with AutoGrid (grid box dimensions) (Salha et al., 2020). In AutoDock v4.2.6, active interaction sites of minocycline, demeclocycline, and tetracycline antibiotics and bromelain were investigated by applying various insertion parameters. Lamarckian Genetic Algorithm (LGA) v4.2 was used for the insertion. Defaultly, the rate of gene mutation was 0.02, the rate of crossover was 0.8, and it was used with various configurations such as $2.5*10^6$ evaluate number. Target coordinates were used as a 126, 126 and 126 points grid box where values were counted along the x, y, and z axes for minocycline. These coordinates were 124, 126, 126 points for demeclocycline and 116, 126, 126 points for tetracycline along the x, y, and z axes, respectively. Grid point spacing was 0.375 Å. For all conformations, bromelain was preserved during the insertion process, while antibiotics were flexible. Ten iterations were performed for each calculation.

RESULTS AND DISCUSSION

Molecular Docking of Bromelain and Antibiotics

Bromelain is a proteolytic enzyme and has a structure containing various functional groups. When bromelain comes into contact with surfaces, it has the potential to generate features like pockets or clefts on the surface, creating suitable areas for tetracycline to adsorb. The binding sites formed by bromelain may be suitable for formation hydrogen bonding, van der Waals interactions, and electrostatic interactions. Since tetracycline has multiple functional groups, it can easily adsorb to these binding sites on both bromelain and the surface. As a result of this interaction, the presence of bromelain can amplify the adsorption of tetracycline onto the surface (Chakraborty et al., 2021; Ke at al., 2022). Bromelain inhibits bradykinin formation at the inflammatory site through depletion of the plasma kallikrein system. It is also known that inhibition of the arachidonic acid pathway inhibits plasma exudation. The beneficial anti-inflammatory effects of bromelain have also been reported in the literature in Human Immunodeficiency Virus (HIV) and cancer patients. Thirty-six patients with chlamydia infection were treated with tetracycline-bromelain complex or doxycycline for a period of 14 days. At the end of the seventh day, improvement was observed in 66.7% of the patients treated with bromelain-tetracycline complex, while 55.6% improved in those administered doxycycline (Romm et al., 2010). Although the mechanism by which bromelain increases antibiotic absorption in the human body is not fully understood, some experimental and clinical applications have made predictions. Dighe et al., in their study, thought that by using bromelain, a proteolytic enzyme, it enables the food to be easily broken down in the digestive system (especially the stomach) and thus increases the absorption of antibiotics. In addition, since bromelain is a proteolytic enzyme, it can

affect the speed of digestion in the stomach and the rate of passage of the contents into the small intestine. Similarly, in a rapidly emptied stomach, antibiotics may reach the small intestine in a shorter time and their possible absorption may increase (Dighe et al., 2010; Banihashemrad et al., 2020). The interactions of the bromelain molecule with minocycline, demeclocycline, and tetracycline antibiotics were monitored by molecular docking studies. Preparation and minimization of molecules before docking operations were performed at UCSF Chimera software (v1.16, developed by the UCSF Resource for Biocomputing, Visualization, and Informatics (RBVI), University of California, San Francisco, CA, USA). The dock prepped molecules are shown in Figure 1.

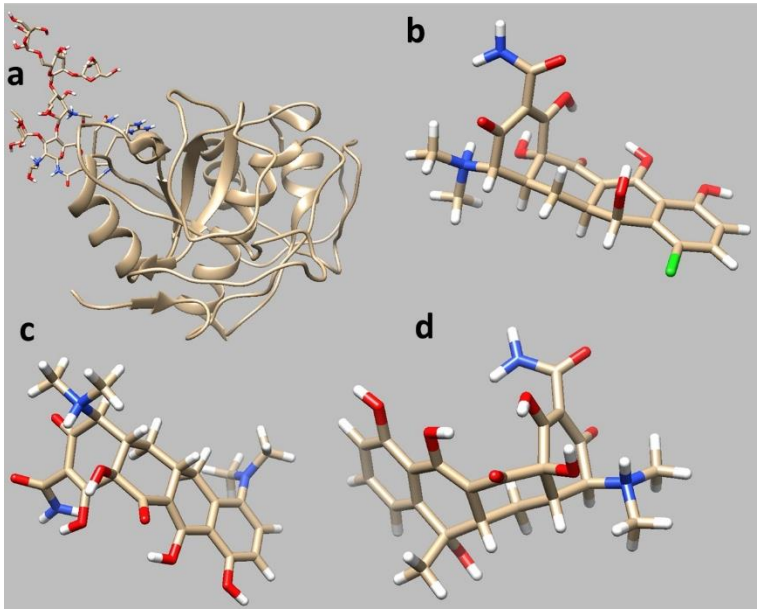

Figure 1. Molecular structures of Bromelain (a), demeclocycline (b), minocycline (c), and tetracycline (d)

The number of distinct conformational clusters were calculated according to the cluster analysis made with the molecular docking of bromelain and antibiotics. This value was found as 8 for each antibiotic in 10 runs using root-mean-square deviation (RMSD) tolerance of 2.0 Å. These clusters were evaluated according to their binding energies. In the clustering histogram, the lowest binding energies between bromelain and antibiotic molecules were -8.26, -8.61, and -8.48 kcal/mol for minocycline, demeclocycline, and tetracycline, respectively. The binding energy value refers to the energy released by the interaction of molecules, and the lower binding energy, the greater the interest in binding (Table 1).

These clusters were evaluated according to their free energy of statistical mechanical analysis. It was seen that the lowest free energy (-1371.31 kcal/mol) between bromelain and demeclocycline at 298.15 ºK. The free energies of the other interactions were found as -1371.13 and -1370.47 kcal/mol for tetracycline and minocycline, respectively at 298.15 ºK. These values indicated that the interaction between bromelain and demeclocycline, minocycline, and tetracycline antibiotics were energetically favorable. Table 1 summarizes the theoretical lowest binding and free energies of bromelain and three different antibiotics.

Table 1: The calculated lowest binding energy and free energy data of bromelain and antibiotics

	Bromelain	
	Lowest Binding Energy (kcal/mol)	**Free Energy (kcal/mol)**
Demeclocycline	-8.61	-1371.31
Minocycline	-8.26	-1370.47
Tetracycline	-8.48	-1371.13

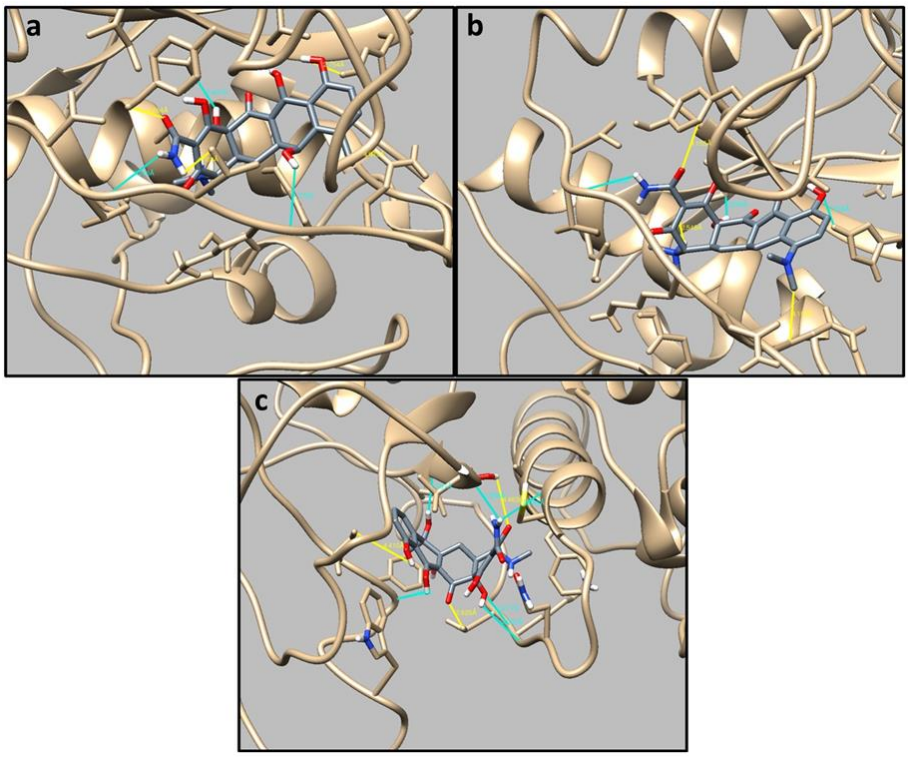

Figure 2. Molecular modelling of bromelain to demeclocycline (a), minocycline (b), and tetracycline (c)

It is important to note that the interactions between bromelain and antibiotics are complex and can vary depending on factors such as the specific antibiotic, dosage, bromelain formulation, and individual variations in digestion and metabolism (Chisci & Fredianelli, 2022; Pavan et al., 2012). The binding sites of bromelain to demeclocycline, minocycline, and tetracycline antibiotics are shown in Figure 2. Molecular interactions between bromelain and antibiotics mainly occurred through hydrogen bonding and hydrophobic interactions. Hydrogen bonds are shown in blue in figure 2 and other interactions are shown with yellow lines. Table 2 summarizes these interaction points and intermolecular distances. In particular, the presence of hydrogen bonds has been evidence of the strong interaction between antibiotics and bromelain. In addition, hydrophobic interactions may contribute to the interaction of bromelain and its hydrophobic structure with demeclocycline, minocycline, and tetracycline antibiotics. As seen in Figure 3, the surface of bromelain is an interacting hydrophobic solid surface. This view has a "hydrophobicity surface" preset. In this preset, hydrophilic areas are identified as dodger blue and hydrophobic areas are orange-red-white. The area where antibiotics bind to bromelain appears to be both hydrophilic and hydrophobic (depends on their dodger blue and orange colors) (Azarkan et al., 2020; Pang et al., 2020). Both the conformation in Figure 3 and the negative binding free energy values given in Table 2 indicate that antibiotics are of molecular interest in bromelain.

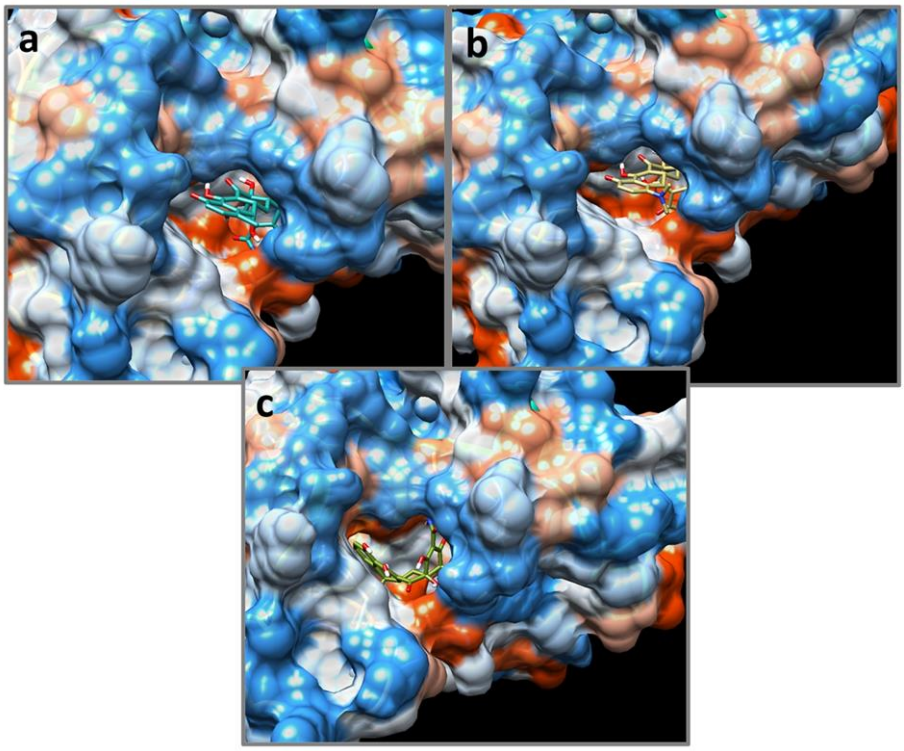

Figure 3: Conformation of demeclocycline (a), minocycline (b), and tetracycline (c) antibiotics with bromelain interactive hydrophobicity solid surface. (Dodger blue signs more hydrophilic surface and orange-red- white signs more hydrophobic surface)

In recent years, it has been understood that bromelain can enhance the tissue permeability of penicillin and tetracyclines following oral using. This results in increased absorption and improved diffusion when these antibiotics are administered intramuscularly. This approach leads to higher levels of antibiotics in both serum and tissues, while also reducing potential side effects. One remarkable study conducted by Neubauer involved 53 hospitalized patients with various conditions such as pneumonia, bronchitis, staphylococcus infections, thrombophlebitis, pyelonephritis, and rectal abscesses (Maurer, 2001). Among them, 23 patients had previously undergone antibiotic therapy without success. Interestingly, when treated with a combination of bromelain and antibiotics, 22 of these patients responded positively. In all disease cases, a significant decrease in morbidity was observed compared to antibiotic treatment alone.

Table 2. Molecular interactions of bromelain with demeclocycline, minocycline, and tetracycline antibiotics

Molecules	H-bonds	Intramolecular Distances
Bromelain – Demeclocycline (LIG)	THR15.B O - LIG H20 LYS 18.B O - LIG H14	VAL 17.B CGA – LIG H19 (4.108 Å) TYR 185.B HH – LIG O8 (4.264 Å) H21 – LIG HIS 158.B (2.754 Å) GLN 20.B – LIG C11 (4.94 Å)
Bromelain – Minocycline (LIG)	THR 15.B O - LIG H20 THR 161.B OG1 - LIG H8 HIS 158.B – LIG O6	TYR 185.B CD1 – LIG O7 (4.155 Å) GLN 20.B CB – LIG C22 (4.195 Å) ALA 33.B – LIG HN (2.545 Å)
Bromelain – Tetracycline (LIG)	CYS 26.B O - LIG H23 ALA 159.B O - LIG H23 LYS 18.B O – LIG H9 THR 161.B O - LIG H10 GLY 184.B O - LIG H19 LYS 18.B NH – LIG O1	VAL 17.B C61 – LIG O3 (2.820 Å) ILE 186.B CD1 – LIG H24 (4.410 Å) THR 161.B HG1 – LIG O6 (4.463 Å)

Similarly, Ryan's double-blind clinical study on acute sinusitis revealed that 83% of patients who received bromelain achieved complete resolution of nasal mucosal inflammation, as opposed to 52% in

the placebo group (Maurer, 2001). All these results proved that there is a favorable interaction between bromelain and tetracycline antibiotics. Looking at the clinical research reports and experimental research in the literature, it has not been precisely explained how bromelain and antibiotic interactions occur. Therefore, thanks to this molecular modeling study, it has been shown how and from which regions the interactions of bromelains and tetracycline antibiotics take place.

CONCLUSION

Especially in recent years, the rapid improvements of biotechnological developments has become both inevitable and mandatory. The basis of this requirement is the desire to advance clinical studies. In experimental studies, process optimization sometimes takes months, which causes both loss of work and high costs (Olivera et al., 2021). At this point, the importance of using computer-based modeling and advanced forecasting has been understood. With these techniques, many experimental and clinical applications can be simulated and evaluated quickly under desired conditions. In this way, pharmaceutical technology, biotechnological innovative approaches, biomaterial applications and medical research studies have gained momentum. Protein-ligand or protein-protein interactions are evaluated in molecular modeling studies (Jones et al., 2021).

In this study, molecular modeling of the interaction between bromelain molecule and demeclocycline, minocycline, and tetracycline antibiotics was performed. It is known that bromelain increases the effectiveness of these three antibiotics in the tetracycline antibiotics group and causes excessive absorption in the body. The structures were seen with free binding energy values calculated for AutoDock computerized molecular modeling studies, where the interactions of bromelain and antibiotics occur spontaneously. No changes were observed in the folding of the two subdomains of the bromelain molecule during the interaction. The interaction mechanism between bromelain and antibiotics was investigated by using the conformational information obtained by molecular modeling studies and the binding energy calculations.

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